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EDITORIAL

Nephroprevention in the oldest old with chronic kidney disease: Special considerations

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Abstract

Nephroprevention strategies are crucial for handling chronic kidney disease (CKD) complications, and slowing its progression. However, these preventative measures should be guided by major geriatrics principles in order to help nephrologists to adequately handle the oldest old with CKD. These geriatric concepts consist of taking into account the relevance of choosing an individualized therapy, handling clinical frailty, and keeping a geriatric perspective which means that a good quality of life is sometimes a more important therapeutic objective in octogenarians than merely prolonging life. Even though nephroprevention strategies for treating the oldest old with CKD are basically similar to those applied to younger patients such as low sodium and protein diet, optimized hemoglobin levels, blood pressure and metabolic control, the treating physician or care provider must at all times be ready to make fundamental adjustments and tweak patient care paradigms and objectives if and when the initial therapeutic options applied have caused unintended clinical consequences and complications. Additionally, the sarcopenia status should also be evaluated and treated in very old CKD patients.

Key words: Oldest old; Very old; Nephroprevention; Chronic kidney disease; Chronic nephropathy

Core tip: Even though nephroprevention in the oldest old is basically similar to those applied to younger patients, it should be performed applying a geriatric perspective, where good quality of life is sometimes a more important therapeutic objective than merely prolonging life.

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INTRODUCTION

Nephroprevention strategies are crucial for handling chronic kidney disease (CKD) complications, and slowing its progression[1,2]. The term "CKD" is inter-

preted as a estimated glomerular filtration rate < 60 mL/min per 1.73 m² measured by the modification of diet in renal disease equation and/or presence of proteinuria, at least 1+, on dipstick urinalysis[3]. The term "nephroprevention" is defined as all those habits, diets, and medications which are currently proposed as useful therapeutic tools for achieving this purpose, such as avoidance of sedentary lifestyles, smoking, high sodium and high protein diets, as well as effectively managing disease states such as hypertension, dyslipidemia, hyperglycemia, and hyperparathyroidism[1,2]. However, these preventative measures should be guided by major evidence-based geriatrics concepts in order to help nephrologists to adequately handle oldest old people with CKD[2,4-9]. In this article, the "oldest old" is defined as people older than 79 years, according to the definition adopted by the most relevant literature in this field[10,11]. The first geriatric concept involved in this care model consists of the relevance of choosing an individualized therapy since treatment outcomes in the oldest old are influenced by many clinical variables which can persuade nephrologists to use alternate therapeutic approaches for treating patients in this category. Such variables include changes secondary to ageing (immunesenescence, reduced glomerular filtration rate and reduced hepatic metabolism), polypharmacy (use of $\geqslant$ 6 medications), prevailing elderly diseases (depression, visual and hearing impairment), and the concomitant presence of other geriatric syndromes (delirium, falls, and postration)[7,12-16].

The second geriatric concept consists of prescribing treatment paradigms under a geriatric perspective. This means that a good quality of life is sometimes a more important therapeutic objective in octogenarians, than merely achieving a lower mortality[2]. This does not mean that very old patients should be undertreated but that their treatment should be adjusted to their real biological expectations, while being cognizant of the increased potential for therapeutic adverse effects[2]. Finally, the third geriatric concept refers to the imperative for taking into account the notion of clinical frailty in the elderly. Frailty is an entity which appears as a consequence of many causes and it is characterized by a reduction in strength and endurance, making people prone to lose autonomy and to die[9]. Such therapeutic strategies are based on the prescription of low intensity resistance and aerobic physical exercises, together with adequate nutrition, appropriate vitamin D supplementation, and the avoidance of polypharmacy, all of which measures may help prevent or delay the onset of this syndrome[9,15,17].

In the present article, we have expanded on the following particular therapeutic targets to include the following-dietary salt, serum hemoglobin, blood pressure, glycemic control and lipid management-in the oldest old with CKD patients (Table 1).

Table 1 Therapeutic targets for oldest old chronic kidney disease patients

	Targets
Diet	Low-normal sodium Low-normal protein
Hemoglobin (g/dL)	11-12
Blood pressure (mmHg)	150/140-80
Hemoglobin A1C (%)	7-8.5

DIETARY SALT

There is a trend to sodium urine loss in the elderly due to their reduced sodium reabsorption capability at the thick ascending limb of the loop of Henle and collecting tubules[18]. Consequently, it is important to take into account that when the oldest old become salt restricted (50 mmol/d), they may develop hyponatremia (senile sodium leakage hyponatremia), volume depletion (ortostatism, hypotension), and even acute renal failure[19,20]. Whereas low sodium diet is one of the cardinal features of nephroprevention[19], this paradigm of care, when applied to the oldest old should be followed by monitoring blood pressure, serum sodium level, and renal function in order to rule out any of the above mentioned complications. If and when such unintended consequences are detected, a normal sodium diet would then be a better prescription for the specific oldest old patient with CKD[19,20].

SERUM HEMOGLOBIN

It has been reported in the literature that the presence of anemia can exacerbate several existing geriatric syndromes together with exaggerating neurocognitive dysfunction[21]. Therefore the oldest old often do not tolerate reduced serum hemoglobin levels as such low hemoglobin levels could negatively impact on gerontological functional test [activities of daily living (ADL) and instrumental ADK], and furthermore lead to an increased tendency to develop delirium and/or falls[22,23]. Thus, the application of a target serum hemoglobin level of 11 g/dL, or less, as part of nephroprevention strategy in the oldest old must be followed by monitoring their cognition status and gait pattern in order to exclude de novo appearance of those geriatric syndromes that are enhanced by anemia[21-24]. If this situation is documented in a particular older old with CKD, a higher serum hemoglobin target (11.5-12 g/dL) will therefore represent a reasonable alternative of care.

BLOOD PRESSURE

Meta-analysis of observational studies indicate that the incidence of stroke, myocardial infarction, and overall mortality increased with increasing blood pressure in

old and vey old patients, although the observed relative risk decreased with increasing age[25]. Additionally, the INVEST study highlighted a J-shaped relationship between systolic and diastolic blood pressure and outcomes in hypertensive old people suffering from coronary arterial disease[25]. The risk of mortality in patients aged ⩾ 80 years increased when systolic blood pressure was < 140 mmHg or diastolic blood pressure < 70 mmHg[25]. Although it has been documented that anti-hypertensive treatment in the oldest old was associated with a reduction in the frequency of strokes and major cardiac events, there was however no benefit in cardiovascular death nor in general mortality[26]. Furthermore, the evidence-base provided by several studies (INVEST, STONE, HYVET) is reassuring regarding targeting relatively higher blood pressure levels in the very elderly-blood pressure target < 150-80 mmHg-although these aforementioned studies did not specifically address CKD patients[25]. Nonetheless, it has been recommended that target blood pressure in the oldest old with CKD should be < 150/90 mmHg in non-albuminuric patients, and < 140/80 mmHg in abuminuric ones[25]. Very importantly, these blood pressure goals should be reached gradually, and the treating physician must always take into account each individual patient´s comorbidities[7,17]. This is to avoid the interdependence phenomenon between diseases (comorbidities worsen each others) usually observed in the elderly and their reduced tolerance to medication; since treatment of hypertension in the oldest old can induce orthostatic hypotension, falls with bone fractures, and the exacerbation of renal failure which sometimes then is not reversible on drug discontinuation[20,27-46]. In this sense, it has been reported in the literature a clinical entity termed "normotensive acute renal failure" which consists of an acute GFR deterioration in CKD elderly when their blood pressure is reduced to normal range. This phenomenon has been attributed to reduced kidney perfusion secondary to senile renal dysautonomy[47]. Moreover, it is worth noting here that concomitant sodium sensitivity and endothelial dysfunction are increased in the very elderly population, and therefore low sodium diet (used with caution) and exogenous nitric oxide donors are often useful for treating resistant hypertension in this group[17]. Other antihypertensive drugs such as thiazides, angiotensin converting enzyme inhibitors (ACEI), angiotensin Ⅱ receptor blockers (ARB), and aldosterone antagonists should be used with caution in this population, more so when GFR is below 30 mL/min per 1.73 m² due to the risk of further GFR reduction precipitating renal failure which may be irreversible, together with the complicating electrolytes and acid-base disorders[20,25-48]. The syndrome of rapid onset end stage renal disease (SORO-ESRD), a new syndrome of unanticipated acute yet irreversible ESRD, which we first described in 2010, is known to be more prevalent in the older CKD patient, and is associated with exposure to nephrotoxic agents including ACEIs and ARBs[37,38,42,43].

HEMOGLOBIN A1C

Elderly people are at high risk for developing diabetes mellitus because of the following two mechanisms: insulin resistance and pancreatic islet senile dysfunction[49,50]. Besides, ageing alters the counter-regulatory responses to hypoglycemia in non-diabetic people; furthermore during hypoglycemic episodes, symptoms begin at higher levels of glycemia and they are more intense in young people, while psychomotor coordination is more affected in old individuals[50] Additionally, diabetes mellitus is usually associated with high comorbidity in old people, and this subgroup cannot obtain cardiovascular benefit from strict glycemia control[49,50]. Moreover, therapeutic strategies with less stringent A1c levels are therefore needed in the oldest old diabetic patients since this subgroup of patients assist double to the hospital due to hypoglycemia episodes than the general diabetic patients, and it has also been documented that hypoglycemia is related to cognitive impairment in the elderly[49,50]. Thus, the consensus recommendation is a hemoglobin A1c target < 8% for elderly patients (not < 7% as is usually recommended for young adults) or for those patients with major complications and/or comorbid conditions[50]. Finally, a hemoglobin A1C target of 8%-9% has been recommended for patients with low life expectancy (⩽ 5 years)[50].

LIPID METABOLISM

Regarding lipid lowering therapy in this population, an interesting study in very elderly patients documented a 15% reduction in coronary events with pravastatin. This suggests, that this drug can be prescribed in the oldest old suffering from diabetes mellitus except in those with very poor life expectancy[50].

MISCELLANEOUS-PROTEIN DIET AND EXERCISE

Although energy needs decline with age, very elderly people can be exposed to malnutrition because of anorexia, impaired taste and smell, chewing and swallowing problems, geriatric syndromes and senile prevalent comorbidities which lead to difficulties for cooking and eating[24,50]. Because of the above predicated reasons, caution must be employed when overly restrictive eating patterns (including renal sparing low protein diet) are applied since such practices may further contribute to malnutrition in the oldest old with CKD. The interventions for improving nutritional status in the oldest old patients consist of using smaller but more frequent and fortified portions of food, and/or adding nutrition supplements between meals[24,50]. Additionally,

senile sarcopenia is a prevalent entity which can worsen with low protein ingestion, as well as with other ageing-associated comorbidities, such as diabetes mellitus[51]. Conversely, adequate physical activity, adjusted to the individual patient´s clinical situation, can further improve functional status even in patients with poor health status[50,51].

OVERALL MORTALITY IN VERY ELDERLY PEOPLE

Even though, there is some limitation of ascribing a single pathology as the cause of death in very elderly people since concomitant multiple diseases are very common in this age group, many studies have documented cardiovascular (31%-54%), oncologic (20%-25%), and respiratory (10%-15%) diseases as the first, second, and third causes of death, respectively[10,24]. However, studies performed in centenarians (people older than 90 years) have documented respiratory disease (48%-52%), particularly pneumonia, as the main cause of death. Besides, it has been observed that the higher cardiovascular causes of death in the very elderly people are acute myocardial infarction, and cardiac insufficiency secondary to cumulative damage from ischemic heart disease. Regarding oncologic diseases, malignancies of the digestive tract: primarily gastric, esophageal, and colorectal cancer, are the most frequent oncologic causes of death in very old people. Additionally, the remaining major causes of death in very elderly people are: cerebrovascular disease, Alzheimer´s disease and related dementias of later life[10,24].

CONCLUSION

Even though nephroprevention strategies for treating the oldest old with CKD are basically similar to those applied to younger patients, it is recommended that the managing physician must always individualize patient care. The treating physician or care provider must at all times be ready to readjust and tweak care paradigms and objectives if and when the initial therapeutic options applied have caused unintended clinical consequences and complications.

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REVIEW

Combined functional and anatomical diagnostic endpoints for assessing arteriovenous fistula dysfunction

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Abstract

Failure of arteriovenous fistulas (AVF) to mature and thrombosis in matured fistulas have been the major causes of morbidity and mortality in hemodialysis patients. Stenosis, which occurs due to adverse remodeling in AVFs, is one of the major underlying factors under both scenarios. Early diagnosis of a stenosis in an AVF can provide an opportunity to intervene in a timely manner for either assisting the maturation process or avoiding the thrombosis. The goal of surveillance strategies was to supplement the clinical evaluation (*i.e.*, physical examination) of the AVF for better and earlier diagnosis of a developing stenosis. Surveillance strategies were mainly based on measurement of functional hemodynamic endpoints, including blood flow (Q_a) to the vascular access and venous access pressure (VAP). As the changes in arterial pressure (MAP) affects the level of VAP, the ratio of VAP to MAP (VAPR = VAP/MAP) was used for diagnosis. A Q_a < 400-500 mL/min or a VAPR > 0.55 is considered sign of significant stenosis, which requires immediate intervention. However, due to the complex nature of AVFs, the surveillance strategies have failed to consistently detect stenosis under different scenarios. VAPR has been primarily developed to detect outflow stenosis in arteriovenous grafts, and it hasn't been successful in accurate diagnosis of outflow lesions in AVFs. Similarly, AVFs can maintain relatively high blood flow despite the presence of a significant outflow stenosis and thus, Q_a has been found to be a better predictor of only inflow lesions. Similar shortcomings have been reported in the detection of functional severity of coronary stenosis using diagnostic endpoints that were based on either flow or pressure. This limitation has been associated with the fact that both pressure and flow change in the presence of a stenosis and thus, hemodynamic diagnostic endpoints that employ only one of these parameters are inherently prone to inaccuracies. Recent attempts have resulted in development of new diagnostic endpoints that can combine the effects of pressure and flow. These new hemodynamic diagnostic endpoints have shown to be better predictors of functional severity of lesions as compared to either flow or pressure based counterparts. In this review article, we discussed the advantages and limitations of current functional and anatomical diagnostic endpoints in AVFs.

Key words: Arteriovenous fistula; Dysfunctional arteriovenous fistulas; Stenosis; Surveillance; Flow rate;

Pressure

Core tip: Current surveillance strategies are based on either flow (Q_a) or pressure (VAPR) measurements. The Q_a has only shown to be a good predictor of inflow stenosis in arteriovenous fistulas (AVFs). The VAPR was primarily developed to detect outflow stenosis in arteriovenous grafts and has shown to be a poor predictor of stenosis in AVFs. These limitations have been associated with the fact that both pressure and flow change in the presence of a stenosis and thus, hemodynamic diagnostic endpoints that employ only one of these parameters are inherently prone to inaccuracies. Thus, diagnostic endpoints that can combine both effects of pressure and flow can provide better assessment of stenosis severity in AVFs.

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INTRODUCTION

Around 600000 Americans have end-stage renal disease, among whom approximately 415000 patients are being treated by hemodialysis through surgically created vascular access (VA)[1]. Failure in maintaining a functional VA has been the leading cause of hospitalization in the hemodialysis population and has resulted in more than $1 billion annual cost to the health care system in the United States[1]. The most preferred form of the VA is the arteriovenous fistula (AVF); however, this type of access has still a significantly high failure rate (20% to 50% in the United States). AVF failure requires placement of central venous catheter which is the least desirable form of VA due to its significant morbidity and mortality[2,3].

Thrombosis is the major cause of failure in AVFs, which requires endovascular or surgical intervention of the access[4-7]. The majority of AVFs with thrombosis had an underlying stenosis[8-10]. A developing stenosis gradually reduces the blood flow to the access and alters the pressure in the AVF. The National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF-KDOQI)[11,12] has recommended routine surveillance to detect the stenosis early enough to allow preemptive interventions. Current surveillance strategies[13] include device-based measurements, such as blood flow to AVF or venous access pressure. Under the current KDOQI guidelines[12], an AVF with blood flow rate < 400-500 mL/min or a ratio of venous access pressure to main arterial pressure > 0.55 has to be referred to fistulography for assessing the grade and location of the stenosis.

The accuracy of the current surveillance strategies for detecting a stenosis is debatable, especially in AVFs[14-17]. These limitations can be associated with specific pathophysiology of AVFs[8,10]. AVFs can have a significant stenosis and still maintain a relatively high blood flow[18]. In such scenarios, blood flow to AVF gradually reduces over time. Thus, sequential (longitudinal) measurements of Q_a over time were expected to provide better clinical decision making than a single Q_a measurement. Also in case of a significant outflow stenosis, the venous access pressure may retain its normal levels at the cannulation site due to development of downstream collateral pathways[8,10,15,19]. It is noteworthy that similar shortcomings have also been reported in functional (hemodynamic) diagnosis of coronary stenosis. Such diagnostic endpoints for coronary artery disease were either based on pressure or flow; however this neglects the fact that both flow and pressure change in the presence of a stenosis. Recent studies have attempted to shift the current paradigm into introducing new diagnostic endpoints that can account for both pressure and flow variation for assessing the functional severity of a stenosis[20-24]. This review article describes the advantages and limitations of current surveillance strategies including functional and anatomical diagnostic endpoints in AVFs.

This review has covered the most important and pioneering studies that have reported the use of hemodynamic and anatomical endpoints for assessing the AVF functionality. We performed a literature search using PubMed, Medline, and Google Scholar for studies written in English from 1995 to 2014. We used the following search terms: arteriovenous fistula; surveillance strategies; stenosis; flow rate; pressure; coronary flow reserve; fractional flow reserve; pressure drop coefficient; resistance index, in combination with the exploded term "diagnosis". The references were included based on their relevance and contribution to address the challenges and future directions in the diagnostic field of stenosis linked to AVF.

AVF MATURATION, FUNCTIONALITY, AND DYSFUNCTION

According to the guidelines of NKF-KDOQI[11,12], the venous segment of a matured AVF should follow the rules of sixes: a blood flow > 600 mL/min, a diameter > 6 mm, a depth of around 6 mm, and at least 6 cm of a straight segment for cannulation. Normally, a minimum of 28 d (d: days) should be allowed for AVF maturation before performing the first needling; however, this time could be extended if the AVF fails to mature. Once an AVF is used for cannulation, it should be able to provide a minimum flow rate of 350-450 mL/min during 3-5 h of dialysis without recirculation, a characteristic that defines a functional AVF. A dysfunctional AVF is, however, defined as an access that is not able to provide the minimum flow during dialysis and is clinically identified by variations in thrill/bruit, difficult cannulation, recirculation, excessive bleeding from the venopuncture sites and

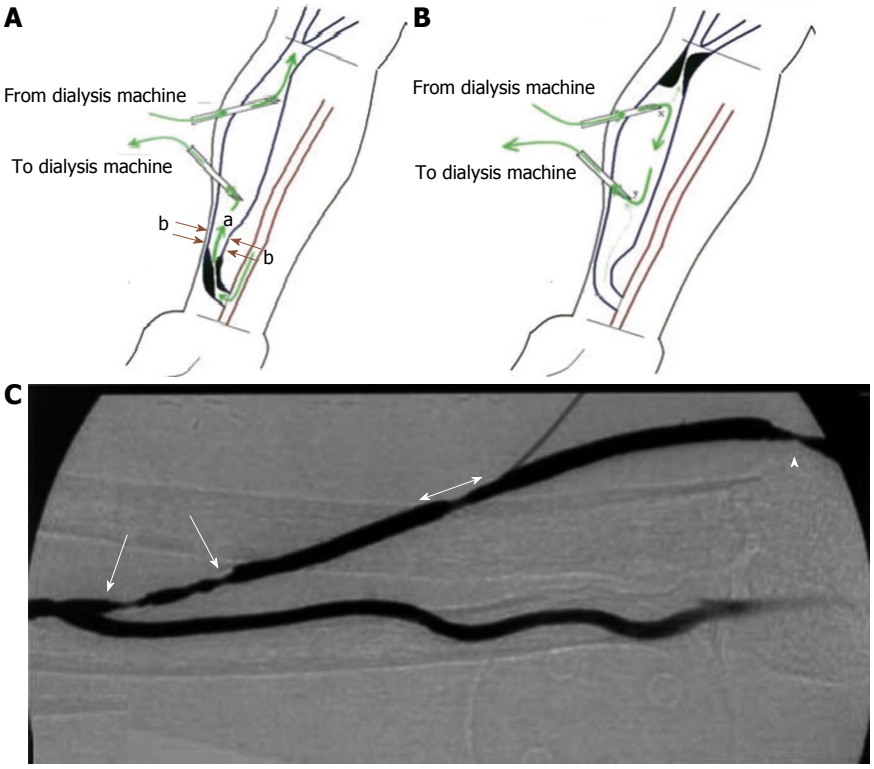

Figure 1 Schematic of the locations of (A) inflow stenosis, and (B) outflow lesion with respect to the anastomosis and cannulation sites[27]. The (green) arrows in A and B represent the direction of blood flow within the arteriovenous fistulas (AVF) and the dialysis needles; B: Depicts a recirculation condition under which due to significant outflow stenosis the blood flow from dialysis machine returns back to dialyzer; C: An angiographic picture of an AVF with multiple inflow stenoses (single head arrows) and outflow stenosis (double head arrow and the arrow head). Reprinted from Asif *et al*[25] and Fahrtash *et al*[27], with permission.

ultimately thrombosis. Thrombosis, the main cause of access failure, is usually preceded by the development of an underlying stenosis. Consequently, the detection of stenosis in AVFs before thrombosis could offer a strategy to improve AVF survival by early intervention.

Stenosis in vascular access

Stenosis in vascular access can be categorized into two main groups of inflow and outflow stenoses[9,25]. An inflow stenosis is a lesion that occurs around the anastomosis and proximal to the venous needle, while an outflow stenosis is located further from anastomosis and distal to the venous needle. The stenosis location has been shown to be dependent on the type of access. Radio-cephalic AVFs are more prone to inflow stenosis, whereas outflow stenosis is more likely to occur in the brachio-cephalic AVFs. In contrast to AVFs, the arteriovenous grafts (AVGs) mostly develop outflow stenosis[26]. Figure 1A[27] and 1B[27] show the schematics of an inflow and outflow stenosis with respect to the anastomosis and cannulation sites, respectively, while Figure 1C[25] shows an angiographic picture of an AVF with multiple inflow and outflow lesions. All the clinical monitoring and surveillance programs have been designed to predict the development of a significant stenosis early enough to allow preemptive corrections of AVFs. Despite the importance of stenosis severity, its definition is still controversial[27,28]. A significant stenosis has been defined as a local reduction of > 50% in luminal diameter as compared to the adjacent normal vessel. This definition is inherently biased and is dependent on the location of the reference cross-section in the adjacent normal vessel (Figure 2[27]). Also, imaging techniques such as Doppler ultrasound only provides a 2D illustration of a 3D lesion, while other imaging modalities such as computed tomography (CT)-scan or magnetic resonance imaging (MRI) are expensive and not readily accessible. Despite these drawbacks, the stenosis severity still serves as the most important endpoint to direct the clinical decision making for the timing of further interventions.

MONITORING AND SURVEILLANCE PROGRAMS

Monitoring strategies mainly include physical examination (PE) and other clinical evidences of access dysfunction for stenosis detection, while surveillance programs were intended to supplement clinical monitoring by measuring variations in blood flow rate and venous access pressure. The PE, backbone of all screening programs, is a readily available and cost-effective tool to detect inflow and outflow stenosis in AVFs. PE has proved to be an accurate predictor of venous stenosis, and several studies have concluded that PE should be the part of all screening programs[29]. The only drawback of PE is the need for

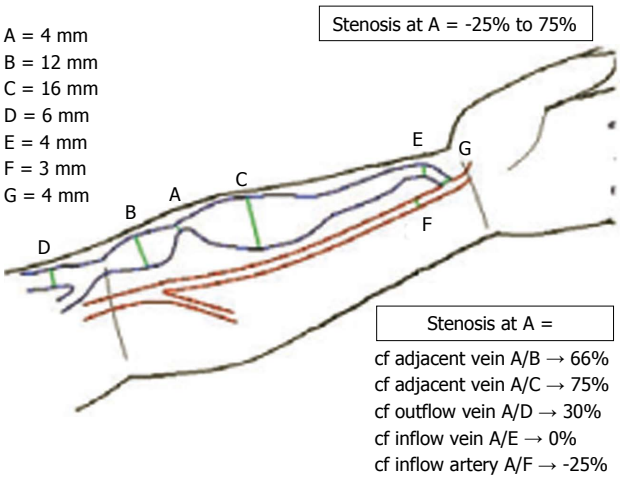

Figure 2 Dependency of stenosis severity to the location of reference cross sec-tion[27]. In this figure stenosis is located at A. Depending on the location of the reference cross-section, one can calculate a stenosis severity ranging from -25% to 75%. The -25% diameter changes represents a case in which the minimum diameter in the arteriovenous fistulas circuit has happened elsewhere than cross-section A. Reprinted from Fahrtash *et al*[27], with permission.

trained and experienced dialysis personals, leading to variability in decision making.

FUNCTIONAL (HEMODYNAMIC) ENDPOINTS

A developing stenosis eventually reduces blood flow and alters pressure profiles in the vascular access. Effects of stenosis on hemodynamic (blood flow and pressure) profiles are dependent on the type of vascular access (AVF or AVG) and the location of stenosis (inflow, outflow, or both). Therefore, monitoring the changes in flow and pressure can provide useful functional information on the severity of the underlying stenosis. NKF-KDOQI guidelines have recommended that both AVGs and AVFs undergo routine surveillance for blood flow and venous access pressure measurements.

Flow surveillance

The blood flow (Q_a) measurement is currently the gold standard of all surveillance programs. The Q_a measurement has been shown to be fairly reproducible both within and between the dialysis sessions. Blood flow rate can be measured by either indirect techniques such as ultrasound dilution or direct methods such as Doppler ultrasound, or MRI. The latter can provide valuable information about the location and severity of stenosis; however, it has the disadvantage of being more expensive and time consuming. Figure 3[8,9] shows a sample velocity pulse obtained from Doppler ultrasound as well as an example of detected stenosis using such technique. Although Q_a has been widely used as the most reliable surveillance strategy, there are numerous different Q_a thresholds for clinical decision makings in AVFs[30]. A wide range of Q_a from 300 mL/min to 900 mL/min[31-35] has been reported in different studies as the threshold for intervention. Under current KDOQI guidelines, an AVF should be referred for fistulogram when Q_a < 400-500 mL/min.

Polkinghorne *et al*[15] looked at the effectiveness of the KDOQI guideline for Q_a (< 500 mL/min) to detect a significant stenosis in 137 patients with AVF. These patients were randomly assigned to a control group receiving standard-of-care clinical treatment and Q_a surveillance group that received the same treatment as the control group plus monthly Q_a measurement. Under the normal treatment, an AVF was referred to fistulography if any of the following occurred: (1) raised venous dynamic pressure; (2) reduced blood pump flow; or (3) excessive bleeding from venopuncture site. They showed that the likelihood of stenosis detection in the Q_a surveillance group was two times but insignificant in relation to the control group, with a trend for a significant stenosis to be detected earlier. However, they also showed that over reliance on only blood flow threshold < 500 mL/min could misdiagnose some cases with positive sign of stenosis under standard-of-care treatment and angiography. They concluded that this misdiagnosis could be due to lack of understanding of the relationship between the blood flow in the vascular access and a developing stenosis.

Tessitore *et al*[33] tested different surveillance techniques such as PE, venous access pressure ratio, recirculation, and Q_a on a random population of 119 matured AVFs to find the ability and accuracy of these methods in detecting a significant stenosis. In addition to the surveillance methods, all patients underwent angiography to identify the grade of stenosis in the AVFs. Almost 50% of the AVFs had a significant stenosis either upstream of venous needle (inflow stenosis) or downstream of venous needle (outflow stenosis) or at both sites. A combination of PE and Q_a < 650 mL/min was able to provide a moderate-to-excellent tool to detect inflow stenosis with sensitivity of 85% and specificity of 89%. However, Q_a was not determined to be an adequate predictor of outflow stenosis. Therefore, they concluded that accuracy of Q_a to detect a significant stenosis is strongly dependent on the location of lesion.

Moreover, a randomized study[36] on 58 patients showed that preemptive intervention for AVFs with a Q_a > 500 mL/min results in 3-fold reduction in thrombosis and loss of vascular access as compared to the KDOQI guideline (Q_a < 400-500 mL/min) that is more suitable to detect a hemodynamically significant stenosis. Therefore, the current Q_a surveillance for AVF needs modification for improved detection of a significant stenosis well before it adversely affects the functional or hemodynamic condition.

Pressure surveillance

Besarab *et al*[37,38] proposed the use of venous access pressure to predict the stenosis severity in AVGs. Figure 4[38] shows the schematic for the measurement of venous access pressure using pressure transducers at the

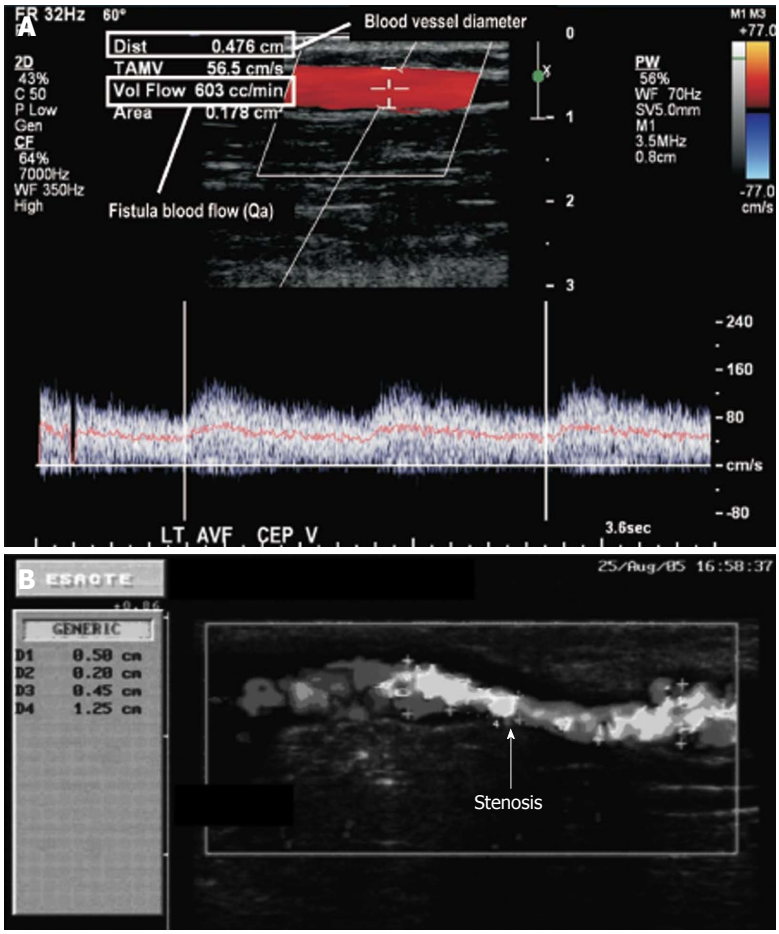

Figure 3 (A) Velocity pulse from Doppler ultrasound, and (B) detection of stenosis and estimating its grade using Doppler ultrasound[8,9]. AVF: Arteriovenous fistula. Reprinted from Campos *et al*[8] and Feddersen *et al*[9], with permission.

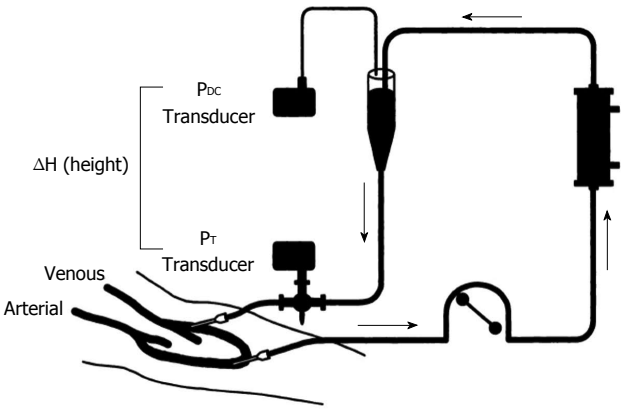

Figure 4 Schematic for measurement of venous access pressure in an arteriovenous graft using pressure transducers located at the venous needle and drip chamber[38]. Reprinted from Besarab *et al*[38], with permission.

venous needle and also at the drip chamber of dialysis circuit. Further with the development of this technique, the ratio of venous access pressure (VAP) to the mean arterial pressure (MAP) was used for detecting the stenosis severity (VAPR = VAP/MAP). A VAPR > 0.55 was associated with a clinically significant stenosis. In a prospective study, Besarab *et al*[39] monitored the variation of VAPR on 832 patients among whom 80% of accesses were AVG. The VAPR > 0.55 was found to be an excellent criterion for angiographic referral and intervention of a clinical stenosis in AVGs. It should be noted that VAPR was primarily developed to detect outflow stenosis in AVGs and by design was unable to detect an inflow stenosis[10]. It should be noted that AVGs are more prone to develop an outflow stenosis than an inflow lesion. In case of an inflow stenosis pressure drops in the access and consequently, VAPR remains below the cut-off level. Thus VAPR has been unsuccessful to detect the inflow lesions in AVGs.

Although, the VAPR is a promising parameter in AVGs[13,18,39,40], it has failed to show much advantage in assessing the functionality of AVFs[8,9,15]. This assertion originates from the fact that there is a fundamental difference between AVFs and AVGs. The AVG is essentially a single tube that connects the artery to vein, and thus, all the blood that enters the arterial anastomosis has to exit from the venous anastomosis. Therefore, any abnormal elevation in the pressure can be associated with the formation of stenosis mainly in the outflow segment. In contrast, the VAPR may show lesser variation while a stenosis is developing in an AVF because of the collateral pathways (accessory or collateral veins) that provide an alternative route to bypass the significant stenosis. Consequently, the flow in

the upstream venous segment of AVFs may not change even in the presence of downstream significant stenosis. In other words, the VAPR may undergo minimal changes as the flow can occur due to presence of downstream collateral channels.

The effect of stenosis on the flow and pressure fields is dependent on the location of stenosis. In general, an outflow stenosis causes an increase in the venous access pressure, while the access flow decreases over time. This is particularly more evident in an AVG than an AVF. In such scenario, an AVF can maintain a relatively high flow rate with almost unchanged pressure levels due to the development of collaterals. In the case of an inflow stenosis venous access pressure either remains stable or can decrease with the reduction in access flow under adverse remodeling. Therefore, pressure monitoring alone may not be able to detect such inflow stenosis, while it can be detected by sequential flow measurements and PE.

ANATOMICAL ENDPOINTS

Once an access is diagnosed for a significant stenosis either based on the standard-of-care clinical treatment or any of the functional surveillance strategies, a fistulogram is acquired to determine the grade and location of stenosis. However, as discussed earlier, there are a few criticisms to the current measurement protocol of the stenosis severity based on a fistulogram. These include: (1) fistulogram provides only a 2D illustration of 3D vessel; and (2) stenosis definition is biased to the location of the reference cross-section in the adjacent normal vessel for which the diameter varies a lot.

For example in a recent study, Fahrtash *et al*[27] criticized the current definition of stenosis severity and showed that, stenosis can have a wide range of severity from -25% to 75% reduction in luminal diameter based on the location of reference cross-section (Figure 2). Therefore, they hypothesized that a significant stenosis can be determined based on the absolute minimum diameter in the AVFs. They divided 170 radio-cephalic AVFs into two groups: dysfunctional (n = 93) and functional (n = 77) AVFs. The absolute minimum diameters of two groups were measured using grayscale and color ultrasound. They found that a diameter of 2.7 mm can be a good cutoff value to distinguish a functional radio-cephalic AVF from a dysfunctional one with 90% sensitivity and 80% specificity. Thus, it was concluded that a minimum diameter can be a more accurate measure to decide on the dysfunctionality of an AVF. However, this study was limited to only radio-cephalic AVFs and thus, more studies are needed to determine the critical minimum diameter for other types of AVFs.

Other studies[19,41-43] have primarily introduced the diameter as a pre-operative factor to predict if an AVF will mature. Current guidelines suggest a minimum diameter of 2 mm for successful AVF creation at wrist, but agreement on minimal diameter for other sites is lacking[19]. Lauvao *et al*[42] evaluated 158 patients undergoing initial dialysis access creation with native AVF. Three types of AVFs were created in these subjects including posterior radiocephalic AVF (n = 24), wrist radiocephalic AVF (n = 72), and brachiocephalic AVF (n = 62). Using multivariate logistic regression analysis, a vein diameter > 4 mm was found to be the only independent predictor of AVF maturation. However, a previous study by Wong *et al*[44] on 46 patients with initial radio-cephalic fistula suggested a diameter > 1.6 mm as the predictor of successful maturation of AVF. Therefore, despite its significance, there is still not a uniform agreement on the minimum diameter of AVF that can assist the clinical decision makings.

OTHER FUNCTIONAL ENDPOINTS

In addition to flow, pressure, and anatomical endpoints, wall shear stress has also been shown to have a strong correlation with functionality of AVFs. A multifold increase in blood flow rate after the AVF placement results in an extensive raise in the wall shear stress (WSS) levels acting on the luminal surface of the fistula. In order to accommodate for the marked increase in the hemodynamic stresses, the arterial and venous segments of the AVFs undergo structural changes such as vasodilation and wall hypertrophy[45-48]. These compensatory responses, also known as remodeling, attempt to regain the baseline levels of hemodynamic stresses (pre-surgery condition) in the vessels. Arterial remodeling in AVFs is mainly characterized by dilation and intima-media hypertrophy (outward hypertrophic remodeling)[49]. However, this adoptive remodeling in the venous segment can be interrupted by aggressive formation of neointimal hyperplasia, which can result in an undesired hypertrophy (thickening of the venous wall in the inward direction) and later venous stenosis, the major cause of failure in the AVFs. Therefore, monitoring the WSS levels can provide useful information on the functionality status of the AVFs.

Rajabi-Jaghargh *et al*[50] studied the linkage between the longitudinal changes in WSS and the luminal dilation for the AVFs in a pig model. Changes in the WSS levels within the AVFs were evaluated from the computational fluid dynamics models of the fistulas that were developed based on the acquired CT-scan and Doppler ultrasound data of the pigs at 2 d, 7 d, and 28 d post surgery time points. It was found that the slope of changes of WSS over time [τ' = ($WSS_{28\ d\ or\ 7\ d}$ - $WSS_{7\ d\ or\ 2\ d}$)/(time difference)] can be used to assess the functionality status in AVFs. The τ' for the AVFs with favorable remodeling (FR), as shown in Figure 5A[50], was negative between all the successive time-points representing a consistent decrease in the WSS levels over time. In contrast, for the AVFs with adverse remodeling (AR), Figure 5A, the τ' at all the successive time-points were positive showing that WSS levels were

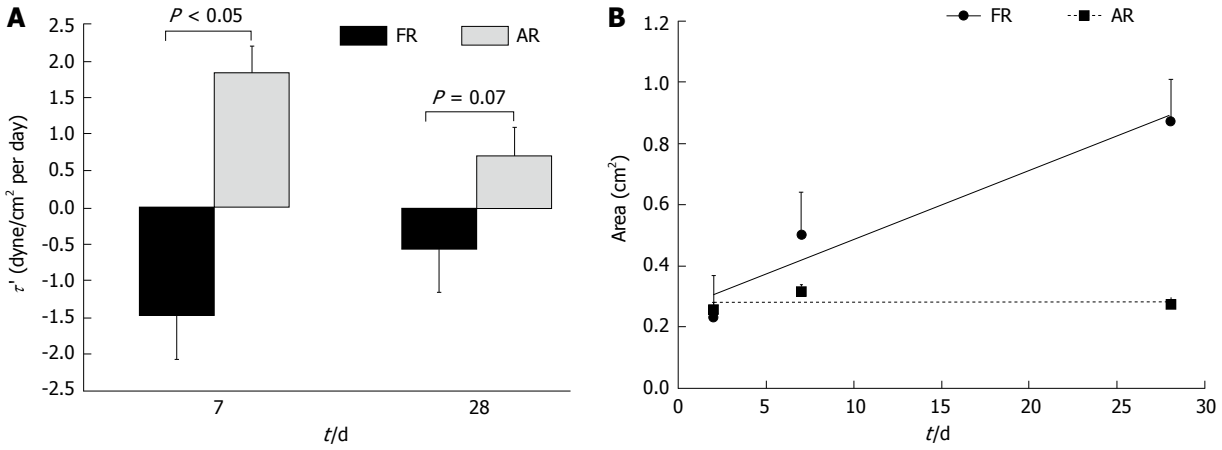

Figure 5 Variation in (A) temporal gradient of wall shear stress and (B) temporal gradient of luminal area of the venous segment for arteriovenous fistulas with favorable remodeling and adverse remodeling over time[50]. FR: Favorable remodeling; AR: Adverse remodeling. The *t*/d in the x axis stands for time (days). Reprinted from Rajabi-Jagahrgh *et al*[50], with permission.

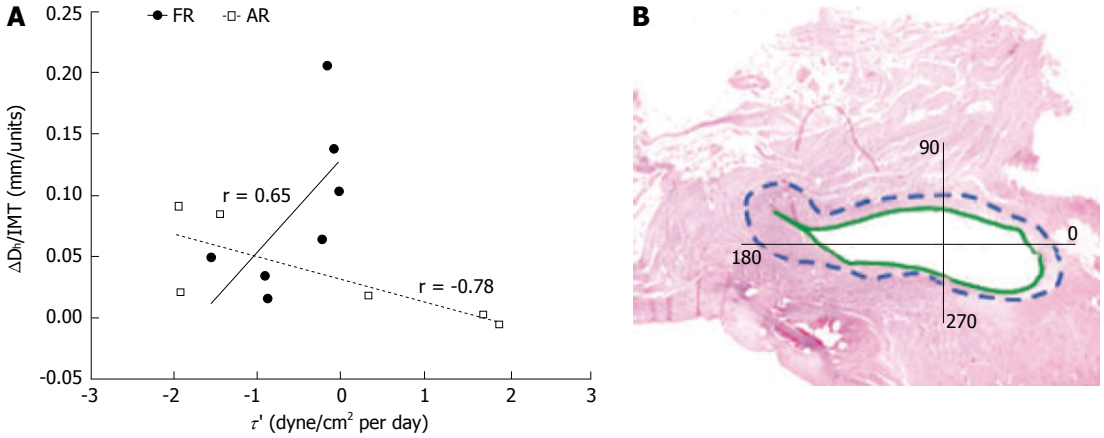

Figure 6 (A) Variation in morphological changes of the venous segment with respect to corresponding temporal gradient of wall shear stress (τ') for arteriovenous fistulas with favorable remodeling and adverse remodeling[51]. Morphological changes were quantified by calculating the ratio of differences in luminal diameter of venous segment over time (ΔD_h) to the corresponding amount of intima-media thickening (IMT). The IMT was calculated from (B) histology analysis using hematoxylin and eosin (H and E). The IMT was calculated as the average of intima-media thicknesses at four quadrants of each H and E staining slide. FR: Favorable remodeling; AR: Adverse remodeling; AVFs: Arteriovenous fistulas. Reprinted from Rajabi-Jagahrgh *et al*[51], with permission.

increasing over time for this group. These opposite patterns of WSS over time were accompanied with distinct remodeling behaviors within the two groups. The luminal area of the venous segment for the FR increased over time (Figure 5B), while for the AR the luminal area remained unchanged or reduced.

Also, in another study[51] by the same group, they showed that the temporal changes in shear stress can be correlated with morphological changes in the venous segment (Figure 6A). The morphological changes were quantified with the ratio of differences in diameter between successive time points (ΔD_h) by the amount of intima media thickness (IMT). The IMT was obtained from histology analysis (Figure 6B). The τ' showed distinct correlation between the AVFs with FR as compared to the ones with AR (Figure 6A). The positive τ' in the AR group was associated with the largest amount of IMT and lowest or negative ΔD_h, which also revealed stenosis formation in the venous segment. In contrast, the negative τ' was shown to be associated with relatively larger ΔD_h and larger IMT. This showed that the IMT in the AVFs with FR was in the outward direction as compared to the inward hypertrophy in AR group. Therefore, it was concluded that the increase in WSS of the venous segment of an AVF over time can be associated with adverse remodeling and reduced functionality, while the decrease in WSS over time can be considered a sign of favorable remodeling.

Although WSS has a key role on remodeling behavior and functionality of AVFs, the complexities and limitations associated with the accurate calculation of WSS under clinical settings have made it an undesirable clinical endpoint for surveillance strategies. Therefore, the main focus of this review is to introduce new diagnostic tools that can be readily available under current clinical settings such as flow, pressure, and anatomical endpoints. These endpoints, especially flow and pressure, have also been the main focus of surveillance strategies. However, as mentioned earlier the ability of current surveillance strategies to predict the functionality

status of AVFs has been controversial. This limitation can be associated with the fact that Q_a is based on only flow measurements, while the VAPR is a pressure based parameter. However, it may be noted that both flow and pressure change under a developing stenosis. Therefore, relying on parameters that are either based on pressure or flow can result in inaccurate and less than optimal decision making outcomes. Consequently, there is a need for new functional diagnostic endpoints that can combine both effects of pressure and flow.

FUNCTIONAL DIAGNOSTIC PARAMETERS FOR CARDIOVASCULAR AND RENAL STENOSIS

Adequacy of diagnostic tools that are based on either pressure or flow has been one of the major challenges for assessing the functional severity of stenosis in vasculatures such as coronary and renal arteries[52,53]. In this section, the limitations of current hemodynamic (pressure or flow) based endpoints for detecting the cardiovascular and renal stenoses will be discussed. Also, the combined functional (pressure and flow) endpoints for better detection of stenosis in vasculatures will be introduced.

Existing cardiovascular and renal diagnostic endpoints

Currently, fractional flow reserve (FFR; a pressure ratio) and coronary flow reserve (CFR; a flow ratio) are the two gold standards to assess the functional severity of a stenosis in coronary arteries[54]. FFR is the ratio of mean pressure distal to a stenosis to the mean proximal pressure under hyperemic condition. CFR is the ratio of blood flow rate to a diseased vessel under hyperemic condition to the corresponding basal (non-hyperemic or resting) flow. The values of FFR and CFR decrease as the stenosis severity increases. However, both FFR and CFR are affected not only by the stenosis severity but also by distal microvascular flow resistance that can increase under left ventricle hypertrophy, chronic or acute ischemia, diabetes mellitus, and other disease conditions[55-57]. If microvascular resistance is abnormal (high), then CFR decreases while FFR tends to increase. Under such scenario, FFR may be incorrectly above the cut-off (0.75-0.8) range despite the existence of a significant stenosis[58,59]. This may lead to inaccurate diagnosis which can result in either delay or missing of intervention procedure. Similar limitations exist for current KDOQI surveillance strategies to detect an outflow stenosis. Also, in the presence of collateral channels both CFR and FFR increase which result in uncertainty in diagnostics[60]. This is also similar to the scenario of an outflow stenosis with developed collateral channels in AVFs. These limitations have been associated with the inherent inaccuracies of current diagnostic parameters to detect stenosis severity that are based on either pressure or flow measurements. Such gap has resulted in recent attempts to introduce better diagnostic tools that can combine the effects of pressure and flow[61-64]. The new diagnostic endpoints rely on fluid dynamics principals that are based on non-linear relationship between pressure and flow in the presence of a developing stenosis.

Pressure-flow relationship in stenosed vessels

Based on fluid mechanics fundamentals, pressure drop for an incompressible (blood) flow inside a vessel is a function of frictional forces (viscous losses) and losses due to momentum changes. The latter can be induced by variation in luminal diameter (or area) of the vessel (*i.e.*, as a result of a stenosis), or presence of bends, bifurcations, anastomosis, and, *etc*. The viscous forces are linear function of flow, while the momentum changes have quadratic (non-linear) relationship with flow. In general, the pressure drop-flow relationship can be written as below:

$$\Delta p = Av + Bv^2 \quad (1)$$

where A and B represent the coefficients of viscous losses, and losses due to momentum changes, respectively. Equation 1 can be also written in a more general form as below:

$$\Delta p = kv^n \quad (2)$$

where n can vary between 1 and 2. The exponent in this relationship is of specific importance. For a fully developed laminar flow in a straight vessel, viscous losses are the main component of pressure drop. For such flows, n is nearly equal to 1. However, if the momentum of flow changes due to change in luminal diameter (*i.e.*, as a result of a stenosis), or due to existence of bends, bifurcations, or anastomosis, then the exponent of Δp-v relationship will become greater than 1. As the exponent becomes closer to 2, the contribution of momentum changes to pressure drop become more pronounced.

Using analytical formulations, Rajabi-Jaghargh *et al*[65] have shown that at early stages of stenosis, viscous losses are the most dominant component of pressure drop and n stays closer to 1. However, as the stenosis severity increases from 64% to 90% area stenosis the contribution of momentum changes to pressure drop becomes more pronounced and n will be > 1.5. Figure 7[65] shows the contribution of viscous losses and losses due to momentum changes to the total pressure drop in a stenosed artery with the increase in the percentage area stenosis. The n is equal to 1.5 at the point where the viscous losses and momentum changes contribute equally to the total pressure drop. For n < 1.5, the contribution of viscous losses are more, while losses due to momentum changes are more pronounced for n > 1.5. In case of an AVF, anastomosis segment imposes a local pressure loss due to momentum changes (blood flow acceleration in the bend), and thus Δp-v relationship is expected to have an exponent > 1. Although the Δp-v relationship does not directly help us to evaluate the functionality of an AVF, analyzing the exponent of Δp-v

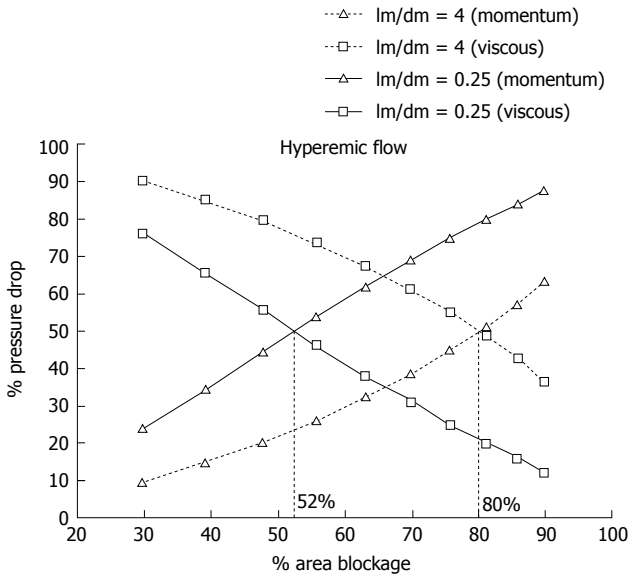

Figure 7 Contribution of viscous losses and losses due to momentum changes to the total pressure drop in a stenosed coronary artery with the increase in the percentage area stenosis[65]. Here, the pressure drop values at different stenosis severity were ob-tained under the corresponding hyperemic flow for coronary arteries. Also, lm and dm represent the length of stenosis throat and diameter of throat, respectively. Reprinted from ref. [65], with permission.

relationship can identify the contribution of viscous or momentum losses to the total pressure drop and the underlying geometrical variations (diameter or area change) in the AVF.

COMBINED FUNCTIONAL DIAGNOSTIC ENDPOINTS

Based on the pressure-flow relationship in stenosed vessels, the new functional diagnostic parameters can be defined as (1) resistance index, which represents the linear scaling of pressure drop with the velocity at the proximal artery ($R = \Delta p/v$); and (2) pressure drop coefficient, which is a pressure drop normalized by the dynamic pressure at the proximal artery ($C_p = \Delta p/(0.5\rho v^2)$, where ρ is blood density). The resistance index would be more helpful to predict a developing stenosis in early stages where *n* is closer to 1, while the pressure drop coefficient becomes more important as the stenosis becomes more severe over time in which case *n* is closer to 2. Both R and C_p have been shown to be better predictors of the functional severity of coronary stenosis under the limiting scenarios (*i.e.*, micro-vascular disease and collateral channels) as compared to FFR and CFR, the current gold standards[66-69].

Combined functional diagnostic endpoints in AVFs

The new functional diagnostic endpoints (resistance index, *R*, and pressure drop coefficient, C_p) have been recently[70] used in a pilot study on a pig model. It was shown that these parameters are capable of detecting the very early signs of a developing stenosis in AVFs. In this study, six AVFs were created between the femoral arteries and veins of 3 pigs, each pig having two AVFs on either limb. The variation in flow rates and geometries of the AVFs were studied at three post-surgery time points (2 d, 7 d, and 28 d) over about one month using Doppler ultrasound and CT-scan techniques. Also, computational fluid dynamics were used to calculate the pressure and velocity profiles for all the AVFs at every time points. Time averaged pressure difference between the proximal artery and outflow vein in conjunction with the average velocity at proximal artery were used to calculate C_p and *R* in AVFs. During the first week, all AVFs attained favorable remodeling[50,51] characterized by dilation, significant increase in flow rate, unchanged pressure drop, reduction in severity of local stenosis, and some amount of thickening due to the development of intimal hyperplasia. These changes were associated with the reduction in C_p and R levels over the first week (Figure 8).

In contrast, from 7 d to 28 d some of the AVFs showed significant dilation, while others experienced minimal changes in the mean diameter. During this period, the amount of thickening was also doubled in all the AVFs. Therefore, the minimal dilation and high amount of IMT from 7 d to 28 d for some AVFs resulted in adverse remodeling characterized by inward hypertrophy in those AVFs. On the other hand, the AVFs with significant dilation and venous wall thickening underwent positive remodeling with outward hypertrophy. Over this time period (7 d-28 d), the increase in average diameter and flow rate were minimal as compared to the first week, while the pressure drop increased for 50% of its baseline value. Also, the severity of the local stenosis, measured by area reduction, in AVFs (Figure 8A[70]) increased to 41.7% ± 8.3% which was below the clinically significant level (= 75% area stenosis). Corresponding to these changes, C_p and *R* (Figure 8B[70]) increased considerably over this time period. It was concluded that assessing the AVF functionality based on only diameter or flow rate could be misleading because they may not reveal complete information regarding the developing stenosis. However, C_p and *R* significantly increased over this time period and showed better delineation of stenosis severity. Thus, C_p and *R* could better assess the functionality of an AVF. However, this study was limited to relatively small number of data points and thus, studies with larger population and longer duration are needed to better determine advantages of the new combined pressure-flow diagnostic endpoints over the current gold standards.

Bedside measurement of new functional diagnostic endpoints

It should be noted that all the pressure-flow parameters that are needed to calculate C_p or *R* can be measured under current clinical settings. Velocity at the proximal artery can be measured through Doppler ultrasound probes and the pressure drop can be measured from the pressure readings at the cannulation sites during

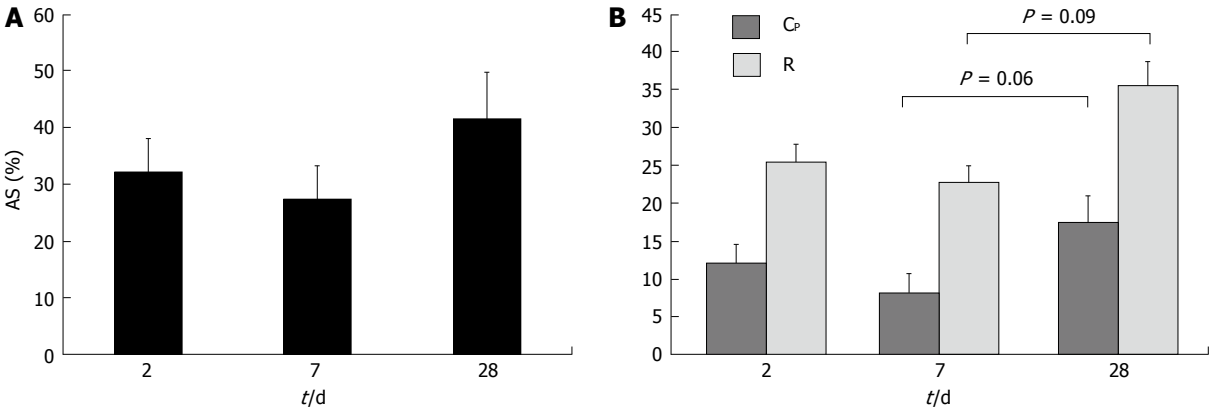

Figure 8 Variation of (A) percentage area stenosis and (B) pressure drop coef-ficient and resistance index over time for arteriovenous fistulas created in a pig model[70]. AS: Area stenosis; C_p: Pressure drop coefficient; R: Resistance index. The *t*/d in the x axis stands for time (days). © 2014, Copyright the Authors. Artificial Organs, © 2014 International Center for Artificial Organs and Transplantation and Wiley Periodicals, Inc.

the dialysis treatment. In the case of an inflow stenosis both *R* and C_p increases as pressure drop increases and flow rate decreases. Thus, both *R* and C_p are capable of detecting a developing inflow stenosis. If stenosis occurs downstream of the venous cannula (outflow stenosis) in AVFs, the pressure readings can show either lower or unchanged values of pressure drop over time, which does not reflect the presence of downstream stenosis. This is because of the development of collateral channels at the outflow stenosis area in AVFs. This shortcoming has been the major criticism to the current pressure surveillance program in which the AVF patency is assessed based on only pressure ratios at the outflow venous cannula and proximal artery. In such scenarios, if the venous flow rate decreases, both C_p and *R* begin to increase. It is noteworthy that irrespective of Δp status (numerator), as C_p has inverse quadratic relation with flow, any reduction in flow shows non-linear and pronounced increase in C_p as compared to *R*. Therefore, *R* and C_p are also capable of detecting an outflow stenosis. Thus, combining the flow and pressure data in the functional diagnostic endpoints could improve the ability of these parameters in assessing the patency of AVFs.

Limitations

The new functional diagnostic endpoints have not been tested for patient population. Thus, multi-central randomized studies on human subjects are needed to evaluate the potential advantages of the proposed diagnostic parameters for longitudinal assessment of AVF functionality.

CONCLUSION

According to KDOQI guidelines, a blood flow (Q_a) < 400-500 mL/min and a ratio of venous access pressure to main arterial pressure (VAPR) > 0.55 are associated with the existence of a significant stenosis which needs immediate interventional care. However, an AVF can maintain a relatively high blood flow rate and a normal VAPR despite the presence of a significant stenosis. The Q_a has shown to be a strong predictor of an inflow stenosis, whereas VAPR has shown to be a poor predictor of stenotic lesions in AVFs. These shortcomings have been mainly attributed to the fact that under a developing stenosis both pressure and flow profiles change, and thus, relying on only one of these parameters to detect a significant stenosis could be inadequate. Similar shortcomings also have been reported in detection of coronary and renal stenosis based on diagnostic endpoints that are either based on flow or pressure. In the context of coronary or renal stenosis, it has been shown that the diagnostic endpoints that combine the effects of pressure and flow can better predict the functional severity of a stenosis. This similarity inspired us to bridge between the advances in diagnostic field of coronary and renal stenosis with the diagnosis of stenotic lesions in AVFs. The new functional diagnostic endpoints are based on fundamental fluid dynamics concepts and are primarily presented in two major forms including: (1) resistance index (a ratio of pressure drop by flow); and (2) pressure drop coefficient (the pressure drop normalized by the dynamic pressure). We believe that these endpoints are capable of better distinguishing changes in the hemodynamic variations (pressure and flow) and thus, could be promising diagnostic tools to detect the functional severity of stenosis in AVFs.

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REVIEW

Aging and uremia: Is there cellular and molecular crossover?

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Abstract

Many observers have noted that the morphological changes that occur in chronic kidney disease (CKD) patients resemble those seen in the geriatric population, with strikingly similar morbidity and mortality profiles and rates of frailty in the two groups, and shared characteristics at a pathophysiological level especially in respect to the changes seen in their vascular and immune systems. However, whilst much has been documented about the shared physical characteristics of aging and uremia, the molecular and cellular similarities between the two have received less attention. In order to bridge this perceived gap we have reviewed published research concerning the common molecular processes seen in aging subjects and CKD patients, with specific attention to altered proteostasis, mitochondrial dysfunction, post-translational protein modification, and senescence and telomere attrition. We have also sought to illustrate how the cell death and survival pathways apoptosis, necroptosis and autophagy are closely interrelated, and how an understanding of these overlapping pathways is helpful in order to appreciate the shared molecular basis behind the pathophysiology of aging and uremia. This analysis revealed many common molecular characteristics and showed similar patterns of cellular dysfunction. We conclude that the accelerated aging seen in patients with CKD is underpinned at the molecular level, and that a greater understanding of these molecular processes might eventually lead to new much needed therapeutic strategies of benefit to patients with renal disease.

Key words: Aging; Uremia; Apoptosis; Autophagy; Senescence; Telomeres; Mitochondria; Post-translational protein modification; Klotho

Core tip: This review presents evidence that suggests that the morphological similarities between uremia and physiological aging are underpinned by similarities at a cellular and molecular level. Several of the classical cellular features of aging such as mitochondrial dysfunction and altered proteostasis have been observed in the cells and tissues of uremic humans and animals, and in *in vitro* models of uremia. There are also many shared features between aging and uremia in terms of

cell death and survival pathways. These commonalities may present new targets for the future management of patients with chronic kidney disease.

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INTRODUCTION

Observation alone suggests that patients with end stage kidney disease (ESKD) are biologically older than their unaffected peers. As a group, ESKD patients have a morbidity and mortality profile similar to that of the geriatric population, and the pathophysiology of the uremic syndrome has interesting parallels with the aging process. Based on these thoughts it has been posited that kidney failure results in accelerated, pathological aging[1]. Indeed there are striking analogies between the effects of aging and uremia on the structure and function of the heart and vasculature, with similar changes seen in pulse contour, pulse wave velocity, and impedance, and similar structural abnormalities with wall thickening, decreased elastin, and increased collagen content[2].

Aging is characterized by a progressive loss of physiological integrity, leading to impaired function and increased vulnerability to death[3]. Dialysis dependent patients of any age have an increased risk of mortality when compared to those with a functioning transplant and healthy controls of the same age[4], and are more susceptible to disease, particularly that of the cardiovascular system: a 25-34-year-old dialysis patient has a relative risk of cardiovascular mortality similar to that of a > 75-year-old in the general population[5]. Furthermore, the prognosis for chronic kidney disease (CKD) patients is still extremely poor and has not improved greatly despite many treatment advances: CKD patients receiving dialysis aged 50 and under are likely to live 30 years less than age-matched people without CKD[5]. Whilst survival rates have slightly improved they have not kept pace with the rises seen in the normal population without CKD, with the result that relative survival in age-specific patients with CKD actually decreased between 1977 and 2007[6]. There is thus a need to identify if CKD is inducing an aging-like cellular and molecular dysfunction, and if so whether any novel potential therapy might be derived from an increased understanding of the pathways that are induced by both CKD and aging.

ESKD confers a greatly increased risk of infectious morbidity and mortality, whilst simultaneously being a chronic inflammatory state, a pattern of immune dysfunction also associated with aging[7]. These abnormalities also seem to be reflected at a cellular level, with preferential loss of cells belonging to the lymphoid cell lineage, and inflammation and expansion of proinflammatory immune cells[8].

There is a high prevalence of the frailty syndrome amongst dialysis patients, a phenotype partly defined by weight loss, muscle weakness, and fatigue, which is associated with adverse outcomes in geriatric patients[9]. In the original study that developed this definition, 6.9% of participants ⩾ 65-year-old were classified as frail; in a more recent study of dialysis patients 44% of those under 40-year-old were found to be frail[10]. Cognitive impairment is also highly prevalent in the dialysis-dependent population and occurs in comparatively young patients[1,11].

Whilst much has already been written about the intriguing similarities that appear to exist between the aging process and CKD[1,8,12,13], comparatively little work has been undertaken looking at the cellular and molecular hallmarks of aging in the context of the known evidence concerning uremia-induced cellular and molecular pathways. Therefore in this review, in order to try and fill this perceived gap in the literature, we have first briefly outlined what the main cell death pathways are and by what means these processes interact with each other, followed by an analysis of published research concerning the mechanisms of aging and uremia-induced cell death and their common molecular pathways and cellular characteristics. Lastly we provide an assessment of how this knowledge may lead to benefits in both nephrology and gerontology.

CELL DEATH AND SURVIVAL PATHWAYS

An outline of cell death

Since the first descriptions of apoptotic cell death appeared more than 40 years ago[14] the study of cell death has become a substantial and important area. The main cell death pathways have been reviewed exhaustively in the literature and it is not the aim of this review to repeat this information. What is pertinent here is how much our understanding of cell death has changed and evolved in recent years. This is because cell death and survival pathways are now being assessed more as molecular processes and less as a series of morphological characteristics. One of the most fundamental changes is that each death pathway is no longer considered in isolation and there is an appreciation that cell death can no longer be considered as a choice between apoptotic, autophagic or necrotic death. Pathways once thought of as discreet have been found to be closely interconnected with others whilst some pathways have needed to be recategorized. In addition several completely novel pathways have been described. An example of reclassification is that necrosis is now subdivided into two distinct forms, one being programmed necrosis that

is usually termed necroptosis or regulated necrosis, and accidental or non-regulated necrosis which is more in line with the original concept of necrosis. Another example of recent developments is that apoptosis has now been split into four different classes whilst a total of 13 functional classes of regulated cell death have been described[15]. So whilst this review is focusing on the most established and described death and survival pathways they must not be considered as being complete. Lastly, the role of autophagy in cell death has been recently challenged[16,17] whilst its role in cell survival[18] asserted.

Uremia induced apoptosis

Although apoptosis and uremia have been studied extensively both separately and together, a clear picture of how uremia induces apoptosis has yet to be established. Instead a large number of studies using experimental models and human subjects have shown that uremia is associated with apoptosis in a wide range of cells and tissues such as skeletal muscle[19,20], myocardium[21], platelets[22,23], monocytes[24], neutrophils[25], lymphocytes[26], leukocytes[27] and vascular endothelial cells[28]. The kidney has also been shown as a target for apoptosis in uremia with both podocytes[29] and proximal tubular cells identified as having increased apoptotic cell death[30]. Furthermore, it has become known that it certain circumstances dialysis itself can be an activator of apoptosis[20,26]. It is unclear if the apoptosis seen in the kidney is the cause or the effect of CKD. However, it does seem probable that acute kidney injury (AKI) induced apoptosis can subsequently lead to the activation of interstitial fibroblasts *via* transforming growth factor beta (TGF-β) resulting in CKD[31,32]. In fact expression of TGF-β has been found to be elevated in nearly all human and experimental forms of CKD[33] and demonstrated to be directly associated with age in healthy human subjects[34].

Uremia induced necroptosis

Uremia induced necroptosis (or programmed necrosis) has yet to feature prominently in the literature although this is possibly due, at least in part to previous cell death descriptions not being classified correctly according to current definitions (see aging induced apoptosis below).

Aging induced apoptosis and necroptosis

The induction of apoptosis in aging in most tissues awaits clarification. However, in skeletal muscle at least there is clear evidence that muscle mass decreases with age[35-37] with apoptosis being known to be elevated in the skeletal muscle of aged subjects[38-41]. It has been suggested that aging increases cell death by caspase independent mechanisms. There is also some evidence that terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining is greater the kidneys of aged in mice[42] but TUNEL staining has been shown not to be specific for apoptosis[43]. It seems plausible that at least some of the examples for age induced apoptosis in the literature instead reflect increases in necroptosis.

Apoptosis and necroptosis crosstalk

It is now appreciated how significantly involved the apoptosis machinery is in other cell death and survival pathways. Many of the described apoptotic death receptors such as tumor necrosis factor receptor 1 and FAS are now also known to be able to induce necroptotic cell death[44,45]. Caspase-8, a key component of receptor mediated apoptosis is now thought to regulate the activation of necroptosis[45]. Inhibitor of apoptosis (IAP) are endogenous caspase inhibitors and therefore play a role in controlling apoptosis. When IAP levels are reduced this leads to caspases being activated which results in apoptotic cell death. Another IAP, X-Chromosome-linked IAP has been shown to be reduced in the muscle of CKD mice and *in vitro* in muscle cells treated with serum obtained from CKD mice[46].

The activation of autophagy is known to breakdown IAPs and lead subsequently to the induction of necroptosis. Furthermore, in conditions where IAPs are suppressed or absent and caspase activity is inhibited can lead to the activation of necroptosis *via* receptor-interacting protein1 (RIP1) and its downstream kinase (RIPK1)[47]. It has been postulated that RIP1 together with RIP3, cIAP, Caspase-8 and cFlip act as essential components of the ripoptosome, a signalling platform that can switch modes between apoptotic and necroptotic cell death[48]. Recent work indicates that it is RIPK3 activity that determines whether cells die by necroptosis, or in its absence, by caspase-8 mediated apoptosis[49] whilst another group have suggested that necroptosis can be induced in the absence of RIPK1 and without the formation of a functioning ripoptosome[50], the complex considered essential for necroptosis to occur.

Autophagy

Autophagy is the dynamic, multistep cellular process wherein portions of cytoplasm, including organelles, are sequestered into double-membrane vesicles (termed autophagosomes) and delivered to lysosomes where they are degraded, with eventual recycling of the resultant macromolecules[51]. By removing excessive and aberrant organelles and proteins, autophagy contributes to cellular homeostasis and protein quality control, and functions as a source of energy for the cell[52]. Autophagy is up-regulated and has a protective function in the face of cellular stressors such as starvation[53] and ischemia[54].

Autophagy and apoptosis crosstalk

It is perhaps not surprising that autophagy and apoptosis exhibit crosstalk as both pathways play such significant roles in development, homeostasis and pathology[55]. Evidence of this crosstalk has been plentiful[56-60] and indicates that the pathways can interact in an additive or antagonistic fashion and that the molecular machinery

for both can combine *via* p27[56], p38[57], p53[58] and beclin-1[59,60]. It is likely that these overlapping pathways are involved in uremia and aging induced dysfunction. For example in autophagy-deficient mice the onset of ischemia/reperfusion injury resulted in greater proximal tubular apoptotic injury with significantly elevations in serum urea and creatinine compared to wild type animals. This indicates that autophagy maintains proximal tubular homeostasis and protects against ischemic injury[61]. In another study using a dietary adenine-induced chronic renal failure model a high phosphate diet was found to increase apoptosis in vascular smooth muscle cells (VSMC) and that this rise could be reduced by autophagy inhibition. However, reducing autophagy was associated with an increase in calcium deposition in VSMC. The study concluded that autophagy might be an endogenous protective mechanism against phosphate-induced vascular calcification[62].

Autophagy and necroptosis

In addition to necroptosis crosstalking with apoptosis *via* IAP (see apoptosis and necroptosis crosstalk) there is also evidence of autophagy and necroptosis crosstalk in a similar fashion. Using a novel chalcone derivative as an anti-cancer agent it was found that Jun N-terminal kinases-mediated autophagy was able to cause IAP degradation followed by necroptosis[63]. It seems likely therefore that there is a therapeutic potential for autophagy to be exploited by anticancer agents to provoke cancer cell death. However, it should be noted that the molecular interactions between the two processes is still largely unknown and indeed there is evidence that autophagy activation can block necroptosis in several cell lines[64,65].

Autophagy in aging

Beyond its function at a cellular and organ level, autophagy has been heavily implicated in the aging process and the determination of life span. Normal and pathological aging are associated with failing proteostasis and reduced autophagic activity[3], and genetic inhibition of autophagy produces degenerative changes in mammalian tissue resembling those seen in aging. Caloric restriction, which has been shown to promote longevity in model organisms, stimulates autophagy, as do some pharmacological interventions and genetic manipulations that increase life span in model organisms, and inhibiting autophagy attenuates this effect[66].

Autophagy in uremia

Much work has been published describing the role of autophagy in the pathophysiology of AKI and CKD, but very little has been published looking at the effects of uremia on autophagy in other tissues. Chen *et al*[67] assessed autophagy activation in leukocytes isolated from peripheral blood samples, which had been taken from stage 5 CKD patients and healthy controls after overnight fasting and 2 h after breakfast. Overnight fasting induced conversion of microtubule-associated protein light chain 3 (LC3) Ⅰ to Ⅱ (as detected by western blot as increased quantities of the latter, and signifying autophagosome formation) in healthy subjects. mRNA levels of autophagy-related gene 5 (*Atg5*) and beclin-1 also increased in fasted healthy subjects but not in CKD patients. Interestingly there was no difference between CKD patients receiving or not receiving hemodialysis. Furthermore, a negative association was found between LC3Ⅱ and left atrium size, Atg5 transcription and left ventricular end-diastolic diameter, and beclin-1 transcription and mitral inflow E- and A-wave sizes. The authors conclude that autophagic activation is impaired in CKD patients and is not reversed with hemodialysis, and that this impairment is related to cardiac abnormalities.

Siedlecki *et al*[68] assessed the effect of rapamycin administration in a murine model of normotensive uremic cardiomyopathy. Treatment of surgically induced renal injury mice with rapamycin blocked the development of cardiac hypertrophy and fibrosis when compared with vehicle-treated animals. The experimenters suggest that this protective effect is mediated by the extracellular signal-regulated kinase and mammalian target of rapamycin (mTOR) pathways. They do not speculate on the possible involvement of autophagy, but rapamycin is known to stimulate autophagy *via* mTOR, and has been shown to have anti-aging effects in mammals[69]. The authors raise the interesting question of whether renal transplant recipients taking rapamycin as an immunosuppressant exhibit reversal of uremia-induced cardiac changes beyond that associated with successful transplantation.

In summary, the principle cell death and survival molecular pathways consisting of apoptosis, necroptosis and autophagy are strongly interrelated and crossover at many points. Whilst our current knowledge on how these interacting pathways are controlled and regulated is far from complete our appreciation of how similar many of the molecular signalling induced by uremia and aging appears to be growing pathways.

SHARED CELLULAR CHARACTERISTICS OF AGING AND UREMIA

Cell senescence, telomere shortening and stem cell exhaustion

Cellular senescence can be defined as stable arrest of the cell cycle coupled to classic phenotypic changes[70]. This was originally described by Hayflick *et al*[71] in serially passaged human fibroblasts, which undergo a certain number of divisions before entering a senescent phase (the "Hayflick limit"). This phenomenon was subsequently shown to be due to telomere shortening[72], but can be triggered by non-telomeric aging-associated stimuli such as DNA damage and excessive mitogenic signaling[3].

Senescent cells accumulate in aged organisms, although senescence *per se* does not cause aging,

having a protective effect by preventing the propagation and causing the removal of damaged and potentially oncogenic cells from tissues. A failure to clear senescent cells and replace these with new ones may, however lead to their accumulation[3]. Senescent cells are known to possess large amounts of proinflammatory cytokines and matrix metalloproteinases (the "senescence-associated secretory phenotype") which may in themselves contribute to aging[73].

Senescent cells have a flattened and enlarged morphology, and express a different set of genes such as p16, p21, p53, and retinoblastoma protein (pRb)[74]. Senescence-associated β-galactosidase (SA-β-gal) is a frequently used biomarker of cell senescence *in vivo* and *in vitro*[75].

Jimenez *et al*[76] looked at markers of senescence in circulating immune cells in uremic pre-dialysis, hemodialysis-dependent and transplanted patients. Abnormal telomere shortening was seen in a subpopulation of lymphocytes in pre-dialysis patients. In hemodialysis patients who dialyzed with cellulosic membranes, a subset of mononuclear cells demonstrated telomere shortening and exhibited increased levels of intracytoplasmic proinflammatory cytokines, which were released in response to substimulatory doses of lipopolysaccharide and bacterial DNA *in vitro*. The authors postulate that these senescent mononuclear cells both result from and contribute to chronic inflammation in such patients. A subpopulation of lymphocytes with shortened telomeres was also found in transplant patients with near normal renal function. It was suggested that these resulted from chronic activation due to major histocompatibility complex incompatibility and immunosuppressive therapy.

Tsirpanlis *et al*[77] measured the activity of telomerase (the enzyme that preserves telomere length and structure and thus prevents senescence[78]) in peripheral blood mononuclear cells in hemodialysis-dependent patients and non-renal failure subjects. Telomerase activity was reduced in hemodialysis patients compared to healthy controls, and was lower in long-term than in short-term dialysis patients. These findings indicate that defence against senescence is reduced in this cell type and associated with chronicity in hemodialysis patients.

Several groups have looked at the role of senescence in the endothelial dysfunction associated with cardiovascular disease in uremia. Adijiang *et al*[79] administered indoxyl sulphate, a uremic toxin, to hypertensive and normotensive rats, and examined their aorta for histological and immunohistochemical evidence of senescence. The indoxyl sulphate-treated animals showed significantly increased aortic calcification and wall thickness, and significantly increased expression of SA-β-gal, p16, p21, p53 and pRb in cells embedded in the calcification area. The same group went on to demonstrate that indoxyl sulphate stimulated senescence of cultured human aortic smooth muscle cells *via* an oxidative stress mechanism[74].

Carracedo *et al*[80] evaluated the effects of uremia on low-density lipoprotein (LDL) carbamylation and the effect of carbamylated LDL (cLDL) and oxidized LDL on the number, function, and genomic stability of endothelial progenitor cells (EPCs) obtained from healthy volunteers. EPCs were exposed to cLDL generated after incubation of native LDL (nLDL) with uremic serum from patients with CKD stages 2-4. Compared with cLDL, nLDL induced an increase in oxidative stress, depolarization and senescence in EPCs, and a decrease in EPC proliferation and angiogenesis. The authors hypothesize that cLDL triggers genomic damage in EPCs resulting in premature senescence, and that this contributes to atherosclerotic disease in uremia.

Klinkhammer *et al*[81] demonstrated that bone marrow mesenchymal stem cells (MSCs) isolated from uremic rats (both surgically induced and adenine diet) showed signs of premature senescence, and failed to accelerate healing of glomerular lesions when injected into the left renal artery of rats with acute anti-Thy1.1-nephritis when compared to MSCs obtained from control rats. The authors conclude that CKD leads to a sustained loss of *in vitro* and *in vivo* functionality in MSCs, possibly due to premature senescence. Stem cell exhaustion and the resultant decline in tissue regenerative potential has been noted as one of the hallmarks of aging[3].

In summary, aging and uremia share many important cellular characteristics such as increases in cell senescence, telomere shortening and exhaustion of stem cells. This provides further evidence that supports the contention that uremia can be considered as a form of accelerated aging[1].

Klotho

The *klotho* gene was originally identified as being involved in the suppression of aging in transgenic mouse studies[82]. Defective klotho expression resulted in mice having a premature aging phenotype, which had striking similarities to that of CKD patients, including reduced life span, arteriosclerosis, hyperphosphataemia and high concentrations of plasma fibroblast growth factor-23 {FGF23, a bone derived hormone that promotes renal phosphate excretion and reduces serum levels of 1,25-dihydroxyvitamin D3 [1,25-(OH)2VD3][83]}. This observation, coupled with the fact that, although found in multiple tissues, klotho expression is highest in the kidney (predominantly in the distal convoluted tubules[84]), suggested that CKD might be a state of klotho deficiency, and this might contribute to the accelerated aging phenotype of uremia[85].

Through alternative splicing klotho exists in membrane-anchored and soluble, secreted forms, the latter being found in mammalian cerebrospinal fluid, blood and urine[84]. These forms have distinct functions. Membrane klotho forms a complex with FGF receptors and functions as a co-receptor for FGF23. Soluble klotho functions

as an endocrine factor, and has a role in a number of processes including modulation of ion transport[86] and counteraction of the renin-angiotensin system[87]. Klotho suppresses 1α-hydroxylase in the kidney to regulate calcium metabolism[88], and participates in in the regulation of parathyroid hormone synthesis in the parathyroid gland by FGF23[84,89].

Both physiological aging and CKD are associated with reduced klotho levels. Lower renal klotho protein expression has been shown in aging rodents compared to young ones[90], and plasma klotho concentrations were found to be two-fold higher in normal children than in adults[91]. Renal klotho RNA has been shown to be reduced in CKD kidneys[92], as have urinary klotho levels[85]. Klotho concentrations in plasma, urine and kidney were found to be decreased in parallel in a rodent CKD model[85].

Klotho may influence cell death and survival pathways *via* its anti-senescence and oxidation effects. Liu *et al*[93] analysed various tissues and organs from klotho$^{-/-}$ mice and demonstrated a decrease in stem cell number and an increase in progenitor cell senescence. Tissues from klotho-deficient animals showed evidence of increased Wnt signalling. *In vivo* and *in vitro* Wnt exposure triggered by the absence of klotho accelerated cellular senescence. The authors conclude that klotho might act as a secreted Wnt antagonist and that a decrease in klotho concentration leads to an increase in Wnt signalling and this may play a role in aging.

de Oliveira *et al*[94] generated a klotho-knockdown human fibroblast, in which premature senescence was seen alongside an increase in p21 expression. p53 knockdown in klotho attenuated cells restored normal growth and replicative potential. These results suggest that klotho regulates cell senescence by suppressing the p53/p21 pathway. Ikushima *et al*[95] demonstrated that purified recombinant klotho protein could attenuate apoptosis and senescence in human umbilical vein endothelial cells. The same group went on to show that this occurred *via* mitogen-activated kinase and extracellular signal-related kinase pathways[96].

Klotho may exert an anti-aging effect by suppressing the inflammatory effect of substances secreted by senescent cells. Liu *et al*[97] have shown that cellular klotho interacts with retinoic acid-inducible gene-Ⅰ (RIG-Ⅰ) and that this interaction inhibits the RIG-Ⅰ induced expression of interleukin 6 (IL-6) and IL-8 both *in vivo* and *in vitro*.

Thus the deficiency in klotho seen in uremia and aging might underpin the enhanced cell senescence, apoptosis and stem cell depletion common to both states[81]. Given that tissue klotho expression is greatest in the kidneys a common mechanism is perhaps to be expected. Indeed recent data indicate that kidney tissue klotho expression greatly effects systemic concentrations and they concluded that the kidney is the prime mediator of klotho function[98]. Therefore klotho, a recognised anti-aging factor, is under the control of the kidney and thus lends further support to there being a molecular basis for the observed shared phenotype between uremia and aging.

Post-translational protein modification

Spontaneous post-translational protein modifications result from the non-enzymatic attachment of reactive molecules to protein functional groups. This process occurs in healthy individuals with aging, but is increased in certain disease states. Alterations to protein structure may result in functional changes, which can be pathogenetic[99]. Carbamylation is one form of post-translational protein modification specifically associated with CKD and uremia. Cyanate, a dissociation product of urea, binds to proteins and free amino acids, resulting in abnormal cellular responses that may contribute to inflammation and atherosclerosis. As carbamylation results from a direct product of uremia it may serve as a quantitative biomarker of time-averaged urea concentrations in addition to its potential use in risk assessment[99].

One of the most widely studied and publicised forms of post-translational protein modification is glycation. Advanced glycation end products (AGEs) are formed by the non-enzymatic modification of tissue proteins by physiologic sugars. AGEs accumulate in tissues as a function of increased production (*e.g.*, in diabetes mellitus), decreased renal removal of AGE precursors (*e.g.*, in advanced CKD) and time (as occurs in physiological aging)[100]. Covalent cross-linking occurs in affected proteins, leading to increased stiffness of the protein matrix, thus impeding function, and increased resistance to proteolytic removal, thus affecting tissue remodeling[101]. This contributes, for instance, to the histological and functional changes seen in diabetic glomerulosclerosis and atherosclerosis[102]. AGE accumulation also stimulates cytokine and reactive oxygen species (ROS) production through AGE-specific receptors, modifies intracellular proteins[100], and has been shown to promote senescence[103] and apoptosis[104] in the cells of affected tissues, contributing to cell death and tissue dysfunction.

Significantly elevated serum levels of AGEs are present in ESKD, with no differences between patients with and without diabetes[105], and uremic patients are known to be exposed to high levels of oxidative stress[106]. Taki *et al*[107] demonstrated that plasma levels of pentosidine, an AGE, was correlated and independently associated with coronary artery calcification score in hemodialysis patients. Pentosidine formation is accelerated by oxidative stress[108], and in this study was correlated with indoxyl sulphate. The authors thus conclude that indoxyl sulphate may enhance oxidative stress, which in turn enhances AGE generation.

Increased oxidative stress and AGE generation are known to play a role in the pathophysiology of aging[100], and both of these events are present in patients with CKD[105,106] and therefore represent two further potential crossovers between uremia and the aging process.

Table 1 Events common to aging and uremia covered by this review

Aging	Uremia
TGF-β ↑	TGF-β ↑
Autophagy ↓	Autophagy ↓
Apoptosis ↑ (muscle)	Apoptosis ↑
Senescence ↑	Senescence ↑
Telomere shortening ↑	Telomere shortening ↑
Stem cell exhaustion ↑	Stem cell exhaustion ↑
Klotho ↓	Klotho ↓
AGEs ↑	AGEs ↑
Mitochondrial dysfunction ↑	Mitochondrial dysfunction ↑

TGF-β: Transforming growth factor beta; AGEs: Advanced glycation end products.

Mitochondrial dysfunction

According to the mitochondrial free radical theory of aging, progressive, age-related mitochondrial dysfunction results in increased production of ROS, which causes further mitochondrial deterioration and cellular damage[109]. Recent data have questioned the idea that ROS have an entirely deleterious effect in aging, suggesting that they represent a stress-induced survival signal which acts to activate homeostatic responses to cellular stress and damage. As these accumulate with aging ROS eventually pass a threshold and aggravate the damage[110].

Dysfunctional mitochondria can contribute to aging independently of ROS[3]. Damaged mitochondria have an increased tendency to permeabilize in response to stress, leading to apoptotic cell death[111] and inflammation[112]. Aging associated mitochondrial dysfunction arises *via* several mechanisms[3]. For example, mitochondrial decline occurs as a consequence of telomere attrition in telomerase-deficient mice with subsequent p53-mediated repression of peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PCC1a) and PGC-1b[113], and can be partially reversed in wild-type mice by telomerase activation[114]. Sirtuins, a group of nicotinamide adenine dinucleotide-dependent protein deacetylases[115], also play a role in controlling mitochondrial function. Silent information regulator two protein 1 modulates mitochondrial biogenesis *via* the transcriptional co-activator PGC-1a[116] and the removal of damaged mitochondria by autophagy[117]. SIRT3 targets many enzymes involved in energy metabolism[118], and may directly control ROS production by deacetylating manganese superoxide dismutase, a mitochondrial antioxidant enzyme[119].

Mutations and deletions in mitochondrial DNA are known to accumulate with aging[3]. One of the most common and abundant mitochondrial DNA mutations is a 4977 base pair deletion between nucleotide positions 8470 to 13,477 (mtDNA4977)[120], which is known to accumulate in a variety of human tissues with age and has been demonstrated to be associated with several neurodegenerative diseases (including Alzheimer's) and atherosclerosis[121,122]. Defective quality control by mitophagy (organelle-specific autophagy that targets abnormal or worn out mitochondria for degradation) leads to reduced clearance and turnover of ineffective and toxic mitochondria[123]. The net result of these processes is that there is a reduction in the formation of healthy mitochondria, an increased incidence of mitochondrial damage, and a failure to clear and recycle abnormal organelles, with consequently increasing bioinefficiency, inflammation and cell death with aging.

Patients with advanced uremia are recognised to have low body temperatures, reduced stamina and low basal energy expenditure, suggesting a hypometabolic state[124]. Thompson *et al*[125] examined the forearm muscles of patients with ESKD using ^{31}P-magnetic resonance spectroscopy. They noted increased phosphocreatine depletion and increased glycolytic ATP production during exercise, suggesting mitochondrial dysfunction due to either limitation of oxygen supply, reduced mitochondrial content or an intrinsic mitochondrial defect. Exercise-related abnormalities remained despite anemia correction with erythropoietin[125].

Lim *et al*[126] demonstrated a high frequency of mtDNA4977 in the skeletal muscle of chronically uremic patients, and that this correlated with enhanced oxidative damage to DNA, lipids and proteins of mitochondria compared to healthy controls. Liu *et al*[127] found that the incidence and proportion of mtDNA4977 in hair follicles was significantly higher amongst hemodialysis patients compared to age matched controls. Therefore mitochondrial abnormalities, contributing and consequent to high levels of oxidative stress in uremia, are strongly suspected to play a role in the causation of pathological aging in CKD, acting as a nexus for several processes, including defective bioenergetics, telomere attrition, DNA mutations, autophagy, inflammation and cell death. Mitochondrial abnormalities therefore represent a further crossover point between aging and the uremia.

DISCUSSION

In this review we have sought to draw the reader's attention not just to the morphological similarities between advanced aging and uremia, but also to their shared characteristics at a cellular and molecular level (see Table 1). Experimental evidence has been provided to suggest common involvement of established cell death and survival pathways (apoptosis, necrosis, necroptosis and autophagy), and the presence of several of the recognised cellular and molecular features of the aging process in patients with ESRD and in experimental models of uremia. These include mitochondrial dysfunction, damage to genetic material, telomere shortening, impaired proteostasis, cell senescence, stem cell loss, oxidative stress, AGE accumulation, and klotho deficiency. Based on this evidence it could be posited that the physical resemblance between advanced age and uremia is underpinned by shared cellular and molecular

"abnormalities". These observations also reinforce the idea of the "uremic syndrome", in which dysfunctions in multiple body systems arise due to a pervasive defect at a cellular level.

Information gathered by research into aging pathways and "anti-aging therapies" might inform interventions to avoid, slow the progression of or even reverse some of the pathological changes seen in uremia. Given that these pathways are seen throughout most tissues and cell types it is also possible that a single intervention might treat several pathologies. However, the aging process remains incompletely understood in healthy individuals, and those pathways that are known are complex and heavily interconnected. Disentangling these in the uremic syndrome, in which multiple co-existing and interdependent metabolic abnormalities arise, will be a challenge. Additionally, many of these pathways have known (and possibly unknown) protective mechanisms (against malignant transformation, for example), thus blocking them may have unwanted and deleterious effects. What could be more immediately practicable would be employing some of the therapies known to be effective in improving the health of elderly patients, such as exercise.

The concept of accelerated aging in uremia is an intriguing and complex one that may yield important therapeutic targets and strategies to improve health outcomes in patients with CKD. Much work, however, remains to be done in understanding its cellular and molecular basis before any potential benefits can be realised.

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REVIEW

Complement activation in progressive renal disease

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Abstract

Chronic kidney disease (CKD) is common and the cause of significant morbidity and mortality. The replacement of functioning nephrons by fibrosis is characteristic of progressive disease. The pathways that lead to fibrosis are not fully understood, although chronic non-resolving inflammation in the kidney is likely to drive the fibrotic response that occurs. In patients with progressive CKD there is histological evidence of inflammation in the interstitium and strategies that reduce inflammation reduce renal injury in pre-clinical models of CKD. The complement system is an integral part of the innate immune system but also augments adaptive immune responses. Complement activation is known to occur in many diverse renal diseases, including glomerulonephritis, thrombotic microangiopathies and transplant rejection. In this review we discuss current evidence that complement activation contributes to progression of CKD, how complement could cause renal inflammation and whether complement inhibition would slow progression of renal disease.

Key words: Complement; Innate immune system; Chronic kidney disease; Transplantation; Proteinuria; Fibrosis

Core tip: Complement activation occurs in progressive chronic kidney disease and may contribute to the chronic inflammation that is characteristically found in the kidney. It is therefore possible that inhibiting complement activation would reduce inflammation, lead to reduced fibrosis and preservation of renal function.

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INTRODUCTION

Chronic kidney disease (CKD) is recognised worldwide as a major public health problem[1]. In 2007 the United Kingdom age-standardised prevalence of CKD stages 3-5 was 8.5% (10.6% in females and 5.8% in males)[2] and similar prevalences have been described in other countries. In 2012 in the United Kingdom, the number of new patients requiring renal replacement therapy was 6891, equating to 108 patients per million population, with diabetes and glomerulonephritis being the two most common diagnoses in incident dialysis patients. Although not all patients with CKD will progress to renal failure, all stages of CKD are associated with increased

morbidity and mortality[1].

Tubulointerstitial inflammation and fibrosis is a major factor in the progressive loss of renal function in most kidney diseases[3]. The process is complex due to the number of interacting pathways which ultimately result in the replacement of functioning nephrons with scar tissue. Cellular stress and injury induces an inflammatory and pro-fibrogenic response involving growth factors[4-6] and pro-inflammatory cytokines as well as activation of the renin-angiotensin system. This leads to a chronic inflammatory cell infiltrate, increasing numbers of activated fibroblasts (myofibroblasts) and excessive matrix deposition. There is evidence from preclinical models that the immune system is important in the development of renal fibrosis[7,8]. A component of the innate immune system that may be important in driving renal inflammation is the complement system, which can directly affect cell function and also influence the adaptive immune response.

COMPLEMENT SYSTEM

The complement system is a biochemical cascade made up of approximately 30 serum and membrane-bound proteins and represents a major part of the innate immune system. Complement was identified in the late 19th century by German scientist Paul Ehrlich as a heat-labile blood serum component with non-specific antimicrobial activity that "complements" other immune functions. As part of the innate immune system, the complement system responds rapidly to defend the host against a variety of invading microorganisms[9]. The complement system can also participate during the inductive phase of the acquired immune response by contributing to the recognition and presentation of non-self antigen, triggering antigen presenting cell activation, maturation and proliferation[10,11].

The primary location for biosynthesis of complement is the liver. Although Erlich and Morgenroth suggested the liver as the main source of complement production in 1900, it was only confirmed in 1976 by Alper *et al*[12] who described recipient to donor C3 allotype conversion after liver transplantation. This is also supported by studies of hepatocyte cell function[13]. In addition, evidence for extrahepatic synthesis of complement increased and it is now known that extrahepatic complement synthesis contributes approximately 10% of circulating C3. The alternative sites for complement production include epithelial cells, fibroblasts, lymphocytes and macrophages derived from different organs, including the kidney[14]. In the kidney, local complement production has been shown to occur at different sites along the nephron and may be further enhanced by the presence of cytokines and infiltrating immune cells during acute inflammation[15-17].

ACTIVATION OF THE COMPLEMENT SYSTEM

Activation of the complement cascade is triggered by one of three distinct pathways: the classical pathway, the alternative pathway and the mannose-binding lectin (MBL) pathway (Figure 1). All three pathways converge to cleave complement component C3, which subsequently initiates activation of the terminal complement pathway and formation of the membrane attack complex (MAC). The classical pathway is initiated by the activation of the C1 complex when C1q binds the Fc region of IgG or IgM. There is sequential cleavage of activation of C4 and C2, leading to the assembly of the classical pathway C3 convertase.

Activation of the alternative pathway is dependent on the spontaneous low level hydrolysis of the internal thioester bond of C3 to C3(H_2O). C3(H_2O) resembles C3b and can bind to factor B (FB). FB is activated by factor D forming the alternative pathway C3 convertase. The alternative pathway also amplifies the classical and lectin-binding pathways and is therefore critical for the full activity of complement. The third complement activation pathway, the lectin-binding pathway, is homologous to the classical pathway except that it is activated by the binding of a lectin to carbohydrates on microbial surfaces. The C3 convertase cleaves C3 resulting in assembly of the C5 convertase and sequential binding of C6, 7, 8 and 9 to form C5b-9, the membrane attack complex.

The main purpose of complement activation is to remove invading pathogenic organisms such as bacteria. This is achieved directly through the formation of the MAC or indirectly by opsonisation and stimulation of phagocytosis. Products of C3 and C4 activation on the surface of pathogens are recognised by the complement receptors CR1 and CR3 present on macrophages and neutrophils leading to phagocytosis of the opsonised target. Complement activation results in production of the small, biologically active anaphylatoxins, C3a and C5a. These readily diffusible complement components have a variety of functions, including chemotaxis and release of histamine from mast cells, mediated through binding to specific receptors[18]. These receptors, and also CRs1-4 (Table 1), are present on many immune cells and provide links between complement and the adaptive immune system[19,20].

The complement system contains proteins, both membrane bound and fluid phase, which regulate activation to prevent damage to host cells. They act by promoting decay of the convertase complexes, act as cofactors for the enzymatic degradation of the active proteins and by preventing the assembly of the MAC. The importance of these regulators is seen when their function is impaired, resulting in excessive complement activation and tissue injury.

COMPLEMENT ACTIVATION IN RENAL DISEASE

Complement activation is known to occur in immune mediated glomerular diseases (lupus nephritis, membranous nephropathy and post-infectious glomerulo-

Table 1 Properties of complement receptors

Receptor	Alternative name(s)	Location	Specificity	Role
CR1	CD35	Macrophages Neutrophils B-cells Some T-cells Renal epithelium	C3b C4b	Binding of opsonised immune complexes for transport to phagocytes
CR2	CD21	B-cells Some T-cells Dendritic cells Epithelia	C3d	Link between innate and acquired immune response on B-cells Presentation of immune complexes to B-cells
CR3	CD11b/18	Macrophages Natural killer cells Neutrophils	iC3b	Cellular-extracellular matrix linkage Promotes phagocytosis of opsonised complexes
CR4	CD11c/18	Macrophages Neutrophils	iC3b C3dg	Receptor for iC3b-opsonised particles
C3aR	-	Renal epithelium Macrophages	C3a	Mediation of inflammation
C5aR	CD88	Neutrophils	C5a	
C1qR	CD93	Leukocytes Platelets Monocytes Neutrophils	C1q	Up-regulation of phagocytic capacity Protein chaperone

Adapted from Morgan *et al*[94] 1999.

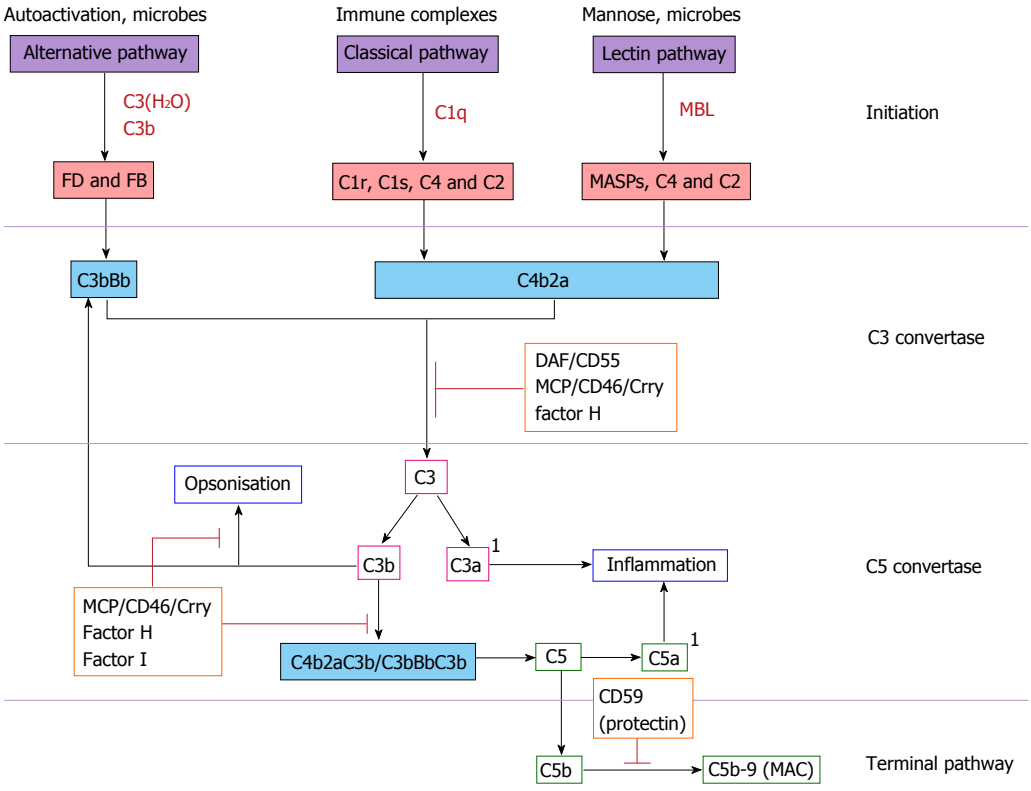

Figure 1 Complement activation pathways. Complement activation is triggered *via* activation of either the alternative, classical or lectin pathways, all three of which converge to cleave central component C3. Briefly, activation of the alternative pathway occurs following the spontaneous hydrolysis of C3 to C3(H_2O). C3(H_2O) binds factor B (FB) to form C3bB which is then cleaved by FD leaving the C3bBb complex. C3bBb is stabilised by properdin to form the alternative pathway C3 convertase. C3 is subsequently cleaved to C3a and C3b to form the C5 convertase C3BbC3b. Activation of the classical complement pathway occurs when immunoglobulin-bound antigens bind to and activate the C1 complex (consisting of C1qr2s2). Activated C1qr2s2 cleaves C4 to C4a and C4b. C4b becomes membrane-bound and binds to pro-enzyme C2, which is then cleaved to C2a and C2b fragments by C1s. C2a remains bound to C4b, forming the classical C3 convertase C4b2a. C3 is cleaved to C3a and C3b to form the C5 convertase C4b2aC3b. The lectin complement pathway is homologous to the classical pathway, with the exception that it is activated by the binding of lectin to microbial cell surface carbohydrates (mannose). Surface-bound lectin activates MBL-associated serine proteases (MASPs), which directly activate C3 and directly cleave C2 and C4. Activation of the terminal complement pathway occurs when the alternative and classical C5 convertases C3bBbC3b or C4b2aC3b cleave C5 in to C5a and C5b. C5b binds to C6 and C7, forming C5b67, which associates with an adjacent membrane. C5b67 then binds to C8 and multiple C9 molecules forming the transmembrane pore C5b-9, also known as the MAC. [1]Denotes the anaphylatoxins C3a and C5a. Orange boxes highlight regulatory complement proteins. MBL: Mannose-binding lectin; MAC: Membrane attack complex; DAF: Decay accelerating factor; MCP: Membrane cofactor protein.

nephritis), atypical haemolytic uraemic syndrome and during antibody mediated rejection. However, what is less clear is whether complement activation contributes to the non-disease specific inflammation, tissue injury and fibrosis that are characteristic of progressive nephropathies.

COMPLEMENT ACTIVATION IN CLINICAL PROTEINURIC DISEASE

The association between proteinuria, tubulointerstitial fibrosis and declining renal function is well established, however, the mechanism by which proteinuric glomerular disease casuses interstitial injury is uncertain. Complement proteins will be filtered when glomerular permselectivity is impaired and enter the tubular compartment. Complement activation products can be found in the urine of patients with a wide variety of proteinuric diseases; diabetic nephropathy, membranous nephropathy, IgA nephropathy and focal segmental glomerulosclerosis (FSGS)[21]. In some cases this may be due to spill over of complement activated in the glomerulus, however, complement activation products can be found in diseases where glomerular complement activation is not a major feature, for example diabetic nephropathy and FSGS[21,22]. This implies that complement is activated within the tubular compartment.

The tubular epithelium activates complement on its apical surface[23,24], which occurs primarily *via* the alternative pathway[25,26]. There are several explanations for this. It may be related to urinary pH[21] or ammonia production from stressed epithelial cells[27] directly activating C3. There may be enzymes with convertase-like activity in the apical brush border of the proximal tubule which is also known to be relatively deficient in complement regulatory proteins[28]. Properdin, which stabilises the alternative pathway convertase binds to the glycosaminoglycans on the apical surface of tubular epithelium[26]. Factor H also binds but at a different site[29,30], suggesting a balance between complement activation and inhibition which may be disturbed in proteinuria as albumin reduces Factor H binding[31]. Whatever the explanation, proteinuria provides a source of complement proteins to a host cell surface which is unable to control activation.

Demonstrating that complement activation is the cause of tissue injury in clinical proteinuric disease is difficult. Mosolits *et al*[32], studying patients with proteinuria, found a spatial and quantitative relationship between renal MAC deposition and inflammatory cell infiltrate and tubulointerstitial expansion. Urinary MAC concentrations are increased in proteinuric renal diseases. The concentration can relate to disease activity[33] although this is not always the case[34,35] and is further complicated when there is glomerular immune complex deposition.

COMPLEMENT ACTIVATION IN PRECLINICAL MODELS OF PROTEINURIA

Pre-clinical models have significantly contributed to our understanding of the role of complement in proteinuric disease. Aminoglycosides (puromycin and adriamycin) disrupt glomerular epithelial function leading to glomerulosclerosis, proteinuria and tubulointerstitial fibrosis in rodents. Induction of disease in complement deficient mice has shown that complement activation contributes to glomerulosclerosis and tubulointerstitial fibrosis and that activation occurs *via* the alternative pathway[36,37]. These studies have also demonstrated the important function of complement regulatory proteins in controlling complement activation, as their deficiency exacerbates injury[36]. Evidence from studies in both mice and rats suggests that both MAC[38] and the anaphylatoxins may be responsible for the damage that occurs[36,39].

Other pre-clinical models have also been used to investigate how complement influences the development of tubulointerstitial injury including a model of mesangioproliferative glomerulonephritis[40] and the remnant kidney model[41,42]. Disease severity is reduced if an intact complement system is absent. These models also allow testing of the potential for therapeutic targeting of complement. There is strong evidence that either complement depletion[43] or inhibition[40] can reduce the severity of proteinuria-related tubulointerstitial disease. There is the potential to target therapeutic complement inhibition at the tubular epithelium. A recombinant protein with an antibody portion directed at the tubular brush border linked to a complement inhibitor reduced interstitial injury and preserved renal function in rats with puromycin nephrosis[44].

COMPLEMENT ACTIVATION IN NON-PROTEINURIC KIDNEY DISEASE

Less is known about the role of complement in non-proteinuric renal disease. Unilateral ureteric obstruction (UUO), induced by ligation of one ureter, is the most commonly used preclinical model of progressive renal disease. The injury is characterised by the gradual development of interstitial inflammation and fibrosis, macrophage, T cell and fibroblast infiltration and eventual loss of functioning nephrons, closely resembling the pathology of chronic renal disease observed in patients. The first study to address the role of complement in UUO used rats deficient in C6 which are unable to assemble MAC. No difference was seen in disease severity in normal or C6 deficient animals, indicating no role for MAC in disease development[45].

Boor *et al*[46] found that interstitial fibrosis and macrophage infiltration were significantly reduced in C5 deficient ($C5^{-/-}$) mice after five days of UUO. Similarly,

significant reductions in fibronectin, vimentin, platelet-derived growth factor (PDGF)-B and PDGF-D mRNA were observed in $C5^{-/-}$ mice. A protective effect was also observed when UUO mice were treated with a C5aR antagonist. In a more recent study of UUO in C3 deficient mice there was a significant reduction in interstitial fibrosis and tubular atrophy in the absence of complement activation[47]. It is clear from the above animal studies that activation of C3 and C5 contribute to the development of progressive renal fibrosis during experimental obstructive nephropathy, however, the mechanism by which this occurs remains largely uncharacterised. There are no corresponding clinical studies to support these pre-clinical observations.

COMPLEMENT FUNCTION IN DIABETIC NEPHROPATHY

Although diabetic nephropathy is initially glomerular, progression of disease is associated with both glomerulosclerosis and tubulointerstitial fibrosis. There is evidence that complement may be involved in susceptibility to and progression of diabetic nephropathy. The serum concentration of mannose-binding lectin is variable and determined by polymorphisms in the gene promoter and coding sequence. Higher MBL concentrations early after the onset of type 1 diabetes may predict the development of nephropathy many years later[48]. Serum MBL concentrations are higher in type 1 diabetics with albuminuria and overt nephropathy[49,50] although this does not appear to relate to genotype. The effect of MBL on the development of diabetic nephropathy has been studied in MBL deficient mice with streptozotocin induced diabetes. The absence of MBL retards the development of glomerular disease and albuminuria[51], although not in all mouse strains[52].

MAC can be detected in the glomeruli of the patients with diabetes. Deposition may be increased by the glycosylation and loss of function of CD59, an inhibitor of MAC assembly[53]. In diabetic disease there is differential regulation of complement genes in both the glomerulus and tubular compartment[54], suggesting a possible role for complement proteins synthesised within the kidney. In addition, complement activation may be involved in the coronary and renal vascular disease associated with diabetes[53,55] and again the MBL pathway may be involved[56].

POLYCYSTIC KIDNEY DISEASE

In patients with inherited cystic kidney disease cysts develop within the nephron disrupting normal renal structure and function. It is evident that inflammatory and fibrotic changes occur in renal tissue surrounding the cyst and this may in part be responsible for the loss of renal function that occurs. In mouse models of cystic kidney disease there is increased expression of complement genes in cyst epithelium, particularly C3, and also evidence of complement activation[57,58]. To investigate a role for complement in cystogenesis $Cys1^{cpk/cpk}$ polycystic kidney disease mice were crossed with C3 deficient mice which lack the main effector functions of complement[58]. Mice deficient in C3 developed fewer cysts and had reduced renal volume, suggesting that complement, possibly by modifying inflammation, has a role in cyst development. This is supported by studies of complement inhibition in two different animal models of cystic disease[59]. Complement inhibition reduced kidney volume, cyst number and reduced the inflammatory infiltrate in tissue adjacent to cysts. Critically complement inhibition also preserved renal function.

Proteomic analysis of cyst fluid from patients with autosomal dominant polycystic kidney disease (ADPKD) identified complement proteins within the cysts[60]. Increased concentrations of complement proteins, including C3, Factor B and C9, can also be found in the urine and by immunostaining along the cyst epithelium, suggesting activation of the alternative pathway[59,61]. Song *et al*[62] described the pattern of gene expression in ADPKD kidneys. Complement genes were consistently up regulated suggesting that a proportion of the complement proteins in the cyst fluid may be derived from local synthesis.

COMPLEMENT AND PROGRESSIVE LOSS OF TRANSPLANT FUNCTION

Complement is important at many stages during the course of a kidney transplant: complement polymorphisms may alter outcome after transplantation[63], complement gene expression is increased in pre-implantation biopsies and this can predict outcome[64], complement has a role in the development of ischaemia reperfusion injury[65] and augments the alloimmune response[66]. All of these factors may impact upon long-term transplant outcome, however it is the role of complement in antibody mediated rejection (AMR) that has generated most interest recently.

Antibody binding to graft endothelium will activate complement, initially *via* the classical pathway. Complement can then alter endothelial function to enhance thrombosis and leukocyte chemotaxis. When this occurs acutely, most frequently seen in pre-sensitised or ABO incompatible transplants, it can lead to rapid graft loss. The most compelling evidence that complement is important in acute AMR is the reported experience of complement inhibition with monoclonal anti-C5 in the treatment[67,68] or prevention of AMR[69].

The role of complement in chronic AMR, increasingly recognised as a cause of graft failure, is less clear. When the classical pathway is activated C4 covalently binds the endothelium. It is degraded to limit activation leaving a biologically inert fragment, C4d, attached to the endothelium. This can persist in the glomerulus and peri-tubular capillaries and act as a biomarker of

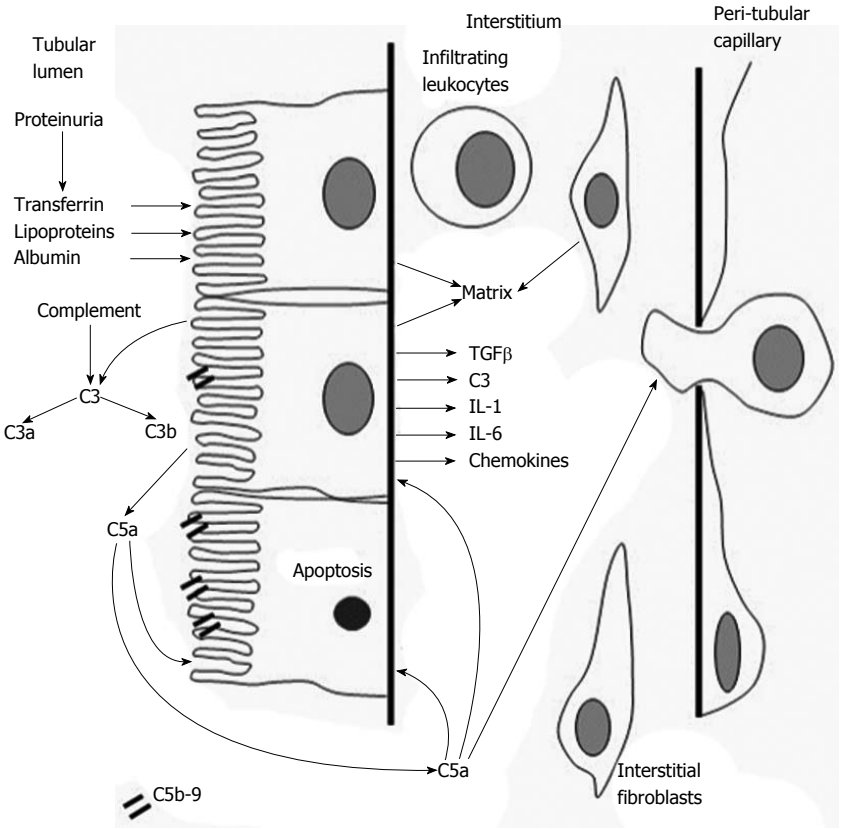

Figure 2 Complement-mediated renal injury. Complement proteins can access the tubulointerstitial compartment from the tubular lumen, the circulation or due to local synthesis. Activation can injure and activate the tubular epithelium inducing the synthesis of pro-inflammatory and pro-fibrotic cytokines. This results in an increase in interstitial inflammatory cells and fibroblasts, finally resulting in the deposition of excess matrix proteins. TGFβ: Transforming growth factor beta; IL-1: Interleukin 1.

antibody binding to endothelium. Detection of C4d in a transplant biopsy is now routinely used in the diagnosis of AMR and when present predicts a poor long-term outcome. Antibody can directly affect endothelial function[70] and whether complement further contributes to injury is less clear. There is currently a trial ongoing (NCT01327573) to assess the efficacy of anti-C5 treatment in treating chronic AMR which will answer this question.

EFFECT OF COMPLEMENT ON RENAL CELL FUNCTION

Complement activation, through generation of anaphylotoxin and sub-lytic concentrations of MAC can alter cell function. This may be the mechanism by which complement influences the progression of renal disease (Figure 2). Complement activation by the alternative pathway and deposition of sub-lytic concentrations of MAC on tubular epithelial cells stimulates the synthesis and release of pro-inflammatory cytokines (including tumor necrosis factor α and interleukin 6)[71], reactive oxygen species[25] and increases the synthesis of matrix proteins[72]. Complement can also induce expression of major histocompatibility antigens on tubular cells, allowing these cells to drive T cell proliferative responses, potentially augmenting allo and autoimmunity[73].

Renal epithelial can also respond to the anaphylatoxins, expressing both C3a receptor (C3aR)[74] and C5aR[75]. Tubular cells exposed to C3a, possibly synthesised from the tubular cells themselves[76], increase collagen synthesis[74] and adopt a more mesenchymal phenotype[39]. Similar effects can be seen in tubular epithelial cells exposed to C5a. The effect of C3a and C5a on tubular cells may in part be indirect due to increased activation of transforming growth factor beta and other growth factors that can be induced by the anaphylatoxins[77]. In an attempt to mimic more physiological conditions, the tubular cell line HK2 was grown on a microfluidic device allowing flow across its apical membrane. When the medium contained normal human serum or C3a the cells underwent mesenchymal transition and migrated into the supporting basement membrane[78].

LOCAL RENAL COMPLEMENT SYNTHESIS

The first study to demonstrate that human renal proximal tubular epithelial cells synthesised and secreted complement component C3 *in vitro* was published by Brooimans *et al*[15] more than 20 years ago. It is now

evident that resident renal cells, including tubular[79-82] and glomerular epithelial cells[16,83], mesangial cells[16,84,85] and endothelial cells[17] can synthesise many, if not all complement proteins[86]. Complement production is stimulated by pro-inflammatory cytokines and gene expression is increased in biopsies form patients with inflammatory glomerulonephritis[87], acute rejection[88] and chronic kidney disease[89]. Making use of polymorphisms in C3 it is possible to quantify the amount of protein produced from a transplanted kidney when the donor and recipient are mismatched for these polymorphisms. Renal derived C3 can contribute up to 10% of circulating C3 when the transplant is undergoing acute rejection[90].

Several pre-clinical studies have highlighted the importance of intrarenal synthesis of complement as an important mediator of local tissue. Renal complement production is increased in models of renal disease[91]. A strategy of renal syngenic or allogeneic transplantation in knockout mice can create a mouse which has a deficiency only in local complement synthesis. Application of this strategy has demonstrated a role for local C3 synthesis in transplant rejection[66], ischaemic reperfusion injury[92] and tubulointerstitial injury in proteinuric disease[93].

CONCLUSION

Chronic, non-resolving inflammation drives fibrosis in the kidney leading to a progressive loss of renal function. Complement activation occurs in the kidney during the progression of a broad range of renal diseases and could contribute to the inflammatory environment in which fibrosis occurs. There is increasing from pre-clinical models that complement activation may be linked with fibrosis, with some evidence for this from clinical studies. However, further work is required to define the role of the complement system in clinical disease progression.

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REVIEW

Immune profiling and cancer post transplantation

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Abstract

Half of all long-term (> 10 year) australian kidney transplant recipients (KTR) will develop squamous cell carcinoma (SCC) or solid organ cancer (SOC), making cancer the leading cause of death with a functioning graft. At least 30% of KTR with a history of SCC or SOC will develop a subsequent SCC or SOC lesion. Pharmacological immunosuppression is a major contributor of the increased risk of cancer for KTR, with the cancer lesions themselves further adding to systemic immunosuppression and could explain, in part, these phenomena. Immune profiling includes; measuring immunosuppressive drug levels and pharmacokinetics, enumerating leucocytes and leucocyte subsets as well as testing leucocyte function in either an antigen specific or non-specific manner. Outputs can vary from assay to assay according to methods used. In this review we define the rationale behind post-transplant immune monitoring assays and focus on assays that associate and/or have the ability to predict cancer and rejection in the KTR. We find that immune monitoring can identify those KTR of developing multiple SCC lesions and provide evidence they may benefit from pharmacological immunosuppressive drug dose reductions. In these KTR risk of rejection needs to be assessed to determine if reduction of immunosuppression will not harm the graft.

Key words: Immune-profiling; Immunosuppression; Kidney; Malignancy; Transplantation

Core tip: Kidney transplant recipients (KTR) with cancer have different leukocyte compartmentalisations and immune cell functions than KTR with no cancer. These differences can be used to determine KTR at risk of developing cancer and identify those who do not mount a reaction to their graft. Indicating there is a group of KTR that may benefit from pharmacological immunosuppressive drug dose reductions.

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INTRODUCTION

Kidney Transplant Recipients (KTR) have a 3 to 12-fold increased risk of developing Non-Lymphoid or solid organ cancers (SOC) when compared to the general population[1-4]. Cancers in KTR have poorer prognoses for a given stage/grade than the general population, which leads to higher mortality[5-9]. In Australia, it is observed that 20% of KTR will develop SOC within 15 years post transplantation (the median graft survival). Over a 5 year period (2007-2011) 267 KTR (or 31%) of all KTR died with a functioning graft (ANZDATA, 2012).

Additionally, KTR have a 60 to 250-fold increased risk of developing a Non-Melanoma Skin Cancer (NMSC), which includes; squamous cell carcinoma (SCC), basal cell carcinoma, Kaposi's sarcoma, Merkel cell carcinoma, and adnexal tumours[1,7,10]. SCC is the most common cancer in KTR with 50% of KTR who are 15 years post transplantation developing an SCC[11]. The disease progression of SCC is much more aggressive than the general population and is exemplified by the development of multiple SCC lesions and metastatic potential, phenomena that rarely occur in the immune competent[5,6,12].

The cumulative risk of subsequent SCC tumours is 30%-32%, 60%-62% and 75%-80% over 1, 3 and 5 years after first tumour, respectively[13]. Compounded, this equates to approximately 10% of KTR having > 5 tumours within 5 years of their first tumour, with some individual KTR reaching 40 primary SCC tumours during recipient life[14]. A single SCC lesion is a risk factor for subsequent SCC development with 60%-80% of KTR with one or more tumours developing another tumour within 1-3 years[15]. SCC tumour characteristics that are risk factors of metastatic SCC and include: size[16], depth[16,17], thickness[17], diameter[18] and poor differentiation[17]. Depth > 2.8 mm has a three-fold greater risk of metastasizing in KTR than the general population[19].

Further evidence of tumour aggression is the invasive potential of SCC in KTR, with more perineural and lymphatic invasion that the general population[20]. Metastatic incidence increases by 5%-8% with every SCC tumour accrued in KTR[14]. Due to SCC lesions mainly located in ultra violet (UV) exposed areas, *e.g.*, the neck, face and scalp there is a possibility of invasion into subcutaneous cranial nerves in the perineural space, leading to extensive surgery and perhaps death[21]. Reports observed an incident mortality of 1%-18%[22,23]. Observational studies have showed a 37% incidence of SCC metastasizing[18] which leads to the median KTR survival after diagnosis being only 2 years[24]. Furthermore, it has been observed that a previous SCC is a risk factor for multiple SCC and even development of SOC[11,13,19]. This is probably due to the exposure of pro-carcinogenic agents as well as the compounding effects of cancer induced, and pharmacological administrated, immunosuppression.

Therefore there are various risk factors and clinical parameters that influence the development of post-transplant cancer. The next section will introduce some of these factors and the rationale behind why they are factors of risk.

IMMUNOSUPPRESSION TYPE

There are limited and conflicting data on the use of different types on immunosuppressive drugs and the associated cancer risks. The conflict mainly due to the multiple confounding factors associated to cancer, immunosuppressive drugs in particular have the dual capacity to suppress both anti-graft and anti-cancer immunity. The immunosuppressive drug types introduced in this section include; azathioprine (AZA), mycophenolate mofetil (MMF), calcineurin inhibitors (CNI), steroids and mammalian target of rapamycin inhibitors (mTORi). These immunosuppressants are rarely used in monotherapies and are therefore hard to compare one another; instead modes of action and evidence for cancer development are presented.

AZA

AZA is catabolised to 6-mercaptopurine, which directly affects the synthesis of purines and has the ability to incorporate into DNA[25,26]. Lymphocytes rely heavily on *de novo* purine synthesis making AZA an effective immunosuppressant. AZA was originally used as an anti-cancer therapy however some cancers intrinsically have, or gain, purine scavenging and are, or become, resistant to AZA treatment[27]. When incorporated, the metabolite and the DNA form a complex that can block DNA repair, is photosensitive and produces reactive oxygen species (ROS) under UV exposure[25,27]. These work synergistically to affect DNA repair which form lesions[26,27]. One case-controlled study identified that AZA increased risk of developing SCC by 5-fold. However, in the same study calcineurin inhibitors (CNI) and steroids were also identified as risk factors[28].

MYCOPHENOLATE

MMF is a pro-drug of mycophenolic acid (MPA), which directly affects purine synthesis and is classified as an anti-proliferative drug[29]. The reaction of MPA is reversible and does not interfere with the DNA structure as AZA does[29]. One study showed a decrease photosensitivity when a cohort was randomised onto a MMF from AZA suppression regimen[30]. In another study comparing MMF to AZA usage in organ transplant recipients showed that the MMF group had a 27% adjusted risk reduction[31]. Conversely, a 3 group randomised control trial of 133 KTR; 45 KTR randomised to AZA treatment, 44 KTR randomised to 3 g daily of MMF and 44 KTR randomised to 3 g daily of MMF with no differences in cancer incidences between all three groups[32].

CALCINEURIN INHIBITORS

Cyclosporine A (CsA) forms a complex with cyclophilin which inhibits calcineurin, making CsA and CNI[33]. Calcineurin de-phosphorylates nuclear factor of activated T cells (NFAT), which translocates to the nucleus. It is in the nucleus where NFAT activates pro-inflammatory cytokines such as interleukin 2 (IL-2)[34]. Therefore CsA indirectly affects pro-inflammatory cytokine IL-2 transcription. An isotype of cyclophilin is expressed in the mitochondria which releases apoptotic signals under oxidative stress. CsA blocks this signal transduction and allows cells to by-pass apoptosis when under oxidative stress, including ROS and UV-damage, contributing to carcinogenesis[35,36]. Other tumorigenic side effects of CsA are direct or in-direct suppression of p53, production of transforming growth factor beta (TGF-β) and vascular endothelial growth factor (VEGF)[37-39].

When investigating this in the clinic, a retrospective analysis of 1000 KTR showed that KTR on CsA based regimens had greater cumulative incidence of tumours than those on an AZA based regimens[40]. In another retrospective study any regimen with CsA had an Odd Ratio of approximately 4.5[41]. Inversely, A CsA based mono-therapy was shown to be less carcinogenic than a MMF and prednisone dual-therapy[42,43]. Another CNI, tacrolimus (TAC), inhibits calcineurin by forming a complex with FK506-binding protein 12 (FKBP12) and outcompetes calmodulin therefore still inhibiting IL-2 transcription. TAC does not target cyclophilin, so avoids all interference with the mitochondria that CsA has. In a retrospective study of 609 liver transplant patients, TAC had a higher incidence rate for *de novo* cancers than CsA[44]. However in most database analyses, TAC-based immunosuppressive regimens have either no significant difference or a reduced risk of cancer incidence and/or risk over CsA-based immunosuppression regimens[45-48].

CORTICOSTEROIDS

Corticosteriods are mainly utilised for treatment of auto-immunity, inflammatory disorders and transplantation rejection. Corticosteriods function by inhibiting transcription of IL-1, IL-2, IL-6, interferon (IFN)-γ and tumor necrosis factor (TNF)-α and transcription factors such as nuclear factor-κB[49-54]. Inhibition of these Th1 cytokines promotes a Th2 response, which provides another indirect immunosuppressive function[55]. Corticosteroids induce TGF-β and can increase the incidence of Kaposi's sarcoma cell proliferation[56,57].

MAMMALIAN TARGET OF RAPAMYCIN INHIBITORS

Both Sirolimus (SIR) and Everolimus (EVO), like TAC, bind to FKBP12. However the formed complex inhibits mTOR's *via* mTORC 1 subunit (Raptor) binding and are considered mTORi. mTORi can also be classified as anti-proliferatives as they induce apoptosis *via* p53 dependent and independent pathways. This and mTORi's ability to prevent IL-2 signalling cause it to have both anti-cancer and anti-rejection properties. Additionally, mTORi affect protein synthesis, including VEGF which inhibits metastatic potential in murine models[58,59]. SIR has been used to treat patients with renal cell carcinoma (RCC) and EVO has shown to benefit patients with metastatic RCC who do not response to mainstream treatment[60-62]. Sirolimus Conversion from CNI based regimens, is beneficial in Kaposi sarcoma and SCC involution[63-66] However it can often lead to increased adverse reactions and increases in rejection episodes if performed too early post-transplant[67,68].

ANTI-THYMOCYTE GLOBULIN INDUCTION THERAPY

Anti-thymocyte globulin (ATG) is either horse- or rabbit-derived antibodies directed against human T cells, given as an induction therapy of transplant recipients. The T cells that reconstitute have a regulatory phenotype and return much faster than other T cells[69]. There is an association with prolonged CD4 lymphopenia and ATG as well as CD4 lymphopenia and cancer[70]. Without knowing cause and effect it is speculative to say that ATG is associated with cancer.

Despite the various functions of immunosuppressive types each playing a role with cancer in KTR, overall immunosuppressive load or immunosuppressive dose can also have detrimental effects and promote cancer development.

IMMUNOSUPPRESSION DOSE

There is an association between immunosuppression dose and cancer incidence. KTR have 3-fold increased cancer risk compared to dialysis patients, in a retrospective registry based study[71]. Furthermore, heart transplant patients have higher levels of immunosuppression than KTR and also have corresponding increases in cancer (100% compared to 88% 5 year incidence, respectively[14]). Additionally, KTR randomised to a low dose CsA base regimen had reduced incidence of cancer following reduction, with the caveat that they had higher rejection rates[72].

IMMUNOSUPPRESSION DURATION

Maintained immunosuppression increases the risk of cancer over time which is evident in the steady increase in KTR that accrue cancer in the years post-transplant. Australian KTR SCC incidence is 20%, 50% and 80% at 5, 15 and 30 years post transplantation respectively[11,73]. Included in the duration of immunosuppression would be the age and aging of the KTR.

AGE AND GENDER

Age is a risk factor of cancer development, independent of immunosuppression duration[74]. This is exemplified in a retrospective study that showed both Age and male gender were risk factors[41]. When comparing KTR to the general population in an aged matched cohort of median age 39 years old, there was a 12-fold increased risk of developing non-skin cancers[4]. Age and gender can influence other parameters of cancer risk. This is particularly the case in Australia were certain, culturally male-orientated, jobs may involve higher exposure to UV radiation.

ULTRA-VIOLET RADIATION

It is evident that UV exposure increases the risk of skin cancer, including NMSC, by the observations recorded by clinicians of the locations of tumours. Cumulative sun exposure, including outdoor occupation, latitudinal residence and even childhood burning events all increase risk of post-transplantation cancer development[75-77]. These increases in carcinogenesis are in part to the aforementioned AZA-UV interactions but mainly *via* direct UV-related mutagenesis. Due to the structure of DNA, it absorbs of UV-A (315-400 nm) and UV-B light (280-315 nm), in doing so the DNA itself forms cyclobutane pyrimidine dimers in two adjacent pyrimidines of the same DNA strand, which alters the structure of DNA and restricts transcription[78,79]. A single point mutation can lead to transcriptional arrest[79]. A study found that invasive SCC contained mutations of the tumour suppressor gene P53[80]. An important conclusion from this study is that P53 mutation could have happened in childhood, as most UV exposure happens in childhood[81].

In addition to direct DNA mutagenesis, UV exposure can also have local and systemic effects on the immune system. It is thought that the local effect involves antigen presenting cells (APC)'s, including resident keratinocytes and Langerhans cells[82,83]. Whereas the systemic immunosuppression may come from splenic cells, migrated Langerhans cells, dendritic cells. Increased expression of IL-4, IL-10, prostaglandin E2, IL-1α and TNF-α with polarisation of immunity to a Th2 response also plays a role in systemic immunosuppression[83-85]. In combination with this, co-stimulation is effected on both APC and T cells[86]. Other cell types that are affected by UV irradiation are innate immune cells and suppressor cells[87-91]. Regulatory T cells (Tregs) that are induced by UV express lymph node homing molecule CD62L and may provide systemic immunosuppression[87,88].

The DNA damage and immune suppression of UV can be reversed by IL-12 dependent induction of nucleotide excision repair protein[92]. Also immunity can be restored by the administration of IL-12[93], activating APC's, increasing IFN-γ and thus balancing Th1-Th2 polarisation[93,94].

Other clinical parameters are associated with cancer risk that are also orientated by human behaviour, apart from UV exposure, are communicable diseases such as oncogenic viral infections that remain latent in the immune competent.

VIRAL INFECTION

Human papillomavirus (HPV) is a group of more than 150 viruses with some types associating with anogenital, oropharyngeal and skin cancers[95,96]. It has been speculated that HPV infection may prevent UV light-induced apoptosis[97]. Between 65% and 90% of SCC lesions from transplant recipients are positive for HPV DNA[98].

Epstein barr virus (EBV) is associated with: sino-nasal angiocentric T-cell lymphoma, Hodgkin lymphoma and nasopharyngeal carcinoma[95]. There are data that EBV associates with mononucleosis, Burkitt lymphoma and post-transplant lymphoproliferative disorder in KTR[99,100].

Chronic Cytomegalovirus virus (CMV) infection can cause graft rejection, but with malignancy however it does have indirect associations with cancer[99]. A prospective study followed 63 KTR and retrospectively included 131 KTR, with convincing data that CMV positive KTR with increased $\gamma\delta$ T cell proportions, the $V\delta2^{neg}$ sub-population in particular, had decreased cancer incidence[101]. This case-control study compared 18 short-term KTR (median 3 years post Tx), who developed 12 skin and 6 solid tumours over the prospective period and compared to 45 KTR who did not develop cancer. The skin nor solid organ tumour types were not disclosed.

IMMUNE PHENOTYPING

The association with cellular markers and cancer has been previously studied. The identification of immune cell populations and sub-populations in patient blood is called immune phenotyping. Measurement of CD4 T cells in 150 KTR revealed that KTR with skin cancer had 330 $CD4^+$ cells/μL of blood in comparison to KTR with no cancer who had 565 $CD4^+$ cells/μL ($P < 0.01$). Additionally KTR with cancer had non-significant increases in CD8 and CD19 lymphocytes[102]. Another study involving 250 KTR over a 10 year period showed a mean of $CD4^+$ lymphocytes of < 600 $CD4^+$ T cells/μL for those with cancer and > 700 $CD4^+$ T cells/μL for those with no cancer, however there was no useful threshold found using receiver operator curve (ROC) analysis[103]. Additionally, $CD8^+$ T cells and $CD19^+$ B cells were also investigated in the same study; there was no difference between KTR with SCC when compared to KTR without SCC[104]. It was noted however, that immune phenotype was more pronounced in KTR with SOC compared to KTR with SCC: CD4 count: 234 cells/μL *vs* 543 cells/μL, $P < 0.001$; CD8: 328 cells/μL *vs* 640 cells/μL $P = 0.100$; CD19: 19 cells/μL *vs* 52 cells/μL, $P < 0.001$[104]. All these

studies showed an association with CD4 lymphopenia and cancer, however the majority of the cohorts underwent ATG induction therapy. However they did not define $CD4^+$ subsets or other lymphocytes that may be affected by cancer.

While these studies provide some evidence that cancer may influence the peripheral immune cells, there was no investigation into sub-types of these cells, primarily because multi-parameter flow was not common place. Recently, it was reported that high numbers of $CD4^+$ Regulatory T cells (Tregs, *i.e.*, $CD4^+FOXP3^+CD127^{Lo}CD25^{Hi}$) and low numbers of Natural Killer (NK cells, *i.e.*, $CD56^+CD16^+$), in peripheral blood associated with and predicted recurrent SCC in KTR[105]. This study also showed an increase in $CD8^+CD28^-$. These CD8 T cells co-localise with Tregs within cancer tissue and have been shown to be suppressive from patients with cancer, and therefore abbreviated to $CD8^+$ Tsupps[106]. Furthermore, there was a decrease in $CD8^+CD45RA^-CD62L^+$ CD8 central memory T cells ($CD8^+$ Tcm), which has been shown to decrease in KTR using the corticoid steroid prednisolone, despite cancer status[105]. This indicates that immunosuppression may affect immune phenotype and warrants investigation.

Operationally tolerant organ transplant recipients have increases in Regulatory T cells, B cells (particular naïve B cells), Vδ1 γδ T cells and decreases in $CD3^+$ proportions (B:T ratio), NK cells, Vδ2 γδ T cells within their peripheral blood[107]. Transplant patients have increased Regulatory T cells, B cells (memory B cells), $CD8^+$ γδ T cells and $CD8^+$ $CD27^-CD28^-$ T cells and decreases in CD4 counts, NK cells and $CD8^+$ Tcm[105,108].

REGULATORY T CELLS (TREGS)

Immune suppressor cell existence has been debated from the early 1970's through to the mid 1990's[109-112]. The pivotal paper adoptively transferred $CD4^+CD25^+$ T cells in CD25 depleted mice, which mitigated the autoimmune diseases that manifested[112]. However, CD25 is also expressed on activated lymphocytes with only the highest proportion being suppressive *in vitro via* competitive absorption of IL-2[112-115]. The discovery and transfection of the transcription factor forkhead box protein 3 (FOXP3) into naïve T cells helped identify FOXP3 and its function as the master regulatory gene[116,117] and CD127 inversed expression to FOXP3 expression has given Tregs the current phenotype $CD4^+FOXP3^+CD25^{hi}CD127^{lo}$[114].

Tregs are required in a healthy immune system to maintain self-tolerance and immune homoeostasis during immune reactions, pregnancy and disease. Uncontrolled immune reactions and organ failure result when mutations in FOXP3 occur, as observed in the scurfy mouse models and similarly Immunodysregulation, Polyendocrinopathy, enteropathy, and X-linked (IPEX) syndrome observed in humans[118-120]. Both IPEX and X-linked Autoimmunity-Allergic Dysregulation syndrome cause multi-organ failure due to mass lymphocyte proliferation of self-reactive effector cells [119].

$CD4^+$ TREG SUBSETS

The $CD4^+$ Treg in the periphery, defined by $FOXP3^+CD25^{hi}CD127^{lo}$, contain two subsets: those that originate from the thymus, known as natural Tregs (nTregs), and those that are induced in the periphery, known as induced Tregs[121]. The Ikaros family transcription factor, Helios is expressed in 100% of all $CD4^+FOXP3^+$ thymocytes of mice and approximately 70% of Tregs in the periphery of both mice and humans[122]. Though the premise that Helios only defines nTreg is currently under debate, nonetheless, it may provide evidence of *in vivo* activated Tregs[101,123]. Despite the debate it seems that KTR with cancer have similar Helios expression than KTR without cancer[108].

TREG MODES OF ACTION

Treg apoptosis induction requires cell contact with co-stimulatory molecule Cytotoxic T cell Late Antigen-4, Fas/Fas ligand interaction and release of Perforin and Granzyme B[124-126]. Indirectly, Tregs can down-regulate B7 Co-stimulation molecules CD80/CD86 on APC[127]. In addition, prostaglandin E2 (PGE2) excreted by Tregs, mediates expression of indoleamine 2,3-dioxygenase in APCs causing tryptophan starvation and leading to impaired lymphocyte proliferation[128]. Another form of suppression is the formation of localised adenosine by cleaving phosphate groups from ATP, ADP and AMP by ecto-NTPDase-1 (CD39) and ecto-5'-nucleotidase (CD73) cell surface enzymes[129]. Expression of CD39 and CD73 has been shown on murine and human Tregs[129]. Human Tregs also may work in concert with other CD73 expressing cells to elicit a regulatory response. Adenosine has been shown to act *via* Adenosine receptors (A1, A2a, A2b and/or A3), with A2a receptor being the dominate receptor on effector cells[130,131]. The adenosine formed by the hydrolysis of ATP can regulate lymphocyte proliferation in autoimmune disease, transplantation and cancers[132-134]. Additionally, it has been shown that adenosine and PGE2 in Tregs co-operate when regulating immune responses[133]. Other regulatory cells are $CD4^+$ helpers that have suppressive function are classified by the ability to secret of IL-10 (Tr1) and TGF-β (Th3) which they are also induced by, respectively.

TREGS IN VIRAL INFECTIONS

EBV antigen specific Tregs, mainly IL-10 secreting Tr1 and recruited nTregs, can inhibit the EBV-specific immunity permissive in tumour progression[100,135]. Thus reduction in Tregs may be beneficial in treatment of chronic viruses. Interestingly, Treg depletion in a herpes simplex virus (HSV) mouse model decreased paralysis onset, indicating that Tregs have an early role in protective immunity to HSV infection, similarly observed

in Lymphocytic Choriomenigitis virus mouse model, shown in the same study[136].

TREGS AND TRANSPLANTATION

In regards to transplantation, when isolated $CD4^+CD25^-$ cells are administered to BLABc *nu*/*nu* mice grafted with C57BL/6 skin there is a swifter rejection rate than administering untouched lymphocytes of the same source[112]. This indicates $CD4^+CD25^-$ T cell subpopulation has greater cytotoxicity when absent from $CD4^+CD25^+$ T cells and that $CD4^+CD25^+$ T cells are possible inducers of tolerance.

In KTR, Tregs can differ in accordance with the situation of the patient. Two different studies on clinically tolerant, chronic rejection, stable, minimally suppressed KTR and healthy controls, showed tolerant KTR and minimally suppressed KTR had similar $CD4^{+-}$ $CD25^+FOXP3^+$ and $CD4^+CD25^{hi}$ cells with similar FOXP3 transcription levels when compared to the healthy controls[137,138] and that chronically rejecting KTR had lower $CD4^+CD25^{hi}$ cells with low FOXP3 transcripts, indicating that Tregs may be protective or involved with tolerance[137,138]. An additional study supported this in liver transplant recipients which showed increased FOXP3 mRNA expression in $CD4^+CD25^{hi}$ T cells of tolerant patients compared to patients who had rejection episodes after cessation of immunosuppression[139]. Thus induction of Tregs for suppression of allograft cellular rejection episodes[140] and possible induction of tolerance[141] seem like an attractive substitute to immunosuppression. However Tregs that co-express CD25 and CD39 have been denoted as a memory subtype of Treg (mTreg) and are associated with cellular rejection episodes[142] in KTR. Increases in Tregs are also associated with cancer in the general population[143] and KTR[105].

TREGS IN CANCER AND IMMUNE SURVEILLANCE

It has been shown that the percentage of $CD4^+CD25^{high}$-$FOXP3^+$ Tregs and Tr1 cells are increased in Head and Neck Squamous Cell Carcinoma (HNSCC) patients in comparison to healthy controls[144,145]. Ectonucleotidase activity contributed by CD39 and CD73 is also increased on Tregs in this cohort[133]. CD39 has been shown to down-regulate IL-17 production, decreasing Th-17 cell linage. This particular Treg subtype, in the same study, has been shown to be down-regulated in autoimmune Multiple Sclerosis[132]. It has been shown that high levels of Treg in HNSCC patients from the general population associate with a poor prognosis[146-148].

Cancers and Tregs not only have commonalities between each other but they also promote each other. TGF-β and IL-10 secretions from tumours activate Th3 and Tr1 regulatory cells respectively, consequently regulating surrounding cancer cytotoxic lymphocytes[145]. Also tumour cells recruit Tregs with a series of chemokines such as C-X-C Ligand 12 and C-C motif 20 and 22 (CCL20/22)[100]. CD39 and CD73 have been shown to be expressed on Tr1 and tumour cells alike[129,149]. Cancer progresses by the tumours' ability to secrete these soluble factors into its microenvironment. PGE2 is a product of Cyclooxygenase 2 (COX-2) and is involved in aiding immune escape. COX-2 is expressed on Tr1 and over-expressed on cancer cells[145,150,151].

In a post-transplant cancer setting, it has been shown that Tregs ($CD4^+FOXP3^+CD25^{hi}CD127^{lo}$) in blood from KTR with a history of SCC can predict the risk of developing a subsequent SCC lesion[105]. Another study has shown that Tregs alone can predict cancer onset and associate to the severity of the cancer developed[108]. In this same study Hope *et al*[108] shows prospectively that Tregs increase in KTR when the cancer becomes apparent and then decreases post-resection of tumour tissue.

NK CELLS IN CANCER AND IMMUNE SURVEILLANCE

Carroll *et al*[152] revealed that NK cells, which have cytolytic ability to kill cancerous and pre-cancerous cells, are decreased in KTR with cancer. NK cells are a part of the innate immune system that identify abnormal cells and supply the signals to undergo apoptosis thus "killing" abnormal cells. The identification process involves Major Histo-incompatibility Complex (MHC) class I down regulation, which some viruses and cancerous cells adopted to avoid the adaptive immune system[153]. It is an important step in metastatic cells to successfully invade the host[154]. Once the cell has been identified the NK cell only activates if there is an imbalance of CD94: NKG2A and the killer-cell immunoglobulin-like receptors (KIR) family. Once activated internal granules locate to the synapse that is created between the NK cell and target cell[152]. During the effector stage the granules are released out of the NK cell and into the synapse and onto the target cell. These proteins include Perforin, granzyme A and B. It is these proteins that play their role in the killer phase of NK cells[155]. Perforin creates pores in the membrane that granzyme B can enter and activate the caspase kinase pathway and cause the target cell to undergo apoptosis[155]. This cytotoxic ability to kill cancer cells can be inhibited by Tregs but also cancer cells themselves[156,157]. This NK-Treg interaction is a TGF-β and cell-cell contact mechanism of down-regulation NKG2D and induction of apoptosis, respectively[158,159]. This leads to decreased NK cell numbers and function in the peripheral blood of cancer patients that have elevated TGF-β[160,161]. There are two other types of NK cells: those that express CD1-d restricted T cell receptor, NK T cells and those that lack Fc receptor CD16 and over express CD56, $CD56^{bright}$ NK cells[162-164]. Both these cells can interact with the adaptive immune system and enhance

anti-tumour ability by direct and indirect mechanism respectively[162,164].

CD8 SUBSETS IN CANCER AND IMMUNE SURVEILLANCE

Another cell type with anti-tumour properties is $CD8^+$ cytotoxic T lymphocytes (CTL). $CD8^+$ CTL are in the effector arm of the adaptive immune system. CTLs use the ability to lyse tumour cells using Fas-Fas ligand as well as perforin-IFN-γ granules similar to NK cells[165]. It has been shown that antigen specific CTL are defective in cancer patients and that removal of Tregs can restore cytolytic function[166-168].

CD4 and CD8 T cells follow an immunogenic pathway to immune senescence. T cells exiting the thymus are naïve since they express both CD27 and CD28 co-stimulation molecules and home to the lymphoid organs[169,170]. When antigen is presented they become CTL, clear the threat, and the majority apoptose with the minority homing to lymphoid organs as central memory T cells or extra-lympoid sites as effector memory T cells[169,170]. Upon subsequent exposures the cells become exhausted and lose expression of co-stimulation molecules and are termed T effector memory CD45RA+ or TemRA cells[169,171]. These cells are loosely phenotyped as $CD8^+CD28^-$ and shown to be regulatory in cancer patients and may associate with poor prognosis[106]. Tumours themselves may induce this loss of CD28[106,172] and they are also expanded in patients with CMV infection[173]. It has been shown that Memory T cells and (NK cells have anti-tumorigenic properties and that Tregs regulate both of these lymphocyte subsets[158,174]. Thus, an excess of Tregs is associated with poor prognosis in cancer and is thought to aid cancer cells evade this immune surveillance.

IMMUNE CELL FUNCTIONS

Kidney transplant recipients (KTR) with cancer have increased numbers and proportions of Regulatory T cells (Tregs) and decreased numbers and proportions of NK cells[105,108]. However, the immune system's effectiveness cannot be gauged by cell numbers and proportions alone; this chapter investigates the immune function of KTR with cancer.

It has been shown that Tregs isolated from tumour tissue and the peripheral blood of KTR with cancer have higher suppressive function than Tregs from the blood of normal donors[145,175,176]. Importantly, the stage and grade of HNSCC are associated with greater numbers and greater suppression capacity of the Tregs on a cell-per-cell basis than healthy controls[177] and, as such, also associate with poor cancer prognosis in the general population[176].

In the Transplant population it is known that CNI regimens are associated with reduced numbers and proportions of Tregs and how mTORi maintain these Treg parameters[178,179]. Furthermore, Tregs numbers and proportions are increased by mTORi usage in KTR with no cancer and CNI usage decreases Tregs in KTR with cancer. A proposed mechanism is CNI's ability to reduce Nuclear Factor of Activated T cells (NFAT), decreasing production of IL-2 which is vital for function and homeostasis, in mice[180]. Molecular interactions between NFAT and FOXP3 show that NFAT acts as a molecular switch between immune stimulator and immune regulator, thus down regulation decreases FOXP3 expression and FOXP3's ability to form these regulatory complexes[178,181]. Additionally, FOXP3 mRNA transcription was decreased in CNI treated peripheral blood mononuclear cells compared to Rapamycin in an allo-stimulated mixed lymphocyte reaction[182]. There is also an inverse correlation to CNI level and Treg function[183].

Tregs promote cancer survival whereas NK cells have anti-cancer abilities. The function or dysfunction of NK cells plays an important role in the apoptosis of pre-cancer and cancerous cells. Patients with genetically (*MCM4* or *GATA2* mutations) related NK cell deficiencies in either number or function, have increased risk of infections, in particular: Herpes viruses, HPV, CMV and EBV (reviewed elsewhere[184]).

NK cells are large granular lymphocytes that lack the CD3 T cell complex. They function by identifying and spontaneously causing apoptosis in cancerous and infected cells without prior antigen presentation[152,185]. The identification process requires abnormal cells to display stress signals such as down-regulation of "self" surface proteins: Major Histo-incompatibility Complex (MHC) class Ⅰ and regulatory KIR[154,155,186]. The down regulation of MHC-Ⅰ, reduces the effectiveness of cytotoxic CD8+ T cells and adaptive immune responses but makes the cells more sensitive to NK and innate immune responses[187]. Once an NK cell identifies this down-regulation, it binds and activates, expressing a type Ⅱ transmembrane glycoprotein CD69 and other surface markers of activation[188]. Internal granules locate to the immune synapse that is created between the NK cell and the target cell and the effector molecules (perforin, TNF-α, granzymes and interferons) are released into the synapse and onto the target cell. Upon degranulation, Lysosome-Associated Membrane Protein 1 (CD107a) is exposed on the surface of the NK cell[155]. The released perforin creates pores in the target cell membrane through which granzyme B can enter the target cell and initiate apoptosis *via* the caspase kinase pathway. Therefore there are several ways to measure NK cell activity including: CD69 up-regulation in the activation stage, CD107a in the effector stage, release of cytokines (perforin, granzyme B, IFN-γ) in the killing stage, and total cytolysis of the target cells.

Cancer cells have greater metabolic demands than normal cells[189], utilising glycolysis and lactate pathways, *via* Lactate Dehydrogenase (LDH), causing an 18-fold increase in glucose utilisation, even under aerobic conditions[190]. This LDH can be measured as a cytotoxic assay (first described in 1988[191]). Additionally, in *in*

vitro assays, NK cells undergo apoptosis when they are exhausted from their last kill. Recently, it has been shown that the loss of NK cells from an *in vitro* assay with a set number of NK cells, can relate to the amount of target cells killed. This loss has been termed "target induced NK cell loss" (TINKL). These two assays have been chosen for clinical application. LDH is a single platform, self-contained, non-radioactive, sensitive assay that can be used in any laboratory. TINKL is a flow-based assay that can be readily implemented in clinical flow laboratories.

It is widely accepted that NK cell function is decreased in cancer patients however it is not reported if KTR with cancer have further reduced NK cell function. The effect immunosuppression has on NK cells have been investigated both *in vitro* and *in vivo*[192,193]. Immunosuppressive drugs: AZA, MMF, CNI, and prednisolone all have individual effects. These effects depend on the how the NK cells are stimulated and how NK function is measured. One particular study showed only a decrease in NK function in short term KTR compared to healthy controls, which was not observed in long term KTR[192]. Both IFN-γ and CD107a expression have been shown to decrease when NK cells were co-cultured in the presence of clinically relevant concentrations of a variety of immunosuppressive drugs[193].

TREATMENT OPTIONS FOR KTR WITH CANCER

The aforementioned assays give clinicians the ability to objectively identify patients that may develop premetastatic cancer with relatively high sensitivity and specificity. However they do not inform clinicians if KTR will benefit from cancer prevention therapy.

A randomised control trial randomised pre-transplant KTR to a standard level CNI regimen and a CNI sparing regimen[72], thus investigating the benefit of reduced immunosuppression as primary cancer prevention. However, those with reduced CNI had increases in rejection episodes[72]. Other studies investigated converting CNI based regimens to mTORi based regimens as secondary prevention therapy, as mTORi are used as anti-cancer therapies[194,195]. There was a benefit, however not all conversions were successful (30%) and an additional 30% did not tolerate the mTORi side effects[14,196,197]. Furthermore, immune phenotype has revealed that those who maintain high levels of Tregs after mTORi conversion (> 20 Tregs/μL) do not benefit from conversion and may benefit from immunosuppressive drug reduction. To perform immunosuppressive drug reduction as secondary cancer prevention, risk of graft rejection will need to be measurable.

Pre-transplant anti-Human Leukocyte Antigen (HLA) and IFN-γ ELISPOT associate post-transplant with antibody and cellular mediated rejection episodes[198-200]. Monitoring HLA molecules and Donor Specific Antibodies (DSA) routinely has decreased antibody mediated rejection episodes dramatically[201,202]. IFN-γ ELISPOT has been used to predict 6-mo graft function and rejection episodes[200]. Additionally it has been used pre-transplant to categorise patients into CNI or mTORi maintenance therapy[203]. These studies are limited in clinical application as donor specific cells were used to stimulate the mixed lymphocyte reactions, requiring use of precious or non-existent deceased donor material. This restricts the utility of ELISPOT to live recipient/donor pairs. An IFN-γ ELISPOT assay has been developed that utilises a variety of unrelated HLA disparate material to measure total allo-response and is termed "Panel of Reactive T cells"[204]. This assay has been shown to have potential to determine post-transplant risk of rejection when measured pre-transplant. However there are no current studies utilising IFN-γ post-transplant as a form of rejection prediction in long-term KTR.

The IFN-γ ELISPOT may be extended to guide immunosuppression reductions[205,206]. There are a few studies utilising a viral peptide stimulated IFN-γ ELISPOT to discriminate KTR who may benefit from reduced immunosuppressive drugs as a form of treatment[205,206]. KTR with unresolved BK pathogenesis also had a non-significant decrease in EBV peptide and phytohaemagglutinin mitogenic IFN-γ ELISPOT responses[205,207]. This may share a link with development of malignancy as they are both considered manifestations of over-immunosuppression.

When KTR have a cancerous lesion, surgical resection is the recommended treatment. There are no randomised control trials investigating the effect of tumour resection and minimal evidence of benefit in KTR when reducing immunosuppression. However, treatment in the general population is associated with a decrease in Tregs. Failure of Tregs to fall after tumour excision, chemo or immunotherapy is due to incomplete resection or predicted relapse of disease[208,209].

When switching or reducing immunosuppression, adequate precautions must be used. Currently there are no assays that reliably determine cancer risk although there is an immune phenotype that can predict time to next tumour in KTR with a history of SCC[105]. CNI avoidance or reduction results in increases of rejection; one way to potentially avoid these rejection episodes is to identify those KTR with cancer who have evidence of a potential alloresponse and exclude them from dose reduction. In order to reduce immunosuppression safely, both the cellular and humoral alloresponses need to be assessed.

PRE-TREATMENT ALLORESPONSE MEASURES

Assessment of allo-responses would be needed to assess risk of rejection episodes for it to be possible to reduce immunosuppression. Currently cytokines and HLA antibodies can be measured by Enzyme Linked Immuno SPOT (ELISPOT) and Luminex technologies respectively[198,210]. Inflammatory cytokines such as IFN-γ are secreted by Th1 effector T cells and are a predictor of

acute rejection and infection[200,204]. A National Institute of Health funded Clinical Trials in Organ Transplant consortium approved ELISPOT has been able to detect 6-mo post-transplant acute rejection in pre-transplant patients[211,212]. Additionally a similar assay has been used to run CNI avoidance maintenance therapy with a 3-fold reduction in acute rejection as shown in literature[203]. The humoral aspect of the immune system is already routinely assessed in most transplant programmes by solid phase alloantibody detection systems[202]. HLA DSA are clinically relevant and observed DSA presence has informed clinicians to alter immunosuppression regime of patients[199,201]. However both these techniques have not been measured in long-term kidney transplant recipients with a history of cancer.

CONCLUSION

Long-term immunosuppression increases the risk of cancer development. The dose of immunosuppression can be increased by closely monitoring graft function and survival. In this review we present that there are several emerging immune monitoring tools that are available to potentially help reduce immunosuppression. Future studies may be undertaken to determine if these assays can help identify those at risk of cancer development and if reduction of immunosuppression is of benefit.

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REVIEW

Biomarkers in chronic kidney disease, from kidney function to kidney damage

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Abstract

Chronic kidney disease (CKD) typically evolves over many years, with a long latent period when the disease is clinically silent and therefore diagnosis, evaluation and treatment is based mainly on biomarkers that assess kidney function. Glomerular filtration rate (GFR) remains the ideal marker of kidney function. Unfortunately measuring GFR is time consuming and therefore GFR is usually estimated from equations that take into account endogenous filtration markers like serum creatinine (SCr) and cystatin C (CysC). Other biomarkers such as albuminuria may precede kidney function decline and have demonstrated to have strong associations with disease progression and outcomes. New potential biomarkers have arisen with the promise of detecting kidney damage prior to the currently used markers. The aim of this review is to discuss the utility of the GFR estimating equations and biomarkers in CKD and the different clinical settings where these should be applied. The CKD-Epidemiology Collaboration equation performs better than the modification of diet in renal disease equation, especially at GFR above 60 mL/min per 1.73 m^2. Equations combining CysC and SCr perform better than the equations using either CysC or SCr alone and are recommended in situations where CKD needs to be confirmed. Combining creatinine, CysC and urine albumin to creatinine ratio improves risk stratification for kidney disease progression and mortality. Kidney injury molecule and neutrophil gelatinase-associated lipocalin are considered reasonable biomarkers in urine and plasma to determine severity and prognosis of CKD.

Key words: Chronic kidney disease; Estimated glomerular filtration rate; Kidney damage; New biomarkers; MicroRNA

Core tip: Until more accurate equations are developed the chronic kidney disease (CKD) epidemiology collaboration appears to be superior to other glomerular filtration rate (GFR) estimating equations. In circumstances where CKD requires confirmation estimated GFR based on the combined creatinine-cystatin C equation is recommended. The recent advances in molecular biology have resulted in promising biomarkers for CKD detection and prognosis; however more research is needed before applying them into clinical practice.

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INTRODUCTION

Chronic kidney disease (CKD) has become a public-health problem. The definition of CKD was introduced by de National Kidney Foundation (NFK/KDOQI) in 2002 and latter adopted by the international group Kidney Disease Improving Global Outcomes (KDIGO) in 2004. The definition of CKD requires a decrease in kidney function with a glomerular filtration rate (GFR) of less than 60 mL/min per 1.73 m^2 and/or kidney damage for 3 mo or more. Kidney damage refers to pathologic abnormalities documented by biopsy or imaging, alterations in urinary sediment or proteinuria (proteinuria/creatinuria > 200 mg/g, albuminuria/creatinuria > 30 mg/g)[1].

One important aspect about of classification of CKD is that it can usually be detected with non invasive testing. CKD classification is relevant as it has been associated with outcomes such as kidney disease progression, cardiovascular disease and all cause mortality. It is also important as it can allow therapeutic interventions in earlier stages to slow disease progression reduce complications related to decreased estimated GFR (eGFR), cardiovascular (CVD) risk and improve quality of life and survival[2-4]. GFR is the most important marker of kidney function. Unfortunately GFR cannot be easily measured in most clinical or research settings (see below), and therefore estimating equations are based on filtration markers such as serum creatinine (SCr) and cystatin C (CysC). Other biomarkers such as albuminuria may precede kidney function decline and have demonstrated to have strong associations with disease progression and outcomes. New potential biomarkers have arisen with the promise of detecting kidney damage prior to the commonly used markers of kidney disease. The aim of this review is to summarize the most recent findings of most biomarkers in CKD and its implications in clinical practice.

KIDNEY FUNCTION MEASUREMENT

Kidney function estimation was commonly made using SCr concentration, blood urea nitrogen (BUN) level and urine analysis[5]. However accumulating evidence has demonstrated that these biomarkers are not optimal to detect kidney disease in early stages[6-9]. The KDIGO recommends that CKD be diagnosed, classified, and staged by GFR[10]. In clinical practice GFR is crucial for diagnosis, management, drug dosing and prognosis, in addition to its utility for research and public health[11-13]. GFR is the volume of fluid filtered from the glomerular capillaries into the Bowman's capsule per unit time[14,15]. GFR values are associated with age, sex and body surface and are 120 and 130 mL/min per 1.73 m^2 in young men and women, respectively (GFR declines with age)[16-18].

mGFR

Establishing the true GFR is difficult because the filtration process simultaneously takes place in millions of glomeruli and filtrate composition and volume change when passing through the kidney. GFR is measured (mGFR) indirectly as the clearance of filtration markers that are eliminated by the kidney only by glomerular filtration. Clearance can be measured as either plasma or urinary methods that record the clearance of endogenous or exogenous substances by the kidney[11]. As such, an ideal substance is one that is freely filtered at the glomeruli and neither secreted nor reabsorbed by the renal tubules[15,18]. Inulin is an exogenous filtration marker derived from a fructose polymer and is a physiologically inert substance and is considered an ideal substance for mGFR[19,20]. Although inulin clearance is considered the gold-standard method for mGFR[20,21], the need for continuous infusion, multiple blood samples and urine collection, make it cumbersome and expensive to measure and has led to research of alternative methods with other biomarkers[10,21-24].

Other methods for mGFR have also been validated. Soveri *et al*[24] reported that kidney excretion of 51Cr-EDTA or iothalamate, and plasma removal of 51Cr-EDTA or iohexol, using inulin clearance as reference, were sufficiently accurate (P30 > 80%) methods to measure GFR[24]. Among these iohexol is the most recent biomarker for mGFR, it is a non-ionic and non radioactive contrast agent, its molecular weight is 821 Da, has a small extra renal clearance and could be measured only as plasma clearance without the need of urine collections[25]. Some of its other advantages are low expense, wide availability, stability in biologic fluids, and rare adverse reactions when given in a small dose (5 mL of 300 mg/mL iodine)[26,27]. In addition, iohexol does not require a continuous Ⅳ infusion and can be given as an intravenous bolus injection. It can be measured by several different techniques, the most used is the high-performance liquid chromatography (HPLC). However, HPLC requires a great deal of effort which limits its usefulness in the clinical setting[28]. Capillary electrophoresis (CE) a technique in which electrophoretic separations are performed in capillary tubes and is easier and faster than HPLC[29]. Shihabi *et al*[30] demonstrated that the iohexol determination by CE correlates well with HPLC.

However all these methods still require the need of continuous infusion or bolus administration of the marker (subcutaneous or intravenous) and like inulin, their complexity limits their application in clinical practice and epidemiological studies, mostly for the length of time that the procedure entails.

Routinely, GFR is usually estimated from prediction equations which are based on endogenous serum markers like creatinine or CysC in addition to demographic variables such as age, sex and race[13,16,31]. Measured GFR is reserved for situations where eGFR may be inaccurate such as patients in non-steady state, or individuals that

possess different characteristics compared to those where the estimating equation was created such as old age, loss of muscle mass (malnutrition, amputation, paraplegia) obesity, chronic illness or in situations where precise GFR is important, like kidney definition[12,32-34].

GFR estimation

Given the limitations of creatinine as a marker of kidney function, implementation of prediction equations has been widely used to eGFR from endogenous filtration markers without the need of clearance calculation[32]. As mentioned above, SCr and CysC are the most commonly used endogenous filtration markers for eGFR.

Creatinine: SCr derives from creatine degradation with a weight of 113 Da[35]. It is freely filtered but is not reabsorbed or metabolized however a significant percentage of creatinine in the urine derives from proximal tubular secretion[16,36]. One of the requirements for utilizing estimating equations based on SCr is stable kidney function. In addition, non-GFR determinats, such as variation in production associated to dietary intake, or changes in muscle mass, variation in tubular secretion and extrarenal creatinine excretion (associated with advanced kidney disease) need to be accounted when utilizing creatinine[13,32,37,38].

Another important factor that limits the accuracy of equations is the variability in SCr measurement[39]. In a study that examined frozen samples from 554 participants, where creatinine was measured with different assays, the SCr changed on average 0.23 mg/dL. This difference can result in substantial variations in GFR estimation when the SCr concentration is relatively normal[40]. The recognition that small variations in SCr translates in significant changes in kidney function has prompted to standardize creatinine determinations throughout clinical laboratories. In 2006 a standard method was introduced as a reference and was used in combination with the isotope-dilution mass spectrometry method in order to achieve better consensus among methods[41,42].

CysC: CysC has come to light as another marker of kidney function during the past decade. However, its clinical use worldwide remains limited compared with that of SCr[43]. CysC is a non-glycosylated protein produced by all nucleated cells. CysC is freely filtered, reabsorbed and completely metabolized in tubular cells and therefore is not subjected to tubular secretion[44,45]. Compared to creatinine, CysC has a more stable rate of production with less intra variability; however CysC serum levels are also influenced by non GFR determinants, such as uncontrolled thyroid disease, corticosteroid use, age, sex, ethnicity, smoking and adipose tissue[46-48]. In a recent meta analyses, the reciprocal value of CysC was more closely related to GFR (correlation coefficient 0.82 *vs* 0.74) and higher area under de curve (0.93 *vs* 0.84)[49].

In addition, CysC predicts outcomes and the association is stronger than SCr. Shlipak *et al*[50] reported CysC level to have an important association with mortality across the GFR range, including individuals with GFR between 60 and 90 mL/min per 1.73 m^2, grouped as "preclinical kidney disease"[50]. These findings have been reproduced in other studies in older adults where CysC has been shown to be a better predictor of adverse cardiovascular and non cardiovascular outcomes compared to to SCr[51-56]. Potential explanations for these findings may be accounted by the fact that compared to SCr, CysC is not influenced by muscle mass and reflect a better marker of GFR in this population[53]. In addition, these findings have also been reproduced in the general population and CysC estimated GFR has consistently provided a stronger association with outcomes than equations based on SCr eGFR[57].

Estimating equations

Since Effersoe in 1957 developed the first equation to estimate GFR[58], more de 20 equations have been developed. Most of the equations incorporate demographic and clinical variables[39]. The most commonly used equations include Cockroft Gault (CG)[59], 4-modification of diet in renal disease (MDRD)[60,61], 2009 CKDEPI[62] and more recently the equation that combines creatinine and CysC[63]. Since the standardization of creatinine, the CG equation is barely used in clinical practice[39].

CG: The CG formula was created almost thirty years ago in order to estimate creatinine clearance. It was developed in a population of white men and therefore the equation does not take into consideration sex, race and body surface area. Until recently, CG equation was solely utilized for drug dosing however the equation has been recently compared to the widely used equations with similar findings[59,64].

MDRD equation: The MDRD equation was developed in 1999 from a study including 1628 mostly white and non diabetic patients with CKD stages 3 and 4. The original equation included 6-variables and was further abbreviated in year 2000 to a four variable equation that included age, sex, ethnicity, and SCr[60]. In 2006 it was adapted to be used with standardized creatinine[61]. The four variable equation demonstrated to have similar performance compared to the six variable equation[65]. Although the MDRD has demonstrated to have high accuracy for individuals with CKD, the equation underestimates GFR in healthy individuals resulting in false positive diagnosis of CKD in this population[66].

CKD-epidemiology collaboration equation: The CKD epidemiology collaboration (CKD-EPI) was developed in 2009 and resulted from a study that included 8250 participants and was validated in similar cohort of 3900 subjects. Compared to the MDRD coh–ort, the CKD-EPI had higher GFR (68 mL/min per 1.73 m^2 *vs* 40 mL/min per 1.73 m^2), younger

age, included diabetics, blacks and kidney transplant recipients[39,62,67]. Linear regression was employed to estimate the logarithm of measured GFR from standardized SCr concentrations, gender, race, and age. The main objective for the CKD-EPI was to develop an equation that was superior to the MDRD, especially amongst those subjects with GFR > 60 mL/min per 1.73 m^2. Indeed, the same variables were used in CKD-EPI and MDRD equations but CKD-EPI performed better in those with GFR > 60 mL/min per 1.73 m^2. In subjects with GFR > 60 mL/min per 1.73 m^2 the P30% was 88.3% (86.9%-89.7%) and 84.7% (83%-86.3%) for CKD-EPI and MDRD, respectively, while in subjects with GFR < 60 mL/min per 1.73 m^2 the P30% for CKD-EPI was 79.9% (78.1%-81.7%) and for MDRD was 77.2% (75.5%-79%). Furthermore the CKD prevalence was estimated using the CKD-EPI and MDRD Study equations among 16032 adults from the NHANES cohort. Median eGFR by CKD-EPI was almost 10 mL/min per 1.73 m^2 higher than by MDRD. As a result, the CKD-EPI resulted in a significantly lower estimated CKD prevalence than the MDRD equation in the g (11.6% *vs* 13.1%, respectively)[62].

CysC and combined CysC and creatinine equations: In order to overcome the imprecision of creatinine estimating equations, Stevens *et al*[48], developed three eGFR equations for CysC (using CysC alone, CysC with demographic factors, and CysC with SCr and demographic factors) and compared them with mGFR iothalamate and 51-EDTA in 3418 patients. The equation that included CysC with SCr yielded the most accurate GFR estimates (P30 of 89%)[48]. Segarra *et al*[68] found that CysC-based GFR equations performed better than the CKD-EPI equation in a study of 3114 hospitalized patients because creatinine generation is dependent on the presence of muscle mass and malnourishment[68]. Similarly CysC-based GFR was superior than the CKD-EPI equation in certain subgroups of patients in which SCr level may be insensitive to capture reduced kidney function such as patients with chronic liver disease, frail elders, AIDS and malignancy[69-74].

Inker *et al*[63] developed a new GFR estimating equation that was based on CysC alone or in combination with creatinine in a cohort of 5000 subjects and was further validated in a cohort of 1119 subjects with measured GFR. The authors developed two new equations involving CysC (2012 CKD-EPI cys, and 2012 CKD-EPI Cys-cr) and compared them to the 2009 CKD-EPI equation. Bias was not different between the three equations however precision and accuracy was improved with the combined CysC-cr equation. Also in subjects whose eGFRcr was of 45-59 mL/min per 1.73 m^2, the combined equation reclassified correctly 17% to a no CKD category (GFR > 60 mL/min per 1.73 m^2). The authors concluded that the combined equation performed better than equations based on either CysC or SCr and should be used in those subjects where CKD needs to be confirmed[63].

Ongoing studies include the eGFR-C study which is a prospective longitudinal cohort study of 1300 adults with stage 3 CKD that will be followed for 3 years with reference iohexol mGFR. The objective of the study is to evaluate the performance of GFR-estimating equations, including the new equations that incorporate CysC in addition to albuminuria, in order to monitor GFR progression in this populations. Data will be analyzed to assess the impact of race, proteinuria and diabetes on equation performance[75].

Equations, their performance and their implications

When we evaluate the performance of an equation we should take into account bias, precision, and accuracy. Bias has been defined as a median difference between the measured and estimating GFR, precision this is the repeatability or reproducibility of the measurement and accuracy is defined as percentage of eGFR within 30% of measured GFR. Accuracy is probably the best single measure for comparing equations because it incorporates bias and precision. The 2002 KDOQI guidelines concluded that an eGFR within 30% of an mGFR was satisfactory for clinical interpretation, and as a performance metric for accuracy, the guidelines recommended that > 90% of participants in the validation population have eGFR within 30% of the measured GFR (P30 > 90%)[76]. Although accuracy in GFR assessment has significantly improved and bias was decreased with the CKD-EPI equation, precision has not substantially improved. This imprecision is due to random error secondary to variation in non-GFR determinants and GFR measurement error, whilst bias reflects differences between the development and validation populations in measurement methods for GFR, assays for filtration markers, or the relationship of the surrogates to the non-GFR determinants of the filtration marker[13].

In one study conducted by Michels *et al*[77] that included 271 patients with a mean SCr of 1.2 mg/dL, the CG, MDRD, and CKD-EPI equations were compared with mGFR using the I-iothalamate filtration marker (median mGFR 78.2 mL/min per 1.73 m^2), to assess the agreement between equations and examine whether the agreement was influenced by other known variables such as age, weight, body mass index and level of GFR. In general this study concluded that the CKD-EPI equation gives the overall best GFR estimation however the performance was close to MDRD[77].

One of the largest studies where MDRD and CKD-EPI were compared with the aim to assess performance was performed in a population of 12898 individuals from North America, Europe and Australia. The P30 ranged from 59%-95% and was higher for the CKD-EPI than for the MDRD equation in most studies, bias varied according to level of eGFR, was smaller for the CKD-EPI than for the MDRD equation at higher eGFR, but larger at lower eGFR. Table 1 shows the performance comparison of the equations in these populations. Authors from this study concluded that equations did

Table 1 Performance comparison of creatinine-based estimated glomerular filtration rate in North America/Europe/Australia

Ref.	Country	Patients, *n*	mGFR (value mL/min × 1.73 m^2, SD)	eGFR (equation)	Results		
					[1]Bias (95%CI) mL/min × 1.73 m^2	[2]Precision (95%CI)	[3]P30 (95%CI), %
Murata *et al*[180]	United States	5238	I-Iothalamate, urine (55.9, SD 29.7)	MDRD	-4.1	ND	77.6
				CKD-EPI	-0.7		78.4
Levey *et al*[62]	United States	3896	I-Iothalamate, urine and others (68, SD 36)	MDRD	-5.5 (-5.0 to -5.9)	0.274 (0.265-0.283)[4]	80.6 (79.5-82.0)
				CKD-EPI	-2.5 (-2.1 to -2.9)	0.250 (0.241-0.259)[4]	84.1 (83.0-85.3)
Lane *et al*[181]	United States	425	I-Iothalamate, urine (50, IQR 29 to 69)	MDRD	-1.0	15.0[5]	75
				CKD-EPI	-1.7	13.8[5]	80
Michels *et al*[77]	The Netherlands	271	I-Iothalamate, urine (78.2, SD 33)	MDRD	14.6 mL/min	19.9[6]	81.2
				CKD-EPI	12.3 mL/min	12.1[6]	84.5
Tent *et al*[182]	The Netherlands	253 before donation, 253 after donation	I-Iothalamate, urine (115, SD 20) and (73, SD 13)	MDRD	-22 mL/min (20-25)	20 (14-26)[5]	73 (68-79)
				CKD-EPI	-14 mL/min (11-16)	18 (14-22)[5]	89 (85-93)
				MDRD	-15 mL/min (14-16)	12 (9-15)[5]	71 (65-76)
				CKD-EPI	-11 mL/min (9-11)	12 (10-16)[5]	89 (85-93)
Kukla *et al*[183]	United States	107 on steroid-free early post tranplantation 81 on steroid-free at 1 yr	I-Iothalamate, urine (55.5, SD 17) and (56.8, SD 17.7)	MDRD	8.23	17.9[4]	71.7
				CKD-EPI	13.30	21.1[4]	58.5
				MDRD	2.40	15.8[4]	75.0
				CKD-EPI	6.91	17.3[4]	66.7
White *et al*[184]	Canada	207	Tc-DTPA, plasma (58, SD 22)	MDRD	-7.4	14.4[5]	79 (73-84)
				CKD-EPI	-5.2	15.7[5]	84 (78- 88)
Pöge *et al*[185]	Germany	170	Tc-DTPA, plasma (39.6, IQR 11.8 to 82.9)	MDRD	4.49	10.0[6]	71.8
				CKD-EPI	8.07	10.9[6]	64.1
Jones[186]	Australia	169	Tc-DTPA, plasma (75, IQR 5 to 150)	MDRD	-3[7]	ND	81
				CKD-EPI	-1.5[7]		86
Cirillo *et al*[187]	Italy	356	Inulina, plasma (71.5, SD 36.3)	MDRD	-5.2	14.9[6]	87.4
				CKD-EPI	-0.9	13.2[6]	88.2
Eriksen *et al*[188]	Norway	1621	Iohexol, plasma (91.7, SD 14.4)	MDRD	1.3 (0.4-2.1)	18.2 (17.2-19.5)[5]	93 (91-94)
				CKD-EPI	2.9 (2.2-3.5)	15.4 (14.5-16.3)[5]	95 (94-96)
Redal-Baigorri *et al*[189]	Denmark	185	Cr-EDTA, plasma (85.1, SD 20.3)	MDRD	0.81 (IQR, -1.56 to 3.19)	16.49[6]	88.6
				CKD-EPI	1.16 (IQR, -0.76 to 3.09)	13.37[6]	89.7

[1]Computed as estimated GFR minus measured GFR. Positive numbers indicate overestimation and negative numbers indicate underestimation of measured GFR. Smaller absolute values indicate lesser bias; [2]Lower values indicate greater precision; [3]Higher values indicate greater accuracy. Among the 3 studies (14, 18, 19) that reported alternative measures of accuracy, results were consistent with P30 in all. In addition to P30, references 14, 18, and 19 reported P10; Reference 14 also reported P20; [4]Evaluated as the root mean square error for the regression of estimated GFR on measured GFR; [5]Evaluated as the IQR for the differences between estimated and measured GFR; [6]Evaluated as the SD of the differences between estimated and measured GFR; [7]Converted to raw scale by multiplying percentage of bias by measured GFR. CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; mGFR: Measure glomerular filtration rate; eGFR: Estimated glomerular filtration rate; IQR: Interquartile range; MDRD: Modification of diet in renal disease; ND: Not documented; P30: Percentage of estimated GFR values within 30% of measured GFR; Tc-DTPA: Technetium-diethylene-triamine-pentaacetate; Cr-EDTA: Chromium-ethylenediamine-tetraacetic-acid. Adapted from Earley *et al*[39].

not perform as well in regions outside North America, Europe, and Australia. In Asia and Africa, equations were less accurate (P30 ranged from 29%-94%). Equation performance can be improved by deriving local "race/ethnicity" coefficients; however, the new equations are more accurate in the Caucasian populations. The coefficients also do not seem to be generalizable beyond the local population presumably reflecting differences in SCr generation due to racial, ethnic, and regional variations in muscle mass and diet, and use of non standardized SCr[39].

Thus far the new equation CKD-EPI Cys-cr has been evaluated in diverse populations. The berlin initiative study (BIS) included 610 older adults with a mean SCr level

of 1.0 mg/dL, and mean CysC level of 1.15 mg/L. The study intended to assess the performance of the CKD-EPI Cys-cr equations compared to the mGFR by iohexol. A major finding of this study was that CysC had a stronger association with mGFR than creatinine and the best GFR estimation was derived from a combined Cys-cr equation (named BIS-2)[78]. The combined CKD-EPI Cys-cr equation performed well in Japanese and Chinese individuals[79-81]. One recent study compared the CKD-EPI Cys-cr and other four approved equations in a cohort of 788 adult Chinese patients and a Tc_DPTA mGFR of 76 mL/min per 1.73 m^2. Compared to other equations, the CKD-EPI Cys-cr had less bias, (-4.11 mL/min per 1.73 m^2) and higher accuracy (P30% of 77.03%)[80]. In a population of almost 700 kidney transplant recipients the performance of the CKD-EPI Cys-cr was superior showing less bias and better accuracy compared with 2009 CKD-EPI, using inulin mGFR as reference[82].

In addition, it is important to mention that the performance of the equations is affected not only by demographic and clinical factors but by the reference method considered as the gold standard to measure GFR in different populations[83-85].

From the epidemiological standpoint, CKD prevalence was assessed in diverse populations comparing the MDRD and CKE-EPI equation[62]. For example, the Atherosclerosis Risk in Communities Study reclassified 43.5% to a higher eGFR category compared with CKD stage 3 for MDRD[86]. The AusDiab (Australian Diabetes, Obesity and Lifestyle) study reclassified 266 participants identified as having CKD with MDRD to no CKD with CKD-EPI, decreasing the prevalence of CKD in adults > 25 year 1.9% in Australia[87]. The kidney early evaluation program included 116321 individuals where 17.5% and 2.7% were reclassified to higher or lower eGFR categories, respectively, when compared with MDRD[88].

Reclassifying subjects to a higher GFR has demonstrated to translate in a lower risk for outcomes. In a recent meta-analysis, the CKD-EPI and MDRD equations were compared with respect to CKD stage and risk prediction in a 1.1 million adults from distinct cohorts followed over seven years. Outcomes included mortality, cardiovascular mortality, and kidney failure. In this study CKD-EPI reclassified to a higher and lower estimated GFR category 24.4% and 0.6% respectively, compared with the MDRD, and when the CKD-EPI equation was used, the prevalence of CKD was reduced by 2.4 percent. Furthermore, in individuals with MDRD eGFR of 45-59 mL/min per 1.73 m^2, the CKD-EPI creatinine equation reclassified 34.7% to eGFR of 60-89 mL/min per 1.73 m^2 and 1.2% to eGFR of 30-44 mL/min per 1.73 m^2. Individuals reclassified to a higher eGFR category had 0.80, 0.73, and 0.49 lower adjusted risks for death, cardiovascular disease, mortality, respectively, than those not reclassified. Overall net reclassification favored the CKD-EPI over the MDRD for the three outcomes[86].

Rule *et al*[89] evaluated the association of CKD risk factors (urine albumin, lipid profile, uric acid, hypertension, diabetes and smoking) with eGFR based on Cr and/or CysC and compared them with iothalamate mGFR in 1150 subjects with a mean age 65 year and mean mGFR of 80 mL/min per 1.73 m^2. Authors concluded that the association between most of the risk factors was stronger for CysC than SCr and CysC was a better predictor for risk stratification and management of CKD than SCr eGFR[89].

These data demonstrates that the CKD-EPI equation is superior for GFR estimation leading to fewer false-positive diagnoses of CKD. In addition the CKD-EPI equation translates in a decreased prevalence of CKD and is associated with a more precise risk prediction for outcomes and prognosis. The KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease, based on this evidence recommends that CKD be diagnosed, classified, and staged by eGFR and suggests CKD-EPI should be utilized as the preferred equation[1].

Other endogenous biomarkers for kidney function

Blood urea nitrogen: BUN increases as GFR declines however is less valuable than the SCr since the BUN can vary independently of the GFR. The production rate of urea is not stable and increases with rich protein diets or tissue breakdown such as bleeding, muscle trauma or steroid administration. On the other hand a very low protein diet or liver failure can decrease BUN without affecting GFR[32,90].

B2-microglobulin: B2-microglobulin (B2-M) is a small molecule of 11.8 kDa and constitutes a class Ⅰ HLA, is present in all nucleated cells in the body, and has a large quantity of immune cells like lymphocytes and monocytes. It has the characteristic that it is freely filtered in the glomeruli and is reabsorbed and metabolized in the proximal tubule[91]. Levels of B2-M are elevated in kidney disease, in addition to other conditions such as malignancies, autoimmune diseases, infections and aging[92]. There is data to demonstrate that plasma B2-M is a good endogenous marker of GFR and that in the context of GFR decline the increase of serum B2-M occurs prior than SCr. B2-M has been associated with death in a cohort of 1034 elderly subjects and appeared to be superior than CysC, even after adjustment for known risk factors[93,94]. Lack of further studies in the last decade however has limited the utility of this biomarker in clinical practice.

KIDNEY DAMAGE

The kidney damage refers to pathologic abnormalities documented by biopsy or imaging, alterations in urinary sediment or proteinuria (proteinuria/creatinuria > 200 mg/g, albuminuria/creatinuria > 30 mg/g). Damage usually precedes alterations in functions. For instance it is known that albuminuria precedes the decrease in

eGFR, hence the importance to count with markers of renal damage in stages that are blind for current markers of renal function decline. In theory this could facilitate early diagnosis, guide interventions and monitor disease progression.

Albuminuria

Albumin excretion rate (AER) can be determined in 24 h urine collections or in spot collections. Increases should be confirmed in at least two of three samples, within a period from 3 to 6 mo[11]. Microalbuminuria, or incipient nephropathy, is defined as an AER of 20-200 μg/min in timed samples, or 30-300 mg/24 h in 24 h samples, however spot collections are accurate enough that they can replace 24 h collections and these are now strongly recommended by the most recent guidelines[1,95].

The corresponding values that define microalbuminuria in a urine sample are AER > 30 mg/24 h or an albumin-creatinine ratio (ACR) of 30-300 mg/g (0.3-3 mg/mmol). Higher values indicate macroalbuminuria, also called clinical nephropathy[1]. Taking these values into account the prevalence of microalbuminuria in 4101 individuals of NHANES (1999-2000) with ACR 30-300 mg/g and ACR > 300 mg/g was 7.3% and 1.7% in men and 10.4% and 0.9% in females, respectively[96].

The threshold of ACR > 30 mg/g to define kidney damage has been validated as a risk factor for adverse events in different populations. In high risk patients for CKD, the ACR > 30 mg/g is has demonstrated to be a risk factor cardiovascular (CV) death and all cause mortality, progression of kidney disease, acute kidney injury (AKI) and kidney failure[97,98]. Likewise, these findings have been reproduced in low risk cohorts. In more than 1 million participants from 21 cohorts, ACR > 30 mg/g and ACR > 300 mg/g were associated with higher risk for death (HR of 1.6 and 2, respectively). Moreover the risk for CV mortality was two-fold higher with ACR > 30 mg/g compared to those with ACR of 5 mg/g and this risk persisted after adjustment for GFR and other known risk factors. This risk also applies to ACR levels < 30 mg/g. In study of Waheed *et al*[99], ACR of 10 mg/g compared to 5 mg/g was associated with all cause mortality. This however may not necessarily reflect kidney damage and may be a marker of endothelial dysfunction.

On the basis of the linear association of albuminuria with progression of CKD, end stage renal disease (ESRD), and all cause of mortality independent of eGRF, albuminuria staging has been added in the 2012 KDIGO guidelines.

Combination of biomarkers

Combining albuminuria with eGFR improves the prediction of CKD progression. This was demonstrated in the Nord-Trondelag Health (HUNT-2) study that included 65589 participants, where albuminuria and eGFR independently predicted kidney disease progression and the combination of both markers was superior to predict those subjects at highest risk for ESRD development[100]. In a large prospective cohort involving more than 26000 subjects, the authors evaluated whether combining eGFR creatinine, CysC, and urine ACR could improve risk prediction when compared with eGFR alone. In this cohort the adjusted mortality risk was six fold higher in patients with CKD identified by all three markers and was also three fold higher in patients with CKD defined by both eGFR Cys-cr, compared to those with CKD defined by eGFR creatinine alone. The risk for CKD progression to kidney failure was higher among patients with CKD defined by all three markers. The authors concluded that adding CysC to SCr and ACR was superior for prediction for kidney disease progression and death[101].

New biomarkers for kidney damage

Although albuminuria is a powerful biomarker, it may occur after the damage has occurred or may not be present in other types of kidney damage such as tubulointerstitial disease and hypertensive kidney disease. This has led to the search for new biomarkers that are also non-invasive and could better correlate with the etiology of the kidney disease. Moreover; early identification of patients with CKD could allow implementing early interventions to reduce CVD or CKD progression. In the next few paragraphs we describe the most promising biomarkers in CKD (Table 2) and its utility (Table 3).

Kidney injury molecule

Kidney injury molecule (KIM-1) is a transmembrane protein is a type 1 transmembrane protein whose expression has been upregulated after kidne injury[102,103]. KIM-1 is an early biomarker for proximal tubular damage since it is expressed in the urine during the first 12 h of the tubular injury[104]. Experimental and clinical studies have demonstrated high KIM-1 expression in areas of fibrosis and inflammation. In murine models with polycystic kidney disease, KIM 1 is highly expressed in renal tubules, it associates with interstitial fibrosis in human allografts and in type 1 diabetes mellitus regression of microalbuminuria has been associated with lower urinary levels of KIM-1[105-108].

Persistent expression of KIM-1 has been associated to inflammation characterized by high monocyte chemoattractant protein-1 (MCP-1) levels[109]. In contrast, in experimental models, mice with mutant KIM-1 are protected from fibrosis and had lower inflammatory markers[110]. In a retrospective analysis of 107 diabetic type 1 with CKD stages 1-3 (AER > 500 mg/24 h) followed for 5-15 years, 63% of those subjects with higher KIM-1 levels (> 97 pg/mL) progressed to ESRD whereas only 20% of patients with lower levels progressed. In addition baseline plasma KIM-1 levels correlated with rate of eGFR decline after adjustment for baseline urinary albumin-to-creatinine ratio, eGFR, and Hb1Ac[111]. KIM-1 may represent a promising marker for the future. Larger

Table 2 Novel biomarkers in chronic kidney disease

Biomarker source	Ref.	Population/type of study	Commentaries
u-LFABP Urinary	Nielsen *et al*[190]	227 newly diagnosed type 1 diabetic patients/longitudinal	Baseline u-LFABP levels predicted development of microalbuminuria (HR = 2.3, 95%CI: 1.1-4.6), and predicted mortality (HR = 3.0, 95%CI: 1.3-7.0)
NAG Urinary	Kern *et al*[191]	87 type 1 diabetics with microalbuminuria and 174 controls/ longitudinal	Baseline NAG independently predicted microalbuminuria (OR = 1.86, $P < 0.001$) and macroalbuminuria (OR = 2.26, $P < 0.001$) but risk was attenuated in multivariate models
CTGF Urinary	Nguyen *et al*[192]	318 type 1 diabetic patients and 29 control subjects/cross sectional	U-CGTF was significantly higher in diabetic nephropathy than micro o normoalbuminuria. U-CGTF correlated with albuminuria and GFR
IL-18 Kidney tissue	Miyauchi *et al*[193]	12 type 2 diabetes with overt nephropathy and 7 patients with MCD/ cross sectional	IL-18 expression in tubular cells was observed highly observed (83%) in patients with diabetes but only observed in 14.3% of MCD
ApoA-Ⅳ Plasma	Boes *et al*[194]	177 non-diabetic patients with mild to modetare renal CKD/longitudinal	Baseline ApoA-Ⅳ was a significant predictor of disease progression (HR = 1.062, 95%CI: 1.018-1.108) and patients with level above the median had significantly faster progression compared with patients with level below median ($P < 0.0001$)
CD14 mononuclear cells Urinary	Zhou *et al*[195]	16 patients with autosomal dominat polycystic kidney disease/longitudinal	Baseline urinary CD14 mononuclear cells correlated with 2 yr change in total kidney volume in males
NGAL	Bolignano *et al*[121]	33 patients with glomerulonephritis and proteinuria > 1 g per day/cross sectional	u-NGAL was higher in glomerulonephritis compared with controls and significantly correlated with serum creatinine and urinary protein excretion
Urinary	Smith *et al*[124]	158 patients with CKD stages 3 and 4/longitudinal	u-NCR was associated with a higher risk of death and initiation of renal replacement therapy
Urinary	Bolignano *et al*[125]	96 white patients with CKD/ longitudinal	Baseline urinary and serum NGAL were predictors of CKD progression
Urinary/serum	Shen *et al*[119]	92 patients with chronic glomerulonephritis CKD stage 2-4, and 20 control subjects/longitudinal	s-NGAL levels were higher compared to controls and negatively correlated with the eGFR Patients with sNGAL level > 246 ng/mL had a poor 2 yr renal survival compared with the control group
Serum	Bhavsar *et al*[123]	286 participants from the ARIC and 143 matched controls/longitudinal	Higher quiartiles of NGAL (but no KIM-1) were associated with incident CKD
KIM-1 Serum	Krolewski *et al*[111]	107 diabetic type 1 with CKD 1-3 (AER > 500 mg/24 h)/longitudinal	Baseline plasma KIM-1 levels correlated with rate of eGFR decline KIM-1 levels (> 97 pg/mL) correlated with progression to ESRD
Urinary	Peters *et al*[109]	65 patients with Proteinuric IgAN and 65 control subjects/longitudinal	In patients with IgAN uKIM-1 excretion was significantly higher than controls uKIM-1 is independently predictor of ESRD
FGF-23	Nakano *et al*[134]	738 Japanese patients with CKD stages 1-5/longitudinal	Levels of FGF-23 associated with kidney function decline or initiation renal replacement therapy
Serum	Fliser *et al*[137]	227 non diabetic patients with CKD stages 1-4/longitudinal	FGF-23 was an independent predictor of CKD progression
	Lee *et al*[138]	380 patients with type 2 diabetes/ longitudinal	Levels of FGF-23 was associated with increased risk of ESRD and was a significant risk factor for all cause mortality

u-LFABP: Liver-type fatty acid-binding protein; NAG: N-Acetyl-b-O-glucosaminidase; CTGF: Connective tissue growth factor; IL-18: Interleukin-18; ApoA-Ⅳ: Apolipoprotein A-Ⅳ; NGAL: Neutrophil gelatinase associated lipocalin; MCD: Minimal change disease; ARIC: Atherosclerosis Risk In communities; IgAN: IgA nephropathy; u-NCR: u-NGAL to creatinine ratio; eGFR: Estimated glomerular filtration rate; FGF-23: Fibroblast growth factor 23; CKD: Chronic kidney disease; KIM-1: Kidney injury molecule; AER: Albumin excretion rate; GFR: Glomerular filtration rate; U-CGTF: Urinary-connective tissue growth factor; u-NGAL: Urinary-NGAL; s-NGAL: Serum-NGAL; ESRD: End stage renal disease.

Table 3 Utility of new biomarkers in chronic kidney disease

Biomarker	Origin	Outcome assessed
Urinary liver-type fatty acid-binding protein	Proximal tubule	Diabetic Nephropathy: Microalbuminuria and mortality
Urinary N-Acetyl-b-O-glucosaminidase	Proximal tubule	Diabetic Nephropathy: Albuminuria
Urinary connective tissue growth factor	Proximal tubule	Diabetic Nephropathy: Glomerular filtration rate decline
Interleukin-18	Tubulointerstitial	Diabetic Nephropathy: Albuminuria
Apolipoprotein A-Ⅳ	Intestinal enterocytes	CKD: CKD Progression
Urinary CD14 mononuclear cells		Polycystic kidney disease: Kidney volume
Neutrophil gelatinase associated lipocalin	Proximal and distal tubule	Glomerulonephritis: GFR and proteinuria CKD: CKD progression, renal replacement therapy and mortality
Kidney injury molecule-1	Proximal tubule	CKD: CKD progression and renal replacement therapy
Fibroblast growth factor-23	Osteocytes and osteoblasts	Diabetic Nephropathy and others CKD: CKD progression and mortality
Urinary retinol binding protein 4	Proximal tubule	Congenital or acquired tubular dysfunction: Proximal tubule dysfunction

CKD: Chronic kidney disease.

studies however are still warranted before KIM-1 could be applied routinely in clinical practice.

Neutrophil gelatinase-associated lipocalin: Neutrophil gelatinase-associated lipocalin (NGAL) is a lipocalin iron-carrying protein of 25 kDa and is part of the well-defined super family of proteins called lipocalins, is expressed by tubular renal epithelial cells following tubulointerstitial injury[112-114]. NGAL has been an established marker for acute kidney injury however its role in CKD is less studied[115-119]. In patients with IgA nephropathy urinary NGAL level was higher compared to controls and was also associated with disease severity[120]. In patients with glomerular proteinuria above 1 g/24 h and in patients with polycystic kidney disease, NGAL levels were higher compared to controls and significantly correlated to SCr[121,122]. NGAL has also been associated to incident CKD progression in adults. In a community based population of 286 subjects, NGAL was evaluated as an independent risk factor for incident CKD. Those in the highest quartile of NGAL had a higher risk for incident CKD, effect that was attenuated after adjustment for creatinuria and albuminuria[123]. In a cohort of 158 adults with stage 3 or 4 CKD, urinary NGAL to creatinine ratio was associated with mortality and renal replacement therapy and this risk was independent of kidney and CV risk factors[124]. Similar results were found in a cohort of 96 CKD patients followed for 18.5 mo where plasma and urinary NGAL predicted CKD progression after adjustment for eGFR[125].

Thus far there is evidence to support that NGAL levels either in plasma or urine can predict kidney disease progression independent of GFR, however the data is limited by the number of participants and larger studies are needed before establishing this biomarker in clinical practice.

Fibroblast growth factor 23: Fibroblast growth factor 23 (FGF-23) is 32-kDa phosphaturic protein secreted by bone osteocytes. Among its functions is to promote phosphate excretion, decrease calcitriol production and suppress parathyroid hormone[126-128]. In CKD the increase of FGF-23 level precedes the decline in vitamin 1,25-$(OH)_2$ vitamin D3 and the increase of PTH level. Although FGF-23 is higher in patients with moderate to severe CKD, there is data to support that the rise of FGF-23 occurs earlier in the disease. In the past decade several studies have found an association between high FGF-23 levels, kidney disease progression and mortality in subjects with CKD[129-132]. In a cohort of 227 non diabetic patients with CKD followed for more than 4 years, FGF-23 was an independent risk factor for kidney disease progression. Likewise Semba *et al*[133] in 701 healthy women (mean eGFR 60 mL/min × 1.73 m^2), and Nakano *et al*[134] in 738 Japanese patients with CKD stages 1-5 (mean eGFR 35 mL/min × 1.73 m^2) reported that increasing levels of FGF-23 associated with decline in kidney function or initiation renal replacement therapy after a follow-up of 2 and 4.4 years, respectively. In addition, in patients undergoing renal replacement therapy, elevated FGF-23 levels have been associated with CV outcomes such as left ventricular hypertrophy and increased risk of mortality[133-138]. It is important to mention that this association has been independent of phosphate levels and CKD stage.

Asymmetric dimethylarginine: Asymmetric dimethylarginine (ADMA) is an aminoacid of 202 Da, it is normally synthesized intracellularly and eliminated through the urine. One of its adverse effects is the inhibition of the nitric oxide synthases and this mechanism has been associated to adverse cardiovascular side effects[139,140]. As kidney function deteriorates ADMA levels increase and this has been associated to kidney parenchymal damage through the decrease in dimethylarginine-dimethylamino-hydrolase[141,142]. ADMA has been associated to CKD progression. In the diabetic and non diabetic population, ADMA levels are higher as GFR declines and are associated with rapid kidney function decline[143,144]. In a recent study of 164 CKD patients followed for one year, elevated ADMA and markers of oxidative stress were strong predictors of progression in patients with CKD stages 3-4[145]. Moreover, ADMA has been associated to death and CV events in the CKD population[146,147]. Some authors had considered ADMA to be the "missing link" between cardiovascular disease and CKD[139]. Whether counteracting the effects of ADMA in CKD should be explored as a strategy to prevent cardiorenal complications would need to be confirmed in larger studies.

MCP-1: MCP-1 belongs to the group of inflammatory chemokines[148,149]. Expression of MCP-1 is up regulated in kidney diseases that have a sustained inflammatory response, such as in diabetic nephropathy and lupus nephritis[150,151]. Studies have demonstrated glomerular and tubular kidney cells release MCP-1 in response to high glucose levels and urine levels of MCP-1 are increased in diabetic nephropathy[152,153]. Likewise MCP-1 levels in urine are over expressed in active lupus nephritis[151-154]. Emerging evidence suggest that MCP-1 has a significant role in the pathogenesis of many kidney diseases and urinary MCP-1 is a promising biomarker with diagnostic and prognostic implications[155-157].

Urine retinol-binding protein 4: Urine retinol-binding protein 4 (uRBP4) is a 21 KDa protein derived of plasma RBP4 (pRBP4), is an integrant of the lipocalin family and is produced mainly in the liver but also in the adipose tissue where it performs as an adipokine that has been linked to insulin resistance and obesity[158,159]. Unlike other biomarkers such as NGAL and KIM-1, uRBP4 is currently the most sensitive functional biomarker of proximal tubule. pRBP4 is filtered at the glomerulus and completely reabsorbed in the proximal tubule. In addition, it is known that variation levels of pRBP4 (secondary to nutrition, vitamin A levels, liver disease and infection) have small effect on uRBP 4 as a

biomarker[160]. Sensitivity for uRBP4 however decreases as kidney function declines due to false positives that occur in the presence of glomerular disease[161]. This marker was been useful in several diseases related with proximal tubule dysfunction, either hereditary, such as Fanconi syndrome, dent type 1 syndrome and lowe syndrome[162], or acquired conditions that directly affect proximal tubule such as drug toxicity in human immunodeficiency virus, cadmium toxicity, plasma cell dyscrasias, AKI diagnosis and other renal tubulointerstitial diseases[163]. Amer *et al*[164] assessed the prognostic value in renal transplantation of a panel of urinary proteins in 221 patients at 1 year post transplant and reported that patients with glomerular lesions had higher albuminuria than patients with normal histology, and in patients with tubulointerstitial disease, uRBP4 has over expressed. In addition, uRBP4 was a risk factor for long term allograft loss and this risk was independent of kidney biopsy histology and albuminuria[164].

Future directions

Advances in technology during the last decade have enlightened our knowledge regarding genetic regulatory pathways. A fast growing arena are the microRNAs (miRNAs), the current number of miRNAs in humans are estimated to be between 700 and 1000, and they have been implicated in several physiological events as well pathologic process, including kidney disease[165]. miRNA have selective expression by different organs, and the kidney expresses mostly miRNA 192, 194, 204, 215 and 216 which have been implicated in proliferation, migration and structure of renal cells[166,167]. Little changes in these molecules have implications in kidney function, for instance it is know that deletion of the miRNA 30 family decreases renal cells, affects blood pressure and develop vascular damage and extensive fibrosis[168]. Other miRNAs are related with diverse pathophysiologic process, miRNA 155 is associated to blood pressure control through down regulation of type 1 angiotensin Ⅱ receptor[169,170], miRNA 192 and 200 families are related to fibrotic damage in diabetic nephropathy manly by regulation of transforming growth factor beta[171], miRNA 15, 17 and 31 are associated with cystogenesis in polycystic kidney disease[172], and finally miRNA 142, 155 and 223 are increased in acute rejection related to activation of epithelial cells and blood mononuclear cells[173], and can discriminate between acute humoral rejection and cellular rejection[174]. MiRNA expression pathways have also been evaluated as diagnostic biomarkers in other pathologies. In a study of lupus nephritis patients miRNA 27 and 192 in urine could identified in renal biopsies of lupus patients with nephritis[175]. The knowledge of miRNA in health and disease remains with several questions concerning its regulation, production and specific target. In addition most studies have measured miRNA in tissue and therefore become cumbersome to measure in clinical practice. Studies evaluating its utility in plasma and urine are urgently needed. Nonetheless this is a rapidly growing field and future research may provide a better understanding of the pathophysiology in kidney disease and may reveal potential diagnosis and therapeutic options.

Not only in the area of proteomics (NGAL, KIM-1, *etc.*) and transcriptomics (miRNAs) have the kidney markers evolved, the latest piece added to the puzzle corresponds to metabolomics, and as it name points out, is the measure of end products of basic metabolic molecules. These end products could improve the utility of other type of biomarkers[176]. Currently, metabolomics in kidney disease have mainly been studied in uremia, renal cell carcinoma, glomerulonephritis, diabetes mellitus, polycystic kidney disease and drug related nephrotoxicity. For instance in patients with drug related nephrotoxicity, end products from amino acids and simple sugars increase in urine before tissular changes become apparent. The latter has been described with antibiotics[177], and immunosupression therapy, for example, the increase of metabolomic end products during the first month after cyclosporine predicts kidney damage[178]. Similarly metabolomics has been associated to several metabolic profiles (mainly amino acids, derivatives of sugar and phospholipids) that could be useful in the diagnosis and prognosis of different types of renal disease as diabetic nephropathy, IgA nephropathy and other glomerulonephritis, in addition to diagnosis, metabolomics offers a promising future in the area of pharmaco-metabolomics, which could lead to personalized therapeutic targets[179]. At this point metabolomics main limitation is related to problems with specificity and technical variability and is not ready to be implemented in clinical practice.

CONCLUSION

During the last century, SCr has been the most used biomarker to screen and diagnose kidney disease. SCr however has several limitations and should be utilized only in estimating equations. The CKD-EPI is more generalizable and performs better than the MDRD estimating equation, especially in the healthy population. More recently the GFR estimating equation that combines SCr and CysC has demonstrated to be superior than equations that use either SCr or CysC alone, and is recommended in specific conditions, such as when confirmation of CKD is required. Albuminuria remains one of the strongest risk factors for outcomes and the combination of SCr, CysC and urinary albumin to creatinine ratio improves risk stratification predicts CKD progression and mortality.

In the last decade several other promising biomarkers have emerged. However, although these biomarkers are highly sensitive and specific and have allowed an earlier diagnosis of kidney disease with promising results; none of them have been validated to make clinical decisions upon their positivity. These biomarkers should have the potential to indicate injury type or the specific site of harm. It is improbable however that one biomarker would be sufficient to guide intervention upon their result. Larger and long term studies are warranted before applying these biomarkers in clinical practice. The CKD

Biomarkers Consortium has 15 ongoing studies with the aim to develop and validate novel biomarkers for CKD. In the meantime current biomarkers in CKD should be cautiously implemented acknowledging its strengths and limitations.

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REVIEW

ACE and ACE2 in kidney disease

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Abstract

Renin angiotensin system (RAS) activation has a significant influence on renal disease progression. The classical angiotensin-converting enzyme (ACE)-angiotensin Ⅱ (Ang Ⅱ)-Ang Ⅱ type 1 (AT1) axis is considered to control the effects of RAS activation on renal disease. However, since its discovery in 2000 ACE2 has also been demonstrated to have a significant impact on the RAS. The synthesis and catabolism of Ang Ⅱ are regulated *via* a complex series of interactions, which involve ACE and ACE2. In the kidneys, ACE2 is expressed in the proximal tubules and less strongly in the glomeruli. The synthesis of inactive Ang 1-9 from Ang Ⅰ and the catabolism of Ang Ⅱ to produce Ang 1-7 are the main functions of ACE2. Ang 1-7 reduces vasoconstriction, water retention, salt intake, cell proliferation, and reactive oxygen stress, and also has a renoprotective effect. Thus, in the non-classical RAS the ACE2-Ang 1-7-Mas axis counteracts the ACE-Ang Ⅱ-AT1 axis. This review examines recent human and animal studies about renal ACE and ACE2.

Key words: Angiotensin-converting enzyme; Angiotensin-converting enzyme 2; Diabetic nephropathy; Kidney disease; Renin angiotensin system

Core tip: In the kidneys, angiotensin-converting enzyme 2 (ACE2) is expressed in the proximal tubules and less strongly in the glomeruli. The synthesis of inactive Ang 1-9 from angiotensin Ⅰ (Ang Ⅰ) and the catabolism of Ang Ⅱ to produce Ang 1-7 represent the main functions of ACE2. Ang 1-7 reduces vasoconstriction, water retention, salt intake, cell proliferation, and reactive oxygen stress, and also has a renoprotective effect. Thus, in the non-classical renin angiotensin system the ACE2-Ang 1-7-Mas axis counteracts the ACE-Ang Ⅱ-AT1 axis. This review examines recent human and animal studies about ACE and ACE2 expression in various renal diseases.

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INTRODUCTION

The renin-angiotensin system (RAS) has a significant influence on renal disease progression. Angiotensinogen

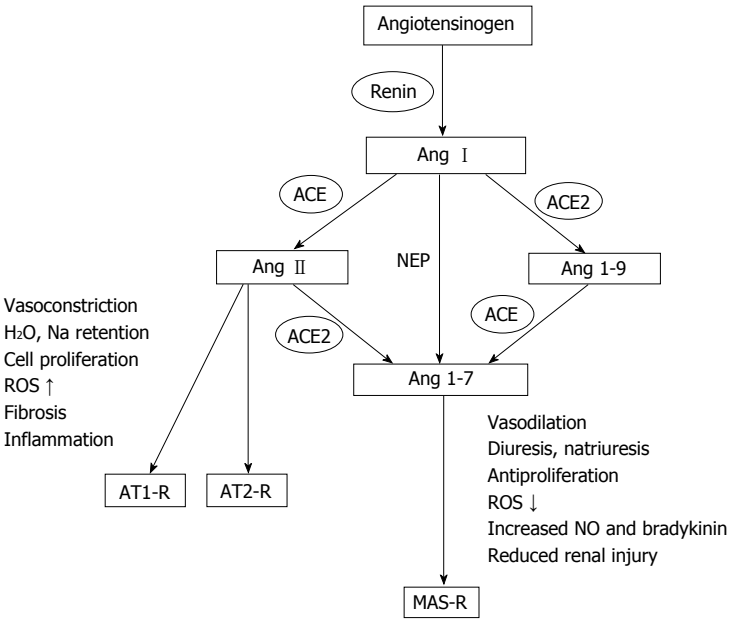

Figure 1 Roles of angiotensin-converting enzyme and angiotensin-converting enzyme 2 in the renin angiotensin system. Angiotensinogen is cleaved by renin to form angiotensin Ⅰ (Ang Ⅰ), which is converted to Ang Ⅱ by ACE. The main function of ACE2 is to synthesize inactive Ang 1-9 from Ang Ⅰ and to produce the vasodilatory and antiproliferative molecule Ang 1-7 from Ang Ⅱ. Ang Ⅰ acts a substrate for neprilysin, which cleaves it to form Ang 1-7. Ang Ⅱ binds to the Ang Ⅱ type 1 receptor (AT1-R) and AT2-R. The Mas receptor (MAS-R) is a specific receptor for Ang 1-7. The ACE2-Ang 1-7-MAS axis counteracts the effects of the ACE-Ang Ⅱ-AT1 axis. ACE: Angiotensin-converting enzyme; NO: Nitric oxide; NEP: Neprilysin; ROS: Reactive oxygen species.

is broken down by renin to give angiotensin Ⅰ (Ang Ⅰ), and angiotensin-converting enzyme (ACE) subsequently converts Ang Ⅰ to angiotensin Ⅱ (Ang Ⅱ). In the kidneys, the ACE-Ang Ⅱ type 1 (AT1) axis (the classical RAS) promotes sodium and water retention, oxidative stress, vasoconstriction, cell proliferation, inflammation, and fibrosis (Figure 1). In 2000, ACE2[1,2] was discovered, which indicated that the RAS is more complex than was previously imagined. The synthesis of inactive Ang 1-9 from Ang I and the catabolism of Ang Ⅱ to form Ang 1-7, which binds to the Mas receptor (its specific membrane receptor) and counteracts the effects of Ang Ⅱ, are the main functions of ACE2[3,4]. Ang Ⅰ is also a substrate for neprilysin, which cleave it to produce Ang 1-7[4]. In the non-classical RAS, the ACE2-Ang 1-7-Mas axis counteracts the effects of the ACE-Ang Ⅱ-AT1 axis. Specifically, it induces natriuresis, reduced oxidative stress, vasodilation, antiproliferative activity, and diuresis by upregulating the concentrations of nitric oxide and prostaglandins. These processes contribute to protecting the kidneys from damage (Figure 1)[4-7]. Ang 1-7 is also metabolized by ACE[4]. Furthermore, accumulating evidence indicates that the ACE/ACE2 ratio regulates the production and accumulation of Ang Ⅱ and that ACE2 deficiency leads to higher Ang Ⅱ concentrations[8-13].

This review examines recent studies about the roles of renal ACE and ACE2 in various conditions (Table 1) and potential treatments for renal disease that target these molecules.

RENAL DISTRIBUTION OF ACE AND ACE2

In mouse kidneys, the apical brush borders of the proximal tubules exhibit colocalization of ACE and ACE2, but this is not the case in the glomeruli[14]. Whilst ACE2 is expressed in podocytes and less strongly glomerular mesangial cells, only endothelial cells have been found to express ACE[14].

In agreement with the findings of animal studies, the apical brush borders of the proximal tubules in human kidneys exhibit marked ACE and ACE2 colocalization, and both ACE and ACE2 were also detected in the glomeruli although at lower concentrations[15,16]. Furthermore, in immunoelectron microscopy studies of human kidneys, it was confirmed that ACE is expressed at the proximal tubule brush borders and in glomerular endothelial cells[17]. ACE has also been detected within renal vascular endothelial cells[18]. On the other hand, no previous studies have used immunoelectron microscopy to examine the distribution of ACE2 in human kidneys.

Glomerular ACE2 expression was found to be markedly weaker than that observed in the proximal tubules in both diabetic and normal control kidneys in all of the species examined in the above-mentioned studies.

RENAL ACE/ACE2 RATIO

ACE2 counteracts the effects of ACE by catabolizing

Table 1 Renal angiotensin-converting enzyme and angiotensin-converting enzyme 2 protein expression in various diseases

Diseases	Species	Glomerular ACE	Tubular ACE	Glomerular ACE2	Tubular ACE2	Ref.	Year
Type 1 DM	Rats	↑	↓	↑	↓	Tikellis *et al*[30]	2003
Type 2 DM	Mice	ND	↓	ND	↑	[1]Ye *et al*[9]	2004
Type 2 DN	Mice	↑	↓	↓	↑	Ye *et al*[14]	2006
Type 1 DM	Rats	↑	↑	↓	↑	Moon *et al* [31]	2008
Type 2 DN	Mice	ND	ND	↓	↑	Chodavarapu *et al*[32]	2013
Type 2 DN	Humans	ND	ND	↑	↑	Lely *et al*[34]	2004
Type 2 DN	Humans	↑	↑	↓	↓	Mizuiri *et al*[15]	2007
Type 2 DN	Humans	↑	↑	↓	↓	Reich *et al*[16]	2007
Primary glomerulopathy	Humans	ND	ND	↑	-	Lely *et al*[34]	2004
IgA nephropathy	Humans	↑	↑	↓	↓	Mizuiri *et al*[36]	2011
Hypertension	Rats	ND	ND	↓[1]		Prieto *et al*[41]	2011
Hypertension	Humans	↑[1]		↓[1]		Koka *et al*[45]	2008
Nephrosclerosis	Humans	-	↓	-	↓	Wang *et al*[22]	2011
Subtotal nephrectomy	Rats	ND		↓[1]		Velkoska *et al*[46]	2009
Subtotal nephrectomy	Rats	↑[1]		↓[1]		Eräranta *et al*[47]	2012

[1]No description about expression pattern of glomerular and tubular ACE and ACE2. DM: Diabetes mellitus; DN: Diabetic nephropathy; ND: Not done; ACE: Angiotensin-converting enzyme; -: No changes.

Ang Ⅱ to produce Ang 1-7. The balance between the effects of these two molecules affects the renal RAS, and hence, the ACE/ACE2 ratio might represent the key parameter that is driving the regulation of the renal RAS[8-13]. In healthy conditions, ACE2 activity rises along with ACE activity, but imbalances can develop under disease conditions[12]. It is suggested that the main priority of the RAS is to achieve an appropriate balance between ACE and ACE2 activity. The ACE-Ang Ⅱ-AT1 axis has been suggested to have detrimental effects on the RAS, whereas the ACE2-Ang 1-7-Mas axis counteracts the ACE-Ang Ⅱ-AT1 axis and seems to play a renoprotective role[19].

Various studies have used kidney injury models to investigate the ACE/ACE2 ratio. For example, Ye *et al*[9] demonstrated that higher ACE2 concentrations and lower ACE protein concentrations in the renal tubules had renoprotective effects in the early stages of the condition experienced by *db/db* mice (rodent model of type 2 diabetes) without nephropathy. In a recent study, significantly increased concentrations of ACE2 mRNA, ACE mRNA, and Ang Ⅱ were detected in the plasma and renal cortical tissue of streptozotocin (STZ)-treated rats (rodent model of type 1 diabetes) compared with the control rats[20]. In addition, the STZ-treated rats exhibited the greater increase in ACE mRNA as opposed to ACE2 mRNA compared with the normal controls[20]. In rats, the consumption of a high salt diet led to a higher glomerular ACE/ACE2 ratio, resulting in kidney damage and oxidative stress[8]. Hamming *et al*[21] reported low sodium intake or ACE inhibition does not affect renal ACE2 despite large changes in renal ACE in healthy rats and they suggested that renal ACE inhibition and dietary sodium restriction affect renal ACE and ACE2 *via* different mechanism.

In humans, kidney biopsies have indicated that hypertensive patients have higher ACE/ACE2 mRNA ratios[10]. In addition, we detected high ACE/ACE2 ratios in patients with type 2 diabetes and overt nephropathy; thus, such changes might play a role in renal damage[15]. In a study of patients with hypertensive nephrosclerosis, Wang *et al*[22] detected a correlation between the glomerular ACE/ACE2 protein ratio and the extent of glomerulosclerosis and an inverse correlation between the glomerular ACE/ACE2 protein ratio and the estimated glomerular filtration rate (eGFR)[22]. Conversely, no associations were detected between the tubulointerstitial ACE/ACE2 ratio and histological or clinical parameters[22].

Batlle *et al*[12] suggested that as ACE and ACE2 are regulated *via* different mechanisms and the ACE/ACE2 ratio could be misleading[12]. Pohl *et al*[23] demonstrated that whilst the ACE2 is expressed along the entire renal tubular segment ACE is only expressed in the brush-border membrane of the late proximal tubules and they suggested that surface expression of ACE and ACE2 differed as a function of endocytosis[23].

Together, the findings of these studies indicate that increases in the ACE/ACE2 ratio induced *via* the ACE-Ang Ⅱ-AT1 axis have a significant influence on the development of severe kidney damage. On the other hand, renal ACE/ACE2 ratio data should be interpreted carefully, as ACE and ACE2 are regulated *via* independent mechanisms.

CHRONIC KIDNEY DISEASE

In dogs, Mitani *et al*[24] found that chronic kidney disease (CKD) kidneys exhibited weaker ACE immunoreactivity than normal kidneys and detected a negative association between ACE expression and renal tissue damage. However, both upregulated and downregulated ACE2 expression were detected in dogs with CKD, and ACE/ACE2 immunoreactivity did not exhibit a close relationship with renal tissue damage[24]. Burrell *et al*[25] suggested

that renal ACE2 deficiency and a lack of cardiac ACE2 activation might influence the progression of cardiac and renal tissue damage in rats with CKD. Furthermore, the detrimental cardio-renal effects of CKD were only partially abrogated by long-term ACE inhibition[25]. However, the CKD rats used in their study might not have been a suitable CKD model, as they were killed 28 days after subnephrectomy. Dilauro *et al*[26] found that renal ACE2 expression was reduced in a mouse model of early CKD, and this led to increased albuminuria *via* an AT1 receptor-dependent blood pressure-independent mechanism.

In a recent study, Roberts *et al*[27] found that hemodialysis CKD patients exhibited lower plasma ACE2 activity than pre-dialysis CKD patients, and female hemodialysis patients displayed lower plasma ACE2 activity than male hemodialysis patients. The lower plasma ACE2 activity exhibited by dialysis patients might slow the catabolism of Ang Ⅱ, which may be responsible for the high prevalence of cardiovascular disease among these patients[27]. Human plasma is known to contain an endogenous ACE2 inhibitor[28]. However, Wysocki *et al*[29] reported that this cannot explain the lower plasma ACE2 activity seen in dialysis patients as the removal of the inhibitor by dialysis resulted in higher plasma ACE2 concentrations. Recently, it has been indicated that plasma ACE2 has renoprotective effects in CKD, but more studies of the plasma ACE2 concentrations of pre-dialysis and dialysis CKD patients and healthy subjects are necessary to confirm this.

Diabetic nephropathy

Diabetic nephropathy is a representative disease of CKD and is linked to activation of the renal RAS, resulting in Ang Ⅱ-induced tubular and glomerular damage. In a study involving diabetic rats, Tikellis *et al*[30] observed reduced renal expression levels of ACE and ACE2 mRNA, higher glomerular ACE and ACE2 protein expression levels, and lower tubular ACE and ACE2 protein expression levels at 24 wk after the administration of STZ. Furthermore, the rats' ACE2 protein expression levels rose after treatment with an ACE inhibitor[30]. In a study examining *db/db* mice without nephropathy, Ye *et al*[9] detected higher ACE2 protein expression and lower ACE protein expression in the animal's renal tubules, which resulted in renoprotective effects. However, as the study had involved young *db/db* mice with early stage diabetes, they could not rule out the possibility that the ACE2 expression levels of the mice might subsequently fall as nephropathy developed[9]. Moreover, they speculated that reduced ACE2 expression and upregulated ACE expression gradually induce kidney damage in diabetes[9]. Ye *et al*[14] also examined the glomeruli of *db/db* mice with established diabetic nephropathy and observed upregulated ACE protein expression and downregulated ACE2 protein expression. In the glomeruli of rats with STZ-induced diabetes, Moon *et al*[31] noted stronger and weaker ACE and ACE2 staining in the glomeruli, respectively, at 8 wk after the administration of STZ, but observed increase in both ACE and ACE2 staining in the tubules compared with the control rats. In a study of *db/db* mice, Chodavarapu *et al*[32] detected reduced glomerular ACE2, increased tubular ACE2 and ADAM17, and suspected ectodomain shedding of active renal ACE2 in the urine.

In humans, we observed downregulated ACE2 expression and upregulated ACE expression in both the glomeruli and tubulointerstitium of diabetic patients with overt nephropathy, which led to the diabetic patients having significantly higher ACE/ACE2 ratios than the controls ($P < 0.001$) (Figure 2)[15,33]. We also detected a positive correlation between the ACE/ACE2 ratio and the serum creatinine, fasting blood glucose, proteinuria, hemoglobin A1c, and blood pressure values and an inverse correlation between and the eGFR ($P < 0.001$)[15]. In addition, Reichi *et al*[16] observed decreased ACE2 expression and increased ACE expression in the glomeruli and tubules in biopsy samples collected from patients with type 2 diabetes-induced kidney disease. Conversely, Lely *et al*[34] detected upregulated ACE2 expression in the glomerular and peritubular capillary endothelia in all types of primary and secondary renal disease as well as renal transplant patients; however, they only examined 8 diabetic patients and did not concentrate on the variation in ACE2 expression between biopsy samples from diabetic nephropathy patients and normal renal tissue from patients underwent surgery for renal tumors. In a real time PCR study in which 8 diabetic patients with overt proteinuria were compared with 66 non-diabetic patients with renal disease, ACE mRNA expression was significantly increased, but ACE2 mRNA expression was not significantly changed in the diabetic patients[35]. The differences between the results obtained in human studies of type 2 diabetic nephropathy and those obtained using *db/db* models of diabetes without nephropathy[9] might have been due to the different stages of diabetes, as no human studies of early stage diabetes have been conducted.

Taken together, human biopsy studies have suggested that upregulated ACE expression and downregulated ACE2 expression are seen at both the glomerular and tubular levels in established diabetic nephropathy. Whilst most animal studies detected associations between diabetes and increased ACE expression and decreased ACE2 expression in the glomeruli, tubular ACE and ACE2 expression were demonstrated to be significantly decreased and increased, respectively.

Primary glomerular disease

In the study by Lely *et al*[34], increased ACE2 expression was detected in the glomerular and peritubular capillary endothelia in all primary renal diseases (including 8 cases of IgA nephropathy, 5 cases of focal glomerulosclerosis, and 18 cases of membranous glomerulopathy), but no

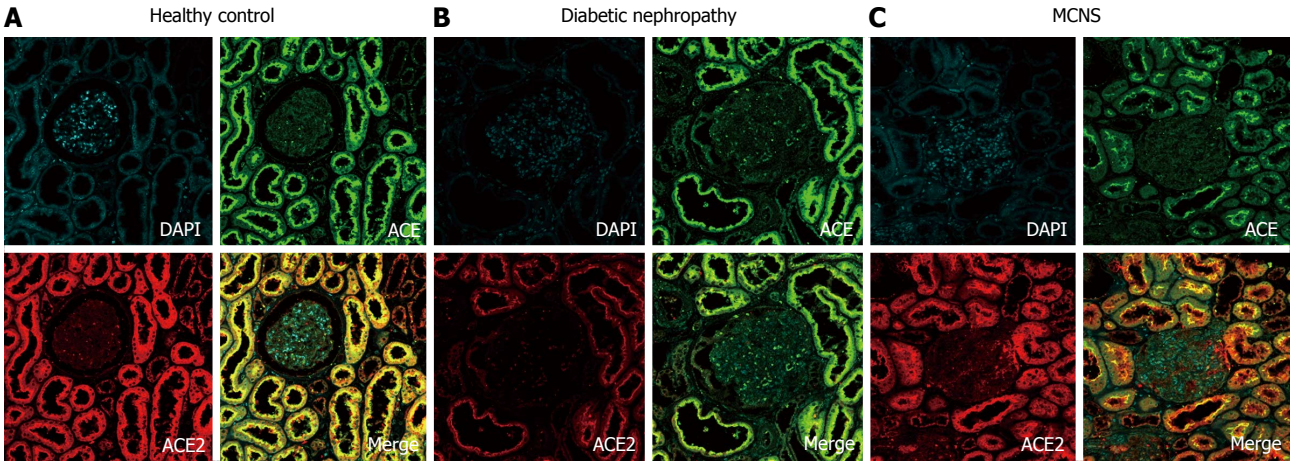

Figure 2 Images obtained with confocal microscopy of triple immunofluorescence staining of angiotensin-converting enzyme (green), angiotensin-converting enzyme 2 (red), and nuclei (DAPI; blue) in kidney specimens. In healthy subjects, marked co-localization of ACE and ACE2 (yellow) was observed in the apical brush borders of the proximal tubules (A). Both ACE and ACE2 were also present in the glomeruli, but the glomeruli exhibited weaker staining than the proximal tubules (A). In diabetic kidneys, stronger ACE expression and weaker ACE2 expression were detected in the proximal tubules, and no marked colocalization of ACE and ACE2 was observed (B); however, similar ACE and ACE2 staining patterns were seen in patients with minimal change nephrotic syndrome (MCNS) and healthy controls (C). Adapted with permission from Mizuiri *et al*[33]. ACE: Angiotensin-converting enzyme.

variations in ACE2 expression were detected between the different renal diseases. In a previous study, we detected lower ACE2 and higher ACE expression in the glomeruli and tubules of 30 patients with IgA nephropathy than in those of 20 healthy controls[36]. In addition, patients with membranous nephropathy, but not those with minimal change nephrotic syndrome, also displayed downregulated tubular ACE2 expression[36]. We excluded patients that were taking ACE inhibitors or AT1-receptor blockers, which might partly explain the differences between our findings and those of Lely *et al*[34]. In a study examining kidney biopsies from patients with focal segmental glomerulosclerosis and patients with chronic allograft nephropathy Reich *et al*[16] demonstrated that the ACE2 and ACE mRNA levels of these samples did not differ from those of the control samples.

We suggest that upregulated ACE expression and downregulated ACE2 expression represent a generalized response to kidney damage[36], but further studies of ACE and ACE2 expression in the kidneys based on human renal biopsy samples are necessary to confirm this.

HYPERTENSION

The renal RAS acts independently of the systemic RAS and is suggested to aid blood pressure control[37]. Imbalances between Ang 1-7/ACE2 and Ang Ⅱ/ACE expression downregulate Ang 1-7 synthesis and promote renal Ang Ⅱ expression and hypertension. Crackower *et al*[38] found that all hypertensive rat strains exhibited significantly downregulated ACE2 mRNA and protein expression. Tikellis *et al*[39] found that the kidneys of spontaneously hypertensive rats demonstrated significantly upregulated ACE2 expression and activity at birth and that at the onset of hypertension tubular ACE2 expression decreased, but glomerular expression paradoxically rose. Furthermore, in ACE2-deficient mice, a link was detected between severe hypertension and the excessive renal accumulation of Ang Ⅱ[40]. In the kidneys of Goldblatt hypertensive rats, upregulated renin expression in the collecting duct was found to be associated with upregulated ANG Ⅱ and ACE expression and downregulated ANG 1-7 and ACE2 expression[41]. Samuel *et al*[37] found that in obese Zucker rats, renal Ang Ⅱ expression was upregulated and ACE2-AT(2)R-MasR axis expression was downregulated by high Na consumption. ACE2 increases the synthesis of Ang 1-7 from Ang Ⅱ, which helps to avoid the excessive accumulation of Ang Ⅱ[42]. Furthermore, strategies such as increasing ACE2 activity and promoting Ang Ⅱ catabolism have been found to be useful for ameliorating hypertension[43]. In addition, recombinant ACE2 has been demonstrated to increase plasma ACE2 after Ang Ⅱ infusion, which results in blood pressure and plasma Ang Ⅱ normalization[44].

In a study of human renal biopsy samples in which total RNA was examined, the ACE to ACE2 ratio was found to be significantly higher in hypertensive subjects than in the controls[10]. In a study involving real-time PCR and immunohistochemistry, Koka *et al*[45] found that hypertensive nephropathy and hypertensive cardiopathy displayed significantly increased ACE expression and decreased ACE2 expression. Furthermore, Wang *et al*[22] reported that hypertensive patients exhibited significantly reduced protein expression levels of ACE and ACE2 in the tubulointerstitium compared with the controls, whereas there was little difference between the glomerular ACE and ACE2 protein expression levels

of the two groups.

The above studies indicate that ACE and ACE2 are important for keeping the RAS in balance, and variations in ACE and ACE2 expression might be associated with hypertension.

SUBTOTAL NEPHRECTOMY

In rats, Velkoska *et al*[46] found that subtotal nephrectomy resulted in the lowering of ACE2 activity; however, these changes were reversed by ACE inhibition. In 5/6 nephrectomized rats, Eräranta *et al*[47] found that dietary phosphate loading led to greater tissue damage and upregulated renal ACE expression. It can be said that renal ACE2 expression is downregulated after subtotal nephrectomy, which might increase the deleterious effects of Ang Ⅱ on the kidneys.

CLINICAL IMPLICATIONS OF CIRCULATING ACE2 AND URINARY ACE2

Only a small number of studies have examined the activity of circulating ACE2 in humans. Soro-Paavonen *et al*[48] demonstrated that type 1 diabetes patients with micro- or macrovascular disease display higher circulating ACE2 activity, suggesting that ACE2 might act to counteract the effects of such conditions. Soler *et al*[49] found that plasma ACE2 activity can be assessed in kidney transplant recipients and is positively correlated with age and the serum levels of urea, γ-glutamyl transferase, glycosylated hemoglobin, creatinine, aspartate transaminase, and alanine transaminase. It is also reported that soluble ACE2 activity correlates with hypertension and soluble ACE concentration decreases, while soluble ACE2 concentration increases in systolic heart failure[50]. The above studies indicate that plasma ACE and ACE2 levels might be useful biomarkers of the RAS in renal and coronary diseases.

In order to identify biomarkers of kidney disease progression, urine is readily collected and available. Soluble ACE2 has been detected in human urine[51], which might have been due to the excretion of the protein from renal cells or from plasma *via* glomerular filtration. Using Western blotting and an enzyme-linked immunosorbent assay, we found that patients with diabetic nephropathy had higher urinary ACE2 protein levels than the healthy controls[52]. In addition, Park *et al*[53] demonstrated that the urinary ACE2 concentration is strongly correlated with type 2 diabetes mellitus and is an independent predictor of microalbuminuria. Renal transplant patients with diabetes have also been found to have increased urinary ACE2 levels[54]. The conversion of membranous ACE2 into its soluble form is partially dependent on tumor necrosis factor-α convertase (ADAM17), which is responsible for increased ectodomain shedding of ACE2 at least *in vitro*[52,55]. Patients with renal disease exhibit increased expression levels of ADAM17[56]. We propose that ACE2 ectodomain shedding is associated with reduced renal ACE2 expression in patients with diabetic nephropathy[52]. A recent study demonstrated that insulin treatment reduced ADAM17 and ACE2 shedding in the kidneys of diabetic Akita mice[57]. The above findings suggest that the urinary ACE2 protein level and ACE2 activity are useful as biomarkers of diabetic nephropathy.

ACE2 AS A THERAPEUTIC TARGET IN KIDNEY DISEASE

ACE1 temporarily downregulates Ang Ⅱ expression, and Ang Ⅱ receptor blockers increase Ang Ⅱ levels. Both types of molecule suppress Ang Ⅱ activity incompletely; therefore, combining RAS inhibitors with ACE2 activators might lead to more complete downregulation of the RAS[12]. Qudit *et al*[58] found that human recombinant ACE2 (hrACE2) slowed the progression of diabetic nephropathy and lowered NADPH oxidase activity and blood pressure. In addition, their *in vitro* study indicated that the protective effect of hrACE2 is derived from upregulated Ang 1-7 expression and downregulated Ang Ⅱ expression[58]. Another study demonstrated that whilst hrACE2 treatment was effective at increasing plasma ACE2, it did not affect renal or cardiac ACE2 activity[44].

Xanthenone (XNT)[59] and diminazene (DIZE)[60] are ACE2 inhibitors. Hernández Prada *et al*[59] found that the acute *in vivo* administration of XNT to spontaneously hypertensive rats led to improvements in their cardiac function and reduced their blood pressure. Jarajapu *et al*[60] proposed that the short-term administration of XNT or DIZE to diabetic patients with complications is not effective at treating diabetic endothelial progenitor cell dysfunction. Moreover, recently Haber *et al*[61] confirmed a lack of enhancement of ACE2 enzymatic activity by XNT and DIZE *in vitro* and *ex vivo* experiments in both mice and rat kidney. Therefore, the suggestion that ACE2 activators have supra therapeutic effects on kidney disease than classical RAS inhibitors is speculative at present, although enhancing ACE2 activity might represent a new therapeutic strategy for Ang Ⅱ overactivity.

CONCLUSION

ACE and ACE2 play significant roles in the RAS, and both enzymes are strongly expressed in the kidneys, where their actions aim to achieve a balance between the ACE-Ang Ⅱ-AT1 axis and ACE2-Ang 1-7-Mas axis. In addition, the renal ACE/ACE2 ratio seems to have a significant impact on a variety of diseases including diabetes, hypertension, IgA nephropathy, and subtotal nephrectomy.

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MINIREVIEWS

Lipid abnormalities in kidney disease and management strategies

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Abstract

Patients with kidney diseases continue to experience significant cardiovascular disease (CVD) morbidity and mortality. Although there are many important risk factors playing a role in the pathogenesis of CVD in chronic kidney disease (CKD) patients, dyslipidemia (elevated triglycerides, elevated oxidized low-density lipoprotein and low/dysfunctional low high-density) represents one of the modifiable risk factors. Renal failure patients have unique lipid abnormalities which not only have complex role in pathogenesis of CVD but also cause relative resistance to usual interventions. Most of the randomized trials have been in hemodialysis population and data from CKD non-dialysis, peritoneal dialysis and renal transplant populations is extremely limited. Compared to general population, evidence of mortality benefit of lipid lowering medications in CKD population is scarce. Future research should be directed towards establishing long term benefits and side effects of lipid lowering medications, through randomized trials, in CKD population.

Key words: Chronic kidney disease; Dyslipidemia; Statins; Cardiovascular disease; Renal transplant recipients; Hemodialysis; Peritoneal dialysis

Core tip: Burden of cardiovascular disease and dyslipidemia continues to be high among patients with kidney diseases. Our review includes unique lipid abnormalities specifically affecting patients with kidney diseases. We have included comprehensive review of the latest evidence of the dyslipidemia treatment for each subgroup [*i.e.*, chronic kidney disease (CKD) not on dialysis, CKD on dialysis and Kidney transplant recipients] and current guidelines from Kidney Diseases: Improving Global Outcomes.

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INTRODUCTION

Chronic kidney disease (CKD) has become a public health problem with a global prevalence of around 8%-16%[1] and with an estimate of more than 10% (*i.e.*, > 20 million) prevalence in the adult United States population[2]. Data from National Health and Nutrition Examination Survey showed that CKD prevalence among ages 60 and above increased from 18.8% in 1988-1994 to 24.5% in 2003-2006[3]. According to United States Renal Data System (USRDS) a total of 112788 patients initiated dialysis in 2011[4]. Cardio vascular diseases (CVD) remain the number one cause of death among patients with kidney diseases[5,6]. USRDS 2013 Annual Data Report indicates that CKD patients not only have higher rates of congestive heart failure, acute myocardial infarction (MI) and cerebral vascular accident compared to non-CKD patients, but they also have lower survival rates compared to non-CKD patients. This survival further decreases with severity of CKD[7]. Similarly, renal transplant recipients (RTR) have elevated CVD mortality with estimated 10-year risk of 21.5%, due to effect of CKD, post-transplant allograft function and effects of various immunosuppressant medications[8,9].

Dyslipidemia is a well-established risk factor for CVD in the general population but this relationship is not straightforward in CKD population. While dyslipidemia is associated with CVD in pre-dialysis CKD[10] and hemodialysis population[11], data regarding its association in peritoneal dialysis patients is lacking[12]. With an ever increasing CKD burden worldwide, providing treatments for modifiable risk factors, like dyslipidemia, becomes an essential component for improving outcomes. In this review, we will examine various lipid abnormalities associated with kidney diseases and current evidence regarding various treatments.

Reverse epidemiology

Relationship between dyslipidemia and survival has not been consistent among patients with kidney disease. "Reverse epidemiology", terminology first coined in 2003, refers to the findings of increase survival among dialysis patients with high body mass index, obesity and hypercholesterolemia[13]. Authors suggested that survival bias, presence of malnutrition and inflammation and time discrepancies of risk factors possibly explain these findings[13]. While some studies have shown that lower cholesterol was associated with an increase in mortality[13,14], other studies have concluded that among dialysis patients without malnutrition/inflammation and among black dialysis patients, hypercholesterolemia is associated with an increase in cardiovascular mortality[15,16]. Chawla *et al*[17] reported data from the cohort of non-diabetic CKD (non-dialysis) patients, cholesterol levels were not associated with CVD mortality.

Lipid profile in kidney diseases

There are both qualitative and quantitative abnormalities seen in the lipid profile of patients with kidney disease[18]. Some of these abnormalities also differ between spectrums of kidney diseases. With impaired renal function and reduced clearance, abnormal removal is major contributor of lipid abnormalities. Common initial abnormalities include hypertriglyceridemia and low high-density (HDL) cholesterol. Elevated triglyceride levels (TG) can be attributed to increased concentration of Apolipoprotein C-Ⅲ[19] and also to the reduced activity of lipoprotein lipase[18].

HDL cholesterol, generally considered as "good" cholesterol, usually plays a role in anti-inflammatory, anti-oxidation and reverse cholesterol transport processes in normal individuals. In CKD patients, these activities are severely affected due to variety of factors[20]. With advanced renal failure, there is decreased production of apolipoprotein A-1 (which leads to decreased HDL levels) and decreased production and activity of lecithin-cholesterol acyltransferase which further decreases HDL levels and maturation of HDL cholesterol[21]. There are functional changes noted in HDL cholesterol in patients with renal failure. Anti-oxidant and anti-inflammatory properties of HDL cholesterol are compromised due to reduced activities of paraoxonase and glutathione peroxidase in renal failure patients[20,21]. Furthermore, oxidative stress can result in dysfunctional HDL which has rather pro-inflammatory effects[22]. Studies in hemodialysis patients have shown that dysfunction of HDL is not only associated with multiple co-morbidities and poor quality of life[23], but also with an increased risk of CVD events and CVD mortality[24]. In summary, patients with renal failure develop certain functional and structural abnormalities in HDL cholesterol which makes them prone to develop atherosclerosis and thus contributing to their CVD burden.

CKD patients also have reduced levels of lipoprotein lipase, hepatic lipase and defective very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) receptors. This leads to accumulation of VLDL, intermediate-density lipoprotein and chylomicron remnants which are susceptible to oxidization. These oxidized products are usually atherogenic and play a role in CVD pathogenesis in this population[20]. CKD patients frequently develop secondary hyperparathyroidism which also has an impact on lipid abnormalities[25]. It has been postulated that this usually occurs due to an increase of intracellular calcium concentration in hepatocytes by elevated parathyroid hormone in CKD patients[26]. Studies have shown a role of parathyroidectomy in reducing triglyceride levels in CKD patients[26,27].

Among renal transplant recipients, it has been seen that lipids and lipoprotein profile ratios were more beneficial when the TG levels were less than 150 mg/dL and apoA1 was greater than 150 mg/dL when compared to the opposite[28]. They are also on immunosuppressive medications, many of which affect the lipid profile adversely. In most renal transplant centers in the United States, kidney transplant patients receive induction

Table 1 Common lipid profile in patients with kidney disease[18,33,34]

	CKD not on dialysis	Hemodialysis	Peritoneal dialysis	Transplant patients
Total cholesterol	Normal or elevated	Normal or low	Elevated	Elevated
Triglycerides	Elevated	Elevated	Elevated	Elevated
LDL cholesterol	Normal or elevated or low	Normal or low	Elevated	Elevated
HDL cholesterol	Low	Low	Low	Normal

CKD: Chronic kidney disease; HDL: Low high-density; LDL: Low-density lipoprotein.

Table 2 Brief summary of randomized clinical trials in patients with kidney diseases[9,35,46,47]

Trial	Study population	Intervention	Follow-up	Major findings
ALERT (2003)	Renal transplant recipients (n = 2102)	Fluvastatin (40 mg/d) *vs* placebo	Mean 5.1 yr	Fluvastatin group had reduced major cardiac events and cardiac death but this was not statistically significant No effect seen on all-cause mortality
4D (2005)	Hemodialysis patients with DM type Ⅱ (n = 1255)	Atorvastatin (20 mg/d)	Median 4 yr	Atorvastatin did not have significant effect on CV death, non-fatal MI, non-fatal stroke and all-cause mortality
AURORA (2009)	Hemodialysis patients aged 50-80 yr (n = 2776)	Rosuvastatin (10 mg/d) *vs* placebo	Median 3.8 yr	Rosuvastatin had no significant effect on CV mortality, non-fatal MI, non-fatal stroke and all-cause mortality
SHARP (2011)	CKD not on dialysis (n = 6247) Hemodialysis (n = 2527) Peritoneal dialysis (n = 496)	Simvastatin 20 mg/d plus ezetimibe 10 mg/d *vs* placebo	Median 4.9 yr	Simvastatin plus ezetimibe significantly decreased major atherosclerotic event but had no major effect on CV mortality or all-cause mortality. Results were available for only entire population (both dialysis and non-dialysis)

ALERT: Assessment of lescol in renal transplantation; AURORA: Assessment of survival and cardiovascular events; SHARP: Study of heart and renal protection; CKD: Chronic kidney disease; CV: Cardiovascular; MI: Myocardial infarction; DM: Diabetes mellitus.

immunosuppression followed by ongoing use of various combination of immunosuppression class of medications including corticosteroids, calcineurin inhibitors (tacrolimus, cyclosporine) and mammalian target of rapamycin (mTOR) antagonists (sirolimus, everolimus)[29]. Steroids commonly cause insulin resistance and hyperinsulinemia which is associated with hypercholesterolemia[30]. Cyclosporine has been noted to decrease hepatic clearance of LDL as well as increase the synthesis of VLDL and decrease the secretion of bile salts causing increase in cholesterol levels. Tacrolimus increases the incidence of new onset diabetes after transplant (NODAT) which in turn is associated with an increased risk of atherosclerotic cardiovascular events[31]. mTOR inhibitors inhibit the activity of lipases, thereby increasing the circulating lipoproteins; they also decrease the fatty acid uptake into the adipose tissue leading to a decrease in plasma lipid clearance adding to the dyslipidemia[32].

Broadly, common lipid abnormalities among the patients with kidney diseases can be summarized in Table 1.

MANAGEMENT STRATEGIES

CKD patients not on dialysis

Statins: In general population, statins are clearly associated with decreasing CVD events and mortality, however results in CKD population have been variable. Most of the data regarding statins in CKD (not on dialysis) comes from subgroup/post hoc analysis and meta-analysis. Only one randomized trial, Study of Heart and Renal Protection (SHARP) trial, evaluated statin therapy with major cardiovascular events[35]. SHARP trial included 6247 patients with CKD not on dialysis, with mean glomerular filtration rate (GFR) of 26.6 mL/min per 1.73 m^2. Patients were randomly assigned to simvastatin 20 mg daily plus ezetimibe 10 mg daily *vs* placebo. The primary outcome was first major atherosclerotic event with median follow up of 4.9 years. Final results were available for the entire study group (both non-dialysis and dialysis), and it showed a significant reduction in the risk of major atherosclerotic event (RR = 0.83, P = 0.0021); non-hemorrhagic stroke (RR = 0.75, P = 0.01) and reduction for the need for revascularization procedure (RR = 0.79, P = 0.0036) in simvastatin/ezetimibe group. There was no significant difference between the two groups for major coronary events and it did not show any significant difference in progression to end-stage renal disease (ESRD) among non-dialysis patients (Table 2).

A 2014 meta-analysis by Palmer *et al*[36], which included 50 studies and 45285 patients, showed that statins consistently reduced CVD events and death rates in CKD patients not on dialysis. It showed that, when compared to placebo, statins reduced overall mortality (RR = 0.79 with 95%CI: 0.69-0.91 in 10 studies and 28276 patients), cardiovascular (CV) mortality (RR = 0.77, 95%CI: 0.69-0.87 in 7 studies and 19059 patients), CV events (RR = 0.72, 95%CI: 0.66-0.79 in 13 studies and 36033 patients), and myocardial infarction (RR = 0.55, 95%CI: 0.42-0.72 in 8 studies and 9018 patients). This meta-analysis did not show any consistent effect of statin on progression of CKD.

Post hoc analyses of three randomized trials

Table 3 Kidney diseases: improving global outcomes recommended doses of commonly used statins, based on doses used in trials, in patients with estimated glomerular filtration rate < 60[9,35,45-47]

	Dose (mg/d)
Fluvastatin	80
Atorvastatin	20
Rosuvastatin	10
Simvastatin/ezetimibe	20/10
Pravastatin	40
Simvastatin	40
Pitavastatin	2

(CARE, LIPID and WOSCOPS) have also shown that pravastatin reduced cardiovascular event rates (HR = 0.77, 95%CI: 0.68-0.86) in patients with moderate CKD; and this was similar to the patients without CKD[37]. Interestingly, subgroup analysis of JUPITER trial showed that rosuvastatin decreased cardiovascular event rates as well as overall mortality in patients with moderate CKD even in the absence of hyperlipidemia (LDL < 130). However, this study originally excluded patients with diabetes and advanced CKD[38]. Other meta-analyses of trials (randomized trials in CKD population plus sub-group analysis of trials of general population) have persistently shown the beneficial effect of statins[39-41].

There has been a suggestion that statins might have been associated with decreased decline in renal function[42]. However, not only majority of data is from secondary analysis; the results have been contradictory as well[43]. As stated above, SHARP trial (only randomized trial in this population) did not show any effect of stain on renal progression. Recent meta-analysis by Nikolic *et al*[44] showed improvement in GFR with statin use with the most benefit observed between year 1 and year 3 of statin therapy.

Recommendations for use: Kidney diseases: improving global outcomes (KDIGO) 2013 guidelines[45] recommend treatment with statins for CKD patients (not on chronic dialysis or had transplantation) $\geqslant$ 50 years of age who have estimated GFR (eGFR) below or above 60 mL/min per 1.73 m^2. For patients between ages of 18-49, KDIGO currently recommends statin therapy if they have known coronary disease, diabetes, prior history of ischemic stroke and if their cumulative 10-year risk of coronary death or non-fatal MI is greater than 10%. Statins are generally well tolerated; main side effects include hepatotoxicity and muscle toxicity including myopathy, myalgia and rhabdomyolysis. The incidence of these side effects has not been higher in CKD population compared to general population. For patients with eGFR $\geqslant$ 60 mL/min per 1.73 m^2, there is no dose adjustments required for CKD patients. KDIGO recommends using doses, used in randomized trials for particular statins, for the patients with eGFR below 60 (Table 3).

Fibrates: Fibrates mainly have effects on reducing triglyceride levels and increasing HDL cholesterol levels. Fibrates can decrease triglyceride levels by 18%-45%[48] and increase HDL cholesterol by 10%[49]. However in patients with CKD, their overall effect on cardiovascular risk has not been proven consistently. K/DOQI guidelines in 2003 recommended use of fibrates for the prevention of pancreatitis in patients with hypertriglyceridemia but in their latest 2013 guidelines, this recommendation has been removed[50].

In post-hoc subgroup analysis of VA-HIT trial, gemfibrozil was evaluated for secondary prevention of cardiovascular events in patients with CKD[51]. Gemfibrozil therapy reduced the composite outcome of coronary death, non-fatal MI and stroke but overall mortality was unchanged. Gemfibrozil group had higher incidence of increase in serum creatinine compared to placebo. Other major trials, evaluating effects of fibrates on cardiovascular risk, either had a very small proportion of patients with kidney disease[52] or CKD patients were entirely excluded[53]. At present KDIGO recommends against use of combination of statin and fibrates in CKD patients due to increased adverse events.

KDIGO recommends therapeutic life style changes in patients with hypertriglyceridemia, although the evidence for this is weak. These include weight reduction, dietary modification, increase physical activity, reduced alcohol intake and treatment of hyperglycemia. KDIGO recommends that fibrates can be considered in patients with triglycerides > 1000 mg/dL[50].

CKD patients on dialysis

Statins: There have been three major randomized clinical trials evaluating effect of statins in dialysis population. First one to be reported was Die Deutsche Diabetes Dialyse study, commonly known as 4D study. In this study, effect of atorvastatin on cardiovascular disease and death was evaluated among 1255 diabetic patients who were receiving maintenance hemodialysis[47]. Groups were assigned to receive atorvastatin 20 mg or matching placebo. At median follow-up of 4 years, despite decrease in LDL cholesterol by 42% within first four weeks, atorvastatin use did not significantly impact the primary endpoints of cardiovascular death, non-fatal MI and non-fatal stroke. Interestingly, atorvastatin group had higher incidence of fatal stroke. Atorvastatin also did not have significant effect on all-cause mortality. Various factors have been attributed to these findings, including the fact that the entire patient population was diabetic, had significant cardiovascular disease burden at baseline, relatively lower dose of atorvastatin, and probable limited role of statin once ESRD occurs. Subsequent post hoc analysis of 4D trial by März *et al*[54] showed that atorvastatin reduced fatal and non-fatal cardiac event and all-cause mortality in the particular group when pre-

Table 4 Kidney disease: Developing global guidelines recommendations for dyslipidemia treatment among chronic kidney disease groups

CKD groups	KDIGO recommendations for dyslipidemia
CKD patients not on dialysis	In adults ≥ 50 yr with eGFR ≥ 60 mL/min per 1.73 m^2, treatment with statins is recommended In adults ≥ 50 yr with eGFR ≤ 60 mL/min per 1.73 m^2, treatment with statins or statins/ezetimibe combination is recommended In adults 18-49 yr, treatment with statins is recommended if they have one or more of the following risk factors: Known coronary disease Diabetes mellitus Prior ischemic stroke Estimated 10-yr incidence of coronary death or non-fatal myocardial infarction > 10%
CKD patients ON dialysis	In adult CKD patients on dialysis, initiation of statin or statin/ezetimibe combination is not recommended In adult dialysis patients who are already on statin or statin/ezetimibe combination at the initiation of dialysis, these agents should be continued
Kidney transplant patients	In adult patients with kidney transplant, treatment with statin is recommended

Adapted from Tonelli *et al*[45]. CKD: Chronic kidney disease; eGFR: Estimated glomerular filtration rate.

treatment LDL is > 145 mg/dL[54].

A Study to Evaluate the Use of Rosuvastatin in Subjects on Regular Hemodialysis: An Assessment of Survival and Cardiovascular Events (AURORA) trial tried to investigate effects of rosuvastatin in hemodialysis patients[46]. This trial included 2776 hemodialysis patients randomly assigned to rosuvastatin 10 mg daily or matching placebo. Primary end-point was composite of death from cardiovascular cause, non-fatal MI and non-fatal stroke. At median follow up of 3.8 years, rosuvastatin did not have any significant association with primary end-point (HR = 0.96, 95%CI: 0.84-1.11, *P* = 0.59). As seen in the 4D study, rosuvastatin group in this study had a 43% reduction in their LDL levels from baseline at 3 mo. No significant effect on all-cause mortality was seen either. Authors suggested several possibilities contributing to these findings which include exclusion of patients < 50 years of age and those who have been on statin, high percentage of patients leaving the study, and lower than expected yearly cardiovascular event rates. In post hoc analysis of this trial among diabetics (*n* = 731), rosuvastatin did not have significant effect on primary end-point although rosuvastatin group had significant decrease in cardiovascular events (HR = 0.68, 95%CI: 0.51-0.90)[55].

SHARP trial[35] included CKD patients on dialysis as well as not on dialysis. It also included patients on both hemodialysis and peritoneal dialysis. Out of 9270 patients, 3023 were on dialysis and among them 2527 were on hemodialysis and 496 were on peritoneal dialysis. As mentioned earlier, there was a 17% reduction in major atherosclerotic event with the use of simvastatin and ezetimibe but the data on individual components of primary endpoints were only available for whole (dialysis + non-dialysis) population, and not separately. The authors suggested that proportional effect on major atherosclerotic event did not differ between dialysis and non-dialysis patients. The results showed that simvastatin and ezetimibe did not have any significant effect on cardiovascular mortality and all-cause mortality. In sub-group of dialysis patients, there were similar numbers of major atherosclerotic events in simvastatin plus ezetimibe group (15%) as in the placebo group (16.5%) with RR = 0.9 with 95%CI: 0.75-1.08, although this trial did not have sufficient power to see any effect in subgroup analysis. Interestingly, lesser number of dialysis patients used lipid lowering therapy and had lower LDL cholesterol levels at baseline compared to non-dialysis patients (Table 2).

Palmer *et al*[56] presented review of statins in dialysis population in 2013. Their review included 25 studies and 8289 patients. Authors reported that at the dose of simvastatin 20 mg/d or equivalent, statin reduced total cholesterol by 46 mg/dL. But they had no significant effect on major cardiovascular events (RR = 0.95 with CI: 0.88-1.03 in 4 studies with *n* = 7084), cardiovascular mortality (RR = 0.94 with CI: 0.84-1.06 in 13 studies with *n* = 4627), and all-cause mortality (RR = 0.96 with CI: 0.90-1.02 in 13 studies with *n* = 4705). They further noted that the data regarding risk of adverse events were not conclusive and there was not enough information to evaluate difference between hemodialysis and peritoneal dialysis population. It was concluded that statins could not be recommended for the prevention of cardiovascular events among dialysis patients.

Recommendations for use: KDIGO 2013 guidelines[45] recommend not to initiate statin or statin plus ezetimibe in dialysis population based on the results of the above mentioned clinical trials. For the patients who are already on statin or statin plus ezetimibe at the initiation of dialysis, there is no conclusive data available. Nevertheless, at this time, KDIGO recommends to continue these agents and periodically review them (Table 4).

Fibrates: There are no specific randomized trials of the use of fibrates in dialysis population. At present, KDIGO recommendations for hypertriglyceridemia and use of

fibrates remain same for both non-dialysis and dialysis populations.

Kidney transplant patients

Kidney transplant patients are unique in that they are not only on multiple long term immunosuppressive medications but are also prone for infections and malignancy therefore one need to be extremely cautious in adding medications in this unique population. Use of any combination of medication in RTR entails one to be vigilant of the side effect profile as well as the drug interactions. Guidelines are developed based on input from several landmark trials; however paucity of trials in managing lipid abnormalities in RTR does pose a challenge.

Assessment of LEscol in renal transplantation (ALERT) Study was a well conducted multicenter randomized double-blind, placebo controlled trial which included 2102 renal transplant recipient who were treated with fluvastatin or placebo and followed for 5-6 years. At the end of the study Fluvastatin group had a significant lowering of their mean LDL cholesterol, total cholesterol and triglyceride levels compared to placebo, with no significant change in the HDL cholesterol level. Even though the study showed a reduction in the primary endpoint of major adverse cardiac events, it was not statistically significant. However the treatment with fluvastatin led to a reduction in the risk of cardiac death by about 38% and non-fatal Myocardial Infarction by about 32% without any significant difference in the adverse events related to the medication dose. Of note, the power of the trial (to achieve its primary end point) was low; along with that there was increased use of statin in the placebo arm towards the end of the study, both of which could have limited the evidence of the full benefits of the statin drug[9,57-59]. An Extension of the ALERT study reinforced the effective reduction of LDL-Cholesterol as well as the major adverse cardiac events without any safety or tolerability issues even in the cyclosporine treated patients[58].

Another multicenter randomized trial looking into the effect of fluvastatin on acute rejection involved 364 RTR who were given fluvastatin 40 mg and were compared to placebo. Individuals receiving fluvastatin had a reduction in LDL cholesterol level by 18%, total cholesterol (TC) by 10% and an augmented increase in the HDL cholesterol by 6%. There was no increase in adverse events from the use of statin and no reduction in the acute rejection rates in RTR[60]. A recent meta-analysis of statin use in RTR included 22 studies and found the inconclusive effects of statin on all-cause mortality and kidney function. However it did show the significant reduction of TC, LDL cholesterol and concluded the possible benefit of statin in reducing cardiovascular events[61].

As mentioned above, various immunosuppressive combinations are used in RTR and fluvastatin appears to have a less lipid lowering effect in everolimus treated patients which is an mTOR antagonist. Given this information alternative statin therapy or a combination of medication may have to be instituted to better optimize the dyslipidemia. A small study involving 12 RTR on everolimus, when switched from fluvastatin to rosuvastatin showed an additional significant improvement in the lipid panel without affecting the safety and tolerability[62].

Smaller trials have assessed the safety and tolerability of various statins in RTR and some have highlighted the pleiotropic effects of statin on graft survival and improving endothelial dysfunction. Atorvastatin and Simvastatin in spite of their involvement in the cytochrome P450 pathway have been shown to be relatively safe overall in RTR[63].

Based on available data, KDIGO recommends (weak recommendation with moderate quality of evidence) use of statin in renal transplant recipients[45].

CONCLUSION

In summary, patients with kidney diseases have unique lipid abnormalities when compared to general population and they have different clinical implications associated with these abnormalities. Over the time, our understanding has evolved regarding dyslipidemia in CKD patients. Statins remain the first line of treatment for dyslipidemia. Majority of current evidence comes from subgroup/post hoc analysis and meta-analysis, especially in CKD (pre-dialysis), peritoneal dialysis and renal transplant population. Prospective interventional studies are needed in this population to identify subsets of patients who will benefit most and also to assess long term toxicity of statins. KDIGO recommendations provide general principles regarding treatment of dyslipidemia but it should be individualized for each patient.

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MINIREVIEWS

Increasing the use of biocompatible, glucose-free peritoneal dialysis solutions

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Abstract

A major concern inhibiting some clinicians from embracing peritoneal dialysis (PD) as the preferred first modality of dialysis is the effects of PD solutions on the peritoneal membrane. These anatomical and functional changes predispose to complications like peritonitis, encapsulating peritoneal sclerosis and ultrafiltration failure. In recent years, "biocompatible" and glucose-sparing PD regimens have been developed to minimize damage to the peritoneal membrane. Can the use of these more expensive solutions be justified on current evidence? In this review of the literature, we explore how we may individualize the prescription of biocompatible PD fluid.

Key words: Individualized prescription; Biocompatibility; Peritoneal dialysis; Glucose degradation products; Peritonitis; Ultrafiltration failure; Residual renal function

Core tip: There is increasing evidence of benefit for using biocompatible and non-glucose based peritoneal dialysis (PD) fluids. However, cost remains an impediment and perhaps there are selected groups of patients where the cost can be justified. We suggest that biocompatible solutions should be considered for patients with residual renal function and/or expected to remain on PD for a long period. They are particularly helpful for patients with drain-in pains. The targeting of diabetic patients for non-glucose solutions is intriguing given the recent IMPENDIA/EDEN study although vigilance is required to minimize unaware hypoglycemia. It remains to be seen if PD nephrologists are willing to take the same leap of faith that our hemodialysis (HD) colleagues took when they moved from Acetate-based HD solutions to Bicarbonate dialysate. It is possible that economies of scale will reduce the cost of the biocompatible solutions if we use them more frequently.

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INTRODUCTION

Peritoneal dialysis (PD) has been a popular modality of renal replacement therapy since it was introduced in 1978[1]. In comparison to hemodialysis (HD), PD provides a more gradual and continuous method of fluid and solute clearance, with improved preservation of residual renal function and minimal cardiac stress. PD is at least equivalent in terms of survival benefits in the initial phase of dialysis vintage[2]. Furthermore, PD is more cost effective than HD, especially when reduced erythropoietin stimulatory agent requirement and patient transport cost savings are considered[3].

Common complications of PD include peritonitis, technique and ultrafiltration failure. It has been proposed that newer "biocompatible" and "non-glucose" containing PD fluids can reduce these complications[4]. However, these newer PD solutions are more expensive, and the potential cost advantage of PD over HD may be attenuated. We have reviewed the literature to determine if the additional cost of these newer solutions can be offset by reducing complication rates.

It is generally accepted that conventional PD fluids alter the functional and anatomical integrity of the peritoneal membrane over time[5,6]. Glucose degradation products (GDPs), high lactate and low pH levels have been implicated in the pathogenesis of adverse dynamic changes in the peritoneal membrane[7], which then predispose to complications like peritonitis, technique failure, *etc*[8].

Biocompatible PD fluids are produced in multi-compartmented bags that separately store the acidic glucose solution and the bicarbonate buffer solution. This allows the glucose component to be heat sterilized at a low pH thus causing minimal or no caramelization and GDP generation[9]. At the point of use, the acidic glucose compartment is mixed together with the buffer solution to produce a more physiological pH solution, with minimal lactate and GDP concentrations.

ALTERNATIVES TO GLUCOSE AS OSMOTIC AGENTS

Glucose remains a popular osmotic agent in conventional PD solutions due to its low cost, relative safety and effectiveness. Increasing glucose concentration allows for greater ultrafiltration due to the larger osmotic gradient. However, increasing glucose concentrations also means increased glucose absorption, which may result in metabolic abnormalities like hyperglycemia, hyperinsulinemia, obesity and hyperlipidemia[10]. Non-glucose based osmotic agents such as icodextrin (used in Extraneal solution) and amino acids (used in Nutrineal solution) are often used in glucose-sparing regimens to reduce the metabolic impact of glucose absorption. The icodextrin molecule is large sized and does not cross the membrane easily, thus producing a prolonged osmotic gradient and sustained ultrafiltration. The enhanced ultrafiltration achieved with Extraneal results in better fluid balance with improved blood pressure control[11], and a reduction in left ventricular mass[12].

Nutrineal is an amino acid based PD solution which is generally considered equivalent to a 1.5% glucose bag with respect to osmotic power. Although the pH of the solution is 5.5 (low), it contains no glucose and hence is considered biocompatible. No study has shown any mortality benefit with this solution but improvements in nutritional parameters like albumin, transferrin and protein catabolic rate has been observed in some malnourished PD patients[13,14]. Both these non-glucose based PD solutions are licensed to be used once a day.

COST OF BIOCOMPATIBLE PD SOLUTIONS

Table 1 illustrates the cost difference between the various PD solutions. For convenience sake we have included the trade name of the PD fluids most commonly used in the United Kingdom. The catalogue prices of the non-conventional solutions are approximately 50% more expensive than the conventional ones. In the United Kingdom, based on these catalogue prices, continuous ambulatory PD compromising of daily 4 exchanges (CAPD × 4) of Dianeal would cost £5650/year, but × 2 Physioneal, Nutrineal, Extraneal would cost £10860/year. The incremental cost of switching a patient on automated PD from Dianeal to biocompatible glucose sparing regimen is similar. The cost incurred using 4 cycles of Dianeal (1.5%) overnight followed by last fill Dianeal (2.5%) is estimated to be £9420/year. A switch to 3 cycles of Physioneal, 1 cycle of Nutrineal and last fill Extraneal would cost an extra £5000/year (Table 2).

When extrapolating to a PD program of 150 patients the additional cost of prescribing biocompatible, glucose sparing regimen equates to £0.75 M/year. This calculation is somewhat spurious as it is based the on United Kingdom catalog prices which is not the actual price charged to the National Health Service. Nevertheless, as a comparator, the annual salary of a Band 6 nurse in United Kingdom ranges between £25700 to £34500. These figures present a significant dilemma as the same PD program could possibly employ 20 additional fully trained nurses at equivalent cost of changing to glucose sparing biocompatible fluids.

Table 1 Catalog prices of different peritoneal solutions

	United Kingdom (£)		Singapore ($)		Pakistan (Rs)	
CAPD fluid						
Conventional CAPD 2 litre bag						
Dianeal (1.5%)	3.87		10.66		774	
Staysafe (1.5%)	4.24		10.98		812	
Biocompatible CAPD 2 litre bag	£	Increment (%)	Sing $	Increment (%)	Rs	Increment (%)
Physioneal	7.32	89	12.5	17	1464	89
Nutrineal	8.5	120	14	31	1785	131
Extraneal	6.6	70	12.3	16	1200	55
Balance	4.63	9	12.1	10	1020	26
Automated PD Fluid						
Conventional APD 5 litre bag						
Dianeal (1.5%)	8.6		28		1400	
Sleepsafe (1.5%)	7.8		28		1450	
Biocompatible APD 5 litre bag		Increment (%)		Increment (%)		Increment (%)
Physioneal	12.2	42	39	39	2200	57
Sleep balance	12.5	60	40.5	45	2350	62

Source: Fresenius Dialysis Product Catalogue 2013 revised (United Kingdom, Singapore and South Asia); Baxter PD Product List 2014 (United Kingdom, Singapore and Pakistan). CAPD: Continuous ambulatory peritoneal dialysis; APD: Automated peritoneal dialysis; PD: Peritoneal dialysis.

Table 2 Estimated annual cost of peritoneal dialysis fluids based on United Kingdom catalog prices

	United Kingdom (£)	Increment (%)
CAPD		
Dianeal (1.5%) × 4	5650	-
2 × Dianeal, Nutrineal, Extraneal	8340	48
2 × Physioneal, Extraneal, Nutrineal	10860	92
APD		
Dianeal: 1.5% (× 4 cycles) with last fill of 2.5%	9420	-
Dianeal, Nutrineal, Extraneal: (× 3 cycles 1.5%, 1 cycle Nutrineal) with last fill Extraneal	11790	25
Physioneal, Nutrineal, Extraneal(× 3 cycles 1.5%, 1 cycle Nutrineal) with last fill Extraneal	14420	53

CAPD: Continuous ambulatory peritoneal dialysis; APD: Automated peritoneal dialysis.

EVIDENCE OF BENEFIT AND USE OF BIOCOMPATIBLE PD SOLUTIONS

Faced with the reality of current financial constraints can we individualise the use of biocompatible PD fluids?

The balANZ trial[15] was a large well conducted RCT exploring the clinical benefits of biocompatible solutions. Using biocompatible fluids, a significant 33% reduction in peritonitis rates was achieved although other studies have not yielded similar results. We have to consider if employing additional nurses would be more cost effective than biocompatible solutions in reducing peritonitis rates[16].

The balANZ study also suggested that biocompatible solutions may better preserve residual renal function (RRF). Although the primary end point did not reach statistical significance, the rate of decline of RRF was lower in the biocompatible PD fluid arm and time to anuria which was a secondary end-point did reach statistical significance. The importance of delaying onset of anuria should not be underestimated and would support using these more expensive solutions in patients with residual renal function.

One of the strongest drivers for the use of biocompatible solutions is the hope that PD membrane will be preserved, thereby delaying PD technique failure and reducing the development of encapsulating peritoneal sclerosis (EPS). Dialysate concentration of Cancer Antigen 125 (CA-125) is proposed to be an indicator of peritoneal mesothelial cell health[17]. There is evidence to suggest that biocompatible solutions preserve CA-125 levels, implying that they might prevent peritoneal membrane damage induced by the bioincompatible nature of the PD solutions[18,19]. Those most at risk of EPS may benefit from using biocompatible solutions. The incidence of EPS complication increases with time on PD[20]. There is consensus that EPS is very rare in people who were on PD for less than 3-4 years. The Pan-Thames EPS study[21] showed that more than 70% of the patients who developed EPS had a PD vintage of more than 5 years. If one is to use biocompatible solutions to reduce EPS risk, it should be prescribed at outset of PD. One might argue that elderly patients with high co-morbidity and short life-expectancy are unlikely to develop this complication. Perhaps more controversially, young patients with good match prognosis

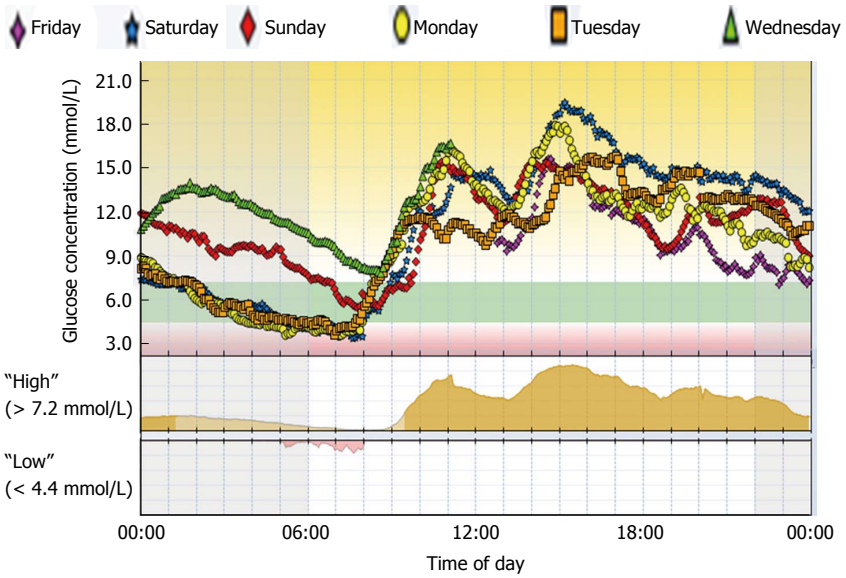

Figure 1 Continuous Glucose monitoring (6 d) of a Diabetic patient on peritoneal dialysis using Extraneal at night (22:00 to 06:00) not only showing hyperglycemia during the day (after 10 am) when glucose peritoneal dialysis solutions used, but also showing significant and regular episodes of hypoglycemia (unaware) suffered by the patient overnight. Continuous Glucose Monitoring demonstrates the merits and risk of using non-glucose based PD solutions (Extraneal). On one hand the overnight Extraneal dwell (from 22:00 to 06:00 h the next day) controlled the blood sugar effectively in comparison to the glucose based PD fluid dwell (from 06:00 till 22:00 h the same day). On the other hand Extraneal is putting the patient at risk of hypoglycemia (between 05:00 and 08:00 h). It is noteworthy that diabetic end stage renal disease patients have an increase incidence of hypoglycemia unawareness. PD: Peritoneal dialysis.

index for transplantation (especially patients with live donors) are also less likely to remain on PD long enough to develop EPS.

Infusion pain with PD fluids is known to affect treatment compliance and quality of life[22]. This pain is ascribed to the low pH of conventional PD solutions and the use of biocompatible PD fluids instead has shown to alleviate this discomfort in a randomized controlled trial[23].

GLUCOSE BASED *VS* NON-GLUCOSE BASED PD FLUIDS

The use of hypertonic 3.86%-glucose bags appears to precede the development of ultrafiltration failure (UF) and impaired osmotic conductance which are important predictors of PD technique failure and EPS[24]. Replacing 3.86% hypertonic solutions with Extraneal would be a reasonable strategy. The role of icodextrin for patients who have high transport characteristics exhibiting UF failure is well established, and recommended in the International Society of Peritoneal Dilaysis guidelines. However, it is not clear if reducing glucose exposure further by substituting Nutrineal for 1.36% glucose solutions will have clinically significant effects on peritoneal membrane preservation. Whilst inadequate solute clearance and ultrafiltration failure are undoubted causes of PD technique failure, patient and carer "burn out" is probably equally important. In this situation, biocompatible solutions will not help but diverting resources to providing more nursing support may be more effective in helping such patients continue on PD.

There are other obvious reasons for minimizing glucose load in the PD solution. Li *et al*[25] (on behalf of the IMPENDIA and EDEN study groups) reported a significant improvement in glycemic and lipid control with the use of glucose sparing PD fluids in the diabetic population. Could better glycaemic control have been achieved through more meticulous diabetic treatment if the additional resources were devoted to providing a comprehensive diabetic service? We suggest an additional caveat: not only should we be concerned about hyperglycaemia but hypoglycemia unawareness might be more dangerous leading to cardiac instability (an association between unaware hypoglycaemia and prolonged electrocardiogram QT-dispersion has been found in non-dialysis patients[26]). Hypoglycaemia unawareness is certainly something that we have found in diabetic patients that undergo routine continuous glucose monitoring. Figure 1 provides an example of diurnal hourly variations in interstitial glucose concentrations in a diabetic patient using nocturnal icodextrin to minimize overnight glucose exposure.

CONCLUSION

It is very ironic to note that HD faced a similar dilemma when a transition from acetate to bicarbonate buffered dialysate was proposed. Prescribing bicarbonate dialysate was equally controversial as it was more expensive and generally all the supportive data came from *in vitro* studies while *in vivo* studies provided very

little support. Nevertheless, a calculated rational leap of faith was taken and over time bicarbonate buffered HD dialysate has become cost-effective. Furthermore, the superiority of bicarbonate over acetate-based buffer was demonstrated during this time. Although we strongly believe in the potential benefits of PD biocompatible fluids, we acknowledge the pragmatic hesitancy of our colleagues due to associated high premium costs. In such a stalemate situation an approach to individualizing the prescription of biocompatible PD solutions is sensible. There is evidence to support its use in selected patients groups such as those with residual renal function with good life expectancy or patients with drain-in pain. The use of non-glucose PD solutions to improve diabetic control is perhaps more controversial but one hopes that cost will fall as uptake of these solutions increase. We are quite hopeful that in the imminent future the story of biocompatible PD fluids will have a similar conclusion to that of the bicarbonate buffered dialysate in HD.

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Role of β2-microglobulin in uremic patients may be greater than originally suspected

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Abstract

The role of beta2-microglobulin (β2M) in dialysis-related amyloidosis as a specific amyloid precursor was defined in the 1980s. Studies in those years were largely related to β2M amyloidosis. In 2005, for what was probably the first time in the available literature, we provided data about the association between β2M and early-onset atherosclerosis in hemodialysis patients without co-morbidities. In recent years, the role of uremic toxins in uremic atherosclerosis and the interest in β2M as a marker of cardiovascular (CV) and/or mortality risk have grown. In the current literature, clinical studies suggest that β2M is an independent, significant predictor of mortality, not only in dialysis patients, but also in predialysis patients and in the high-risk portion of the general population, and it seems to be a factor strongly linked to the presence and severity of CV disease. It is still unknown whether β2M is only a uremic toxin marker or if it also has an active role in vascular damage, but data support that it may reflect an increased burden of systemic atherosclerosis in a setting of underlying chronic kidney disease. Thus, although there have been some inconsistencies among the various analyses relating to β2M, it promises to be a novel risk marker of kidney function in the awareness and detection of high-risk patients. However, more research is required to establish the pathophysiological relationships between retained uremic toxins and further biochemical modifications in the uremic milieu to get answers to the questions of why and how. In this review, the recent literature about the changing role of β2M in uremic patients will be examined.

Key words: Beta2-microglobulin; Carotid atherosclerosis; Cardiovascular disease; Cardiovascular risk; Coronary artery disease; Hemodialysis; Mortality; Uremia; Uremic toxins

Core tip: Previously, the clinical significance of beta2-microglobulin (β2M) in uremic patients was limited to β2M-derived amyloidosis; in recent years, its role and power has changed and expanded. Although there have been some inconsistencies among the various analyses relating to β2M, the data generally support β2M as a promising novel marker of kidney function by predicting cardiovascular (CV) risk, CV events and overall mortality.

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INTRODUCTION

Beta2-microglobulin (β_2M) forms the non-variable light chain of the Class 1 major histocompatibility complex (MHC). It is found on the surfaces of nearly all nucleated cells. It is a non-glycosylated polypeptide with a molecular weight of 11.729Da[1,2]. Due to its small size, β_2M is present in the glomerular filtrate of the normal kidney. It was originally discovered as a component present in the urine of patients with tubular proteinuria. It has been estimated that there are 10^5-10^6 β_2M molecules/cell in human lymphocytes. When MHC is degraded, the MHC-associated β_2M is released into circulation[2-4]. This results in a constant production of free β_2M at a level of 0.13 mg/h × body weight in kilograms under normal conditions[2]. β_2M is found in low concentrations as a conformationally less restricted free monomer in blood and other biofluids, including synovial fluid. The normal physiological function, if any, of the freely circulating β_2M is unknown[2]. The β_2M synthesis rate in healthy individuals ranges from 2 mg/kg per day to 4 mg/kg per day, with a half-life of 2.5 h, and plasma concentrations vary between 1 mg/mL and 3 mg/mL[5].

Elevated β_2M levels are observed in chronic renal failures, lymphoproliferative disorders, inflammations, infections and other conditions as well, with high cell turnover[2]. A relationship has been noted between tumor burden and β_2M[6]. Concerning its use in oncology, β_2M levels correlate with the disease stage and poorer prognosis in patients with multiple myeloma and chronic lymphocytic leukemia[6,7]. It is also the most important predictor of treatment-free survival and overall survival of patients affected by lymphocytic leukemia and in most cases of lymphatic neoplasia. Additionally, serum β_2M levels can help to predict outcome in patients > or = 60 years with untreated acute myeloid leukemia[6,8]. Therefore, it is emphasized that β_2M may be the subject of future target therapy in cancer research[9].

Given that β_2M elimination is achieved *via* glomerular filtration, it is not surprising that plasma levels are inversely related to the glomerular filtration rate. Levels can be elevated as much as 60-fold in anuric patients with end-stage renal disease[5]. β_2M is a well-known, frequently studied representative marker of middle molecule uremic toxins and its role in dialysis-related amyloidosis as a specific amyloid precursor was defined in the 1980s[10]. Previous studies have largely been related to β_2M amyloidosis. However, its relationship with vascular risk was not identified until 2005. That year, to our knowledge for the first time in the available literature, we provided data on this relationship. We showed that, besides well-known cardiovascular (CV) risk factors, β_2M levels were independently related to carotid artery intima media thickness (C-IMT) in non-diabetic hemodialysis (HD) patients who had no clinical evidence of atherosclerosis[11]. Since then, the number of studies concerning this relationship has increased and now β_2M's direct pathophysiological role in vascular disease and its power as a predictor of overall and CV events are more predominant in the literature. In this review, the recent literature about the changing role of β_2M in uremic patients will be examined.

β_2M-DERIVED AMYLOID

In long-term dialysis patients, retention of β_2M produces a disease related to the deposition of β_2M amyloid fibrils around large joints such as shoulders and hips. In 1980, Assenat *et al*[12] were the first to report finding amyloid in the material they had excised from their patients' carpal tunnels and in 1985, Gejyo *et al*[13] established a novel type of amyloidosis in patients undergoing HD. Initially, it was believed to occur only in patients on chronic HD; therefore, it was called "dialysis-associated amyloidosis". However, it soon became clear that amyloidosis could develop in patients on any type of renal replacement therapy and even in uremic and predialysis patients. Therefore, it came to be referred to as β_2M-derived amyloid or in line with general amyloid terminology, as A β_2M-amyloid[5].

Pathogenesis

The exact mechanism of amyloidogenesis in dialysis patients remains unclear; however, elevation of circulating β_2M levels may not be the only cause of β_2M-derived amyloid. Recent studies have emphasized that in addition to substrate retention, biochemical modification of the β_2M molecule (such as oxidative modification) in the uremic milieu may potentiate its pathogenicity[14]. Glycosylated β_2M, a modified microglobulin, has been found in amyloid deposits as advanced glycation end products. This may further enhance the development of the lesions by both stimulating the secretion of cytokines and acting as chemoattractant and an apoptosis-delaying agent for monocytes. However, it remains unknown whether this modification plays an active role or is merely a long-term transformation of long-lived amyloid fibrils[14-17].

One of the other pathogenetic concepts in β_2M-derived amyloidosis is limited proteolysis and partial breakdown of native β_2M[5]. In recent years, a cleavage product form of β_2M that has a deletion of lysine at position 58 on the molecule ΔK58-β_2M and behaves differently from normal β_2M has been demonstrated in the sera of 20%-40% of dialysis patients[18]. Although it is conformationally unstable and amyloidogenic *in vitro*, it was suggested that it could play a role in β_2M amyloid fibrillogenesis[19]. However, it was not detected by 2D electrophoresis in the *ex vivo* amyloid fibrils of

two patients affected by dialysis-related amyloidosis[20]. Based on this result, the authors concluded that the process of amyloid deposition "in a target tissue requires that the fibrillogenic protein attains the amyloidogenic conformation at the right site, at the right time, and at the right concentration". They further speculated that ΔK58-β2M might be more susceptible to degradation than to amyloid deposition. In contrast, a N-terminal truncated species lacking six residues, ΔN6-β2M which was highly amyloidogenic *in vitro* was not detectable in plasma[20]. The list of factors that have been shown to influence the conformation of intact β2M is very long. However, many of the *in vitro* conditions that are highly favorable for amyloid formation from normal β2M are not encountered *in vivo* because of the possibility that several factors may interplay in different ways *in vivo*[5].

Clinical manifestations/diagnosis

This is a systemic type of amyloidosis but clinical manifestations of the disease are largely confined to the musculoskeletal system with carpal tunnel syndrome, spondyloarthropathies, hemarthrosis, joint pain and immobility. Late in the course of the disease, systemic deposition can occur, principally in the gastrointestinal tract and heart. Ninety percent of the patients may have the disease pathologically but not manifest clinical symptoms. Additionally, clinical symptoms are often nonspecific and easily mistaken for other articular disorders. The manifestations of β2M appear gradually over the course of years, between two and ten years after the start of dialysis in the majority of patients[2,5,21].

There are some suggestive findings, but no pathognomonic clinical or radiological findings exist in β2M amyloidosis. The gold standard diagnostic technique to demonstrate positive Congo Red staining and the presence of β2M is biopsy. Diagnostic material usually has to be obtained from synovial membranes or bone lesions[5,21].

Treatment

There have been many studies addressing the effects of different dialysis membranes on serum β2M levels. Generally, it is recommended that non-cuprophane, high-flux dialyers should be used for patients with evidence of or at risk for β2M amyloidosis[21]. High-volume hemodiafiltration and ultrapure dialysate were also reported to be associated with increased β2M removal, lower serum concentrations and reduced inflammation[21-23]. However, dialysis of any kind or with any membrane is incapable of removing sufficient quantities of β2M to completely prevent the deposition of amyloid and, with the exception of kidney transplantation, no currently available therapy can stop the disease progression of β2M amyloidosis or provide symptomatic relief[21].

β2M AND CARDIOVASCULAR RISK

In recent years, progressively more studies have been conducted with the aim of showing the involvement of uremic toxins and endothelial dysfunction in several aspects of uremic atherosclerosis[24]. Related to this, interest has grown in β2M as a marker of kidney function and CV risk.

Pathogenesis

The role of serum β2M in the pathogenesis of CV disease is still not clearly known. However, there have been some suggestions. For example, β2M appears to damage vessels by participating in amyloid formation in the vascular wall[25]. Also, retained uremic solutes, such as β2M advanced glycosylated end products which have been substrates for oxidative injury, seem to further contribute to the proatherogenic milieu of uremia[14]. Additionally, it has been demonstrated that some uremic toxins inhibit endothelial proliferation and wound repair in uremic patients[26]. In the presence of uremic serum, endothelial progenitor cells, which contribute to vessel repair and neovascularization, undergo a decrease in their ability to migrate[27]. The influence of the uremic milieu was confirmed by the observation that high serum levels of β2M and indole-3-acetic acid were associated with low numbers of circulating CD34+CD133+ endothelial progenitor cells[28]. Another study investigated whether β2M was proinflammatory by inducing oxidative burst in leukocytes; β2M was not found to be a factor for induction of leukocyte free radical production[29]. However, the involvement of β2M in the inflammatory process and its association with vascular risk is still an area of interest deserving attention.

Carotid atherosclerosis

In 2005, we investigated the associations of different risk factors with C-IMT, which had been an early marker of atherosclerosis, in "healthy" non-diabetic HD patients who had no clinical evidence of atherosclerosis[11]. In multivariate regression analysis, age, β2M, C-reactive protein and left ventricular hypertrophy were independently related to C-IMT. Elevated levels of β2M were found to be correlated not with the inflammatory markers but with the time patients had been in a uremic state. As we explained, although elevated plasma β2M was a well-known characteristic of chronic renal failure, that correlation may be just an epiphenomenon rather than a causal relationship, or β2M levels may indirectly influence uremia-related CV risk factors, or β2M *per se* may contribute to atherogenesis. As these were probably the first data about the importance of β2M as a CV risk factor in uremic patients, our findings necessitated confirmation in additional, larger scale studies. In 2006, using the same patient group, we assessed the determinants of the progression of C-IMT over the course of one year[30].

As in our former study, β2M was independently related to C-IMT at baseline; however, age and sex were the only independent predictors of the progression in C-IMT from baseline to the 12 mo stage. Subsequent studies in the general population showed that β2M was independently and significantly associated with total mortality and adverse CV outcome in patients with prevalent asymptomatic carotid atherosclerosis.

Peripheral arterial disease

In 2007, Wilson *et al*[25] researched patients in the general population with and without peripheral arterial disease (PAD) and analyzed their plasma. The peak intensity of a 12 kDa protein was higher in patients with PAD. Western blot analyses and immunoaffinity studies confirmed that that protein was β2M and circulating β2M in PAD patients was elevated and correlated with the severity of the disease. Another study found no relationship between β2M and the augmentation index, either in patients with PAD or in healthy subjects. However, it did demonstrate that among patients with PAD, elevated plasma β2M levels were associated with higher aortic stiffness irrespective of CV disease risk factors[31]. Subsequent studies did not support this association between β2M and PAD[32]. Additionally, no changes were found in β2M levels in PAD patients after exercise on a treadmill, thus challenging the initial hypothesis by Wilson *et al*[25] of an increase in β2M levels in patients with PAD due to repeated bouts of ischemia-reperfusion[33]. Although the conflicting results mostly pointed to a non-specific elevation of β2M in patients with a high vascular risk, it was concluded that β2M levels may not indicate the presence of PAD, but may instead reflect an increased burden of systemic atherosclerosis in a setting of underlying chronic kidney disease (CKD)[31,32,34].

Coronary artery disease

In 2007, we evaluated the determinants of coronary artery disease (CAD) other than conventional risk factors in nondiabetic HD patients[35]. Patients with CAD were compared to those without and, although β2M levels were higher in CAD patients (5.4 ± 1.4 mg/d *vs* 4.8 ± 1.5 mg/dL), the difference between the groups was not found to be statistically significant. The association between CV risk markers and arterial calcification in patients with CKD at Stages 3 and 4 had only recently been studied and β2M was found to be associated with coronary artery calcification beyond some other inflammatory biomarkers[36]. In addition, β2M, along with cystatin C and C-reactive protein, were found to predict mortality and improve risk classification and discrimination for a high-risk cohort undergoing coronary angiography[37].

Acute heart failure

In a study evaluating the prognostic role of serum β2M in heart failure, patients with severe renal dysfunction were excluded and a higher baseline serum β2M concentration was found to be the most powerful predictor of cardiac events and cardiac mortality in acute heart failure patients with creatinine ≤ 3.0 mg/dL. Furthermore, the baseline serum β2M concentration had a superior ability to distinguish cardiac event risk in acute heart failure patients compared with creatinine-based renal parameters[38].

Left atrial size

A linear correlation was found between the circulating levels of β2M and cystatin C and left atrial diameters. Additionally, left atrial diameters were negatively related to creatinine clearance in two study groups, one with CAD and the other without[39].

Arterial stiffness

Arterial stiffness occurs due to loss of compliance of the vascular wall. It is a prominent feature of vascular ageing and strongly predicts CV and total mortality. β2M has been shown to be related to arterial stiffness in the general population[40]. In HD patients, β2M levels were found to be positively associated with pulse pressure, which is a result of arterial stiffness. Additionally, β2M levels were positively associated with insulin resistance[41].

β2M AND ALL-CAUSE AND CARDIOVASCULAR MORTALITY

The HEMO study on 1704 HD patients showed that the predialysis serum β2M predicted mortality. After making statistical adjustments for the number of years on dialysis and for residual kidney function, for every 10 mg/L increase in the β2M level, there was a corresponding increase of 11% in mortality. The specific causes of death that account for this increased mortality have not been determined[42]. Another study evaluated the association of β2M levels in 490 HD patients with their clinical outcomes by dividing them into two groups according to their serum β2M levels[43]. Mortality from all causes in the higher β2M group was found to be significantly higher compared to that in the lower β2M group. These results demonstrated that serum β2M was a significant predictor of mortality in HD patients, independent of HD duration, diabetes, malnutrition and chronic inflammation[43].

The impact of β2M was studied in patients with CKD at different stages not yet on dialysis[44]. Baseline β2M levels were associated with vascular calcification but not with arterial stiffness. Higher β2M levels were independently associated with overall and CV mortality, with CV events in the whole cohort, and with CV events in the predialysis cohort. Furthermore, serum β2M was identified as an independent predictor of all-cause mortality in a population-based sample of older adults. Also, β2M was identified as a novel risk marker for adverse CV outcomes in patients with carotid

atherosclerosis[45].

β2M and infectious mortality in hemodialysis patients

The HEMO Study Group examined the association of serum β2M levels and dialyzer β2M kinetics with cause-specific mortality. They focused on cardiac and infectious diseases which were the most common causes of death. There was no statistically significant association in that study between cumulative mean predialysis serum β2M levels and cardiac mortality. However, in the entire cohort, each 10 mg/L increase in serum β2M level was associated with a 21% increase in the rate of infectious mortality[46].

β2M and mortality and graft loss

The association between post-transplant serum β2M and the outcomes following kidney transplantation were investigated. Serum β2M at discharge was a potent predictor of long-term mortality and of graft loss in kidney transplant recipients, providing information on the allograft function beyond that of serum creatinine[47].

ENCAPSULATING PERITONEAL SCLEROSIS

This is a serious complication in peritoneal dialysis patients. β2M was found to be a useful screening test for the onset of encapsulating peritoneal sclerosis and β2M and the accumulation of middle-molecular uremic toxins were thought to be related to the pathophysiology of this disease[48]. Recently, the accumulation of advanced glycation end products and β2M in the fibrotic thickening of the peritoneum in long-term peritoneal dialysis patients was investigated. The proportion of β2M-expressing areas was found to be elevated in long-term peritoneal dialysis patients, which may be a marker of peritoneal injury[49].

β2M AS A NOVEL MARKER OF KIDNEY FUNCTION AND RISK PREDICTION

Recently, there have been studies which evaluated whether novel biomarkers could add any information to improve risk prediction in patients at moderate and high risk. Data have provided that β2M, cystatin C and C-reactive protein predict mortality and improve risk classification and discrimination for a high-risk cohort. β2M and, to a lesser extent, beta trace protein, shared cystatin C's advantage over serum creatinine-based estimated GFR in predicting outcomes, including kidney failure. Thus, β2M shows promise as a novel filtration marker of kidney function for risk prediction of all-cause and CV mortality[50-52].

CONCLUSION

Previously, the clinical significance of β2M in uremic patients was limited to β2M-derived amyloidosis; in recent years, its role and power have changed and expanded. Although there were some inconsistencies among the various analyses relating β2M to clinical outcomes, the data generally support β2M as a promising novel marker of kidney function by predicting CV risk, CV events and overall mortality. The exact role β2M plays in CV events and why it predicts CV and high risk of morbidity and mortality is still unclear. Further studies are needed to clarify the role of β2M in uremic patients.

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Review on renal recovery after anatrophic nephrolithotomy: Are we really healing our patients?

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Abstract

The main goals for urinary stone treatment are to preserve renal function, reduce or avoid complications related to calculi, and to render the patient free of calculi as soon as possible. Anatrophic nephrolithotomy (ANL) is a valid and useful alternative for conventional staghorn calculi excision. Although excellent stone free rates can be achieved with ANL there are some drawbacks that may be of concern. Morbidity related to intraoperative and postoperative complications is one of them. Another, great concern is the possibility of reduction on renal function related to the procedure itself. This may be related to nephron injury during nephrotomy and parenchymal closure or to ischemic injury. In this review we assess functional results after anatrophic nephrolithotomy.

Key words: Anatrophic nephrolithotomy; Kidney lithiasis; Kidney stone disease; Percutaneous nephrolithotripsy; Staghorn calculus

Core tip: Anatrophic nephrolithotomy (ANL) is a valid and useful alternative for conventional staghorn calculi excision. Although excellent stone free rates can be achieved with ANL there are some drawbacks that may be of concern. Morbidity related to intraoperative and postoperative complications is one of them. Another, great concern is the possibility of reduction on renal function related to the procedure itself. In this review we assess functional results after anatrophic nephrolithotomy.

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INTRODUCTION

The main goals for urinary stone treatment are to preserve renal function, reduce or avoid complications related to calculi, and to render the patient free of calculi

as soon as possible. Procedures with low morbidity and rapid recovery are also essential in current practice. Guidelines from American Urological Association and European Urology Association state that conventional excision of staghorn stones must be considered only in exceptional cases and that percutaneous nephrolithtomy (PNL) should be the preferred choice[1,2].

The definition of "staghorn calculus" is related to the calculation that fills at least one caliceal group and, mandatorily, the pelvis. If the calculus fills the renal pelvis but not all the caliceal groups, it is recognized as a "partial staghorn calculus". However, if this kidney stone occupies the renal pelvis and at least three quarters of the pyelocaliceal system, it is labeled as "complete staghorn stone". Computed Tomography based morphometric studies may help classify and predict outcomes for staghorn calculus treatment[3,4], nevertheless, it is implicit that the greater the stone more difficult it is to leave the patient without remaining calculi in the collecting system. Several authors showed the relation between stone size and stone clearance. In a recent study, el-Nahas *et al*[5] showed that the stone-free rate for percutaneous nephrolithotomy as monotherapy was 56% and complete staghorn calculus was an independent risk factor for residual stones[5].

Undoubtedly, the main reason for conventional surgery rates decrease is the improvement of techniques such as extracorporeal shockwave lithotripsy (SWL) and endourological procedures (ureteroscopy and PNL)[6-9]. Yet, even with such technological developments, some special conditions are still best handled with conventional surgery, such as complex collecting system anatomy, extremely large stones, extremely poor function of the affected renal unit, or excessive morbid obesity.

Anatrophic nephrolithotomy (ANL) is one of the most used option for conventional staghorn calculus removal. Smith *et al*[10] described the anatrophic nephrotomy and plastic calyrhaphy a procedure in which stone removal and correction of collecting system anomalies was possible. Although excellent stone free rates can be achieved with ANL there are some drawbacks that may be of concern. Morbidity related to intraoperative and postoperative complications is one of then. Another, great concern is the possibility of reduction on renal function related to the procedure itself. This may be related to nephron injury during nephrotomy and parenchymal closure or to ischemic injury. In this review we assess functional results after anatrophic nephrolithotomy.

ANATROPHIC NEPHROLITHOTOMY PROCEDURE

Smith *et al*[10] identified some factors that may contribute for perpetuating renal inflammatory process after stone surgery: poor drainage, renal parenchymal damage, failure to control infection and inadequate removal of calculi. In order to control those issues and to preserve the maximal number of functional nephrons they described the anatrophic nephrotomy and calyrhaphy. The main steps in this procedure are: control of the main renal artery and obstruction of the posterior segment of renal artery, endovenous infusion of methylene blue to highlight the Brödel's white line, obstruction of the renal artery common trunk and creation of the condition of hypothermic ischemia, nephrotomy along the anterior border of the posterior calyces (approximately 0.5 to 1 cm posterior to Brödel's white line), calculus extraction, reconstruction of the pyelocaliceal system, and closure of the renal capsule[10]. The first 100 consecutive cases using this technique were published by Boyce *et al*[11] and showed 95% stone-free rate. Serum urea nitrogen obtained to assess renal function and serum creatinine has improved or remained stable in all but 2 patients. Other authors also published their results regarding renal function. Thomas *et al*[12] used 131 I hippuran scanning to assess renal function of thirteen patients operated on with classic ANL with a mean follow up of 13.6 mo. Thirteen percent decrease in renal function of the kidneys undergoing ANL surgery was reported. Nonetheless, total renal function assessed by effective renal plasma flow level remained normal in the postoperative stage. Compensatory hypertrophy may explain the unchanged total renal function as a 13% increase in the contralateral kidney was reported.

Studies in patients with solitary kidney may help to understand changes in renal function without the compensatory effect of the contralateral kidney. With a mean follow-up of 6 years, patients with solitary kidneys operated on with classic ANL were evaluated by Stubbs *et al*[13] and associates. No changes in pre- and post-operative serum creatinine was observed. However, creatinine clearance showed a small increase from 52 to 55 mL/min, but it was not statistically significant.

MODIFIED ANATROPHIC NEPHROLITHOTOMY

Several modifications of the classical approach have been described usually without defining the intersegmental plane[14-19]. Kijvkai *et al*[18] compared standard ANL and modified ANL and concluded that the standard procedure preserved more renal function than the modified[18]. Table 1 describes results of modified ANL in regard to renal function assed by scintigraphy.

In 2003, Kaouk *et al*[20] studied laparoscopic ANL for the management of staghorn renal stone in pigs[20]. After injecting polyurethane in the pyelocaliceal system to create a staghorn calculus model the animals were submitted laparoscopic nephrolithotomy. Glomerular filtration rate (GFR) was assessed before and four to five weeks later with diethylene triamine pentaacetic acid (DTPA) renal scans. The mean total GFR rised from 26.4 mL/min to 54.8 mL/min. A case series was first reported by Simforoosh and associates in 2008[21]

Table 1 Renal function after modified anatrophic nephrolithotomy

Ref.	*n*	Parameter	Renal function improvement/stabilization	Renal function decrease	Percent reduction
Belis *et al*[15]	13	131-iodine hippuran	100%	0%	-
Morey *et al*[16]	16	DMSA	18.8%	81.2%	4%
Melissourgos *et al*[17]	24	DMSA	62.5%	37.5%	4%
Kijvikai *et al*[18]	15 (7 St/8 Mod)	DTPA	0%	100%	9% St/27, 2% Mod
Ramakrishnan *et al*[19]	26	DMSA	87%	13%	-

DMSA: Dimercaptosuccinic acid; DTPA: 99mTc-diethylenetriaminepentaacetic acid; St: Standard; Mod: Modified.

with an update in 2013[22]. Stone-free rate was 88%. Mean pre-operative serum creatinine level rised from 1.20 mg/dL to 1.31 mg/dL in the postoperative period, but without statistically significant difference. Researcher described a stone-free rate of 63% in eight patients evaluated. Tree patients were submitted to preoperative 99mTc-DTPA renography to asses renal function 3 mo after surgery. Renal function decreased 4%, 12%, and 4% on the operated kidney of each patient.

Robot-assisted laparoscopic ANL (RANL) has also been described. Ghani *et al*[23] tried to replicate the conventional technique with ice-slush hypothermia. Follow-up at 1 mo demonstrated no change in renal function as estimated by creatinine clearance. King *et al*[24] evaluated seven consecutive patients submitted to RANL. Renal function was estimated by the Modification of Diet in Renal Disease study equation. In five of six patients estimated GFR was unchanged and improved in one patient (19 mL/min per 1.73 m^2 preoperative *vs* 25 mL/min per 1.73 m^2 postoperative).

PERCUTANEOUS NEPHROLITHOTOMY *VS* ANATROPHIC NEPHROLITHOTOMY

Several studies have assessed the impact of PNL on renal function[25-32]. Usually there is an immediate decrease on renal function after surgery with return to baseline on long term. Improvement or stabilization of renal function may occur because of better drainage, infection and inflammation resolution after surgery. On the contrary, renal function may decrease because of several injury mechanisms. Patient comorbidities, direct injury by kidney puncture and tract dilation, ischemia, inflammation and fibrosis are some of the possible mechanisms implicated on renal function deterioration.

Wilson *et al*[33] tried to quantify the level of parenchymal injury after stone treatment in an animal study. Percutaneous nephrolithotomy accounted for the largest amount of microscopic lesions, although, it was less than 2% of total renal volume and did not affected total renal function. Moskovitz *et al*[26] evaluated renal units separately and identified a remarkable reduction in the functional volume of the pole that underwent PNL, nevertheless, regional uptake and total renal function remained unchanged[26].

In cases where the amount of calculi is remarkable multiple access tracts may be required during the PNL procedure. It could be expected that the number of access tracts and ancillary procedures used for complete stone clearance could negatively impact on renal function. In regard to multiple tracts, there are few studies that support this hypothesis. El-Tabey *et al*[34] found that multiple punctures were an independent risk factor for renal function deterioration in a cohort of patients with solitary kidney. Hegarty *et al*[35] and Fayad *et al*[36] also noted that multiple tracts carries a risk of adversely affect renal function. Handa *et al*[37], on the other hand, showed that multiple access tracts does not lead to a more severe reduction in renal function[37].

Ancillary procedures such as extracorporeal shock wave lithotripsy (ESWL) and retrograde intrarenal surgery (RIRS) are frequently required for complete clearance of staghorn stones. The number of ancillary procedures to render the patient stone-free may range from 2.1 in partial to 3.7 in complete staghorn stones[1]. Most of the studies addressing PNL and ESWL do not show decrease in renal function[38-41]. Also, combined PNL and RIRS does not seem to adversely impact renal functio[42,43]. Zeng *et al*[43] reported that only 2.7% of patients had renal function deterioration after combined treatment. Nevertheless, the potential deleterious effect of ESWL on kidney structures is well established[44,45] and the combination of PNL may have a greater impact on renal function. In regard to RIRS parenchymal injury is not so evident, even so, more studies with longer follow-up are needed.

Most of the studies shows that renal function is not greatly compromised after PNL (Table 2). Nonetheless, there are no prospective randomized studies specifically comparing PNL and ANL. A well-designed study comparing PNL and open surgery was published by Al-Kohlany *et al*[46]. Eighty-eight renal units were assed, 43 submitted to PNL and 45 to conventional surgery. Modified ANL, extended pyelolithotomy, and combined pyelolithotomy/nephrolithotomy were included. Renal function was assessed with 99mTc-mercaptoacetyltriglycine (MAG3) scans and no significant decline in the operated renal unit was observed, although, results were not segregated by technique. Shen *et al*[47] also compared PNL and open surgery in a prospective randomized study. Renal function was assessed with serum and urinary b2-microglobulin and they found no difference between groups. As in Al-Kohlany *et al*[46]

Table 2 Renal function after percutaneous nephrolithotomy

Ref.	*n*	Follow up	Parameter	Renal function improvement/stabilization	Renal function decrease
Ekelund *et al*[25]	11	14 d	DTPA	73%	27%
Moskovitz *et al*[26]	88	1.5-24 mo	SPECT/DMSA	Total percent uptake unchanged	Decreased functional volume of the treated region
Tok *et al*[27]	711	12-24 h	eGFR	13% improvement in the geriatric group	2% decreased in the non-geriatric group
Kuzgunbay *et al*[28]	16	51.1 mo	Serum creatinine	75%	25%
El-Nahas *et al*[29]	122	12 mo	Tc99m MAG3	91.5%	8.5%
Nouralizadeh *et al*[30]	94	48 h	eGFR	0%	100%
Akman *et al*[31]	272	37.3 mo	eGFR	79.6%	20.4%
Ozden *et al*[32]	69	45.7 mo	eGFR	85%	15%

DTPA: 99mTc-diethylenetriaminepentaacetic acid; SPECT/DMSA: Single photon emission computed tomography; eGFR: Estimated glomerular filtration rate; Tc99m MAG3: Technichium99 metastable Mercaptoacetyltriglycine.

study, results were not segregated by technique.

DISCUSSION

Renal function improvement may occur after stone treatment. Possible mechanisms related to increase in renal function are the relieve in obstruction, resolution of infection and inflammatory process, and compensatory hypertrophy of the remaining tissue[12]. Nevertheless, the stone-extraction procedure may itself negatively compromise the functional condition of the surgically treated kidney. Decreased renal function after percutaneous nephrolithotomy may occur because of parenchymal damage during needle puncture and tract dilation. Ischemic injury may also arise if there is inadvertent injury to major vessels, although, it is not so common.

In regard to anatrophic nephrolithotomy decrease in renal function may occur because of direct injury to parenchymal tissue, leading to a permanent scar at the site of nephrotomy. Another possible mechanism is the ischemia-reperfusion injury related to occlusion of renal artery and vein. Protection measures as ice-slush hypothermia and mannitol have been used, as well as restriction of ischemia time to no longer than 30 min. However, the impact of those measures on renal function are not fully known.

It seems that the type of methodology used to assess renal damage influences the postoperative results. When functional markers are employed, kidney damage is temporary and usually mild. Examples of functional markers are renal plasma flow, GFR, serum creatinine, and estimated GFR. However when cellular damage and morphological assessment are considered, renal damage becomes more evident. In most surgeries postoperative renal function is preserved and even when renal dysfunction is observed, it is usually negligible. Nevertheless, information about long term follow-up is scarce, as well as the the cumulative impact of multiple procedures.

As previously addressed PNL is the standard treatment for staghorn stones. Nevertheless, there are some limitations with this approach. The Clinical Research Office of the Endourological Society (CROES) PNL Global Study and the British Association of Urological Surgeons Section of Endourology have reported the efficacy of PNL for treatment of patients with staghorn stones[48,49]. The CROES study group analyzed outcomes of 1466 patients with staghorn calculi compared with 3869 patients with nonstaghorn stones undergoing PNL. They found that patients with staghorn stones more frequently underwent multiple punctures (16.9% *vs* 5.0%) and had lower complete stone-free rates (56.9% *vs* 82.5%). The United Kingdom study group reported on 299 patients who underwent PNL for staghorn calculi demonstrating an intraoperative complete stone-free rate of 59% and 47% on formal postoperative imagin[49].

When the number of less invasive procedures exceeds what is considered reasonable, we must consider the conventional surgery[1,2]. With the advances in laparoscopic and robotic assisted methods replication of the open technique is possible with less morbidity. The main drawbacks of open surgery as bleeding, longer recovery and morbidity related to flank incision may be overcome with laparoscopic/robotic approach.

Although a definitive conclusion can not be drawn from the available literature in regard to which one is the best approach to treat complete staghorn stone, percutaneous nephrolithotomy still is the first option. Nevertheless, in carefully selected cases anatrophic nephrolithotomy may achieve optimal outcomes.

CONCLUSION

Although parenchymal damage after anatrophic nephrolithotomy is of concern renal dysfunction is usually clinically insignificant. Comparative studies of the available modalities are scarce as well as long term follow-up and the impact of multiple procedures.

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MINIREVIEWS

Appropriate kidney stone size for ureteroscopic lithotripsy: When to switch to a percutaneous approach

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Abstract

Flexible ureteroscopy (fURS) has become a more effective and safer treatment for whole upper urinary tract stones. Percutaneous nephrolithotomy (PNL) is currently the first-line recommended treatment for large kidney stones ⩾ 20 mm and it has an excellent stone-free rate for large kidney stones. However, its invasiveness is not negligible considering its major complication rates. Staged fURS is a practical treatment for such large kidney stones because fURS has a minimal blood transfusion risk, short hospitalization and few restrictions on daily routines. However, as the stone size becomes larger, the stone-free rate decreases, and the number of operations required increases. Therefore, in our opinion, staged fURS is a practical option for kidney stones 20 to 40 mm. Miniaturized PNL combined with fURS should be considered to be a preferred option for stones larger than 40 mm. Moreover, URS is an effective treatment for multiple upper urinary tract stones. Especially for patients with a stone burden < 20 mm, URS is a favorable option that promises a high stone-free rate after a single session either unilaterally or bilaterally. However, for patients with a stone burden ⩾ 20 mm, a staged operation should be considered to achieve stone-free status.

Key words: Ureteroscopy; Lithotripsy; Laser; Kidney calculi; Nephrostomy; Percutaneous

Core tip: Flexible ureteroscopy (fURS) has become a more effective treatment for large and multiple kidney stones. However, as the stone size becomes larger, the stone-free rate decreases, and the number of operations required increases. We herein review the appropriate kidney stone size for ureteroscopic lithotripsy and for situations when it should be combined with percutaneous surgery. In our opinion, staged fURS is a practical option for stones 20 to 40 mm. Miniaturized percutaneous nephrolithotomy combined with fURS should be considered to be a preferred option for stones larger than 40 mm.

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INTRODUCTION

The technical developments and patient requests for rapid stone removal have led to changes in clinical stone management. In the past 30 years, kidney stone treatment has shifted from open surgery to percutaneous surgery, and this has almost been replaced by shock wave lithotripsy (SWL). However, during the last decade, the limitations of SWL for some situations have become evident, and flexible ureteroscopy (fURS) has become more available. The demand for imperative complete stone removal has led to a shift toward endourology. The fURS and related instruments are still evolving. This evolution has made it possible to treat urinary stones in all locations, while decreasing the morbidity associated with the active intervention. However, as the stone size become larger, the stone-free rate of fURS monotherapy decreases, and the number of operations required increases. A percutaneous approach should be considered preferentially for larger stones. In this review, we discuss the possibilities and limitations of ureteroscopic lithotripsy in terms of the kidney stone size and stone number.

INDICATION FOR ACTIVE TREATMENT OF KIDNEY STONES

In general, there is a consensus that small stones may be treated with conservative management. In contrast to ureteral stone, most kidney stones are asymptomatic. It is questionable for small stones, especially in the lower pole, if treatment is required. The natural history and the risk of progression of such kidney stones have not been well evaluated. However, stone growth, potential obstruction, associated infection and pain are clear indications for the treatment of such kidney stones. Several authors have reported a significant rate of incidents during the follow-up of kidney stones (Table 1). For example, Glowacki *et al*[1] have reported that symptomatic events developed in 31.8% of patients, and spontaneous passage occurred in 15.0%, while surgical interventions were required in 16.8%. Burgher *et al*[2] reported that 77% of asymptomatic kidney stones became larger, and 26% required surgical intervention. Hübner *et al*[3] reported that an infection developed in 68% of asymptomatic kidney stones, and 45% had become larger after 7.4 years of follow-up. They also suggested that 83% of kidney stones require surgical intervention within the first five years after the diagnosis. Inci *et al*[4] observed that 33.3% of lower pole kidney stones had become larger within 52.3 mo of follow-up, while only 11% required surgical intervention. In a prospective randomized controlled trial with a 2.2-year of follow-up, Keeley *et al*[5] reported that there is no significant difference between SWL and observation, when they compared with asymptomatic kidney stones < 15 mm regarding the stone-free rate, symptoms, requirement of intervention, and renal function. Although some authors recommended prophylactic treatment for these asymptomatic kidney stones, conflicting data have been reported about the issue[6]. Other indications for active removal of kidney stones are shown in Table 2.

URETEROSCOPIC LITHOTRIPSY FOR LARGE KIDNEY STONES

Percutaneous nephrolithotomy (PNL) is currently the first-line recommended treatment for large kidney stones ⩾ 20 mm[7,8]. PNL yields an excellent stone-free rate for large kidney stones. However, its invasiveness is not negligible due to its considerable major complication rates. The puncture and dilation of a nephrostomy tract, although it is an essential process in PNL, may induce renal parenchymal damage, blood loss, or visceral injury. A recent global study of PNL reported the major complication rates, which included significant bleeding in 7.8%, renal pelvis perforation in 3.4%, and hydrothorax in 1.8%[9]. Blood transfusions were necessary in 5.7% of the patients. Postoperative high-grade fever occurred in 10.5%. The conventional prone position during the surgery may induce the respiratory problems.

Recently, fURS has become an effective treatment for kidney stones throughout all renal calyces. URS is an endoscopic surgery performed through the natural orifice. Thus, renal parenchymal damage is unlikely after URS[10]. The flexible ureteroscopes and their peripheral equipment have rapidly improved over the past few years. URS with holmium laser lithotripsy yields a same or better outcome than SWL for ureteral stones, as well as small kidney stones[7,8]. Some authors have reported the treatment outcomes of fURS for large kidney stones. For example, Grasso *et al*[11] reported their treatment outcomes of 45 patients with kidney stones larger than 20 mm, with a 76% stone-free rate after the first session of fURS. Second sessions were needed in 15 patients, and the stone-free rate increased to 91% without major complications. This primary remarkable result was supported by additional studies with similar findings[10,12-15]. We summarized the results of the updated studies in Table 3. For larger stones (> 20 mm), fURS monotherapy has achieved an excellent stone-free rate, although its outcome depends on the operator's skills and it may require staged procedures.

We previously reported the treatment outcome of large kidney stones (mean cumulative stone diameter: 31 mm) with an overall 90% stone-free rate after a mean 1.4 session of fURS[10]. In particular, we satisfactorily achieved a 100% stone-free rate in a cohort of

Table 1 Natural history of asymptomatic kidney stones

Ref.	Study type	No. of patients	Follow-up	Disease progression (stone growth)	Symptomatic episode	Need for intervention
Glowacki *et al*[1]	Retrospective	107	31.6 mo	NA	31.8%	16.8%
Hübner *et al*[3]	Retrospective	80	7.4 yr	45%	68%	83%
Keeley *et al*[5]	Randomized prospective	200	2.2 yr	NA	21%	10%
Burgher *et al*[2]	Retrospective	300	3.26 yr	77%	NA	26%
Inci *et al*[4]	Retrospective	24	52.3 mo	33.3%	41.7%	11%

NA: Not available.

Table 2 Indications for active stone removal of kidney stones

Kidney stones
Stone growth
Patients at high risk for stone formation
Obstruction caused by stones
Infection
Symptomatic stones (*e.g.*, pain, macrohematuria)
Stones ⩾ 15 mm
Stones < 15 mm, if observation is not the option of choice
Patient preference
Comorbidity
Social situation of the patient (*e.g.*, profession or travelling)

14 patients with kidney stones 20-40 mm, that included 64% (9/14) of cases with complete stone-free status. Our overall stone-free rate is favorable and equal to that of PNL. In our study, three patients (15%) developed a high-grade fever after the surgery. One patient with a struvite stone developed sepsis after the first session. It is impossible to completely avoid postoperative infections because the bacteria spread into the irrigation fluid during the surgery. Thus, surgeon should make an effort not to push up the intrarenal pressure and to keep the proper drainage flow, as well as the administration of antibiotics[10]. In our opinion, too long operation also apparently increases complication rates. When the operation time goes over 120 min, we usually terminate the surgery and retry the next session. This strategy was supported by a recent report which analyzed large patient cohort from a Japanese nationwide database. The authors suggested that longer operation time (> 90 min) increased the risk of complication[16].

Consequently, staged fURS is a practical option for the treatment of large kidney stones. Staged fURS has little blood transfusion risk, and is associated with a short hospitalization and few restrictions on daily routines. Moreover, the latest digital ureteroscopes, whose image quality is excellent, can promise better treatment outcome[17]. However, as the stone size becomes larger, the stone-free rate of fURS monotherapy decreases, and the number of operations increases. In our study, the stone-free rate for kidney stones > 40 mm dropped down to 67% after a mean 1.8 session, compared with a 100% stone-free rate for stones 20-40 mm after a mean 1.3 session[10]. Therefore, in our opinion, the percutaneous approach should be considered to be a preferred option for stones larger than 40 mm.

MINI-PERCUTANEOUS NEPHROLITHOTOMY COMBINED WITH FLEXIBLE URETEROSCOPY

Kidney stones larger than 40 mm should be treated primarily by PNL. In recent years, the new surgical technique named as "miniperc-PNL (mini-PNL)" or "tubeless PNL," which utilizes a smaller nephrostomy tract (⩽ 18 Fr), was developed. It is expected to prevent the major complications which frequently occurred in conventional standard-PNL (24-30 Fr). Jackman *et al*[18] reported the efficacy of a 13 Fr "miniperc" technique using a ureteroscopy sheath for nine adult patients. They concluded that the "miniperc" can offer advantages associated with hemorrhage, postoperative pain and the hospital stays. This report has been supported by several experts[19-23]. Knoll *et al*[23] evaluated the outcome of standard-(26 Fr) *vs* mini-PNL (18 Fr). They reported a prospective, nonrandomized series of consecutive 50 patients with a solitary kidney stone (lower pole or renal pelvis). After mini-PNL, if uncomplicated, the patients was not left a nephrostomy. Alternatively, a double-J catheter was placed anterogradely and the nephrostomy tract was closed with thrombin-matrix. After standard-PNL, all patients were left 22 Fr nephrostomies. While the stone-free rates were comparable (mini-PNL, 96% *vs* standard-PNL, 92%), mini-PNL showed the advantages of a shorter hospital stay and less postoperative pain. Although the benefits of mini-PNL are still controversial[24], this new less-invasive type of PNL can replace standard PNL for the treatment of large kidney stones, as well as complete staghorn stones. In addition, ultra-mini PNL (11-13 Fr) and micro-PNL (4.85 Fr) were developed and reported their effectiveness of the treatment for 10-20 mm sized kidney stone by some experts[25,26]. These new developed miniaturized PNL are expected to be new standard treatment options.

Furthermore, the simultaneous approach with fURS and PNL in the Galdakao-modified supine Valdivia (GMSV) position has been reported. The double approach (retrograde and antegrade) is expected to be superior to a single antegrade approach with PNL[27,28]. The advantages of the GMSV position enables the good versatility of stone manipulation along the whole upper urinary tract. The GMSV position can make use of combined or subsequent transurethral and percutaneous access to the urinary tract. The GMSV

Table 3 Treatment outcomes of ureteroscopy for large kidney stones

Ref	Study type	No. of patients	Mean stone diameter	Mean number of operation	SFR after the 1st operation	SFR after the 2nd operation
Ricchiuti *et al*[12]	Single center, retrospective	23	3.1 cm	1.43	56.5%	73.9%
Breda *et al*[13]	Single center, retrospective	15	2.2 cm	2.3	60%	86.6%
Riley *et al*[14]	Single center, retrospective	22	3.0 cm	1.82	23%	86.4%
Hyams *et al*[15]	Multi center, retrospective	120	2.4 cm	1.18	83%	97.5%
Takazawa *et al*[10]	Single center, retrospective	20	3.1 cm	1.4	65%	95%

SFR: Stone free rate.

position does not need to change the patient position. Also, it provides better descending drainage, retrieval of the stone fragments from percutaneous tract, and decompression of the intrarenal pressure. Scoffone *et al*[27] reported their experiences with 127 patients who were treated by a simultaneous approach with fURS and standard-PNL (ECIRS: Endoscopic Combined Intra-Renal Surgery) in the GMSV position. The tract was conventionally dilated to 24 Fr or 30 Fr. The mean length of the operation was 70 (range 25-225) min. The stone-free rate was 81.9% after the first session and 87.4% after the second session. Although the overall complication rate was relatively high (38.6%), there was no visceral injury and no anesthetic problems. The anatomical changes related to the supine position do not increase the risk of PNL complications. Although there were some difficulties in the surgeon's manipulations, which are associated with the longer access tract and more limited access field, supine PNL may have some benefits over prone PNL.

A synchronous approach with fURS and mini-PNL (ECIRS) has been suggested to be useful. Hamamoto *et al*[29] reported their treatment outcomes of mini-ECIRS (in the prone split-leg position), mini-PNL (18 Fr tract) and conventional standard-PNL (30 Fr tract). Although their study was nonrandomized and the patient position was prone, the stone-free rate of mini-ECIRS (81.7%) was superior to mini-PNL (38.9%) and standard-PNL (45.1%). Blood loss during the surgery was significantly lower in mini-ECIRS and mini-PNL than standard-PNL. Mini-ECIRS has a good versatility and will be an effective treatment for large kidney stones.

URETEROSCOPIC LITHOTRIPSY FOR MULTIPLE KIDNEY STONES

From some reports describing the outcome of SWL, about 20%-25% patients have multiple stones[30-32]. The stone-free rates after SWL for multiple stones are significantly lower than for a single stone, which dropped down from 70% to only 40%[30]. Many authors reported that the stone number was a significant predictor for the stone-free rates after SWL in their multivariate analyses[8,30-34]. In recent years, URS has been demonstrated its effectiveness and safety for upper urinary tract stones, and the indication has been expanding[35-37]. URS can directly access to the target stones throughout the whole upper urinary tract, regardless of laterality, and actively clear away the stone fragments[38]. This is a great advantage of URS superior to SWL. Therefore, URS may be an ideal treatment for multiple stones that promises a higher stone-free rate than SWL after a single surgery.

As well as fURS, SWL has been considered to be a recommended treatment for small to intermediate kidney stones[8]. The SWL has some advantages: good patient's acceptance, short convalescence, and little need of anesthesia during the treatment. However, the outcome of SWL is susceptible to many factors: stone size, stone position, stone composition, and the distance from skin to stone[30-34]. Particularly, the "multiple stones" is a strong unfavorable factor that impacts on the stone-free rates as well as recurrence-free rates after the treatment. Abe *et al*[30] described in their large cohort study that the stone-free rates after SWL for multiple stones dropped down to 41% compared with 71% for solitary stone. The "multiple stones" was the strongest adverse factor for stone recurrence in their analyses.

PNL is another treatment option for multiple kidney stones. Multiple kidney stones sometimes grow larger in different calices. In such cases, multiple percutaneous tracts are needed for access to the target stones. However, multiple percutaneous tracts may induce blood transfusion risk and the patient's discomfort[39,40].

Flexible ureteroscopes and their peripheral equipments have rapidly improved over the past few years. Nowadays, fURS yields a same or better outcome than SWL for kidney stones[8,36]. In comparison with SWL or PNL, fURS has some advantages for the treatment of multiple kidney stones. The various shaped nitinol baskets enable the removal of stone fragments safely. After the removal of one stone, we can continue the fragmentation of the next stones. Moreover, the latest flexible ureteroscopes and the smallest laser fiber can access to the lower calyx, where the spontaneous passage of residual fragments are hardly expected after SWL. Also, fURS can approach to bilateral upper urinary tract in a single operation[41,42].

There have been some studies of the management of multiple kidney stones by ureteroscopy[43-45]. We summarized the outcomes of the previous reports in Table 4. Breda *et al*[43] studied the results of 51 patients who had multiple unilateral kidney stones. The mean stone number was 3.1 and the mean stone length was

Table 4 Treatment outcomes of ureteroscopy for multiple kidney stones

Ref	Study type	No. of patients	Mean number of stones	Mean number of operation	SFR after the 1st operation	SFR after the 2nd operation
Breda *et al*[43]	Single center, retrospective, unilateral kidney	51	3.1	1.4	64.7%	92.2%
Herrera-Gonzalez *et al*[44]	Single center, retrospective, unilateral kidney	125	3.59	1	74.4%	NA
Huang *et al*[45]	Single center, retrospective, bilateral kidney	25	5.1	1.5	50%	92%

SFR: Stone free rate.

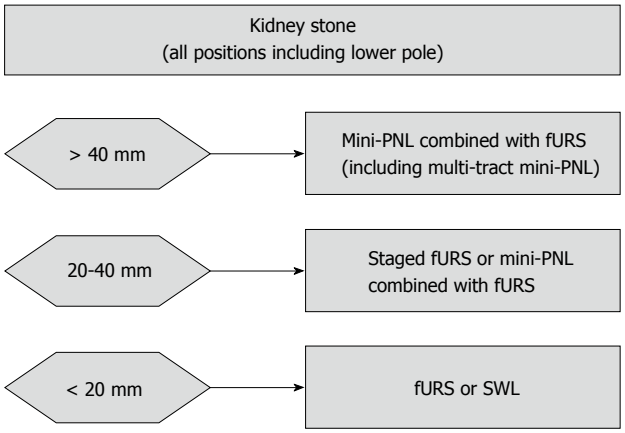

Figure 1 Our proposed treatment algorithm for kidney stones. fURS: Flexible ureteroscopy; PNL: Percutaneous nephrolithotomy; SWL: Shock wave lithotripsy.

6.6 mm. The mean stone burden (cumulative stone length) was 21 mm. The overall stone-free rate was 92.2%, with a mean number of sessions of 1.4. The stone-free rate after one and two sessions in patients with a stone burden ⩽ 20 mm was 79% and 100%, respectively, compared with 52% and 85% in patients with a stone burden > 20 mm. Complications occurred in seven patients (13.6%) including intraoperative bleeding in one, postoperative pyelonephritis in one and a urinary tract infection in three patients.

Herrera-Gonzalez *et al*[44] studied the results of 125 patients with multiple unilateral kidney stones. The mean stone number was 3.59. The mean cumulative stone length was 11.93 mm, and the mean cumulative stone surface was 83.7 mm^2. The overall stone-free rates after a single session was 74.4% The stone-free rates in patients with a cumulative stone surface ⩾ 100 mm^2 was 65.4%, compared with 79.5% in patients < 100 mm^2. Complications occurred in seven patients (5.6%), including urinary tract infections in four, hematuria in two patients, and ureteral perforation in one. The authors concluded that ureteroscopic lithotripsy for multiple kidney stones was an effective treatment.

We also reported the results of 51 patients with multiple stones, although we included both kidney and ureteral stones, either unilaterally or bilaterally, into the study cohort[38]. Our results were equivalent to those in Breda's reports[43]. In our study, the "stone burden" and the presence of "impacted stones" can significantly predict the stone-free rate after the first session of URS, whereas the "stone location" did not significantly influence the outcome. Due to the "impacted stones", if the ureteral mucosa was severely damaged during the procedure, we terminate the surgery in order to prevent a postoperative ureteral stricture. We always place a double-J stent to arrange for the next operation. At the next operation, the access to the residual stones usually becomes easier due to the spontaneous dilation of the ureter by stenting. In our study, we performed same session bilateral URS. We achieved 86% stone-free status after same session bilateral URS with no complication. Some experts has reported the effectiveness of same session bilateral URS[41,42]. Our results supported the adequacy of a same session bilateral URS as a considerable option for bilateral stones when it is performed at the experienced institutions.

We also analyzed our surgical data regarding stone burden[38]. Overall, the mean number of sessions was 1.3, the mean total operative time was 112 min, and stone-free rate after one and two sessions was 80.4% and 92.2%, respectively. The 25 patients with a stone burden < 20 mm had smaller number of sessions, shorter operative time, and higher stone-free rate after the first session than the 26 patients with a stone burden ⩾ 20 mm.

Consequently, fURS is an effective option for multiple stones. Especially for patients with a stone burden < 20 mm, fURS is a favorable option that promises a high stone-free rate after a single session, either unilaterally or bilaterally. However, for patients with a stone burden ⩾ 20 mm, a staged operation should be considered to achieve stone-free status.

CURRENT PROPOSAL FOR ACTIVE REMOVAL OF KIDNEY STONES

Figure 1 shows our proposed treatment algorithm for kidney stones. We select the treatment option with no distinction regarding the stone position (upper/middle pole or lower pole), because the current fURS instruments can easily reach to the all calyces, including the lower calyx, and can clear away the stone fragments by using a basket. Basically, we recommend endoscopic treatment for kidney stones, because residual fragments after SWL frequently do not pass spontaneously and often lead the stone recurrence. Besides, stones composed of calcium oxalate monohydrate, brushite, or cystine are usually resistant to SWL[8]. Depending on the operator's skills

and the stone shape/position/component, stones up to 40 mm can be treated sufficiently by fURS monotherapy, although staged operations may be required. We also recommend using a combination of PNL and fURS for larger stones, especially for staghorn stones, because the fURS can access each calyx, where the percutaneous antegrade approach is difficult. This is associated with a major advantage in terms of clearing the stone burden. Multi-tract PNL has also been evaluated by experts, who reported successful outcomes. However, multi-tract procedures may cause more complications, but if necessary, should be considered for appropriate cases[39,40]. Most upper urinary tract stones should be treated primarily by PNL, URS, SWL or a combination of these techniques. Thus, open or laparoscopic surgery may be a valid primary option in selected cases (*e.g.*, complex stone burden, treatment failed case, anatomical abnormal case.). Recently, the effectiveness of laparoscopic pyelolithotomy for large renal pelvic stone was reported, although further evaluation should be needed[46,47].

CONCLUSION

For large kidney stones, staged fURS is a practical treatment. Staged fURS has little blood transfusion risk, and is associated with a minimal risk of needing a blood transfusion, a short hospitalization and few restrictions on daily routines. However, as the stone size becomes larger, the stone-free rate of fURS monotherapy decreases, and the number of operations increases. Therefore, in our opinion, PNL should be considered to be a preferred option for stones larger than 40 mm. In addition, URS is an effective option for multiple stones. Especially for patients with a stone burden < 20 mm, URS is a favorable option that promises a high stone-free rate after a single session, either unilaterally or bilaterally.

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Time to re-evaluate effects of renin-angiotensin system inhibitors on renal and cardiovascular outcomes in diabetic nephropathy

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Abstract

The use of renin-angiotensin system (RAS) inhibitors, such angiotensin converting enzyme inhibitors/angiotensin-Ⅱ receptor blockers, to slow progression of chronic kidney disease (CKD) in a large group dominated by elderly people in the real world is not supported by available evidence. Large-scale clinical trials had many faults, among them a lack of focus on the elderly. However, it would be difficult to conduct clinical trials of a similar scale in elderly CKD patients. Besides, progression of kidney disease is often slow in elderly persons, and the vast majority of older adults with CKD will die before reaching end stage renal disease. Moreover, since it is not clear that progression of kidney disease, and even of proteinuric diabetic nephropathy, is not inhibited through the use of RAS inhibitors, the most patient-centric goal of therapy for many elderly individuals should be individualized.

Key words: Angiotensin converting enzyme inhibitors; Angiotensin receptor blockers; Dialysis; Chronic kidney disease

Core tip: The use of renin-angiotensin system (RAS) inhibitors, such angiotensin converting enzyme inhibitors/angiotensin-Ⅱ receptor blockers, to slow progression of chronic kidney disease in a large group dominated by elderly people in the real world is not supported by available evidence. Since it is not clear that progression of kidney disease, and even of proteinuric diabetic nephropathy, is not inhibited through the use of RAS inhibitors, the most patient-centric goal of therapy for many elderly individuals should be individualized.

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INTRODUCTION

Renin-angiotensin system (RAS) inhibitors [angiotensin

converting enzyme inhibitors (ACEi) and angiotensin-Ⅱ receptor blockers (ARBs)] have been recommended for reduction of proteinuria and prevention of the progresses of diabetic nephropathy (DN) by national and international guidelines[1-6]. Especially, two landmark trials, the Reduction of Endpoints in non-insulin dependent diabetes mellitus with the Angiotensin Ⅱ Antagonist Losartan (RENAAL)[7] and the Irbesartan Diabetic Nephropathy Trial (IDNT)[8], established the use of ARBs as first-line drugs for hypertensive patients with DN. In line with evidence presented in these trials, ARBs are now widely used for any stage of DN. However, our developed society, examples of which are European countries, the United States, and Japan, is facing a growing elderly population. The evidence referred to in the guidelines was produced more than 10 to 20 years ago when the diabetic population was mainly 50 to 60 years of age. At present, patients with DN are older than previously, suggesting that evidence accumulated earlier does not always hold true. As a general concept, renal function in elderly people is at risk of abrupt and complete inhibition of RAS. Previously, our group proposed that in patients with advanced stage chronic kidney disease (CKD), dose reduction of ACEi was required, especially in elderly patients[9]. In comparison with ACEi, all ARBs are mainly excreted from the bile instead of the kidney[10], supporting the concept that no dose modification is needed for ARBs in advanced stage CKD patients, such as stages 4 and 5 CKD. However, recently, a small dose of ARBs was recommended for patients with advanced stages of CKD[6]. With these issues in mind, this is the best time to reconsider the role of ARBs in the choice of treatment for hypertensive patients with DN. In this mini review, the authors re-examined previous reports that discussed the effects of ARBs on renal and cardiovascular outcomes.

ARE THERE REALLY EFFECTS OF ARBS BEYOND BLOOD PRESSURE LOWERING?

During the past 10 years, in addition to their use for blood pressure reduction, ARBs and ACEi have been administered to reduce proteinuria and to inhibit the progression of renal disease. In both the RENAAL[7] and IDNT[8], unexpectedly, there were very small reductions in blood pressure in patients receiving ARBs compared with patients receiving a placebo. However, in spite of this small reduction in blood pressure, conclusions were drawn regarding factors beyond the blood pressure lowering effects of ARBs on the assumption of a similar blood pressure reduction in both placebo and ARB groups. The possibility cannot be denied that a difference in blood pressure reduction, no matter how small, between groups is important in a large-scale clinical trial[11,12]. Considering these factors, small but not significant average blood pressure changes in a large number of patients cannot be neglected. Between the levels of blood pressure and the frequencies of cardiovascular events[13] there was the log-linear association, indicating that reduction in a systolic blood pressure of 5 mmHg is producing the stroke events by 40% and myocardial infarction by 20% reduction respectively[14]. The cardiovascular endpoints reduction seen in placebo-controlled trials of ACEi or ARBs use is expected from their blood pressure lowering effects, opposing pleiotropic effects of RAS inhibitors on cardiovascular disease (CVD) events. Therefore, it is unlikely that RAS inhibition produces effects beyond lowering blood pressure.

IS THERE A CLOSE RELATION BETWEEN THE LEVELS OF PROTEINURIA OR ALBUMINURIA AND PROGRESSION OF DN?

In the RENAAL, there were a linear relationship between baseline proteinuria and the risk of the primary outcome. Furthermore, every 50% reduction in albuminuria in the first 6 mo produced a reduction of 36% in the primary endpoint and a reduction of 45% in end stage renal disease (ESRD) at the end of study. The authors proposed the renoprotection as reducing proteinuria of losartan but not their lowering blood pressure[15]. Similarly, in the IDNT, every 2-fold increase from the baseline urinary excretion of protein doubled the risk of the primary endpoint. In either treatment groups, this risk was not achieved in half with every 50% reduction in proteinuria at 1 year. These results indicated the amount of proteinuria represented as an intermediate outcome in hypertensive patients with DN[16].

In line with this evidence, an old fashioned dogmatic hypothesis assuming a course of progression of DN stated that, first, microalbuminuria appears as DN and then the estimated glomerular filtration rate (eGFR) starts to decrease[17]. This central dogmatic hypothesis was adopted by the first edition of the CKD guideline of the Kidney Disease Outcomes Quality Initiative[17] and prevailed throughout the nephrology and diabetology world. However, in spite of this guideline, in the real world, general practitioners have been suspicious of this schema. Indeed, Tsalamandris *et al*[18] in 1994 demonstrated that in 40 hypertensive patients with DN followed for more than 7 years, they found 3 different courses of progression of DN over the long term. The first was that in spite of no decrease in the GFR, albuminuria increased; the second was that decreases in the GFR and increases in albuminuria progressed in parallel; and the third was that without any increases in albuminuria the GFR decreased progressively. Similar findings showed that DN is easily able to progress without albuminuria[19]. These data clearly suggest that destruction of other tissue pathways might produce the

decline in renal function. Ten years after the first edition of the CKD guideline, the second version[20] revised and accepted the concept that the levels of albuminuria and progression of DN are not always in parallel and sometimes independently change. This notion should be more greatly emphasized for general practitioners because a larger population of CKD patients with diabetes in the real world is treated by general practitioners than by specialists.

FLAWS IN LARGE-SCALE CLINICAL TRIALS

Onuigbo[21] proposed several serious concerns about randomized controlled trials. First, the discontinuation rates of the trial drugs have been remarkably high. In the RENAAL trial[7], the discontinuation rate of both losartan and placebo was unacceptably high. More than 45% of patients on losartan and more than 50% on placebo discontinued their drug, indicating that the outcome of the trial was not reliable. In contrast, in the ALLHAT trial, only 3.5% of enrolled subjects dropped out throughout the study. In addition to these flaws, both the RENAAL and IDNT trials failed to demonstrate statistically significant reductions in all-cause mortality by ARBs as well as the rate of introduction of dialysis therapy. Secondly, since in the RENAAL there were statistically inconsistence and apparently failed in substantial risk reductions of the doubling of serum creatinine and ESRD and a relatively higher rate of death in the losartan group compared with the placebo group were observed. Thirdly, there has been selection bias for participated patients with preserved renal function at the start of study. Finally, adverse effects, especially potential nephrotoxicity of the trial drug, was not correctly reported.

INCONSISTENCIES IN META-ANALYSES OF RAS INHIBITION IN CKD PATIENTS

Many meta-analyses and review articles have been published with regard to RAS inhibition in CKD patients. Strippoli *et al*[22] evaluated the effects of ACEi/ARBs on renal outcome and all-cause mortality in hypertensive patients with DN. In their analysis, ACEi significantly reduced all-cause mortality (RR = 0.79, 95%CI: 0.67-0.99, P = 0.04) compared with placebo but ARBs did not, although there was strong supportive evidence that ARBs were beneficial, showing a 22% reduction in risk of ESRD and a 42% increase of regression from microalbuminuria to normoalbuminuria. Besides, the effect of all renal outcomes was estimated for favor of ACEi compared with ARBs. Similar findings were reported for CVD outcomes in comparison between ACEi and ARBs. The benefit of ACEi but not of ARBs on all-cause mortality could probably be due to the experimental evidences that bradykinin antagonism of ACEi but not of ARBs, and the selectivity of ARBs could not have an advantage. Despite these findings in 2004, ARBs have been widely used in clinical practice for treatment of patients with DN.

One year after publication of Strippoli *et al*[22], in 2005 Cases *et al*[23] reported a systematic review and meta-analysis of the effect of RAS inhibitors and other antihypertensive drugs on renal outcomes. In their report, comparisons of ACEi or ARBs with other antihypertensive drugs showed a doubling of creatinine (RR = 0.71, 95%CI: 0.49-1.04) and a small benefit on ESRD (RR = 0.89, 95%CI: 0.75-0.99). In hypertensive patients with DN, there was no benefit found in comparative trials of either ACEi or ARBs on the doubling of serum creatinine (RR = 1.09, 95%CI: 0.55-2.15), ESRD (RR = 0.89, 95%CI: 0.74-1.07), GFR, or creatinine values. They proposed that blood pressure lowering effect was a major actions of ACEi/ARBs on renal outcomes conducted as placebo-controlled trials. Therefore, in patients with DN, beyond blood pressure lowering effects still remain unclear. However, considering their data, including data from patients with diabetes in ALLHAT[24], which was not originally designed to investigate the effects of antihypertensive agents for treatment of kidney diseases, it is likely that the mixture of diabetic nephropathy and hypertensive nephrosclerosis could account for the unfavorable effects shown for ACEi. Thus, the importance of the ALLHAT may cancel any effect shown in patients with true DN; therefore, the validity should be cautiously interpreted.

Balamuthusamy *et al*[25] reported a meta-analysis of studies using RAS inhibitors and CVD outcomes in hypertensive CKD patients with proteinuria, which included data from ACEi and ARBs. In that meta-analysis, RAS inhibitors decreased the risk for heart failure (RR = 0.63, 95%CI: 0.47-0.86, P = 0.003) in patients with DN in comparison with the control group. Although there was a decreased risk for myocardial infarction (RR = 0.89, 95%CI: 0.79-1.01, P = 0.06) and an increased risk of stroke (RR = 1.75, 95%CI: 0.96-3.17, P = 0.07) with inhibitors of RAS, the findings were not statistically significant. Based on their analysis, the authors concluded beneficial usage with RAS inhibitors for reduction of the risk of CV outcomes and heart failure in hypertensive patients with DN in comparison with placebo. Moreover, the authors recommended that the RAS inhibitors should be used as the first line antihypertensive drugs for hypertensive patients with diabetes mellitus and proteinuria. However, these results could be cautiously interpreted because a bias with larger numbers affected the findings.

Sarafidis *et al*[26] demonstrated in their meta-analysis that RAS inhibition with ACEi/ARBs in hypertensive patients with DN was related with reductions in the risk for ESRD and the doubling of serum creatinine in comparison with regimens that do not include RAS inhibitors. In addition, these agents did not produce

a reduction of the risk of all-causes mortality was not brought by these agents. In their study, ARBs were reported to reduce the risk of ESRD and the doubling of serum creatinine by 22% and 21% with significance, respectively. In contrast, ACEi were not significantly associated with reduction of 30% for the risk of ESRD but was significantly done with reduction of 29% for the risk of the doubling of serum creatinine. These findings favoring ARBs over ACEi should be interpreted with caution, because the effect on both ESRD and the doubling of serum creatinine were lower in ACEi in comparison with ARBs. These discrepancies might be caused by the two pairs of studies occupying the reported effects of ACEi (Micro-HOPE[27] and DIABHYCAR[28]) and ARBs (RENAAL[7] and IDNT[8]), which are completely different in primary outcomes, participated populations and its study design.

Recently, Sarafidis *et al*[29] summarized that in patients with DN, data from observational analyses and surrogate outcomes (and excluding the data from nondiabetic CKD patients) suggested a blood pressure of < 130/80 mmHg with protein excretion > 0.3 g/d. In non-proteinuric patients with diabetes, the main determinant of blood pressure goals leads to cardioprotection. Diastolic blood pressure < 80 mmHg is warranted, whereas the optimal systolic blood pressure target lies between 130 and 140 mmHg and should be decided on an individual basis, balancing the benefits of stroke reduction and unfavorable risks of hypotension and acute renal failure[30]. However, they proposed that there is no decisive evidence for combined therapy using RAS inhibitors for any type of CKD. Furthermore, sub-analyses from cardiovascular trials suggested no clear-cut benefit of RAS inhibition in hypertensive patients with normo-albuminuria and preservation of eGFR and sometimes produced harm in susceptible individuals.

More recently, Roscioni *et al*[31] postulated that the value of the RAS in the progression of DN has promoted the marketing of a therapeutic strategy to aim every step in the RAS cascade. Blockade of angiotensin Ⅱ by means of ACEi or ARBs is currently considered as the best option to treat DN because the renoprotective capabilities of these agents were well-established.

Among a large number of review articles, the well-designed larger studies dominated the results, whereas small studies had total weights accounting for a small percent of the total results. Thus, even if conclusions of several small studies differed from those of large-scale studies, the results of the large-scale clinical studies would prevail because of the large number of participants.

WHY ARE ARBS NOT RENOPROTECTIVE?

In several reports, Onuigbo of the Mayo Clinic noted that the administration of RAS inhibitors to patients with CKD sometimes produced acute kidney injury (AKI)[32-35]. He could not point out any clear-cut identifiable factors for this phenomenon, although he mentioned that many factors, such as heart failure, hypertension, infections, dehydration, etc. were found to be associated with worsening of renal failure in patients with CKD.

Suissa *et al*[36] assessed the long-term effect of ACEi on the risk of ESRD. They analyzed the data from a population-based cohort of all diabetic patients treated with antihypertensive drugs in the Province of Saskatchewan, Canada, between 1982 and 1986. The patients were followed up to the end of 1997 and identified as cases of end-stage renal failure. Using a nested case-control with the controls matched to each case for age, diabetes type, and duration of follow-up were analyzed. Of 6102 subjects, the 102 cases that developed ESRD were matched to 4129 controls. The adjusted RR of ESRD in relation to thiazide diuretic use, 2.5 (95%CI: 1.3-4.7) for ACEi, 0.8 (95%CI: 0.5-1.4) for blockers and 0.7 (95%CI: 0.4-1.3) for calcium channel blockers were reported. During the first 3 years after the start of follow-up, the RR of ESRD with ACEi use was 0.8 (95%CI: 0.3-2.5), but increased to 4.2 (95%CI: 2.0-9.0) after 3 years. From these data, it is clear that use of ACE-inhibitor use does not reduce the long-term risk of ESRD in diabetes. Their data also suggested that ACEi might actually produce this risk, which contribute to the continuing increases in incidence of ESRD owing to diabetes. These data coming from the real world do not validate the usefulness of ACEi in prevention of progression of DN. In the real world, a recent growth of the proportion of the elderly population is becoming worldwide. Moreover, higher number of elderly patients is brought by the increasing longevity of humans and it is producing subjects with multiple chronic diseases such as hypertension, diabetes, and CKD. These problems increase in morbidity and mortality in the elderly. More than one third of adults in the general population are 70 years over and half of them have CKD[37,38]. Whether evidence supporting current guidelines for the use ACEi/ARBs in patients with CKD can be extrapolated to this large group is unknown. O'Hare *et al*[39] tried to address this question and found that current guidelines addressing ACEi/ARBs use in patients with CKD are funded on evidence with limited relevance to most persons older than 70 years suffered from with CKD. Use of these agents to slow progression in this large group is not supported by available evidence. It is also not clear that slowing the progression of kidney disease represents the most patient-centric goal of therapy for many of these individuals. In elderly persons, renal function is slowly deteriorated and the vast majority of older adults with CKD will die before reaching ESRD[40,41] In a subgroup analysis among patients who were 65 years over and enrolled in the RENAAL trial, losartan was propagated to show renoprotective effect on these older participants. This suggested that this agent has equal efficacy for elderly albuminuric patients. However, the patients in this study was less than 74 years old, indicating that it cannot be applicable for those findings

to patients who are 75 years over[42]. Patients with a mean age of > 65 years were participated in the Telmisartan Randomized AssessmeNt Study in ACE iNtolerant Subjects with Cardiovascular Disease[43]. In this study, the investigators analyzed patients with organ damage but without macroalbuminuria or heart failure who could not tolerate ACEis. Either an ARB (telmisartan) or a placebo were administered to patients in addition to standard treatment and composite renal outcomes (ESRD, doubling of serum creatinine changes in eGFR, or the levels of albuminuria) were examined. Increases in albuminuria were less in patients treated with telmisartan than with placebo (32% *vs* 63%; $P < 0.001$). Furthermore, there was no significant difference in the composite renal outcome between telmisartan and placebo (1.96% *vs* 1.55%). Therefore, it is unlikely that RAS inhibition is effective for patients with DN.

CONCURRENT THERAPY WITH ARBS CAUSES UNRECOGNIZED WORSENING OF RENAL FAILURE IN ADVANCED STAGE OF CKD

Onuigbo *et al*[33] reported that the discontinuation of ACEi and/or ARBs produced reversible AKI in 100 CKD patients and that 75% of these patients were 65 years over, and 23% of these were 80 years over. Also, they examined prospectively the syndrome of worsening renal failure in CKD patients hemodynamically. In 19 of 20 patients the eGFR was increased from 27.8 ± 9.5 to 39.7 ± 14.9 mL/min per 1.73 m^2 after stopping RAS inhibitors. Further, they found that ESRD in the older CKD patients (average age 75.3 years) was frequently coming from patients suffered from unilateral renal artery stenotic lesions with dual kidneys. Similar findings were reported by Ahmed *et al*[44] in 52 patients with advanced stage CKD. Their mean age was 73.3 ± 1.8 years, their average eGFR 16.38 ± 1 mL/min per 1.73 m^2 and urinary excretion of protein 77 ± 20 mg/gCr. Besides, 40 percent had diabetes mellitus. Twelve months after cessation of RAS inhibitors the eGFR increased significantly to 26.6 ± 2.2 mL/min per 1.73 m^2. Of these patients, 61.5% had a more than 25% increase and 36.5% had an increase exceeding 50% in eGFR, although a significant decline in the eGFR slope (-0.39 ± 0.07) in the 12 mo before cessation of RAS inhibitors were found. From these findings in combination, cessation of either ACEi or ARBs could delay the progression of ESRD in the majority of those patients. It is therefore likely that ACEi/ARBs should be used in elderly hypertensive patients with CKD with great caution.

ARE ACEI/ARBS STILL EFFECTIVE IN PREDIALYSIS PATIENTS?

Hsu *et al*[45] examined safety and the adverse effects of ASCi/ARB use for hypertensive patients with advanced CKD and anemia by a population-based longitudinal cohort study. They selected subjects who had a primary diagnosis of CKD and received an erythropoietin stimulating agent. Inclusion criteria was their baseline values for serum creatinine > 6 mg/dL and hematocrit < 28%. From January 2000 through June 30, 2009, 28497 patients were selected. Results showed that use of ACEi/ARB inhibitors significantly reduced the risk of long-term dialysis and the composite outcome, with a hazard ratio (HR) of 0.94 (95%CI: 0.92-0.97) after adjustment for various confounders. In this study, even in patients with DN, ACEi/ARB use reduced the HR of ESRD and the composite outcome of ESRD or death. However, a higher rate of hyperkalemia-associated hospitalization was found among patients treated with ACEi/ARB inhibitors than among nonusers (9.2% *vs* 6.7%), indicating that the use of RAS inhibitors for elderly patients with DN might be dangerous.

ARE ARBS EFFECTIVE IN PATIENTS RECEIVING DIALYSIS?

Heerspink *et al*[46] reported a systematic review and meta-analysis of assessment of blood pressure lowering effects in dialyzed patients. In their analysis, treatment with antihypertensive agents was more closely related with lower risks of CVD events (RR = 0.71, 95%CI: 0.55-0.92, $P = 0.009$), all-cause mortality (RR = 0.80, 95%CI: 0.66-0.96, $P = 0.014$), and CVD mortality (RR = 0.71, 95%CI: 0.50-0.99, $P = 0.044$) than control regimens. Also, their data indicated that there were no differences in blood pressure lowering effects among RAS inhibitors, β blockers, and calcium channel blockers in patients on dialysis. They concluded that the choice of antihypertensive agents might be chosen on the grounds of their tolerability, their side-effect, and other related variables. No specific drugs were recommended. Recently, Iseki *et al*[47] reported that olmesartan, an ARB, did not lower the risks of major CV events or death among patients with hypertension on chronic dialysis. Combining these data, it is suggested that ARBs are not the only antihypertensive drug suitable for patients receiving dialysis. Left ventricular hypertrophy (LVH) is a well-established marker for future occurrence of CVD and an independent predictor of CV events[48-51]. There is some evidence indicating that ARBs could reverse LVH and might confer cardiovascular event risks beyond lowering blood pressure[52-54]. Yang *et al*[55] undertook a meta-analysis to assess the effect of ARBs *vs* placebo or other treatments, as well as ARBs and ACEi in combination, on LVH in patients receiving dialysis. Their study demonstrated that among dialysis patients the ARBs presented a greater regression in the LVM index when compared with the non-ARB users while there was no significant difference in the left ventricular ejection fraction (LVEF) between the two groups. The ARB group had a greater therapeutic value for the left ventricular mass (LVM) index or LVEF without achieving

significance when compared with the ACEi group. No significant alterations were found in the LVM index and LVEF between the ARB and ACEi in combination and the ARB. The authors concluded that ARBs produced a greater reduction in LVH in patients on dialysis. The ARB therapy tended to have favorable effectiveness similar to ACEi; however, the treatment with ARBs and ACEi in combination did not produce additional benefit for LVH in patients on HD. Tai *et al*[56] reported a meta-analysis to examine whether ACEi/ARBs reduced fatal and non-fatal CV events and the LVM in patients receiving HD. In their analysis, in comparison with the control groups, use of ACEi/ARBs did not produce any significant reduction of CV events. ACEi/ARB use resulted in a statistically significant reduction in the LVM (RR = 15.4, 95%CI: 7.4-23.5; $P < 0.001$). From these data, it could be suggested that ACEi/ARBs were effective in reducing the LVM index in patients with CKD accompanied by CVD. These data indicated that ACEi/ARBs are effective to reduce the LVM index in patients receiving HD. While ACEi/ARB use is advocated in peritoneal dialysis (PD) patients, (http://www.kidney.org/PROFESSIONALS/kdoqi/guideline_upHD_PD_VA/index.htm)[57,58] supporting evidence is unclear. Akbari in attempting to answer questions about the efficacy of ACEi/ARBs in patients on PD carried out a systematic review with analysis of randomized controlled trials, in which treatment with ACEi/ARB inhibitors was compared with that with other antihypertensive agents. Their review revealed that there remains no clear cut evidence for the use of ACEi/ARBs for the reduction of mortality and CV events in PD patients; limited data suggested that these agents induce a slow decrease in residual renal function loss. With these facts in mind, ACEi/ARBs can be carefully used in patients on PD.

FUTURE DIRECTIONS

Blood pressure measurements

That measurements of blood pressure in these clinical trials were performed in outpatient clinic might produce erroneous results. Recently, it was shown that blood pressure measurements in medical offices can be considered to be unreliable[59-61] because the mixture of white coat phenomenon and/or masked hypertension cannot be avoided. The recently issued NICE guidelines[62] recommended ambulatory blood pressure monitoring instead of measurement of blood pressure in medical offices[63,64]. Therefore, blood pressure in elderly CKD patients should be measured using home blood pressure[65-68].

Assessment of progression of renal disease

To date, most studies looking at outcomes related to renal disease have not used the renal trajectory as an endpoint. Most previous studies have been employing either a doubling of serum creatinine or time to start of renal replacement therapy. The latter is at most subjective assessment of trajectory[69]. Rosansky[70] proposed the following: change in a patient's eGFR over time (renal function trajectory) is potentially more important when deciding initiation of RRT. In the elderly CKD 4 population with several comorbidities and slow decrease in renal function, the likelihood of death or cardiovascular events prior to the need for RRT should be expected before making arteriovenous access for dialysis.

Newly developed direct renin inhibitor aliskiren

Recently Morishita and Kusano assessed the efficacy of aliskiren on blood pressure control and renoprotection in CKD patients whose proteinuria was not reduced less than 1.0 g daily in spite of administration of ARBs[71]. It is therefore possible that aliskiren produces different action compared with ARBs in hypertensive patients with DN.

CONCLUSION

I would like to propose that it is time for re-evaluation of the use of ACEi/ARBs for patients with DN and that new individualized therapies for elderly people in the real world should be developed.

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ORIGINAL ARTICLE

Case Control Study

Relationship of *MTHFR* gene polymorphisms with renal and cardiac disease

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Abstract

AIM: To investigate the effects of different methylenetetrahydrofolate reductase (*MTHFR*) 677C>T gene polymorphism and hyperhomocysteinemia for the development of renal failure and cardiovascular events, which are controversial.

METHODS: We challenged the relationship, if any, of MTHFR 677C>T and MTHFR 1298A>C polymorphisms with renal and heart function. The present article is a reappraisal of these concepts, investigating within a larger population, and including a subgroup of dialysis patients, if the two most common MTHFR polymorphisms, C677T and A1298C, as homozygous, heterozygous or with a compound heterozygous state, show different association with chronic renal failure requiring hemodialysis. MTHFR polymorphism could be a favorable evolutionary factor, *i.e.*, a protective factor for many ominous conditions, like cancer and renal failure. A similar finding was reported in fatty liver disease in which it is suggested that MTHFR polymorphisms could have maintained and maintain their persistence by an heterozygosis advantage mechanism. We studied a total of 630 Italian Caucasian subject aged 54.60 ± 16.35 years, addressing to the increased hazard of hemodialysis, if any, according to the studied MTHFR genetic polymorphisms.

RESULTS: A favorable association with normal renal function of MTHFR polymorphisms, and notably of MTHFR C677T is present independently of the negative effects of left ventricular hypertrophy, increased Intra-Renal arterial Resistance and hyperparathyroidism.

CONCLUSION: *MTHFR* gene polymorphisms could have a protective role on renal function as suggested by their lower frequency among our dialysis patients in end-stage renal failure; differently, the association with left ventricular hypertrophy and reduced left ventricular relaxation suggest some type of indirect, or concurrent mechanism.

Key words: Homocysteine; Glomerular filtration rate; Renal function; Mediterranean diet; Genetic; Methylenetetrahydrofolate reductase polymorphism; Insulin

resistance; Obesity; Left ventricular hypertrophy; Echocardiography

Core tip: We investigated the effects of different methylenetetrahydrofolate reductase (MTHFR) 677C>T gene polymorphism and hyperhomocysteinemia for the development of renal failure and cardiovascular events, which are controversial, and challenged the relationship, if any, of MTHFR 677C>T and MTHFR 1298A>C polymorphisms with renal and heart function. *MTHFR* gene polymorphisms could have a protective role on renal function as suggested by their lower frequency among our dialysis patients in end-stage renal failure; differently, the association with left ventricular hypertrophy and reduced left ventricular relaxation suggest some type of indirect, or concurrent mechanism.

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INTRODUCTION

Hyperhomocysteinemia is a frequent condition among patients both in end-stage renal disease and on dialysis and may represent an additional risk factor for increased cardiovascular disease[1]. It is recognized that supplementation with folic acid may often reduce, but not always and permanently correct hyperhomocysteinemia. More important, this approach does not reduce cardiovascular events in patients with kidney disease so that Folic acid based regimens are not recommended as a generalized approach in the prevention of cardiovascular events in chronic kidney disease[2]. Some polymorphism of the human methylenetetrahydrofolate reductase (*MTHFR*) gene have been associated with increased homocysteine levels: this was suspected to increase risks of cardio-vascular disease[3] (CVD) especially in the natural story of chronic kidney disease[4]. The more common MTHFR polymorphism (nucleotide 677 C>T) results in a thermolabile enzyme, lower folate levels and an inefficient homocysteine metabolism[5]. In recent years evidence has accumulated that the total homocysteine plasma level of patients under different forms of renal replacement therapy is influenced by a common polymorphism at nucleotide position 677 of the gene coding for 5,10-methylenetetrahydrofolate reductase (MTHFR 677C-->T). Furthermore, compound heterozygosity for the 677T allele and a novel A-->C polymorphism at nucleotide position 1298 of MTHFR was suggested to correlate with a decrease of folate plasma concentrations[6]. Hyperhomocysteinemia appears independent from other risk factors and subsequent reports increased concerns around the related common genetic polymorphism[7] despite earlier studies already challenged this concept[8] since this polymorphism prevalence in the elderly is not lower than in the young[9]. A very relevant question for the putative detrimental role of the allele 677T of the *MTHFR* gene is related to the evidence that this polymorphism is the best explaining protective factor against cervical carcinogenesis[10], and for colonic cancer[11,12], seemingly associated with longer and healthier survival[13]. Nonetheless, according to other studies, MTHFR 677TT homozygous and systolic blood pressure independently influence intima-media thickness[14] as other non-genetic markers[15] and nutritional conditions do[16]. Also mild-moderate renal impairment is associated with mortality, increased left ventricular (LV) myocardial mass[17], lower Ejection Fraction and increased E/A ratio at echocardiography[18]. Insulin resistance accounts significantly for LV mass increase in normotensive individuals[19]. A linear relationship between LV myocardial mass/m^2 (LVMMi) *vs* cardiovascular events, a J-shape relationship between LVMMi *vs* all-cause death[20] and NT-proBNP increase in patients with LV hypertrophy (LVH) suggest a common pathway, through the increase of measured myocardial mass, toward cardiac insufficiency[21]. Relevance of hyper-homocysteinemia stems from many considerations. Among them, in general population with no history of cardiovascular disease, concentrations of homocysteine alone could accurately identify those at high risk of cardiovascular mortality, whereas classic risk factors included in the Framingham risk score do not[22], suggesting the need of intervention[23]. MTHFR polymorphisms[24,25] seemingly intervene, not only inducing hyperhomocysteinemia, within a cluster of different and even interrelated conditions, diseases and indexes. Dietary profiles are the background of any adequate nutrients intake and particularly of a normal B vitamin intake and availability: they can be modified by conditions impairing renal function[26]. *MTHFR* gene-mediterranean diet interaction on homocysteine metabolism was reported: this dietary profile may reduce homocysteine concentrations and consequently influence coronary risk in genetically high-risk individuals by quality and proportion of nutrients[27]. The accompanying body size increase is not invariably detrimental since, actually, patients with established chronic disease benefit of large body size[28]. This finding, defined the obesity paradox, is shared over a variety of cardiovascular, pulmonary, and renal diseases: it challenges the concept about differences for optimal body size in health and disease[29]. The cornerstone is how several metabolic factors affect renal circulation and, as a consequence, renal function. The increase of intra-renal artery resistance, measured by renal artery resistive index (RRI), affects the natural history of atherosclerosis and arterial hypertension, which was found to correlate with LVH and carotid intimal thickening[29], with cardiovascular

risk score and impaired renal outcome and death[30]. Also endocrine factors are very relevant: among them, Parathyroid Hormone intervenes in several mechanisms of disease progression, including LVH[31], impairment of renal function[32] and increase of intrarenal arterial resistance[33,34]. We reported that renal insufficiency in non-diabetic subjects is explained by interactions of MTHFR C677T polymorphism mutation with LVH, high-sensitivity C-reactive protein (hsCRP), intact parathyroid hormone (iPTH), and RRI. Sign of these predictive effects is opposite: subjects with MTHFR 677C>T polymorphism have lower likelihood of renal insufficiency; differently, wild-type MTHFR genotype subjects have lower glomerular filtration rate (GFR) and greater hsCRP, iPTH, RRI, and LVH[35]. Even with the limitations of an observational study, the concept that MTHFR polymorphism could be a favorable evolutionary factor, *i.e.*, a protective factor for many ominous conditions, like cancer and renal failure, appears reasonable and deserving further and more systematic research. A similar finding was reported in fatty liver disease in which it is suggested that MTHFR polymorphisms could have maintained and maintain their persistence by an heterozygosis advantage mechanism[36]. The present article is a reappraisal of these concepts, investigating within a larger population, and including a subgroup of dialysis patients, if the two most common MTHFR polymorphisms, C677T and A1298C, as homozygous, heterozygous or with a compound heterozygous state, show different association with chronic renal failure. Patients considered were on hemodialysis or on maintenance therapy, and GFR, RRI and LVMM and systolic/diastolic function, dietary profile, hsCRP, iPTH, insulin resistance were assessed.

MATERIALS AND METHODS

We studied a total of 630 Italian Caucasian subject aged 54.60 ± 16.35 years. Body mass index (BMI) 27.70 ± 5.76 kg/m^2 consecutively admitted according to the request of their primary care doctors for nutritional assessment and work-up. One hundred and sixty of all subjects were with advanced renal insufficiency, treated by hemodialysis (HD); the other 470 were patients without or with slight-moderate renal impairment, quantified by serum creatinine. These patients were briefly defined No-HD. All patients were managed within a protocol which included medical history, physical examination, nutritional and physical activity assessment, electrocardiogram, Chest X-ray, echocardiography and clinical abdomen and thyroid ultrasound. According to MTHFR genotype, 94 of them were MTHFR C677CC (Wild genotype), 118 heterozygous MTHFR C677CT and 104 homozygous MTHFR C677TT (thermolabile polymorphism) subjects. Of the A1298C subjects 80 were homozygous A1298CC, 76 were heterozygous A1298AC; 158 subjects were with a compound MTHFR heterozygous polymorphism, *i.e.*, both MTHFR A1298AC/C677CT. These data are summarized in Table 1. Routine laboratory tests included virus hepatitis [hepatitis A virus (HAV), HBV, and HCV] and cancer biomarkers (AFP, CEA, Ca125, Ca15-3), thyroid hormones, thyroid stimulating hormone, aspartate Aminotransferase, Alanine Aminotransferase, γ-Glutamyl transpeptidase, ferritin, total protein, and albumin. Human insulin and Folic acid were assayed using immulite 2000 Analyzer, by a solid-phase 2-site chemiluminescent immunometric assay. hsCRP concentrations were assayed by a standard detection limit of 0.175 mg/L (CardioPhase high-sensitivity hsCRP method-Siemens Medical System, Milan, Italy). Homocysteine and B12 Vitamin assay in the blood were performed by ADVIA Centaur® XP Immunoassay (Siemens Medical System, Milan, Italy)[37]. iPTH and NT-proBNP (IMMULITE® 2000 Siemens Medical System, Milan, Italy) were assessed by a solid phase two-site chemiluminescent immunometric assay. PTH values considered normal were < 70 pg/mL for subjects without severe renal insufficiency[38]. Body weight (BW) was measured in light clothing, without shoes, in kilograms, and height (H) was measured in meters, using a scale-integrated stadiometer. BMI was calculated as BW/H2 and patients were categorized as normal weight (< 25.0 kg/m^2), overweight (≥ 25.0 and ≤ 29.9 kg/m^2), and obese (≥ 30.0 kg/m^2). Insulin resistance was assessed by the homoeostasis model-insulin resistance index (HOMA), according to the following formulas: fasting insulin value x fasting blood sugar level/405. The HOMA threshold for insulin resistance is conventionally considered as > 1.7, according to the likelihood ratios for 11-year cardiovascular disease prediction[39]. The Waist-to-Hip (W/H) ratio was assessed in all patients. Ultrasound (US) examinations were performed by echographists unaware of laboratory details at the time of the procedure. An echo-color-doppler machine (Siemens Acuson S2000™, Siemens AG, Muenchen Germany), high resolution, with real-time sectional scan transducers was used. Renal color Doppler echography is performed assessing intra-parenchymal *renal arterial* resistive index, RRI (peak systolic velocity-end diastolic velocity/peak systolic velocity)[40]. First measurement is the size of the left and right kidney. For orientation purposes, perfusion in the whole of the left and right kidneys is then checked using color Doppler ultrasonography and the main trunk of the renal artery is displayed. Three measurements for each kidney are taken by pulsed Doppler within 5 min in the vicinity of the interlobar artery. RRI is calculated as the average value of all measurements taken. RRI threshold to define higher RRI measurements is defined by the 75th percentile derived by measurements of all eligible patients[40]. Echocardiographic studies were performed with two-dimensional guided M-mode echocardiography according to methods established by the American Society of Echocardiography (ASE)[41-44] with transducer frequencies appropriate for body size. Siemens Acuson S2000™, Siemens AG, Muenchen Germany or a GE echo-color-doppler device [GE Logiq7 Expert US, manufactured by GE Medical Systems-Milwaukee-Wisconsin (United States)], high resolution,

Table 1 Differences between methylenetetrahydrofolate reductase groups in all patients

	Wild genotype (*n* = 94)	Heterozygous MTHFR C677T (*n* = 118)	Heterozygous MTHFR 1298 AC (*n* = 76)	Compound heterozygous C677T and A1298C (*n* = 154)	Homozygous MTHFR 1298 CC (*n* = 80)	Homozygous MTHFR 677TT (*n* = 104)	*P*
Age, yr	53.30 ± 11.89	51.59 ± 17.39	56.74 ± 16.55	57.91 ± 17.04	57.85 ± 14.70	50.12 ± 17.09	< 0.0001
BMI, kg/m^2	27.26 ± 5.04	27.28 ± 5.95	28.04 ± 5.84	27.80 ± 6.13	27.01 ± 3.92	28.72 ± 6.61	0.316
GFR	48.84 ± 32.90	66.05 ± 36.70	64.16 ± 36.32	63.31 ± 38.35	61.57 ± 34.91	68.85 ± 27.33	0.002
Triglycerides, mg/dL	109.96 ± 75.73	113.21 ± 57.91	107.63 ± 42.67	128.72 ± 85.29	95.46 ± 37.07	103.94 ± 48.93	0.003
Total cholesterol, mg/dL	191.67 ± 41.71	206.29 ± 52.53	203.84 ± 38.73	198.28 ± 41.37	196.06 ± 54.99	201.54 ± 34.49	0.206
HDL cholesterol, mg/dL	58.11 ± 20.91	55.17 ± 15.75	55.28 ± 18.46	52.52 ± 18.49	52.51 ± 18.50	56.27 ± 16.35	0.175
LDL cholesterol, mg/dL	111.57 ± 34.89	128.47 ± 48.01	127.04 ± 31.67	120.81 ± 35.73	124.46 ± 48.10	124.89 ± 35.30	0.039
AST, U/L	19.50 ± 6.30	23.32 ± 14.23	27.93 ± 17.69	20.49 ± 6.91	21.76 ± 12.26	19.72 ± 5.99	< 0.0001
ALT, U/L	15.82 ± 4.59	16.46 ± 5.38	18.51 ± 5.75	16.46 ± 5.93	16.59 ± 5.74	15.92 ± 5.90	0.031
γGT, U/L	24.63 ± 12.20	33.82 ± 25.96	37.45 ± 38.03	42.71 ± 48.64	28.94 ± 15.17	25.37 ± 16.29	< 0.0001
HOMA	2.00 ± 1.13	3.18 ± 3.49	3.04 ± 2.27	4.04 ± 4.87	2.28 ± 1.07	2.76 ± 2.77	< 0.0001
PTH, pg/mL	84.94 ± 100.37	84.49 ± 170.97	78.37 ± 65.95	86.30 ± 76.75	86.53 ± 95.97	84.83 ± 81.83	0.997
hsCRP, mg/dL	2.58 ± 4.41	2.15 ± 2.79	6.30 ± 13.55	4.30 ± 8.66	3.99 ± 6.42	3.51 ± 4.95	< 0.001
RRI	0.60 ± 0.05	0.59 ± 0.05	0.59 ± 0.04	0.58 ± 0.05	0.59 ± 0.07	0.59 ± 0.06	0.392
EF, %	67.05 ± 8.18	66.94 ± 9.19	65.99 ± 9.15	63.52 ± 12.04	67.15 ± 11.63	66.51 ± 7.26	0.035
E/A	1.15 ± 0.36	1.20 ± 0.26	1.23 ± 0.34	1.09 ± 0.40	1.01 ± 0.26	1.18 ± 0.31	< 0.0001
LVMM/m^2	100.48 ± 54.70	105.44 ± 33.79	107.69 ± 48.47	109.21 ± 41.02	110.38 ± 46.63	97.11 ± 28.69	0.179
AMDS	34.94 ± 2.52	34.97 ± 3.03	33.42 ± 3.88	34.23 ± 3.02	34.93 ± 2.68	34.46 ± 3.18	0.005
Homocysteine μmol/L	17.41 ± 3.00	25.53 ± 8.12	28.58 ± 9.23	18.68 ± 9.01	21.26 ± 9.17	18.83 ± 6.25	< 0.0001

BMI: Body mass index; GFR: Glomerular filtration rate; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; γGT: γ-Glutamyl Transpeptidase; hsCRP: High-sensitivity C-reactive protein; RRI: Renal resistive index; EF: Ejection fraction; LVMM: Left ventricular mass myocardial; AMDS: Adherence mediterranean diet score; MTHFR: Methylenetetrahydrofolate reductase; PTH: Parathyroid hormone; HOMA: Homoeostasis model-insulin resistance index.

with real-time sectional scan transducers were used. An average of two echocardiographic measurements was taken and the cardiologist reading them was blinded to the clinical information of the patient. Measurements were obtained for LV end-diastolic and end-systolic dimension, septal wall thickness and posterior wall thickness in diastole. LVM was calculated with the method of Devereux *et al*[44] and indexed by dividing by body surface area/m^2. All the exams were stored on digital media for subsequent analysis. LV diameters and wall thickness were measured according the ASE guidelines and LV ejection fraction (LVEF) accordingly[41]. LVEF was considered abnormal if < 50%. GFR is assessed as estimated GFR by the modification of diet in renal disease formula in mL/min per 1.73 m^2, according to the Clinical Practice Guidelines for Chronic Kidney Disease KDOQI[38]. Genotypes of the MTHFR C677T and A1298C polymorphisms were detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). DNA was extracted from peripheral blood by a commercially available DNA isolation method (QIAamp DNA Blood Mini Kit QIAGEN, Milan, Italy). Restriction enzyme analysis of amplified product (PCR-RFLP) analysis were carried out for direct genotypes detection of single nucleotide polymorphisms, C667T (rs1801133) and A1298C (rs1801131). PCR products were obtained using specific primers (NCBI Reference Sequence: NG_013351.1): C667T (F5′-GTCCCTGTGGTCTCTTCATCC-3′/R5′-GGTGGCCAAGCAACGCTGTG-3′); A1298C (F5′-CTTCTACCTGAAGAGCAAGTC-3′/R5′-CACATGTCCACAGCATGGAC-3′). Both amplicons were successively digested by HinfI and MboII restriction enzymes for C667T and A1298C respectively, and DNA fragment visualized in a 4% agarose gel stained with Synergy Brand Inc safe (Life Technologies Italia, Monza, Italy); electrophoresis pattern was used to determined MTHFR genotypes[45]. Informed consent was obtained from each patient, relatively also to the use of genetic information, and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee.

Statistical analysis

The fit to the Hardy-Weinberg equilibrium was analyzed. Student's *t* test was used to assess the difference between subject with advanced renal insufficiency, treated by

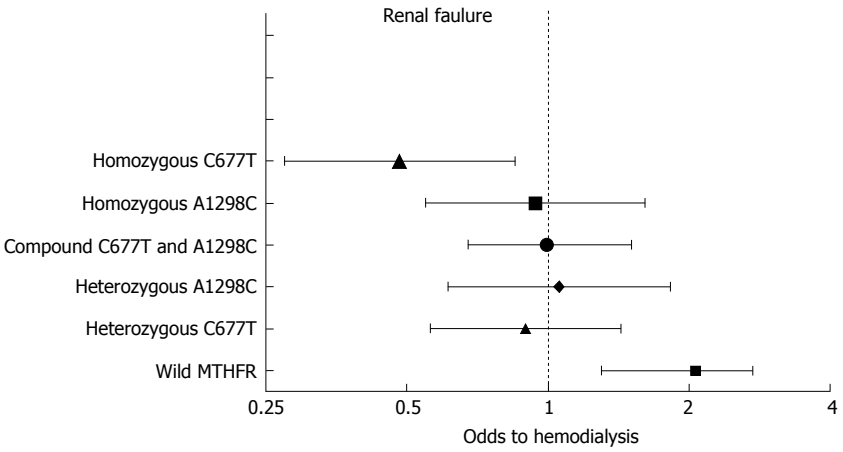

Figure 1 Odds to renal failure-hemodialysis. Comparison of the wild MTHFR genotype in dialysis patients (36/160; 22.5%) *vs* the No-HD group (58/470; 12.3%): $P < 0.003$; OR = 2.062 (95%CI: 1.3-3.273), *i.e.*, the wild MTHFR genotype bears a double risk of renal failure in comparison with all MTHFR polymorphisms and a four-fold risk *vs* the Homozygous C677T MTHFR polymorphism. The individual odds of renal failure, according to the specific MTHFR polymorphism status are as follows (hemodialysis patients 160 *vs* No-HD group 470) are not significant, with the exception of the Homozygous C677T MTHFR polymorphism which exhibits a protective behavior. Heterozygous C677T (28/160 *vs* 90/470); *P* = NS. OR = 0.896 (95%CI: 0.561-1.43); Heterozygous A1298C (20/160 *vs* 56/470); *P* = NS. OR = 1.056 (95%CI: 0.612-1.822); Compound Heterozygous C677T and A1298C (40/160 *vs* 118/470); *P* = NS. OR = 0.994 (95%CI: 0.657-1.505); Homozygous A1298C (20/160 *vs* 62/470); *P* = NS. OR = 0.94 (95%CI: 0.548-1.612); Homozygous C677T (16/160 *vs* 88/470); *P* = 0.015. OR = 0.482 (95%CI: 0.274-0.85). MTHFR: Methylenetetrahydrofolate reductase; OR: Odds ratio; NS: Not significant.

hemodialysis and No-HD group. ANOVA was used to assess the difference in averages between subjects with MTHFR heterozygous, compound and homozygous polymorphism. Descriptive results of continuous variables are expressed as averages (± SD). Two-sided $P < 0.05$ was considered statistically significant. The distributions of MTHFR alleles and genotypes in studied group, *i.e.*, normal-impaired renal function *vs* hemodialysis patients were checked by χ^2 test or Fisher's exact test. Higher quartiles of age, homocysteine, iPTH, RRI, hsCRP and of other continue measures were defined; thereafter, the associations of older age, higher hsCRP, iPTH, RRI, Left Ventricular Hypertrophy (LVMMi $\geqslant$ 135 g/m^2 in men, $\geqslant$ 110 g/m^2 in women[46]) and MTHFR polymorphisms were assessed as odds ratios (ORs) to severe chronic renal failure in hemodialysis with 95%CIs. Statistical analyses were performed using SPSS 18.0 for Windows (SPSS, Chicago, IL), Likelihood Ratio was assessed and sensitivity, specificity and predictivity were calculated by the CEBM Statistics Calculator, by Courtesy of CEBM, and graphs by Prism-Graphpad. Venn Diagram Plotter was used by courtesy of Pacific Northwest National Laboratory.

RESULTS

The differences of averages of measures between patients with MTHFR 677C>T heterozygous and homozygous polymorphism, of heterozygous and homozygous MTHFR 1298A>C polymorphism and of compound heterozygous MTHFR 677C>T/MTHFR 1298A>C polymorphism *vs* wild genotype subjects are shown in Table 1. Glomerular filtration rate is significantly higher in all the polymorphism groups *vs* wild genotype subjects, with figures greater of about 30%-35% more. Difference of age, even significant, are actually minor and, in any case, subjects with polymorphisms are older; homocysteine and low-density lipoprotein cholesterol are slightly higher in the MTHFR 677C>T polymorphism group *vs* wild genotype subjects. There are internal relationships between most measures: a significant linear correlation of GFR *vs* LVMMi (r = -0.37; $P < 0.0001$) is observed. Significant inverse correlation of age *vs* GFR (r = -0.56; $P < 0.0001$) and direct correlations of age *vs* RRI (r = 0.41; $P < 0.0001$), and *vs* LVMMi (r = 0.29; $P < 0.001$) are observed. iPTH shows significant inverse correlation *vs* GFR (r = -0.34; $P < 0.0001$). whereas a direct trend of iPTH is observed *vs* RRI (r = 0.32; $P < 0.001$) and *vs* LVMMi (r = 0.14; $P < 0.05$). No significant correlation is observed both for hsCRP and insulin resistance (HOMA) *vs* GFR, LVMMi and RRI.

Characteristic of study population and differences between HD patients (HD) and No-HD are reported in Table 2.

A significant difference is observed, overall, for the prevalence of wild MTHFR genotype in dialysis patients (36/160; 22.5%) *vs* the No-HD group (58/470; 12.3%): $P < 0.003$; OR = 2.062 (95%CI: 1.3-3.273), *i.e.*, the wild MTHFR genotype bears a double risk of renal failure in comparison with MTHFR polymorphisms and a four-fold risk *vs* the Homozygous C677T MTHFR polymorphism. The individual odds of renal failure, according to the specific MTHFR polymorphism status (hemodialysis patients 160 *vs* No-HD group 470) are not significant, with the exception of the Homozygous C677T MTHFR polymorphism which exhibits a protective behavior (Figure 1).

Likelihood ratio was assessed and sensitivity, specificity and predictivity, which were all very weak and substantially non-contributory: Homozygous C677T MTHFR polymorphism displays a Sensitivity of 0.154

Table 2 Characteristic of study population and differences between hemodialysis patients and no-hemodialysis *n* (%)

	Total (*n* = 630)	Dialysis patients (*n* = 160)	Patients with maintained Renal function (*n* = 470)	*P*
Women	336 (53.3)	72	264	0.014[1]
Obese patients	196 (31.1)	24	172	< 0.000[1]
Patients with GFR < 90	514 (81.6)	160	354	< 0.000[1]
NAFLD patients	256 (40.6)	28	228	< 0.000[1]
MTHFR group				
Wild genotype	94 (14.9)	36	58	0.016[1]
MTHFR C677T	118 (18.7)	28	90	
MTHFR 1298 AC	76 (12.1)	20	56	
Compound heterozygous C677T and A1298C	158 (25.1)	40	118	
MTHFR 1298 CC	80 (12.7)	20	60	
MTHFR 677TT	104 (16.5)	16	88	
Age, yr	54.60 ± 16.35	67.48 ± 14.57	50.22 ± 14.51	< 0.0001
BMI, kg/m^2	27.70 ± 5.76	25.29 ± 3.97	28.52 ± 6.04	< 0.0001
Blood glucose, mg/dL	96.42 ± 26.42	95.33 ± 34.80	96.79 ± 22.91	0.545
Blood urea, mg/dL	52.47 ± 35.74	100.45 ± 41.07	36.13 ± 9.40	< 0.0001
Creatinin, mg/dL	2.36 ± 2.98	6.75 ± 2.99	0.86 ± 0.21	< 0.0001
GFR	62.46 ± 35.32	9.28 ± 3.60	80.56 ± 19.38	< 0.0001
Triglycerides, mg/dL	112.16 ± 64.71	131.90 ± 87.21	105.44 ± 53.48	< 0.0001
Total cholesterol, mg/dL	199.72 ± 44.43	175.80 ± 42.67	207.86 ± 42.05	< 0.0001
HDL cholesterol, mg/dL	54.81 ± 18.10	48.20 ± 15.63	57.07 ± 18.34	< 0.0001
LDL cholesterol, mg/dL	122.75 ± 39.63	101.22 ± 33.04	130.09 ± 39.05	< 0.0001
AST, U/L	21.81 ± 11.16	14.38 ± 4.07	24.34 ± 11.66	< 0.0001
ALT, U/L	16.54 ± 5.63	12.75 ± 4.15	17.83 ± 5.49	< 0.0001
γGT, U/L	33.10 ± 32.11	31.78 ± 19.18	33.55 ± 35.46	0.546
Insulin	11.84 ± 9.73	11.44 ± 10.77	11.98 ± 9.36	0.547
HOMA	3.02 ± 3.30	3.08 ± 3.94	3.00 ± 3.05	0.797
PTH, pg/mL	84.58 ± 105.79	162.38 ± 178.81	57.99 ± 36.85	< 0.0001
hsCRP, mg/dL	3.52 ± 7.01	2.62 ± 2.45	3.82 ± 7.98	0.107
Albumin, g/dL	4.60 ± 0.37	4.64 ± 0.35	4.58 ± 0.37	0.119
Albumin, %	62.39 ± 3.60	62.60 ± 3.03	62.31 ± 3.77	0.388
RRI	0.62 ± 0.06	0.68 ± 0.03	0.60 ± 0.06	< 0.0001
EF,%	65.93 ± 9.99	61.03 ± 12.62	67.87 ± 7.95	< 0.0001
E/A	1.14 ± 0.33	1.03 ± 0.39	1.18 ± 0.30	< 0.0001
LVMM/m^2	104.95 ± 42.10	135.37 ± 55.56	93.84 ± 28.91	< 0.0001
AMDS	34.51 ± 3.09	35.93 ± 1.69	34.02 ± 3.31	< 0.0001
Homocysteine, μmol/L	2.1 ± 5.4	36.8 ± 8.5	21.2 ± 7.7	< 0.0001

[1]Pearson χ^2. GFR: Glomerular filtration rate; BMI: Body mass index; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; γGT: γ-Glutamyl transpeptidase; hsCRP: High-sensitivity C-reactive protein; RRI: Renal resistive index; EF: Ejection fraction; LVMM: Left ventricular mass myocardial; AMDS: Adherence mediterranean diet score; MTHFR: Methylenetetrahydrofolate reductase; PTH: Parathyroid hormone; HOMA: Homoeostasis model-insulin resistance index; NAFLD: Non-alcoholic fatty liver disease.

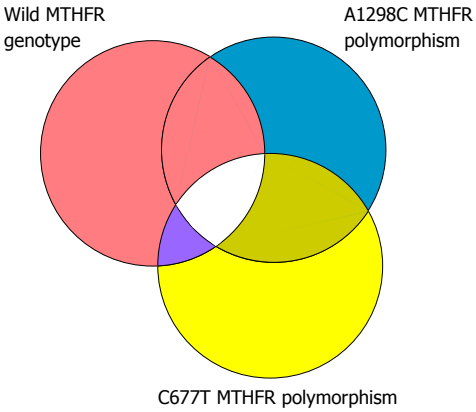

Figure 2 Venn diagram showing proportionally the overlap of methylenetetrahydrofolate reductase genetic polymorphisms A1298C and C677T with the wild one. The three groups have very relevant overlaps in the studied population. MTHFR: Methylenetetrahydrofolate reductase.

(0.097-0.235), with a Specificity of 0.726 (0.687-0.763); the positive predictive value is 0.1 (0.062-0.156) and the negative predictive value is 0.813 (0.775-0.845); the positive likelihood ratio is LR+ 0.562 (0.351-0.901), the negative likelihood ratio is LR- 1.165 (1.057-1.284). Similarly, for the wild MTHFR genotype, Sensitivity is 0.383 (0.291-0.484); Specificity is 0.769 (0.731-0.802); PPV is 0.225 (0.167-0.296); NPV is 0.877 (0.844-0.903); LR+ is 1.655 (1.227-2.233) and LR- is 0.803 (0.68-0.948).

In Figure 2 the polymorphism overlap is displayed by Venn diagram showing proportionally the overlap of MTHFR genetic polymorphisms A1298C and C677T with the wild one. The three groups have very relevant overlaps in the studied population.

Odds to LVH (assessed as increased Left Ventricular Myocardial Mass by Echocardiography), by the comparison of the prevalence of LVH within the wild

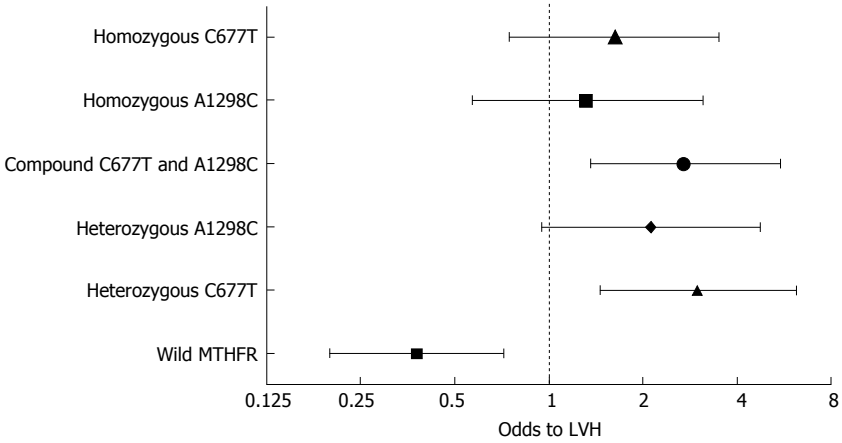

Figure 3 Odds to left ventricular hypertrophy (increased left ventricular myocardial mass assessed by echocardiography). Comparison of prevalence of LVH within the wild MTHFR genotype (12/94; 12.7%) *vs* the polymorphism MTHFR group (131/470; 27.9%): OR = 0.3787; 95%CI: 0.2000 to 0.7171; Z statistic 2.981; *P* = 0.0029, *i.e.*, the wild MTHFR genotype bears a significantly lower risk of LVH in comparison with all MTHFR polymorphisms. The individual odds of LVH, according to the specific MTHFR polymorphism status are as follows: Heterozygous C677T (12/94; 12.7% *vs* 36/118); OR = 3.0000, 95%CI: 1.4581 to 6.1725, Z statistic 2.985, *P* = 0.0028; Heterozygous A1298C (12/94; 12.7% *vs* 18/76); OR = 2.1207, 95%CI: 0.9490 to 4.7393, Z statistic 1.832, *P* = 0.0669; Compound Heterozygous C677T and A1298C (12/94; 12.7% *vs* 44/154); OR = 2.7333, 95%CI: 1.3581 to 5.5012, Z statistic 2.818, *P* = 0.0048; Homozygous A1298C (12/94; 12.7% *vs* 13/80); OR = 1.3259, 95%CI: 0.5676 to 3.0972, Z statistic 0.652, *P* = 0.5146; Homozygous C677T (12/94; 12.7% *vs* 20/104); OR = 1.6270, 95%CI: 0.7475 to 3.5410, Z statistic 1.227, *P* = 0.2199. LVH: Left ventricular hypertrophy; MTHFR: Methylenetetrahydrofolate reductase; OR: Odds ratio.

Table 3 Different prevalence of increased renal resistive index, abnormal left ventricular ejection fraction, normal left ventricular relaxation (E/A > 1), and left ventricular hypertrophy (all patients)

	Wild MTHFR (*n* = 94)	Heterozygous C677T (*n* = 118)	Heterozygous A1298C (*n* = 76)	Compound heterozygous C677T and A1298C (*n* = 154)	Homozygous A1298C (*n* = 80)	Homozygous C677T (*n* = 104)	χ^2	*P*
high RRI	24	30	16	38	28	22	5.746	0.332
EF < 50%	4	4	4	14	4	0	11.188	0.048
E/A > 1	68	100	66	74	42	70	53.497	< 0.0001
LVH (high LVMM)	12	36	18	44	13	20	14.923	0.011

Pearson χ^2. MTHFR: Methylenetetrahydrofolate reductase; RRI: Renal resistive index; EF: Ejection fraction; LVMM: Left ventricular mass myocardial; LVH: Left ventricular hypertrophy.

MTHFR genotype (12/94; 12.7%) *vs* the polymorphism MTHFR group (131/470; 27.9 %) displays an OR = 0.3787; 95%CI: 0.2000-0.7171; Z statistic 2.981; *P* = 0.0029, *i.e.*, the wild MTHFR genotype bears a significantly lower risk of LVH in comparison with all MTHFR polymorphisms (Figure 3). The individual odds of LVH, according to the specific MTHFR polymorphism status, confirm substantially this result, *i.e.*, that MTHFR polymorphisms are associated with LVH. Differences are not significant assessing Ejection fraction and Renal Resistive index. The E/A ratio, *i.e.*, the measurement of left ventricular transmitral filling, and index of overall left ventricular distensibility, is higher, *i.e.*, better, is subjects with the wild MTHFR genotype (Tables 3-5).

DISCUSSION

According to our study, the two most common MTHFR polymorphisms, C677T and A1298C, as homozygous, heterozygous or with a compound heterozygous state, show an association with chronic renal failure patients requiring hemodialysis which suggests some protective role in comparison with the wild MTHFR genotype. Despite the apparent disagreement with the available studied with renal disease patients, this result is less surprising of what can appear at the first glance. Even with the limitations of an observational study, based on the reappraisal of the information available in our data base investigating within a greater population that includes a subgroup of dialysis patients, we find that the concept that MTHFR polymorphism could be a favorable evolutionary factor, *i.e.*, a protective factor for many ominous conditions, like cancer and renal failure, appears reasonable and deserving further and more systematic research. A similar finding was reported in fatty liver disease in which it is suggested that MTHFR polymorphisms could have maintained and maintain their persistence by an heterozygosis advantage mechanism[35].

Homocysteine is settled as a putative risk factor for cardiovascular disease[47] and mechanisms for glomerular injury and progression of renal insufficiency are envisaged[48]. Although high-dose folic acid would slow the progression of atherosclerosis and reduce

Table 4 Different prevalence of increased renal resistive index, abnormal left ventricular ejection fraction, normal left ventricular relaxation (E/A > 1), and left ventricular hypertrophy (chronic renal failure patients-hemodialysis)

	Wild MTHFR (*n* = 36)	Heterozygous C677T (*n* = 28)	Heterozygous A1298C (*n* = 20)	Compound heterozygous C677T and A1298C (*n* = 40)	Homozygous A1298C (*n* = 20)	Homozygous C677T (*n* = 16)	χ^2	*P*
High RRI	20	20	8	28	16	12	10.535	0.061
EF < 50%	4	4	4	12	4	0	9.114	0.105
E/A > 1	24	24	16	8	0	0	72.305	< 0.0001
High LVMM	8	16	4	32	5	4	38.428	< 0.0001

Pearson χ^2. MTHFR: Methylenetetrahydrofolate reductase; RRI: Renal resistive index; EF: Ejection fraction; LVMM: Left ventricular mass myocardial.

Table 5 Different prevalence of increased renal resistive index, abnormal left ventricular ejection fraction, normal left ventricular relaxation (E/A > 1), and left ventricular hypertrophy (normal renal function patients)

	Wild MTHFR (*n* = 58)	Heterozygous C677T (*n* = 90)	Heterozygous A1298C (*n* = 56)	Compound heterozygous C677T and A1298C (*n* = 118)	Homozygous A1298C (*n* = 62)	Homozygous C677T (*n* = 88)	χ^2	*P*
high RRI	4	10	8	10	12	10	6.833	0.233
EF < 50%	0	0	0	2	0	0	5.798	0.326
E/A > 1	44	76	50	66	42	70	19.848	0.001
High LVMM	4	20	14	12	8	16	13.355	0.02

Pearson χ^2. MTHFR: Methylenetetrahydrofolate reductase; RRI: Renal resistive index; EF: Ejection fraction; LVMM: Left ventricular mass myocardial.

cardiovascular events in patients with chronic renal failure, counteracting effects of hyperhomocysteinemia, is still debated and not demonstrated[49]. Differently, there is a good consistency of data that establishes renal involvement and LV hypertrophy as novel risk factors for morbidity and mortality in diabetes mellitus[50]. Cardiac remodeling, also with increase of LVMM, is a premise toward the development of heart insufficiency[51], which could be redefined also encompassing serological biomarkers[52]. The favorable relevance of adherence to healthier nutritional profile and lifestyle changes is well established and warranted in cardiac disease[53,54] and also, by more recent contributions, in renal disease[55]. In earlier studies[56,57] relationship of MTHFR C677T mutation with renal and cardiac involvement was associated with precocious target organ damage. Actually, in younger subjects[58] and in other reports[59] homozygosity for the C677T mutation is not unequivocally associated with increased risk for cardiovascular disease, irrespective of folate intake. This is confirmed by a recent extensive epidemiological study, in which despite lower serum folate and higher homocysteine, MTHFR 677TT genotype, used as a proxy for lifelong high blood homocysteine concentrations, is associated with a significantly lower risk of CVD mortality[60]. Hyperhomocysteinemia is common in patients with severe heart failure, and plasma homocysteine levels are uniformly elevated regardless of the etiology of heart failure. Elevated plasma homocysteine levels are likely a consequence of heart failure-related renal insufficiency[61]. Moreover, high homocysteine levels in patients with end-stage renal disease were not associated with incidence of vascular access thrombosis[62]. In our study, MTHFR C677T mutation occurs in a population which has still a relatively low prevalence of cardiovascular[5] and renal disease[55]. It is possible that this polymorphism, even associated with greater LVMMi, could have maintained its persistence in human populations by an heterozygosis-mutant advantage mechanism exerted over more critical conditions, including the occurrence of renal insufficiency. All-cause and coronary heart disease death rates are low in cohorts with greater adherence to Mediterranean Diet.

In conclusion, *MTHFR* 677C>T and A1298A>C gene polymorphisms could have a protective role on renal function as suggested by the lower frequency of both polymorphisms among our dialysis patients in end-stage renal failure; differently, the association with LV hypertrophy and reduced left ventricular relaxation suggest some type of indirect, or concurrent mechanism related to MTHFR polymorphisms.

ACKNOWLEDGMENTS

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COMMENTS

Background

Hyperhomocysteinemia is a frequent condition among patients both in end-stage renal disease and on dialysis and may represent an additional risk factor for increased cardiovascular disease. It is recognized that supplementation with folic acid may often reduce, but not always and permanently correct hyperhomocysteinemia. More important, this approach does not reduce cardiovascular events in patients with kidney disease so that Folic acid based regimens are not recommended as a generalized approach in the prevention of

cardiovascular events in chronic kidney disease.

Research frontiers

A similar finding was reported in fatty liver disease in which it is suggested that methylenetetrahydrofolate reductase (MTHFR) polymorphisms could have maintained and maintain their persistence by an heterozygosis advantage mechanism. The present article is a reappraisal of these concepts, investigating within a larger population, and including a subgroup of dialysis patients, if the two most common MTHFR polymorphisms, C677T and A1298C, as homozygous, heterozygous or with a compound heterozygous state, show different association with chronic renal failure.

Innovations and breakthroughs

The authors reported that renal insufficiency in non-diabetic subjects is explained by interactions of MTHFR C677T polymorphism mutation with left ventricular (LV) hypertrophy (LVH), high-sensitivity C-reactive protein (hsCRP), intact parathyroid hormone (iPTH), and renal artery resistive index (RRI). Sign of these predictive effects is opposite: subjects with MTHFR 677C>T polymorphism have lower likelihood of renal insufficiency; differently, wild-type MTHFR genotype subjects have lower glomerular filtration rate and greater hsCRP, iPTH, RRI, and LVH.

Applications

MTHFR gene polymorphisms could have a protective role on renal function as suggested by their lower frequency among the authors dialysis patients in end-stage renal failure; differently, the association with LVH and reduced LV relaxation suggest some type of indirect, or concurrent mechanism.

Peer-review

This is a well written manuscript analysing the effect of *MTHFR* gene polymorphisms on renal and cardiac function.

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SYSTEMATIC REVIEWS

Are phosphodiesterase type 5 inhibitors effective for the management of lower urinary symptoms suggestive of benign prostatic hyperplasia?

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Abstract

AIM: To review the efficacy of phosphodiesterase type 5 inhibitors (PDE5-Is) in lower urinary tract symptoms (LUTS) suggestive of benign prostate hyperplasia (LUTS/BPH).

METHODS: A comprehensive research was conducted to identify all publications relating to benign prostate hyperplasia and treatment with sildenafil, vardenafil and tadalafil. To assess the efficacy, the changes in total international prostate symptom score (IPSS), IPSS subscore including voiding, storage and quality of life (QoL), Benign prostatic hyperplasia Impact Index (BII), maximum urinary flow rate (Qmax) and the International Index of Erectile Function (IIEF) were extracted. A meta-analytical technique was used for the analysis of integrated data from the included studies to evaluate the mean difference in the results.

RESULTS: Total IPSS score, IIEF and BII showed a significant improvement in trials in which LUTS/BPH with or without erectile dysfunction (ED) were compared with the placebo. For LUTS/BPH, the mean differences of total IPSS score, IIEF and BII are -2.17, 4.88 and -0.43, $P < 0.00001$, respectively. For LUTS/BPH with comorbid ED, the mean difference are -1.97, 4.54 and -0.52, $P < 0.00001$, respectively. PDE5-Is appear to improve IPSS storage, voiding and QoL subscore (mean difference = -0.71, -1.23 and -0.33, $P < 0.00001$, respectively). Although four doses of tadalafil (2.5, 5, 10 and 20 mg) failed to reach significance in Qmax (mean difference = 0.22, $P = 0.10$), the 5 mg dose of tadalafil significantly improved the Qmax (mean difference = 0.33, $P = 0.03$).

CONCLUSION: PED5-Is demonstrated efficacy for improving LUTS in BPH patients with or without ED and could be considered to be the first line treatment for LUTS/BPH.

Key words: Phosphodiesterase type 5; Inhibitor; Lower urinary tract symptoms; Benign prostate hyperplasia; Tadalafil

Core tip: The efficacy of phosphodiesterase type 5 inhibitor (PDE5-I) in patients with lower urinary tract symptoms (LUTS) and benign prostate hyperplasia (BPH) has been evaluated and prescribed. Regardless of the significant improvement of total International Prostate Symptom Score and storage subscore, there are controversies about the urine flow rate. Also, we do not know the exact mechanism of how it works in the lower urinary tract. From the meta-analytical data, PDE5-I could be an alternative therapy for LUTS/BPH patients whether or not they have erectile dysfunction. Therefore, well designed large scale clinical trials are required to clarify the efficacy and action mechanisms of PDE5-Is in the management of LUTS/BPH.

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INTRODUCTION

Benign prostatic hyperplasia (BPH) is a histopathological diagnosis characterized by epithelial cell and smooth muscle proliferation in the transition zone of the prostate leading to nonmalignant enlargement of the prostate, which may result in lower urinary tract symptoms (LUTS), including storage and voiding symptoms[1-3]. BPH is a common disease of aging men. Moderate to severe LUTS secondary to BPH (LUTS/BPH) is predicted to involve 10% to 25% of the contemporary male population (approximately 900 million men) throughout the world[1-3] and it is considered that presumably 1.1 billion males will suffer from LUTS/BPH by the year 2018[4].

It is widely acceptable that BPH is not the exclusive source of LUTS[1-4]. Over the decades, LUTS/BPH treatment paradigms have shifted from surgical interventions to first-line pharmacotherapy for symptom reduction and improvement in quality of life. However, clinical trials of drugs often enroll men based partially on a clinical diagnosis of non-neurogenic LUTS/BPH.

Pharmacotherapy for LUTS/BPH currently consists of alpha-blockers, 5 alpha-reductase inhibitors or combined therapy[1-4]. Although they are proved to be efficacious, these therapies have potential side effects linked to sexual dysfunction, such as reduced libido and ejaculatory disorders, dizziness and hypotension[5]. These side effects may be exacerbated by combination therapy. Phosphodiesterase type 5 inhibitors (PDE5-Is), consisting mainly of sildenafil, vardenafil and tadalafil, are extensively approved for curing erectile dysfunction (ED)[6,7]. Recently, significant improvement in LUTS/BPH has been reported by a large body of clinical studies on PDE5-Is[8-25]. Although improvement of the PDE5-Is mechanisms in LUTS/BPH have yet not been clearly clarified, proposed contributors include inhibition of PDE5 iso-enzymes, present in the bladder, prostate, urethra and supporting vasculature, and consequently elevation in intracellular nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) concentration which functions to inhibit RhoA/Rho kinase signaling pathways, mediates relaxation of the smooth muscle cells in these structures, improves blood perfusion and reduces afferent signaling in the urogenital tract[26-29]. Understanding these complicated mechanisms shows how PDE5-Is play a role in the treatment of LUTS/BPH and is indispensable for health care professionals to optimize both patient screening and treatment. Nevertheless, recent research has shown that PDE5-Is, either as monotherapy or combined with alpha blockers, also enhance LUTS/BPH, presumably *via* relaxation of smooth muscle in the bladder neck, urethra and prostate induced by the NO/cGMP signal pathway.

With the increasing interests in this efficacy, therefore, we systematically reviewed the literature to explore up-to-date evidence on the efficacy of PDE5-Is in LUTS/BPH.

Epidemiological survey: two common conditions in LUTS/BPH and ED?

Two conditions of LUTS associated with BPH and ED that occur with relatively high frequency in aging have triggered a great deal of concern over the last few decades. As the incidence of histopathological stromal-glandular hyperplasia rises, so does the prevalence of moderate to severe LUTS[30]. Correspondingly, the rate of ED also rises with aging. As such, it is not a surprise that many patients with LUTS will also suffer from ED and vice versa. The link between LUTS/BPH and ED has recently been the subject of significant studies[1,31]. Numerous publications have demonstrated a link between ED and LUTS, the epidemiology of which was summarized in a review[32]. It points out that the majority of well-designed longitudinal studies have been proposed to interpret the relationship between ED and LUTS, including varying NO levels, activated RhoA/Rho kinase and atherosclerosis in the pelvis.

A recent abstract from a larger cross-sectional and multinational assessment of LUTS and sexual function was conducted[33]. Logistic regression analysis showed that patients with severe LUTS were estimated to be twice as likely to suffer from erectile dysfunction (OR = 2.0, 95%CI: 1.4, 2.8) and decreased ejaculate (OR = 1.8, 95%CI: 1, 2.5). Furthermore, patients with severe LUTS were 6 fold as likely to complain of discomfort or pain on ejaculation. Another cross-sectional data analysis is from the multinational survey of the aging male (MSAM-7) in which patients aging fifty to eighty years demonstrated high rates of LUTS/BPH in the

United States and Europe (United Kingdom, France, Germany, Netherlands, Italy and Spain)[34]. In this survey, more than 50% of patients were bothered by ejaculatory dysfunction and it also showed that the link between LUTS and ejaculatory dysfunction still existed after controlling for age and other comorbidities.

Clinical studies of PDE5-Is: Are LUTS/BPH and ED independent?

It was speculated that enhancement in LUTS/BPH could be a result of ED improvement because PDE5-Is significantly mitigated the symptoms of LUTS/BPH and ED. As such, a couple of clinical studies have addressed whether the improvement of BPH symptoms is linked to improved ED symptoms[9,34]. In one study of dose-ranging tadalafil with 716 ED patients and 340 non-ED patients, alterations in LUTS/BPH after 3 mo of medication with distinct doses of tadalafil once daily and placebo was analogous in patients with or without comorbidity of ED, demonstrating that the enhancement in LUTS/BPH did not rely on ED alterations[35]. Another tadalafil study confirmed these finding[36]. As a consequence, they are independent of each other even although the mechanism by which PDE5-Is enhance LUTS/BPH could participate in analogous ways with PDE5-Is enhancing ED.

PDE5-I localization in the prostate

Much evidence from experimental research confirmed that the cGMP-degrading PDE5 as well as NO/cGMP signaling pathway are responsible for the regulation of the normal functions of the prostate, regulating proliferation of glandular epithelial cells and smooth muscle as well as stromal connective tissue[29,37]. As early as 1970, the activity of PDE5-Is isolated from human prostate tissue was confirmed by Kuciel and Ostrowski. However, this method could not tender sufficient data on the PDE5 localization in the prostate.

The golden criteria to detect PDE5 distribution in distinct histopathological portions of the prostate was disclosed by immunohistochemistry (IHC). It was demonstrated that cGMP PDE iso-enzyme localized in the glandular zone, the smooth musculature of stroma and blood vessels by utilization of antibodies[35]. It was also shown that PDE5 is detected in tight conjunction with other critical regulators of NO/cGMP pathway. The concentration of tadalafil in the prostate and plasma was 385.7 ± 83.8 and 305.8 ± 41.1 ng/mL, respectively. In addition, the ratio between tissue and plasma was 1.3[38]. Tadalafil and udenafil significantly enhanced the cGMP and cAMP levels in plasma and prostate tissue[38].

PDE5-Is mechanism of action

Briefly, the current postulated action mechanism in improvement of LUTS/BPH includes: (1) ascending NO synthase/NO activity in the prostate; (2) cGMP mediated protein kinase/endothelin inactivation; (3) decreased autonomic hyperactivity of the afferent nerve in the bladder and prostate; and (4) reduction of pelvic ischemia caused by atherosclerosis of pelvic vessels.

MATERIALS AND METHODS

Identification of studies and study design

We searched the following sources from inception to the specified date: (1) the Cochrane Library; (2) MEDLINE; and (3) EMBASE.

The studies in the present review met the following standards: (1) double blinded, clinical controlled trials; (2) LUTS/BPH was involved; and (3) control groups were given a placebo drug. Studies with PED5-Is monotherapy versus an alpha blocker or combination of both were excluded.

To assess the efficacy of PED5-Is, the outcomes of measurement contain at least one of: (1) International prostate symptom score (IPSS); (2) International index of erectile dysfunction (IIEF) score; (3) maximal urinary flow rate (Qmax); (4) IPSS quality of life index (IPSS-QoL); and (5) IPSS irritative (storage) subscore, IPSS obstructive (voiding) subscore and BPH impact index (BII).

Statistical analysis

The meta-analysis used the review manager (Version 5.3, the Cochrane Collaboration, Oxford, United Kingdom). The heterogeneity test was by χ^2 and I^2 ($I^2 \leqslant$ 50%, low heterogeneity; 50% < $I^2 \leqslant$ 75%, moderate heterogeneity; and I^2 > 75%, high heterogeneity). If the heterogeneity was less than 50%, the fixed-effects model was considered to estimate the integrated effect of the outcomes. For moderate or high heterogeneity, a random-effect was used. The continuous value was used as the mean difference with 95%CI.

RESULTS

Clinical trials with PED5-Is for LUTS/BPH

A total of 16 randomized, double blind and placebo-controlled trials investigated the efficacy and safety of tadalafil (n = 14), sildenafil (n = 1) and vardenafil (n = 1) for LUTS/BPH therapy and comorbidities of LUTS/BPH and ED (5 trials: Brock *et al*[39], 2013, Donatucci *et al*[14], 2011, Egerdie *et al*[15], 2012, McVary *et al*[18], 2007 and Porst *et al*[21], 2009, respectively). The characteristics of the studies are summarized in Table 1. The study designs were analogous, followed by up to 4 wk of washout periods in order to eliminate the medications prior to trials.

Efficacy of PDE5-Is of sildenafil, tadalafil and vardenafil

Sildenafil: In 2007, McVary *et al*[10] first reported that 189 patients given sildenafil had improved significantly in total IPSS score (sildenafil *vs* placebo: -6.3 *vs* -1.93, P < 0.0001), IPSS QoL subscore (sildenafil *vs* placebo: -0.97 *vs* -0.29, P < 0.0001), BII (sildenafil *vs* placebo: -2.0 *vs* -0.9, P < 0.001) and IIEF-EF domain

Table 1 Characteristics and qualities of the studies included in the analysis of tadalafil, sildenafil and vardenafil

Ref.	Sample size	Drug (mg)		Duration (wk)	Run-in period (wk)	Inclusion criteria	Publications
		Trial	Control				
Tadalafil							
Brock *et al*[39]	1089	5	Placebo	12	4	Mean age ≥ 45, LUTS/BPH ≥ 6 mo, IPSS ≥ 13 Qmax 4-15 mL/s	*BJU Int*
Dmochowski *et al*[13]	200	20	Placebo	12	4	Mean age ≥ 40, LUTS/BPH ≥ 6 mo, IPSS ≥ 13	*J Urol*
Donatucci *et al*[14]	427	2.5, 5, 10, 20	Placebo	12	4	Mean age ≥ 45, LUTS/BPH ≥ 6 mo, IPSS ≥ 13	*BJU Int*
Egerdie *et al*[15]	606	2.5, 5	Placebo	12	4	Mean age ≥ 45, LUTS/BPH ≥ 6 mo, IPSS ≥ 13 Qmax 4-15 mL/s	*J Sex Med*
Kim *et al*[16]	102	5	Placebo	12	6	Mean age ≥ 45, LUTS/BPH ≥ 6 mo, IPSS ≥ 13 Qmax 4-15 mL/s	*LUTS*
McVary *et al*[18]	281	5 + 20	Placebo	6 + 6	4	Mean age ≥ 45, LUTS/BPH ≥ 6 mo, IPSS ≥ 13 Qmax 4-15 mL/s	*J Urol*
Oelke *et al*[19]	343	5	Placebo	12	4	Mean age ≥ 45, LUTS/BPH ≥ 6 mo, IPSS ≥ 13 Qmax 4-15 mL/s	*Eur Urol*
Porst *et al*[21]	581	2.5, 5, 10, 20	Placebo	12	4	Mean age ≥ 45, LUTS/BPH ≥ 6 mo, IPSS ≥ 13 Qmax 4-15 mL/s	*Eur Urol*
Porst *et al*[36]	325	5	Placebo	12	4	Mean age ≥ 45, LUTS/BPH ≥ 6 mo, IPSS ≥ 13 Qmax 4-15 mL/s	*Eur Urol*
Porst *et al*[20]	1500	5	Placebo	12	4	Mean age ≥ 45, LUTS/BPH ≥ 6 mo, IPSS ≥ 13 Qmax 4-15 mL/s	*Urology*
Roehrborn *et al*[22]	1058	2.5, 5, 10, 20	Placebo	12	4	Mean age ≥ 45-60, LUTS/BPH ≥ 6 mo, IPSS ≥ 13 Qmax 4-15 mL/s, PVR 150-550 mL	*J Urol*
Roehrborn *et al*[12]	1500	5	Placebo	12	4	Mean age ≥ 45, LUTS/BPH ≥ 6 mo, IPSS ≥ 13 Qmax 4-15 mL/s	*J Urol*
Takeda *et al*[24]	610	5	Placebo	12	4	Mean age ≥ 40, LUTS/BPH ≥ 6 mo, IPSS ≥ 13	*J Urol*
Yokoyama *et al*[25]	460	2.5, 5	Placebo	12	2	Mean age ≥ 45, LUTS/BPH ≥ 6 mo, IPSS ≥ 13 Qmax 4-15 mL/s, prostate volume ≥ 20 mL	*Int J Urol*
Sildenafil							
McVary *et al*[10]	369	50, 100	Placebo	12	4	Mean age ≥ 45, IIEF ≤ 25, IPSS ≥ 12	*J Urol*
Vardenafil							
Stief *et al*[40]	222	10	Placebo	8	4	Mean age ≥ 45-64, LUTS/BPH ≥ 6 mo, IPSS ≥ 12	*Eur Urol*

IIEF: International index of erectile function; IPSS: International prostate symptom; LUTS/BPH: Lower urinary tract symptoms/benign prostatic hyperplasia; Qmax: Maximum urinary flow rate; PVR: Post-void residual volume.

score (sildenafil *vs* placebo: 9.17 *vs* 1.86, $P < 0.0001$) compared to the placebo group after 12 wk of daily treatment (50 mg for 2 wk, then increased to 100 mg). No significant difference of Qmax was observed between two groups ($P = 0.08$); it is possible that relaxation of the urethra and prostate musculature would tend to enhance urinary flow, but relaxation of the bladder could more or less counteract these effects after administration of PDE5-Is (Table 2).

Vardenafil: In one randomized, double blind, placebo-controlled study, Stief *et al*[40] investigated the efficacy of 10 mg vardenafil in LUTS/BPH patients with or without concomitant ED. After 8 wk of therapy, significant improvement in total IPSS score (vardenafil *vs* placebo: -5.8 *vs* -3.1, $P < 0.05$), IPSS voiding subscore, IPSS storage subscore and IPSS QoL score were observed in the vardenafil group compared to the placebo group ($P < 0.0001$, respectively) (Table 2). Although Qmax was enhanced in vardenafil group, there was no significant difference (vardenafil *vs* placebo: 1.6 mL/s *vs* 1 mL/s) (Table 2). Overall, the most frequent adverse events (AEs) consisted of headaches, flushing and dyspepsia, reported in 32 (29.6%) patients in the vardenafil group and 18 (15.9%) in the placebo group. None of the serious AEs was linked to the vardenafil medication. Nevertheless, it is too soon to consider the underlying role for vardenafil in LUTS/BPH therapy because further data clearly needed to ascertain the benefit-risk details relative to the existing treatment options were not provided.

Tadalafil: A total of 14 randomized, double-blind, placebo-controlled studies have showed the efficacy and safety of once daily tadalafil medication in the management of LUTS/BPH. A one year open label trial demonstrated the sustainability of efficacy and safety of once daily tadalafil long term[14]. The efficacy outcomes are summarized in Table 2.

Brock *et al*[39] (2013), investigating the efficacy of once daily tadalafil in the treatment of LUTS/BPH patients with or without ED, first noted that the effects of therapy in men without ED were analogous to that with ED in LUTS/BPH. In patients without ED, the LUTS/BPH total IPSS score (tadalafil *vs* placebo: -5.4 *vs* -3.3, $P < 0.01$), IPSS voiding subscore (tadalafil *vs* placebo: -3.5 *vs* -2.0, $P < 0.01$) and IPSS storage subscore (tadalafil *vs* placebo: -1.9 *vs* -1.3, $P < 0.05$) from baseline to end points was reduced significantly and IPSS QoL (tadalafil *vs* placebo: -1.0 *vs* -0.7, $P < 0.05$) and BII (tadalafil *vs* placebo: -1.4 *vs* -1.0, $P < 0.05$) were significantly improved. However, a small

Table 2 Least squares mean changes from baseline to end-point in lower urinary tract symptoms/benign prostatic hyperplasia in clinical studies for treatment in subjects with erectile dysfunction and without erectile dysfunction

Ref.	Drug	Remarks	Total IPSS		IPSS voiding subscore		IPSS storage subscore		IPSS QoL subscore		BII		Qmax		IIEF	
	mg		T	P	T	P	T	P	T	P	T	P	T	P	T	P
	Tadalafil															
Brock *et al*[39]	5	ED	-5.7	-3.3	-3.5	-1.9	-2.2	-1.3	-1.1	-0.7	-1.6	-0.9				
	5	No ED	-5.4	-3.3	-3.5	-2	-1.9	-1.3	-1		-1.4	-1				
Dmochowski *et al*[13]	20		-9.2	-5.1	-5.6	-2.8	-3.6	-2.3					0.4	0.5		
Donatucci *et al*[14]	2.5		-5.7	-4.1	-3.8	-2.5	-1.9	-1.6	-1.2	-0.9	-1.3	-1.2			5.5	7.2
	5		-5.0		-2.8		-2.1		-1.1		-1.4				5.3	
	10		-5.7		-3.6		-1.8		-1.3		-1.4				3.7	
	20		-4.6		-3		-2.1		-1		1.2				7.6	
Egerdie *et al*[15]	2.5		-6.1	-3.8	-2.7	-2.2	-1.9	-1.6	-0.9	-0.8	-2.1	-1.2			6.5	1.8
	5		-4.6		-3.6		-2.5		-1		-1.6				5.2	
Kim *et al*[16]	5		-5.6	-3.6									2.5	2.3		
McVary *et al*[18]	5		-6.2	-3.9	-4	-2.5	-2.2	-1.4	-0.7	-0.3	-0.7	-0.4	0.5	0.9	6.7	0.7
	5/20		-7.1	-4.5	-4.4	-2.8	-2.7	-1.8	-0.5	-0.2	-1.3	-0.6	0.5		8.4	1.6
Oelke *et al*[19]	5		-6.3	-4.2	-4.1	-2.6	-2.2	-1.6	-1.3	-1			2.4	1.2		
Porst *et al*[21]	2.5		-4.2	-2.1											8.2	2
	5		-4.7												7.9	
	10		-4.7												6.8	
	20		-3.6												5.4	
Porst *et al*[36]	5		-5.6	-3.6	-3.3	-2.3	-2.3	-1.3			-1.8	-1.3			6.7	2
Porst *et al*[20]	5		-7.9	-5.1												
Roehrborn *et al*[22]	2.5		-5.17	-2.27	-2.94	-1.26	-1.96	-0.99	-0.92	-0.49	-1.38	-0.83	1.96	1.24	8.34	2.2
	5		-3.88		-2.23		-2.07		-0.88		-1.4		1.41		7.98	
	10		-5.21		-3.12		-1.58		-0.74		-0.96		1.64		6.97	
	20		-4.87		-3.13		-1.89		-0.86		-1.45		1.58		5.59	
Roehrborn *et al*[12]	5	Qmax < 10	-5.2	-3.6	-3.2	-2.1							2.8	2.4		
	5	Qmax of 10-15	-6.3	-3.8	-3.9	-2.5							1.4	0.9		
	5	Qmax > 15	-6.8	-2.7	-3.9	-1.2							-1.1	-2.7		
Takeda *et al*[24]	5		-6	-4.5	-2	-1.4	-2	-1.4	-2	-1.4						
Yokoyama *et al*[25]	2.5		-5	-3	-3.3	-1.9	-1.7	-1.1	-0.8	-0.5	-1	-0.8	1.3	2.1		
	5		-5.1		-3.72		-1.5		-0.8		-1.1		1.6			
	Sildenafil															
McVary *et al*[10]	50		-6.3	-1.9					-0.9	-0.3	-2	-0.9	0.31	0.16	9.17	1.86
	100															
	Vardenafil															
Stief *et al*[40]	10		-5.8	-3.6									1.6	1		

IIEF: International index of erectile function; IPSS: International prostate symptom; Qmax: Maximum urinary flow rate; QoL: Quality of life; BII: Benign prostatic hyperplasia impact index; ED: Erectile dysfunction; T: Treatment; P: Placebo.

Qmax improvement was still consistent with the poor link between Qmax and LUTS/BPH in the updated BPH guidelines[41]. The limitation of methodology in choosing an ED or non-ED population is when sexually active patients with LUTS/BPH but no ED history were managed in blind, placebo-controlled trials. Therefore, clinical ED determination alone could not fully exclude ED in this reference groups enrolled for LUTS/BPH.

In another multicenter, randomized, double-blind, placebo-controlled clinical trial with LUTS/BPH patients treated once daily with 20 mg tadalafil for 12 wk, Dmochowski *et al*[13] (2010) pointed out that tadalafil significantly improved total IPSS score (tadalafil *vs* placebo: -9.2 *vs* -5.1, $P < 0.001$), voiding subscore (tadalafil *vs* placebo: -5.6 *vs* -2.8, $P < 0.001$) and storage subscore (tadalafil *vs* placebo: -3.6 *vs* -2.3, $P = 0.006$) compared to the placebo group. Qmax from baseline to endpoints showed a small alteration with no significant difference (tadalafil *vs* placebo: -2.1 *vs* 0.1, $P = 0.33$). In addition, several points should be noted when considering these trials. A relatively high tadalafil dose was used without assessing rigorous intent to treat patients. Thus, the magnitude of improvement investigated in these trials in future clinical utilization should be treated with caution.

Donatucci *et al*[14] completed a double blind, placebo controlled, open-label 12 wk trial of tadalafil (2.5 mg, 5 mg, 10 mg or 20 mg once daily) extended to 1 year. The changes from baseline to endpoint in the total IPSS, IPSS voiding subscore, IPSS storage subscore, IPSS health-related QoL and BII were sustained after one year. Besides, the IIEF-EF was also maintained after 1 year. Higher treatment-induced emergent AEs (57.6% of patients) were observed in the higher dose group but 5 mg tadalafil was well tolerated. Although the efficacy of improvement from baseline or 12 wk to endpoint was noted, the changes from baseline to 12 wk were not reported. Qmax was not evaluated in this trial.

Egerdie *et al*[15] conducted a multinational phase 3 (12 wk) randomized, double blind and control-placebo trial to assess the efficacy of tadalafil 2.5 or 5 mg in the management of LUTS/BPH with ED patients. In this study, both doses of tadalafil significantly improved the IIEF-EF (tadalafil *vs* placebo: 6.5, 5.2 *vs* 1.8, both $P < 0.001$). Improvement with 5 mg but not 2.5 mg in IPSS voiding subscore (tadalafil *vs* placebo: -3.6 *vs* -2.2, $P < 0.001$), storage subscore (tadalafil *vs* placebo: -2.5 *vs* -1.6, $P < 0.001$) and BII (tadalafil *vs* placebo: -1.6 *vs* -1.2, $P < 0.001$) was observed but QoL subscore (tadalafil *vs* placebo: -1 *vs* -0.8, $P = 0.082$) failed to reach a significant difference (Table 2).

Kim *et al*[16] reported a 12 wk randomized, double-blind, controlled-placebo trial of LUTS/BPH in Korean men for once daily tadalafil 5 mg. From baseline to endpoint, the total IPSS and Qmax mean changes were numerically but not significantly improved compared with placebo (tadalafil *vs* placebo: IPSS, -5.6 *vs* -3.6, $P > 0.05$ and Qmax, 2.5 *vs* 2.3, $P > 0.05$).

In 2007, McVary *et al*[18] conducted a trial of 281 men allocated randomly to 5 mg tadalafil once daily for 6 wk with a dose escalation to 20 mg for another 6 wk. There was a significant difference in IIEF-EF (tadalafil *vs* placebo: 8.4 *vs* 1.6, $P < 0.001$), total IPSS score (tadalafil *vs* placebo: -7.1 *vs* -4.5, $P < 0.001$), voiding subscore (tadalafil *vs* placebo: -4.4 *vs* -2.8, $P < 0.0001$), storage subscore (tadalafil *vs* placebo: -2.7 *vs* -1.8, $P < 0.001$) and QoL (tadalafil *vs* placebo: -0.5 *vs* -0.2, $P < 0.001$). However, the difference of Qmax was not significant when comparing tadalafil to placebo (tadalafil *vs* placebo: 0.5 *vs* 0.9, $P > 0.05$).

Oelke *et al*[19] investigated the efficacy of 5 mg tadalafil once daily monotherapy through 12 wk of therapy of LUTS/BPH in a randomized, double- blind, international controlled-placebo study. Total IPSS score significantly improved with tadalafil (tadalafil *vs* placebo: -6.3 *vs* -4.2, $P = 0.001$). Significant improvement in voiding subscore (tadalafil *vs* placebo: -4.1 *vs* -2.6, $P < 0.001$) but not storage subscore (tadalafil *vs* placebo: -2.2 *vs* -1.6, $P = 0.055$) and QoL subscore (tadalafil *vs* placebo: -1.3 *vs* -1.0, $P = 0.022$) was observed from baseline to endpoint in this trial. Qmax increased significantly (tadalafil *vs* placebo: 2.4 *vs* 1.2, $P = 0.009$). Nevertheless, this trial was of 12 wk duration to evaluate the efficacy of LUTS/BPH and did not address longer term efficacy of tadalafil on disease progression. Maybe this kind of trial would trigger great interest in the future.

In a phase 2 to 3, multinational, randomized, double-blind, controlled-placebo study, Porst *et al*[21] (2009) randomly assigned patients to tadalafil 2.5 mg, 5 mg, 10 mg and 20 mg once daily for 12 wk. The least square mean difference of IIEF-EF compared to placebo (the value: 2) was significant for all four doses of tadalafil (2.5 mg dose, 8.2; 5 mg dose, 7.9; 10 mg dose, 6.8, and 20 mg dose, 5.4) (all $P < 0.001$). The mean changes of total IPSS score from baseline to endpoint reached a significant difference (tadalafil *vs* placebo: 2.5 mg, -4.2 *vs* -2.1, $P = 0.015$; 5 mg, -4.7 *vs* -2.1, $P < 0.001$; 10 mg, -4.7 *vs* -2.1, $P < 0.001$, and 20 mg, -3.6 *vs* -2.1, $P < 0.001$). However, Qmax failed to reach significance for treatment groups. The limitation could be the absence of a parallel group without LUTS/BPH as a control reference and it could not summarize the minimum times of sexual intercourse monthly before allocation and the trial duration, which could measure the risk-benefit of once daily tadalafil for IIEF-EF improvement.

In a second randomized, double-blind, placebo-controlled 12 wk study, Porst *et al*[36] pointed out that 5 mg tadalafil significantly improved total IPSS score (tadalafil *vs* placebo: -5.6 *vs* -3.6, $P = 0.004$), voiding subscore (tadalafil *vs* placebo: -3.3 *vs* -2.3, $P = 0.020$), storage subscore (tadalafil *vs* placebo: -2.3 *vs* -1.3, $P < 0.002$), QoL index (tadalafil *vs* placebo: -1.0 *vs* -0.7, $P = 0.013$) and BII (tadalafil *vs* placebo: -1.8 *vs* -1.2, $P = 0.029$) from baseline to endpoint. However, uroflowmetry parameters did not show a significant difference at the endpoint. The IIEF-EF in ED men was significantly improved at 12 wk (tadalafil *vs* placebo: 6.7 *vs* 2.0, $P < 0.001$).

In 2013, Porst *et al*[20] pooled data from 4 multinational, randomized, placebo-controlled clinical trials to investigate 5 mg tadalafil once daily for LUTS/BPH for 12 wk. The pooled data confirmed that tadalafil resulted

Table 3 Outcomes of the meta-analysis of total international prostate symptom score, international prostate symptom score storage subscore, international prostate symptom score voiding subscore, international prostate symptom score quality of life subscore, benign prostatic hyperplasia impact index, maximum urinary flow rate, and international index of erectile function score in lower urinary tract symptoms/benign prostatic hyperplasia or lower urinary tract symptoms/benign prostatic hyperplasia and erectile dysfunction patients

Outcome or subgroup	Studies	Participants	Weight	Statistical method	Effect Estimate (Mean difference, 95%CI)	Heterogeneity χ^2	Heterogeneity I^2 (%)	Overall *Z* value	*P* value
Total IPSS in LUTS/BPH	13	9131	100%	Fixed	-2.17 (-2.42, -1.91)	16.44	0	16.75	< 0.00001
Tadalafil	11	8576	95.5%	Fixed	-2.14 (-2.40, -1.88)	13.27	0	16.18	< 0.00001
Sildenafil	1	341	1.0%	Fixed	-4.40 (-6.87, -1.93)			3.48	0.001
Vardenafil	1	214	3.4%	Fixed	-2.20 (-3.57, -0.83)			3.14	0.002
Total IPSS in LUTS/BPH and ED	6	3626	100%	Fixed	-1.97 (-2.43, -1.51)	12.33	3	8.41	< 0.00001
Tadalafil	5	3285	96.6%	Fixed	-1.88 (-2.35, -1.41)	8.49	0	7.90	< 0.00001
Sildenafil	1	341	3.4%	Fixed	-4.40 (-6.87, -1.93)			3.48	0.001
IPSS storage subscore in LUTS/BPH									
Tadalafil	10	6848	100%	Fixed	-0.71 (-0.85, -0.57)	12.64	0	9.96	< 0.00001
IPSS voiding subscore in LUTS/BPH									
Tadalafil	11	7916	100%	Fixed	-1.23 (-1.41, -1.04)	24.7	15	13.28	< 0.00001
IPSS QoL subscore in LUTS/BPH	8	5999	100%	Fixed	-0.33 (-0.40, -0.26)	8.26	0	8.70	< 0.00001
Tadalafil	7	5648	97.7%	Fixed	-0.32 (-0.40, -0.25)	6.26	0	8.38	< 0.00001
Sildenafil	1	351	2.3%	Fixed	-0.68 (-1.17, -0.19)			2.71	0.007
BII in LUTS/BPH									
Tadalafil	5	3504	100%	Fixed	-0.43 (-0.61, -0.25)	3.89	0	4.64	< 0.00001
BII in LUTS/BPH and ED	4	2561	100%	Fixed	-0.52 (-0.74, -0.29)	8.02	13	4.51	< 0.00001
Tadalafil	3	2210	94.8%	Fixed	-0.48 (-0.71, -0.25)	6.59	9	4.11	< 0.0001
Sildenafil	1	351	5.2%	Fixed	-1.10 (-2.08, -0.12)			2.19	0.03
Qmax in LUTS/BPH									
Tadalafil (2.5, 5, 10 and 20 mg)	9	5034	64.9%	Fixed	0.22 (-0.04, 0.49)	13.43	3	1.65	0.10
Tadalafil (only 5 mg)	7	2876	35.1%	Fixed	0.33 (-0.13, 0.80)	8.24	24	2.14	0.03
IIEF in LUTS/BPH									
Tadalafil	2	2009	100%	Fixed	4.88 (3.31, 8.97)	2.28	0	8.96	< 0.00001
IIEF in LUTS/BPH and ED									
Tadalafil	3	1746	100%	Fixed	4.54 (3.75, 5.33)	7.33	18	11.27	< 0.00001

IIEF: International index of erectile function; IPSS: International prostate symptom score; LUTS/BPH: Lower urinary tract symptoms/benign prostatic hyperplasia; Qmax: Maximum urinary flow rate; QoL: Quality of life; BII: Benign prostatic hyperplasia impact index; ED: Erectile dysfunction.

in improvement in total IPSS score from baseline to endpoint (tadalafil *vs* placebo: -7.9 *vs* -5.1, $P < 0.001$), as well as IPSS QoL index and BII (both $P < 0.01$).

Roehrborn *et al*[22] conducted a 12 wk randomized, double-blind, placebo-controlled, dose-finding study in 10 countries. They randomly assigned the patient to tadalafil 2.5 mg, 5 mg, 10 mg and 20 mg once daily for 12 wk. The least square mean difference of IIEF-EF compared to placebo (the value: 2.2) was significant for all four doses of tadalafil (2.5 mg dose, 5.59; 5 mg dose, 6.97; 10 mg dose, 7.98; and 20 mg dose, 8.34) (all $P < 0.001$). The mean changes of total IPSS score from baseline to endpoint reached a significant difference (tadalafil *vs* placebo: 2.5 mg, -3.9 *vs* -2.3, $P = 0.015$; 5 mg, -4.9 *vs* -2.3, $P < 0.001$; 10 mg, -5.2 *vs* -2.3, $P < 0.001$; and 20 mg, -5.2 *vs* -2.3, $P < 0.001$) and the voiding subscore, storage subscore, QoL index and BII all reached a significant difference ($P < 0.01$, 0.001, 0.05 and 0.05, respectively). However, Qmax failed to reach significance for the medication groups.

In the second study by Roehrborn *et al*[12] (2013), with 5 mg tadalafil for the LUTS/BPH for 12 wk, the effects on the Qmax with LUTS/BPH were investigated. Qmax changes were assessed compared to baseline Qmax. For baseline Qmax < 10 mL/s, increases were higher in tadalafil compared with the placebo group (tadalafil *vs* placebo: 2.8 *vs* 2.4, $P = 0.189$); for Qmax of 10 to 15 mL/s, (tadalafil *vs* placebo: 1.4 *vs* 0.9, $P = 0.044$); and for Qmax > 15 ml/s, (tadalafil *vs* placebo: -1.1 *vs* -2.7, $P = 0.246$).

Takeda *et al*[24] (2014) pooled data of randomized, double-blind, placebo-controlled studies of tadalafil 5 mg from 39 sites in Japan and Korea. Total IPSS score significantly improved with tadalafil (-6 *vs* -4.5, $P = 0.001$). Significant improvement in IPSS voiding subscore (tadalafil *vs* placebo: -4 *vs* -3.1, $P = 0.002$), IPSS storage subscore (tadalafil *vs* placebo: -2 *vs* -1.4, $P = 0.002$) and IPSS QoL subscore (tadalafil *vs* placebo: -1.1 *vs* -0.9, $P = 0.038$) was observed from baseline to endpoint in this trial.

Yokoyama *et al*[25] investigated the effects of tadalafil 2.5 mg and 5 mg in a multicenter, randomized, double-blind, placebo-controlled study from 34 sites in Japan, South Korea and Taiwan. Except for Qmax and BII index, the total IPSS score, voiding subscore, storage subscore and QoL subscore reached a significant difference.

The outcomes of meta-analysis of PDE5-Is on LUTS/BPH from integrated studies

The data were pooled for calculations and computed for integrated analysis. Heterogeneity was not observed (I^2 < 30%) and the fixed effect model was used.

For participants with comorbid LUTS/BPH and ED, the total IPSS, BII and IIEF-EF were divided into two subgroups: subgroup with LUTS/BPH and subgroup with LUTS/BPH and ED. Irrespective of overall group or subgroup analysis, PDE5-Is, especially tadalafil, showed an improvement of total IPSS, BII and IIEF domain (P < 0.0001 or P < 0.00001, Table 3). Changes in the storage, voiding and QoL were also reported (P < 0.00001, Table 3). Changes of Qmax for tadalafil at a dose of 5 mg was calculated in LUTS/BPH patients and showed a significant improvement [0.33 (-0.13, 0.80), P < 0.03, Table 3].

COMMENTS

Background

Lower urinary tract symptoms suggestive of benign prostate hyperplasia (LUTS/BPH) are increasingly frequent in aging men. The majority coexist with erectile dysfunction (ED). Irrespective of coexisting ED, LUTS/BPH patients frequently suffer from a poorer quality of life (QoL).

Research frontiers

Until recently, surgical therapy was the cornerstone of management for male LUTS. As early as 1990s, medical therapy became a possible treatment option for voiding problems. Since then, the surgical option has dropped gradually and currently the first option for treatment of male LUTS is medical therapy. 5-alpha reductase inhibitors and α-blockers have dominated the management of LUTS for many years. Nowadays, new drugs have cast a light on the treatment of LUTS, including PDE5-Is and anticholinergics. In the traditional sense, LUTS occurring with aging has frequently been associated with outlet obstruction in the bladder resulting from BPH, whereas the complaint may be explained by the detrusor overactivity. More recently, increasing evidence has shown that phosphodiesterase type 5 inhibitor (PDE5-Is) could exert improvement in LUTS in aging men who frequently suffer from BPH.

Innovations and breakthroughs

PDE5-Is, including mainly tadalafil, sildenafil and vardenafil, were the first line medication to treat ED patients. More and more randomized controlled trials (RCT) have been done to examine the efficacy of PDE5-Is for treatment of LUTS/BPH. As reported, PDE5-Is might have influenced the terminal decision because of distinct pharmacological profiles and side effects and the enthusiasm for PDE5-Is has decreased due to the lack of objective improvement. Furthermore, urodynamic parameters did not change. More important, coherently explaining the disconnection between objective and subjective changes is still pending. Therefore, it is necessary to determine whether PDE5-Is are effective in the treatment of LUTS/BPH on the basis of a systematic review and meta-analysis of published evidence. Meta-analysis has been increasingly utilized since it was introduced to assess clinical data in the urological community by Peter Boyle. In particular, it could give rise to invaluable insights for benefits. To a large extent, even although a large database was available, some predictive characteristics for responders and non-responders could still not be identified. However, all the convincing studies showed that LUTS was significantly alleviated by the regular use of PDE5-Is. In other words, the available studies on the use of PDE5-Is for the treatment of LUTS are promising. Especially in aging males, there is an increased prevalence of LUTS/BPH. Daily PDE5-Is might be a useful treatment for this condition as such a pharmacological strategy has the potential to become the treatment to manage the aging process of the male urogenital tract. Although the present manuscript underscores that PDE5-Is are a promising therapy for LUTS/BPH from other researchers, a couple of questions are still worthy of considering, including patient selection, durability and health economics, in the case of PDE5-Is for treatment of LUTS. In an ideal world, some situations could inevitably be avoided between doctors and patients while using PED5-Is for patients with any given condition. Firstly, the best candidates should be screened with male LUTS patients alone receiving any given treatment. Secondly, patients should be informed about the potential limitations of PDE5-Is during the treatment of their complaints. Thirdly, who is going to have what kind of treatment and when? In addition, the best practice includes the doctor's choice as well as the patient's.

Applications

PDE5-Is significantly improved total international prostate symptom score (IPSS) score, IPSS voiding score, IPSS storage score, IPSS QoL score and international index of erectile dysfunction score (IIEF)-EF score. Significant improvement of total IPSS score and IIEF-EF score was observed in patients with comorbid ED and BPH. As such, PDE5-Is as the first line for management of ED was also demonstrated to be effective for LUTS/BPH. Therefore, well designed clinical studies of large scales are required to ascertain the efficacy and specific mechanisms of action of PDE5-Is for the management of LUTS/BPH.

Abbreviations

PDE5-I: Phosphodiesterase type 5 inhibitor; LUTS/BPH: Lower urinary tract symptoms suggestive of benign prostate hyperplasia; ED: Erectile dysfunction; IPSS: International prostate symptom score; IIEF: International index of erectile dysfunction score; Qmax: Maximal urinary flow rate; IPSS-QoL: IPSS Quality of life Index; IPSS irritative (storage) subscore; IPSS obstructive (voiding) subscore; BII: BPH impact index.

Peer-review

This is an interesting review regarding the efficacy of PDE5-Is in lower urinary tract symptoms and benign prostate hyperplasia.

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REVIEW

Management of patients with a failed kidney transplant: Dialysis reinitiation, immunosuppression weaning, and transplantectomy

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Abstract

The number of patients reinitiating dialysis after a failed transplant increases over time and has more than doubled between the year 1988 and 2010 (an increase from 2463 to 5588). More importantly, patients returning to dialysis have been shown to have a greater than three-fold increase in the annual adjusted mortality rates compared with those with a functioning graft. Continuation of immunosuppression to preserve residual graft function has been implicated to be a contributing factor, seemingly due to immunosuppression-associated cardiovascular and infectious complications and malignancy risk, among others. Nonetheless, maintenance low-dose immunosuppression has been suggested to confer survival benefit in patients returning to peritoneal dialysis. Whether early *vs* late reinitiation of dialysis or whether transplantectomy has an impact on patient survival remains poorly defined. Consensus guidelines for the management of a failed allograft are lacking. In this article, we present a literature overview on the ideal timing of dialysis reinitiation after graft loss, the management of immunosuppression after graft failure, and the risks and benefits of transplantectomy. The authors' perspectives on the management of this special patient population are also discussed.

Key words: Failed kidney transplant; Allosensitization; Immunosuppression weaning; Allograft nephrectomy; Transplantectomy; Dialysis reinitiation after transplant failure

Core tip: The number of patients with a failed allograft returning to dialysis increases over time. Studies suggest that such patients are at increased morbidity and mortality risks compared with their transplant-naïve, incident dialysis patients. This review provides a critical literature overview of the risks and benefits of early *vs* late dialysis re-initiation, immunosuppression weaning, and transplantectomy in patients with a failed allograft. Based on currently available literature, suggested guidelines for the management of this unique patient population are presented.

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INTRODUCTION

Retrospective analysis of the United States Renal Data System (USRDS) database showed that mortality in patients reinitiating dialysis after graft failure was primarily due to cardiac (36%) or infectious complications (17%)[1]. Continuation of immunosuppression has been suggested to play a causative role. Nevertheless, immunosuppression cessation is not without morbidity. Similarly, although transplantectomy would permit immunosuppression withdrawal, it may lead to other unfavorable outcomes.

Clinicians caring for patients with a recently failed allograft are generally faced with three important decisions: timing of dialysis reinitiation, immunosuppression management, and whether to perform transplantectomy[2]. When the cause of graft loss is due to primary nonfunction, arterial or venous thrombosis, hyperacute or early refractory acute rejection, most treating physicians advocate transplantectomy and immunosuppression cessation. In these circumstances, graft rupture or hemorrhage may occur if the graft is left *in situ*. However, when the allograft has been in place for more than 1-2 years, it is common practice to leave the failed allograft *in situ*. Nonetheless, a retained failed transplant has been suggested to be a source of a chronic inflammatory state, potentially leading to unfavorable outcomes. Immunosuppression management in such patients can be challenging. Although maintenance low dose-immunosuppression may preserve residual kidney function, circumvent graft intolerance syndrome, minimize allosensitization, and avoid overt acute rejection, long-term maintenance immunosuppression is not without adverse effects (Table 1). These may include immunosuppression-related malignancy and cardiovascular and metabolic complications. It is also noteworthy that although the number of patients reinitiating dialysis after a failed transplant has more than doubled in the last two decades, studies evaluating the optimal timing of dialysis reinitiation are lacking. A literature overview on the timing of dialysis reinitiation after graft failure, the potential beneficial and adverse effects of low-dose maintenance immunosuppression, and the risks and benefits of transplantectomy are presented.

TIMING OF DIALYSIS REINITIATION

Early studies in the mid 1970s to 1980s suggested that initiation of dialysis in end-stage kidney disease (ESKD) patients with a higher estimated glomerular filtration rate (eGFR) was associated with lower mortality[3]. However, these studies were subsequently criticized for small sample sizes and potential confounding factors. Over the past decade, several observational studies failed to demonstrate the survival benefits of early commencement of dialysis and such practice may even be associated with increased mortality risk[4]. A recent multicenter randomized controlled trial (the Initiating Dialysis Early and Late study) showed comparable mortality rates among early *vs* late dialysis initiation. Eight hundred and twenty eight patients with progressive stage V chronic kidney disease (CKD) (including patients with a failed transplant) were randomized to either start dialysis at eGFR of 10.0 to 14.0 mL/min (early-start group) or to continue routine medical management and start dialysis when eGFR reached 5.0 to 7.0 mL/min (late-start group)[5]. During a median follow up period of 3.59 years, mortality occurred in 37.6% and 36.6% of patients in the early- and late-start groups, respectively (HR 1.04, $P = 0.75$). The frequency of cardiovascular events, infections, or dialysis complications was comparable between the two groups. However, it is noteworthy that in the late-start group, nearly 76% of patients were started on dialysis when the estimated GFR was above the target 7.0 mL/min due to symptomatic uremia. It was thus concluded that planned early dialysis initiation in patients with stage V CKD provided no benefits in terms of survival or clinical outcomes. Similarly, a retrospective USRDS database study ($n = 310932$ patients who were started on dialysis between 2006 and 2008) showed no harmful or beneficial effects of early dialysis initiation on mortality (HR 1.025 per 1 mL/min per 1.73 m^2 for eGFR 5-14 1 mL/min per 1.73 m^2 and 0.973 per 1 mL/min per 1.73 m^2 for eGFR 14-20 mL/min per 1.73 m^2)[6].

Studies on the optimal timing of dialysis reinitiation after a failed transplant are limited. Current guidelines for transplant naïve patients with progressive CKD advocate late-start dialysis (defined as dialysis initiation at an eGFR between 6-9 mL/min). Results of two large registry studies suggested that early compared with late dialysis reinitiation in patients with failed kidney transplants may adversely impact survival. The USRDS registry study ($n = 4741$ patients followed for a median of 15 ± 11 mo after dialysis initiation) demonstrated that nonsurvivors had a significantly higher eGFR at dialysis initiation than their survivor counterparts (9.7 ± 4.8 *vs* 8.0 ± 3.7 mL/min per 1.73 m^2, respectively). Specifically, each 1 mL/min per m^2 higher eGFR at the time of dialysis reinitiation was found to be associated with a 4% higher mortality risk after dialysis reinitiation ($P < 0.01$)[1]. Nonetheless, it is speculated that the sickest patients tended to require commencement of dialysis at higher levels of residual kidney function. This confounding by indication was subsequently addressed in an analysis of the SRTR registry study using propensity score analysis. The study cohort consists of 747 failed kidney transplant patients who had reinitiated dialysis with eGFR < 15 mL/min. A propensity score for early

Table 1 Continuation of immunosuppression after a failed transplant

Potential beneficial effects	Potential adverse effects
Preservation of residual kidney function	Metabolic complications (diabetes, hypertension, dyslipidemia)
Decreased incidence of graft intolerance syndrome and the need for allograft nephrectomy	Steroid-associated adverse effects (*e.g.*, diabetes, cataracts, myopathy, and avascular necrosis among others)
Minimization of allosensitization	Cardiovascular complications
Avoidance of overt acute rejection	Increased susceptibility to infection
Prevention of adrenal insufficiency syndrome	Malignancy (especially skin cancers, Kaposi's sarcoma, non-Hodgkin's lymphoma, and lip cancers)
Prevention of reactivation of systemic disease (*e.g.*, systemic lupus erythematosus, vasculitis)	Costs (particularly when data supporting continued immunosuppression are lacking)

(eGFR > 10.5 mL/min per 1.73 m^2) *vs* late dialysis reinitiation was fitted by logistic regression. Peripheral vascular disease, diabetes mellitus, and male gender were associated with higher odds of early reinitiation of dialysis. In an unadjusted model, each 1 mL/min per 1.73 m^2 higher eGFR at dialysis reinitiation was associated with a 6% higher mortality risk. Such association was not observed in the fully adjusted model. However, there was a trend towards increased mortality risk in patients with a higher eGFR upon reinitiation of dialysis, particularly among the healthiest subgroups of patients identified by the propensity score, including female gender and younger subjects[7].

Whether early dialysis reinitiation in patients with failed transplants adversely impact outcomes is currently not known and warrants further studies. Based on available data, a number of investigators feel that reinitiation of dialysis based on eGFR alone is not justified and could be harmful in some cases[3]. Thus, as with transplant naïve patients, dialysis reinitiation in patients with graft failure may rely on eGFR as a rough guide that must be redefined by patients' comorbidities, nutritional status, and overall wellness.

IMMUNOSUPPRESSION MANAGEMENT

Consensus guidelines for the management of immunosuppression in patients with a failed allograft are lacking. Both continuation of low-dose immunosuppression and immunosuppression withdrawal have their inherent risks and benefits (Table 1)[2].

Continuation of immunosuppression: Potential beneficial effects

Preservation of residual kidney function: In the non-transplant settings, peritoneal dialysis (PD) and hemodialysis (HD) patients with preserved kidney function have been shown to have better survival rates compared with their oliguric or anuric counterparts[8,9]. Similar to the transplant naïve end stage kidney disease population, patient with a retained failed transplant and preserved residual graft function have been shown to have survival advantage over those who lost residual kidney function. A decision analytic model comparing continuation of immunosuppression with immunosuppressant withdrawal in patients returning to PD after graft failure suggests that continued immunosuppression may confer survival benefit over immunosuppressant cessation despite increased malignancy and infection risks (life expectancy: 5.8 years *vs* 5.3 years, respectively)[10]. The survival benefit was apparent even at marginal GFR (defined as an additional GFR of 1.48 mL/min), and incremental at increasing residual graft function. The study results suggest that the loss of residual kidney function may have an adverse impact on survival in patients reinitiating PD. Nevertheless, the study was not without shortcomings. The model hypothesized that continuation of maintenance immunosuppression would preserve residual kidney function, and the beneficial effects of residual graft function are similar to those of the native kidneys. Of interest, results of the USRDS registry analysis demonstrated that compared with hemodialysis, PD was associated with greater survival within the first year after dialysis initiation, but lower after 2 years[11]. It may be speculated that the early survival benefit of PD over HD was due to greater preservation of residual kidney function. Notably, the survival advantage of PD was not seen among patients who initiated PD at lower levels of eGFR. However, neither details on immunosuppression maintenance after graft failure nor data on differential rates of decline in residual kidney over time was provided. A case of well-preserved residual kidney function in a PD patient maintained on low dose dual immunosuppressive therapy after a failed allograft has been described. After return to PD, the patient continued to make 600-1200 cc of urine/day at one-year follow-up[12].

There is currently insufficient evidence to routinely recommend continued immunosuppression in patients returning to PD after graft loss.

Data for any potential survival benefits of continuation of maintenance immunosuppression among patients returning to HD are lacking.

Prevention of allosensitization: Immunosuppression withdrawal after kidney graft failure with or without transplantectomy has been shown to be an independent predictor of allosensitization[13,14]. In a single-center study consisting of 69 patients with confirmed alloantibody

negative at the time of graft loss, more than half (38/69) became sensitized over the following months or years. *De novo* class Ⅰ and/or class Ⅱ anti HLA antibodies (primarily of donor specificity) were detected only in patients whose immunosuppressants were discontinued after graft loss regardless of whether they had a nephrectomy or blood transfusion. Four of fifteen patients without nephrectomy or transfusion developed antibodies after cessation of immunosuppression. In contrast, none of the eleven patients who continued immunosuppressants developed antibodies, seven of whom had an allograft nephrectomy or blood transfusion[14]. In another study, *de novo* donor-specific antibodies (DSAs) appeared in nearly 48% of patients when immunosuppressive therapy was discontinued after graft loss. None of these patients had an allograft nephrectomy[15]. Of interest, it has been shown that a short exposure to the allograft is sufficient to stimulate the immune system and to induce alloantibody production[16]. In a small series of 32 patients who required transplantectomy after early graft loss, DSAs and non-DSA anti-HLA antibodies developed in > 50% of patients whose immunosuppressants were discontinued after transplantectomy (median time between transplantation and transplantectomy was 2.5 d). Histological analysis of explanted allografts showed no features of cellular or humoral rejection. There was no significant difference in the incidence of DSAs among patients receiving transfusions and those who did not[16].

Given current evidence, albeit scant of potential increased allosensitization with cessation of immunosuppression, it may be suggested that patients who are re-allograft transplant candidates be considered for continuation of maintenance therapy, particularly when living donation is a possibility. Whether patients with early graft loss requiring transplantectomy (particularly those anticipated to have a short wait time after early relisting) benefit from continuation of immunosuppression to minimize allosensitization warrant further investigation. In addition, the duration and intensity of maintenance immunosuppression remain to be defined.

Prevention of graft intolerance syndrome and transplantectomy: Graft intolerance syndrome refers to an immunologic intolerance to a retained failed graft, and commonly develops within the first year of dialysis reinitiation. Clinically, patients may present with graft enlargement or tenderness, gross hematuria, fevers, malaise, flu-like symptoms, or any constellation of signs and symptoms thereof. Graft intolerance syndrome may develop in 30% to 50% of patients despite various immunosuppression withdrawal protocols. Although such syndrome may be treated with a short course of high dose corticosteroid, symptom recurrence following immunosuppression weaning generally necessitates transplantectomy. In one single center study, immunosuppression weaning commonly led to symptomatic rejection with fever mimicking infection (93 of 186 study subjects were African Americans). A nearly 7-fold risk (P = 0.017) for admission within six months of graft failure with fever in the absence of infection was observed among African Americans who were tapered from immunosuppression. The majority of these patients ultimately required transplantectomy due to symptomatic rejection or fever of unknown etiology. Notably, fever resolved in all patients after transplantectomy[17].

In a single-center study consisting of 41 patients with graft loss occurring more than 6 mo after transplantation, the need for transplantectomy following immunosuppression weaning was found to correlate with the number of previous acute rejection episodes. In patients who had zero, one, or two or more rejection episodes, transplantectomy was required in 30%, 53% and 83%, respectively. It is suggested that gradual immunosuppression weaning or indefinite low-dose maintenance immunosuppression may prevent the need for transplantectomy[18].

Rapid steroid withdrawal may result in overt adrenal insufficiency variously manifested as hypotension, weakness, fevers, malaise, and weight loss, among others. In severe steroid withdrawal, patients may experience frequent hypotensive episodes during dialysis despite having volume overload[19]. When the graft is left *in situ* for more than one to two years, gradual immunosuppression weaning with close monitoring for clinically overt adrenal insufficiency or acute allograft rejection is advisable.

Continuation of immunosuppression: Potential adverse effects

Infectious, cardiovascular, and metabolic syndrome risks: While low-dose maintenance immunosuppression may be beneficial in preserving residual kidney function in patients who maintain good urine output, such practice is not without adverse effects. In a multi-center cohort study comprising 197 failed allografts in 177 transplant recipients whose allograft functioned for at least 3 mo, low-dose maintenance immunosuppression was associated with an increase in infectious- and cardiovascular disease-related morbidity and mortality[20]. The incidence of infectious complications per patient year was significantly higher in the immunosuppression continuation compared with that of immunosuppression withdrawal groups (1.7 *vs* 0.51, respectively, $P < 0.0001$). Similarly mortality associated with cardiovascular and infectious complications was higher among patients who continued immunosuppression compared with those whose immunosuppression was discontinued [Odd ratio (OR) of 4.9, 95%CI: 1.8-13.5 from cardiovascular disease and OR of 2.8, 95%CI: 1.1-7.0 from infectious complications]. Clinical acute rejection rates (graft tenderness and hematuria, in the absence or presence of non-infectious low-grade fever) were similar between the two groups (P = NS). Based on the study findings, the authors favored immunosuppression withdrawal over low-dose maintenance immunosuppression when patients returned to dialysis. In one single center study, an increase in infection-related complications was

observed among patients whose immunosuppression was weaned over a prolonged period (mean 14 ± 2 mo) compared with those whose immunosuppression was weaned over a shorter (mean 3 ± 1 mo) period (1.34 *vs* 0.87) infections per year, respectively[21]. Furthermore, the longer taper had no advantage over the shorter taper group in forestalling the need for transplantectomy. Mortality associated with disseminated histoplasmosis in a hemodialysis patient maintained on low-dose steroid and azathioprine after graft failure has been described[22].

Similar to early reports, a recent study suggests that although immunosuppression weaning results in a higher risk of allosensitization, maintenance of immunosuppression other than low-dose steroid is associated with a greater incidence of infection and infection-related mortality[17]. In a single center consisting of 186 patients with failed kidney transplants, 44% were hospitalized with fever within six months of graft loss. The rates of hospitalization were comparable between patients who continued immunosuppression and those whose immunosuppression was tapered before hospitalization (45% *vs* 40%, respectively, *P* = NS). However, among febrile hospitalized patients, documented infections occurred in 88% of patients maintained on immunosuppressive therapy compared with 38% of those who had been weaned off of immunosuppression (defined as withdrawal of all immunosuppressive therapy with the exception of ⩽ 10 mg of prednisone daily). Notably, mortality risk was significantly higher in patients with documented infection, with dialysis catheter being the most common infectious source in both groups[17].

Adverse effects associated with long-term steroid use: Well-established adverse effects associated with long-term steroid use include avascular necrosis, osteoporosis, hyperglycemia, cataracts, myopathy and increased susceptibility to infections among others. Nevertheless, it is occasionally necessary to continue steroid to prevent flares of systemic disease such as vasculitis or systemic lupus erythematosus.

Malignancy: Recipients of organ transplants are at increased risk for developing certain neoplasms compared to that of the general population. Kidney transplant recipients receiving low-dose cyclosporine (CSA) was shown to have a significantly lower overall frequency of cancers ($P < 0.034$) and a lower incidence of virus-associated cancers ($P = 0.05$) compared with their normal-dose CSA counterparts[23]. Both the duration and intensity of immunosuppressive agents and their ability to foster the replication of oncogenic viruses have been implicated to play contributory roles in the carcinogenic process[2].

Studies evaluating reversal of cancer risk in patients reinitiating dialysis after graft loss is limited. However, it is noteworthy that studies in ESKD patients who received dialysis or a kidney transplant, and in HIV/AIDS subjects suggest that cancers may be classified into those that are related to ESKD, immune deficiency, non-immune deficiency, or uncertain status (Table 2)[24,25]. Although it is conceivable that immunosuppression cessation has no impact on risk reversal of various "non-immune deficiency-related" cancers, most treating physicians advocate rapid immunosuppression weaning or withdrawal in patients who had a history of cancer, irrespective of malignancy types. In cancers associated with immunosuppression, the risks of continued immunosuppression probably outweigh its benefits.

The Australia and New Zealand Dialysis and Transplantation Registry analysis demonstrated that among all cancers that occur at increased rates in kidney transplant recipients, the pattern of incidence after allograft loss was highly variable[26]. Nonetheless, the incidence of Kaposi's sarcoma and non-Hodgkin's lymphoma decreased markedly upon dialysis reinitiation and cessation of immunosuppression. The study also showed a significant decline in melanoma and lip cancer incidence. Of interest, risk reversal was commonly seen among infection-associated malignancies such as Kaposi's sarcoma with human herpes type 8 and non-Hodgkin's lymphoma with Epstein-Barr virus. The exact cause of increased risk of lip cancer in transplant patients has not been well-established. However, human papillomavirus has been implicated to play a causative role. Although an infectious source has not been identified in transplant patients with melanoma, the association between immunosuppression and its development in kidney transplant recipients has been well described[24].

Costs: Following graft loss and reinstitution of dialysis, the cost of low-dose maintenance immunosuppression should not be overlooked, particularly since data supporting continued immunosuppression are lacking. A typical immunosuppressive regimen consisting of low-dose prednisone and cyclosporine or tacrolimus (or mTOR inhibitors) costs more than two thousand United States dollars annually.

TRANSPLANTECTOMY

While practices differ among centers, most advocate transplantectomy in patients whose allograft failed within one to two years posttransplantation. However, no consensus exists on the timing and indications for transplantectomy when graft loss occurs more than 1-2 years after transplant.

The USRDS registry study demonstrated that transplantectomy was nearly twice as common in patients with early (< 12 mo) compared with late graft loss (⩾ 12 mo)[27]. However, whether transplantectomy was performed electively or for graft-related symptoms could not be determined from the study. A single-center study consisting of 34 pediatric kidney transplant

Table 2 Categorization of cancers in the end-stage kidney disease population

ESKD-related	Kidney
	Urinary tract
	Thyroid
	Myeloma
Immune-deficiency related	Hodgkin's lymphoma
	Non-Hodgkin's lymphoma
	Leukemia
	Melanoma of skin
	Kaposi's sarcoma
	Carcinoma of
	Lip
	Mouth, tongue, tonsil, oropharynx
	Esophagus
	Stomach
	Anus
	Liver
	Larynx
	Lung
	Cervix, uteri, vagina, vulva
	Penis
	Eye, squamous cell carcinoma only
Not-related to immune deficiency	Rectum
	Breast
	Ovary
	Prostate
Of uncertain status	All other cancers

ESKD: End-stage kidney disease.

recipients demonstrated that children with graft failure within one year of transplantation were four-fold more likely to require transplantectomy than those with graft loss after one year (P = 0.04)[28]. Fever, graft tenderness, and an elevated C-reactive protein were significantly more common in children who subsequently underwent transplantectomy than in those who did not. Of interest, one retrospective study suggested that transplantectomy may minimize allosensitization in patients with early (graft survival < 6 mo) but not late graft loss. Patients with early graft loss and nephrectomy demonstrated a decline in PRA at a median follow up of 47 mo (46% at the time of graft loss and 27% at last follow up, P = 0.02). In contrast, PRA remained elevated among those who had a nephrectomy after late graft loss[29]. It is suggested that the time of graft failure and subsequent allograft nephrectomy may play a contributory role in allosensitization.

In general, the decision to perform a failed graft nephrectomy requires careful consideration of potential risks and benefits.

Transplantectomy: Potential benefits

A retained failed allograft has been suggested to serve as a focus for a chronic inflammatory state. In one single-center study, patients with failed kidney transplants returning to hemodialysis were shown to exhibit worse anemia, erythropoietin resistance, and hypoalbuminemia, as well as worse C reactive protein (CRP), erythrocyte sedimentation rate (ESR), and ferritin profiles compared with their transplant naïve hemodialysis counterparts. Furthermore, amelioration of both clinical and laboratory parameters of the chronic inflammatory state was observed following transplantectomy. Although symptomatic patients undergoing transplantectomy had lower baseline hemoglobin and higher CRP, ESR, and ferritin compared with those with a retained graft, the former group of patients had a better hematologic and biochemical profile at 6 mo after transplantectomy compared with the latter[30].

It is noteworthy that hypoalbuminemia and high CRP have been shown to be markers for increased cardiovascular and global morbidity and mortality both in the general population and in ESKD patients on hemodialysis. Some centers favor transplantectomy in patients with biochemical indicators of chronic inflammation before the onset of overt clinical manifestations[31,32].

Retrospective study using the USRDS database (n = 10951 patients returning to long-term dialysis after a failed transplant) demonstrated that transplantectomy was associated with a 32% lower relative risk for all-cause mortality (adjusted HR = 0.68; 95%CI: 0.63 to 0.74)[32]. However, the study was not without shortcomings. Patients who had graft nephrectomy (n = 3451) were younger and in better health condition than their non-nephrectomized counterparts. It is also noteworthy that despite adjustment for confounding factors and likelihood of undergoing transplantectomy, limitations intrinsic to retrospective registry studies remain. In addition, in patients with the failed allograft left *in situ*, it is not known whether low-dose maintenance immunosuppression might be independently associated with increased infectious- and cardiovascular disease-related mortality.

Of interest, in a large retrospective studies consisting of more than 19000 patients with graft failure, transplantectomy in patients reinitiating dialysis was found to be associated with increased mortality among those with early graft loss [graft survival < 12 mo, HR 1.13 (95%CI: 1.01-1.26)] whereas among those with late graft loss (graft survival > 12 mo), transplantectomy was associated with decreased mortality rates (0.89 95%CI: 0.83-0.95)[27]. It is speculated that the association of transplantectomy and mortality risk in patients with early graft loss was due to graft-related symptoms rather than the nephrectomy procedure *per se*. Further studies are needed to determine whether transplantectomy after late graft loss confers a survival advantage over leaving the graft *in situ*.

Transplantectomy: Potential adverse effects

Leaving a failed allograft *in situ* may avoid potential morbidity and mortality associated with the surgical procedure. In addition, in patients with residual kidney function, a retained graft may allow more liberal fluid intake and improve patients' quality of life. In most series reported, transplantectomy-associated morbidity

occurred in 17%-60% and mortality in 1.5% to 14% of patients[33]. The wide variation in the mortality rates reported may be due in part to the timing of surgery, the indication for graft nephrectomy, the patients' condition at the time of surgery, the surgical techniques, and individual centers' practice and experiences[31]. Symptomatic patients who need urgent transplantectomy are more likely to have worse outcomes than those undergoing elective transplantectomy. In one study, patients who underwent graft nephrectomy under suboptimal medical conditions (severe rejection or graft sepsis, hemorrhage from anastomotic suture line), a mortality rate of up to 39% has been reported[34].

Allograft nephrectomy has been shown to be associated with allosensitization, potentially resulting in prolonged wait times for a crossmatch negative kidney in re-allograft candidates. It is speculated that a retained allograft may serve as an antibody sponge, or alternatively, rapid immunosuppression weaning after transplantectomy may promote antibody-mediated allosensitization against the allograft. In one single-center study, *de novo* donor-specific antibodies (DSAs, tested *via* Luminex single-antigen assay) were detected as soon as five days after transplantectomy, suggesting that the antibodies were preformed[15]. Furthermore, the median fluorescence intensity (MFI) of alloantibodies remained stable or declined during follow up. It was hypothesized that if DSAs had appeared because of injury caused by graft nephrectomy, the MFI would have increased during follow up. Whether the detection of preformed DSAs after graft nephrectomy may have important implications in identifying unacceptable antigens for patients awaiting a repeat transplant remains to be studied.

Although post allograft nephrectomy rise in PRAs or DSAs may reflect preformed antibodies, it is also tempted to speculate that transplantectomy may stimulate pro-inflammatory cytokine production and upregulation of HLA alloantibodies. Alternatively, sensitization may occur due to the persistence of antigen-presenting cells or residual donor tissues and vessels[16].

The mechanism(s) or predominant mechanism of *de novo* development of anti-HLA alloantibodies after graft nephrectomy is currently not fully understood. Nonetheless, there has been ample literature showing that transplantectomy leads to an increase in class Ⅰ and class Ⅱ PRA, and DSA and non-DSAs to variable extent[13,16,35-38]. Whether immunosuppression weaning over a prolonged period after graft nephrectomy may reduce the risk of *de novo* anti-HLA alloantibodies development is unknown and warrants further exploration. Prospective studies to assess the potential mechanism(s) of allosensitization after transplantectomy and the impact of such procedure on graft and patient survival as well as on acute rejection rates after a repeat transplant are needed.

Impact of transplantectomy on a repeat transplant

The literature on the impact of transplantectomy on the outcomes of retransplantation have yielded variable and even contradictory results. Selected studies are discussed.

Studies indicating an adverse impact of transplantectomy on various clinical outcomes of a repeat transplant: Early single-center study demonstrated that transplantectomy was associated with a significant increase in PRA levels and a higher incidence of delayed graft function in a repeat transplant[39]. A trend for reduced graft survival was observed among patients whose first grafts failed within the first post-transplant year. However, transplant nephrectomy had no impact on the incidence of acute rejection or renal function of a repeat graft at 3-year follow-up.

In a retrospective study consisting of 192 recipients of a reallograft transplant, nephrectomy of the primary failed graft was shown to have an adverse impact on reallograft transplantation (P = 0.0003)[40]. Multivariate analysis demonstrated a significant relationship between survival of the primary allograft and repeat transplant outcomes. Subgroup analysis performed in patients whose graft functioned more than 6 mo (n = 90) similarly demonstrated that nephrectomy of the failed graft is a risk factor for worse retransplantation outcomes. Other identified risk factors included advanced donor age, longer time interval from transplantectomy to reallograft transplantation, and the lack of induction with Minnesota antilymphocyte globulin.

In a retrospective study comprising 121 patients who had a nephrectomy and 45 who did not undergo nephrectomy prior to repeat transplantation, pre-transplant graft nephrectomy and panel reactive antibody levels greater than 70% were found to be independent risk factors for graft failure after a repeat transplant[41]. Subgroup analysis showed that pretransplant graft nephrectomy adversely affected survival of a subsequent graft among high risk patients defined as those with multiple transplants (≥ 2 transplants) and those who received an allograft from an older donor (> 65 years of age), as well as among European Senior Program patients. However, in the subgroup of patients without "high risk" factors, nephrectomy of a previous graft had no impact on delayed graft function, or graft or patient survival rates after a repeat transplant. Nonetheless, pretransplant nephrectomy was associated with increased rejection rates presumably due to elevated PRA levels.

Studies suggesting a neutral impact of transplantectomy on various outcomes of a repeat transplant: In a retrospective analysis to evaluate graft survival in patients who underwent transplantectomy prior to reallograft transplantation (n = 68) compared with those who did not (n = 21), nephrectomy of a failed graft was found to have no significant impact on the survival

Table 3 Transplantectomy: Potential risks and benefits and impact on a repeat transplant

	Comments
Potential benefits	
A failing graft is a focus of a chronic inflammatory state	
May reduce mortality rates	Variable results, further studies are needed
Potential adverse effects	
Residual kidney function may allow less stringent fluid restriction	
Surgery-related morbidity and mortality	Morbidity 17%-60% in most series reported Mortality 1.5%-14% in most series reported
Allosensitization and the potential for future prolonged wait-times for a compatible crossmatch kidney	
Impact on a repeat transplant	
Mixed reports due to potential confounding factors	
Differences among studies in:	
Immunosuppression withdrawal protocols	
Recipient and donor demographics	
Era of transplantation	
Indications for transplantectomy	
Time on dialysis prior to a repeat transplant	
Causes of prior graft loss	
Allosensitization associated with blood transfusion	
Pre-existing DSA with or without complement-fixing DSA (see text)	
HLA matching of subsequent graft	
Donor type (living *vs* deceased)	
Others	

DSA: Donor-specific antibody.

of a future allograft[42]. Five-year actuarial patient survival were 94.1% and 87.5%, respectively (P = 0.69). PRA levels at the time of retransplantation were comparable between the two groups (37% *vs* 29%, respectively). Multivariate analysis showed a negative impact of PRA levels on graft survival independent of transplantectomy (P = 0.04).

One single-center retrospective study demonstrated that dialysis time was significantly longer in patients who had a graft nephrectomy than those who did not, presumably due to higher PRA levels in the nephrectomy group, making it difficult to obtain a negative crossmatch donor kidney. Nonetheless, acute rejection episodes and one-, five-, and ten-year graft survival rates were not different between the nephrectomy and no nephrectomy group[43]. Univariate analysis demonstrated that PRA levels and the number of acute rejection episodes had no significant impact on graft or patient survival, whether or not the patient had transplantectomy (P = 0.3 for both).

Differential impact of transplantectomy on the outcomes of a future allograft: Retrospective study using the USRDS database (n = 19107 patients returning to dialysis after first graft failure) demonstrated that transplantectomy after early graft loss (graft survival of less than twelve months) was associated with a lower risk of repeat graft failure, whereas transplantectomy for late graft loss (graft survival of $\geqslant$ 12 mo) may be deleterious to repeat transplant outcomes[27]. However, further analysis demonstrated that the protective effect of transplantectomy among those with early graft loss was due to a decrease in death with a functioning graft rather than an improvement in death-censored graft survival. It is speculated that there is a complex association between a retained failed graft and cardiovascular disease. In contrast to early graft loss, leaving the graft *in situ* in patients with late graft loss was shown have some protective effect on a repeat transplant, possibly related to development of tolerance and acceptance of a repeat transplant in the presence of donor antigen. Alternatively, it is suggested that if symptomatic immunological responses prompted a transplantectomy, then primary graft nephrectomy is simply a marker of high immunological risk for repeat transplant failure.

The potential risks and benefits of nephrectomy of a failed graft and its impact on a repeat transplant are summarized in Table 3.

Transplantectomy in patients with graft loss due to BK nephropathy: While some centers advocate graft nephrectomy prior to repeat transplant in patients with graft loss due to BK nephropathy (BKN), re-allograft transplant can be safely performed without original allograft nephrectomy but preferably following BK viral clearance[44]. Nonetheless, successful re-allograft transplant in the setting of severe viremia without concomitant nephrectomy of the allograft in a patient with graft failure due to BKN can be achieved. The patient is a 65-year-old woman who underwent urgent combined liver and repeat kidney allograft transplant due to fulminant hepatic failure and kidney graft failure due to BKN. She received no induction therapy and was maintained on low-dose tacrolimus and prednisone dual therapy. At the time of transplant, plasma BK PCR was 946000 copies/mL. Three months after transplant

plasma BK was undetectable and remained undetectable at 15 mo follow-up (unpublished observation).

Impact of transplantectomy on future retransplantation: The authors' perspectives

The variable and even conflicting results on the impact of transplantectomy on future reallograft transplantation may reflect a multitude of potential contributing factors including but not limited to institution dependent practice on indications for nephrectomy following a failed graft, differences in study design and immunosuppressive withdrawal protocols, donor and recipient demographics, recipient comorbid conditions, era of transplantation, time on dialysis prior to a repeat transplant, the causes of prior graft loss, donor type (living *vs* deceased), quality and HLA-matching of subsequent allograft, alllosensitization associated with blood transfusion, and pre-existing DSA with or without complement-fixing DSA at the time of transplantation, among others. Recent studies have shown that DSA with the ability to bind to C1q and activate complement are associated with greater risk of acute rejection and graft loss than non-complement fixing DSA[45].

While it remains unclear whether transplantectomy after late graft failure has a salutary or harmful effect on a repeat transplant, graft intolerance syndrome refractory to medical treatment is an indication for transplantectomy. In patients with multiple retained failed allografts, graft nephrectomy prior to retransplantation may also be inevitable. Monitoring PRA levels and HLA class Ⅰ/Ⅱ alloantibodies (using Luminex single-antigen assays) prior to and after graft nephrectomy as well as before retransplantation may be invaluable in guiding immunosuppression in re-allograft transplant recipients. In recent years various desensitization protocols have allowed highly sensitized patients to undergo successful retransplantation. Although no consensus exists, graft nephrectomy in patients with erythropoietin resistance and refractory anemia or hypoalbuminemia attributed to the failed allograft may be justifiable. Nonetheless, the decision to perform transplantectomy should be individualized. Effort to reduce cardiovascular and infectious complications undoubtedly improves clinical outcomes after reallograft transplantation whether or not nephrectomy is performed.

MANAGEMENT OF PATIENTS WITH A FAILED KIDNEY TRANSPLANT: THE AUTHORS'OPINION

Clinical studies to support or refute early *vs* late reinitiation of dialysis in patients with a failed kidney transplant are currently lacking. In the authors' opinion, reinitiation of dialysis should not be based solely on an absolute level of residual kidney function. Nonetheless, dialysis reinitiation when eGFR reaches 6-9 mL/min or less seems reasonable. In patients with higher level of residual kidney function, dialysis reinitiation should be based on clinical or laboratory parameters or both. Similar to the nontransplant settings, clinical indications may include symptomatic uremia, volume overload or hyperkalemia refractory to medical treatment, or malnourishment among others. In patients with a failed transplant and significant comorbid conditions such as long-standing diabetes with its associated micro- and macrovascular complications, or infectious or urological complications, weaning of immunosuppression and early return to dialysis seem justifiable.

Although evidence-based recommendations are lacking, continuation of low-dose immunosuppression seems appropriate in pre-dialysis patients and in those with symptomatic rejection to serve as a bridge to allograft nephrectomy. Maintenance low-dose immunosuppression may also be beneficial in patients with anticipated living donor re-allograft transplant or those with residual urine output greater than 0.5 to 1 liter a day. Nevertheless, in the latter group, immunosuppression withdrawal should be considered in high risk patients or those with significant comorbid conditions. These include older age, obesity, diabetes mellitus, neurogenic bladder, recurrent episodes of urinary tract infections or urosepsis, or history of cancers, among others. Proposed algorithm for the management of immunosuppression after allograft loss is shown in Figure 1. Although immunosuppression withdrawal protocols differ among centers, most clinicians advocate immediate discontinuation of antimetabolite (mycophenolate mofetil/mycophenolic acid or azathioprine). Cyclosporine or tacrolimus is generally weaned over several weeks and prednisone over three to six months. At the authors' institution, antimetabolite is discontinued upon return to dialysis, calcineurin inhibitors are weaned over four to six weeks, and prednisone dose is decreased by 1 mg/month until discontinued. Proposed immunosuppression weaning protocols are shown in Table 4.

Transplantectomy is usually performed and immunosuppression rapidly tapered when graft loss occurs within one year after transplant. Whether patients with early graft loss requiring transplantectomy (particularly those with a live donor and those anticipated to have a relatively short wait time after early relisting) benefit from continuation of immunosuppression to minimize allosensitization warrant further exploration. In addition, the duration and intensity of maintenance immunosuppression remain to be defined. At the authors' institution, the graft is usually left in place when graft loss occurs more than one year after transplant. Transplantectomy is generally performed in patients with graft intolerance syndrome or those requiring space for retransplantation. In patients with clinical signs or symptoms suggestive of a chronic inflammatory state, transplantectomy may be considered at the discretion of the treating physician. More importantly, community nephrologists should remain vigilant to the early

Table 4 Suggested immunosuppression withdrawal protocols based on maintenance therapy

CNI + antimetabolite[a] + prednisone	CNI + mTOR inh + prednisone	mTOR inh + prednisone
Discontinue antimetabolite at initiation of dialysis	Discontinue mTOR inh at initiation of dialysis	Taper mTOR inh over 4-6 wk[b]
Taper CNI over 4-6 wk[b]	Taper CNI over 4-6 wk[b]	Maintain same steroid dose at initiation of dialysis x 2-4 wk, then taper by 1 mg/mo (starting from 5 mg daily) until off
Maintain same steroid dose at initiation of dialysis x 2-4 wk, then taper by 1 mg/mo (starting from 5 mg daily) until off	Maintain same steroid dose at initiation of dialysis x 2-4 wk, then taper by 1 mg/mo (starting from 5 mg daily) until off	

[a]Mycophenolate Mofetil (Cellcept®) or Mycophenolic Acid (Myfortic®) or Azathioprine (Imuran®); [b]Taper can be done over a shorter period in slow chronic progressive graft failure but over a longer period when graft failure occurred following recent acute rejection episodes. CNI: Calcineurin inhibitor; mTOR inh: Mammalian target of rapamycin inhibitor.

Table 5 Absolute and relative indications for transplantectomy

Absolute indications (commonly accepted)	Relative indications (controversial)
Primary nonfunction	The presence of hematologic or biochemical markers of the chronic inflammatory state
Hyperacute rejection	Erythropoietin resistance anemia
Early recalcitrant acute rejection	Elevated ferritin level
Early graft loss (generally defined as graft loss within the first year)	Elevated C reactive protein
Arterial or venous thrombosis	Elevated erythrocyte sedimentation rate
Graft intolerance syndrome	Low prealbumin/albumin
Recurrent urinary tract infections or sepsis/urosepsis	Graft loss due to BK nephropathy and high level BK viremia (see text)
Multiple retained failed transplants prior to a repeat transplant	

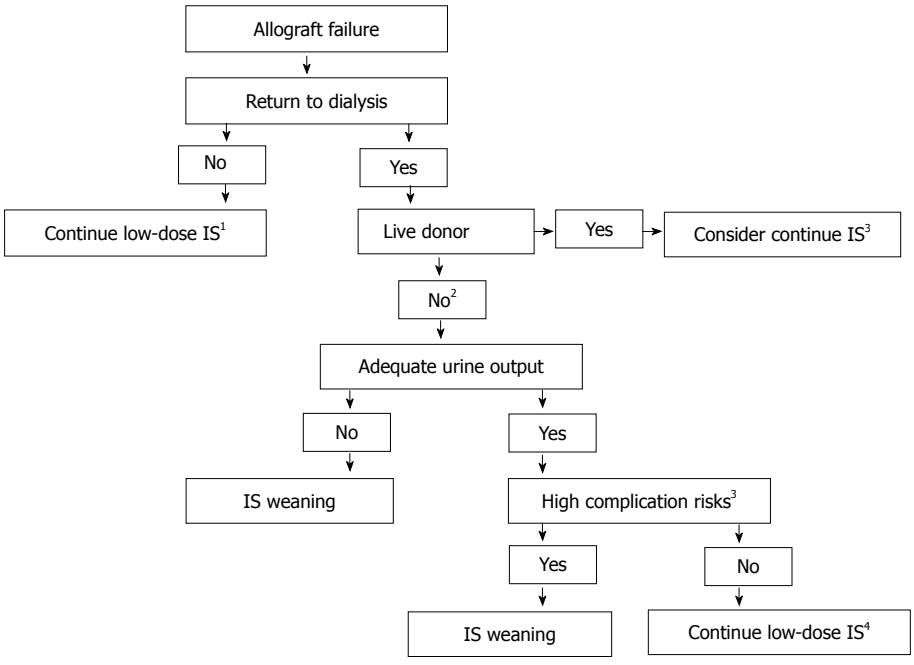

Figure 1 Suggested algorithm for the management of immunosuppression after allograft failure. [1]Continue antimetabolite and low-dose prednisone (usually 5 mg daily), calcineurin inhibitor dose reduction (or mTOR inh dose reduction if used as based-therapy); [2]No live donor or not a re-allograft candidate; [3]See text; [4]Usually prednisone 5 mg daily ± low-dose calcineurin inhibitor (or low-dose mTOR inh if used as based-therapy). IS: Immunosuppression; mTOR inh: Mammalian target of rapamycin inhibitor; ± with or without.

recognition of signs and symptoms of an infected or acutely rejecting allograft for early medical treatment and prevention of emergent transplantectomy given the increased morbidity and mortality associated with the latter. In patients with graft failure due to recent episodes of late acute rejection, gradual weaning of immunosupression is advisable to prevent graft intolerance syndrome and obviate the need to perform urgent transplantectomy. In patients with graft loss due to BK nephropathy, repeat transplant can be safely

performed without prior graft nephrectomy but preferably following BK viral clearance. Suggested absolute and relative indications for graft nephrectomy are shown in Table 5.

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REVIEW

Novel biomarkers of acute kidney injury: Evaluation and evidence in urologic surgery

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Abstract

Patients undergoing urologic surgery are at risk of acute kidney injury (AKI) and consequently long-term deterioration in renal function. AKI is further associated with significantly higher odds of perioperative complications, prolonged hospital stay, higher mortality and costs. Therefore, better awareness and detection of AKI, as well as identification of AKI determinants in the urological surgery setting is warranted to pre-empt and mitigate further deterioration of renal function in patients at special risk. New consensus criteria provide precise definitions of diagnosis and description of the severity of AKI. However, they rely on serum creatinine (SCr), which is known to be an inaccurate marker of early changes in renal function. Therefore, several new urinary and serum biomarkers promise to address the gap associated with the use of SCr. Novel biomarkers may complement SCr measurement or most likely improve the diagnostic accuracy of AKI when used in combinations. However, novel biomarkers have to prove their clinical applicability, accuracy, and cost effectiveness prior to implementation into clinical practice. Most preferably, novel biomarkers should help to positively improve a patient's long-term renal functional outcomes. The purpose of this review is to discuss currently available biomarkers and to review their clinical evidence within urologic surgery settings.

Key words: Acute kidney injury; Urology; Outcome; Renal function; Biomarker; Surgery

Core tip: Patients undergoing renal surgery represent a unique population at risk of acute kidney injury (AKI). AKI is known to be associated with adverse perioperative

outcomes. Therefore, efforts are warranted to promote awareness for AKI. Novel biomarkers promise to improve early and accurate detection of AKI, which may help to provide better patients' outcomes. However, these biomarkers still have to prove their clinical effectiveness prior to their implementation into urologic surgery settings.

Schmid M, Dalela D, Tahbaz R, Langetepe J, Randazzo M, Dahlem R, Fisch M, Trinh QD, Chun FKH. Novel biomarkers of acute kidney injury: Evaluation and evidence in urologic surgery. *World J Nephrol* 2015; 4(2): 160-168 Available from: URL: http://www.wjgnet.com/2220-6124/full/v4/i2/160.htm DOI: http://dx.doi.org/10.5527/wjn.v4.i2.160

INTRODUCTION

Urologic patients are at risk of acute kidney injury (AKI)[1-3]. A recent study evaluating procedure-dependent incidence of AKI in patients undergoing urologic surgery found that AKI was most frequently associated with partial/radical nephrectomy and nephroureterectomy (43.1%), transurethral resection of bladder tumor (15.3%), cystoprostatectomy (3.6%), ureteroscopic lithotripsy (3.6%), transurethral resection of the prostate (2.2%), radical prostatectomy (1.5%) and JJ-stent insertion (1.5%)[4].

Potentially reversible causes of AKI related to urologic surgery may be of pre- (*e.g.*, postoperative bleeding, sepsis) or post-renal (*e.g.*, urinary obstruction, dislocation of ureteric stent, anastomotic leak) origin. However, AKI observed in renal surgery patients is largely related to direct renal damage, resulting in a potentially irreversible decline of renal function. Although partial nephrectomy for renal cell carcinoma aims to preserve renal function, AKI following the direct removal of renal parenchyma and damage of the remaining tissue from hyperfiltration or ischemia is a commonly observed adverse event in these patients[5,6]. Besides the volume of preserved renal parenchyma, type and duration of ischemia during partial nephrectomy remain the most important modifiable factors for renal functional outcome[7].

Ischemic renal injury leads to a robust inflammatory response within the kidney, but also extrarenal manifestations have been observed[8-10]. Furthermore, the impact of renal ischemia-reperfusion injury on tumor propagation, malignant progression, and resistance to therapy is a topic of current investigations[11,12]. In addition, there is evidence demonstrating an impact of postoperative AKI on adverse surgical outcomes[13]. Indeed, AKI is associated with higher complication rates, longer hospital stays, increased mortality, and therefore greater utilization of health care resources and associated costs[14,15]. As patients undergoing urologic oncologic surgery often present with (unknown) pre-existing chronic kidney disease (CKD) at the time of surgery[16,17] an additional perioperative episode of AKI may contribute to worse renal recovery, long-term renal function deterioration and progression of CKD[3,18]. Consequently, urologists need to seek out the risk factors for AKI, identify the present signs and foresee its impact on the perioperative outcome of their patients[13].

While there are excellent reviews highlighting the most promising urinary and serum biomarkers of AKI[19,20], the purpose of this review is to discuss currently available biomarkers and to review their clinical evidence within urologic surgery settings.

DATA ACQUISITION

A non-systematic PubMed/Medline literature search was performed to identify original articles, review articles, and editorials evaluating AKI biomarkers in urologic surgery using the keywords "acute kidney injury, biomarkers, surgery, urology," of the last 3 years (May 30, 2001 to July 31, 2014). The literature search was restricted to English language and availability of full text.

RESULTS

Definition and diagnosis of acute kidney injury

Due to a lack of consensus on the definition of acute renal failure, a wide variation exists in estimates of disease prevalence and mortality[15]. Currently, "AKI" is defined as an abrupt deterioration of kidney function and includes a spectrum ranging from minor renal functional impairment to acute renal failure requiring renal replacement therapy. The Risk, Injury, Failure, Loss, and End-stage kidney disease (RIFLE) staging criteria was the first consensus definition for AKI[21], followed by the Acute Kidney Injury Network (AKIN) classification, which defines AKI as an absolute increase in the serum creatinine (SCr) concentration of $\geqslant$ 0.3 mg/dL from baseline within 48 h[22]. More recently, the Kidney Disease/Improving Global Outcomes (KDIGO) group revised the definition of AKI, retaining AKIN staging criteria by classifying patients according to changes in SCr and urine output[23]. RIFLE, AKIN and KDIGO definitions have emphasized on the non-negligible incidence of AKI and its long-term adverse outcomes[21-23].

Biomarkers of acute kidney injury

Serum creatinine: SCr, is the gold-standard marker for renal function. However, SCr concentrations can be affected by age, gender, and racial differences of body mass as well as dietary factors and volume status[24]. In general, equations that estimate renal function, such as the Modification of Diet in Renal Disease or the Chronic Kidney Disease Epidemiology Collaboration equations[25,26], attempt to overcome the relative inaccuracy of SCr by including these patient characteristics to estimate glomerular filtration rate

Table 1 Baseline reference values of novel biomarkers of acute kidney injury obtained from different studies

Biomarker	Injury	Source	Test	Unit	Healthy controls (range)
Cystatin C	Proximal tubule injury	Serum	Nephelometric immunoassay/ELISA	mg/L	0.53-0.95[38] 0. 85 ± 0.21[39]
		Urine	Nephelometric immunoassay/ELISA	mg/L	0.05[1]-0.28[37] 0.02-0.11[96]
NGAL	Ischemia and nephrotoxins	Serum	ELISA	ng/mL	86.3 ± 43.0 (men)[54] 88.9 ± 38.2 (women)[54] 56.71 ± 17.57[39] 1.7 ± 0.5[55] 0.4-100[56]
		Urine	ELISA	ng/mL	5.7-17.7[55] 11.94 ± 8.09[39] 0.8-28.9 (men)[96] 1.9-316.7 (women)[96]
KIM-1	Ischemia and nephrotoxins	Urine	ELISA	pg/mL	59-2146[70] 395.1 ± 398.8[39] 31.0-1000.0[56] 31.0-1736.5[96]
IL-18	Toxic, delayed graft function	Urine	ELISA	pg/mL	1.4-1.8[80] 3.0-108.6[96] 6.2-311.1[96]
L-FABP	Ischemia and nephrotoxins	Urine	ELISA	ng/mL μg/gCr	3-400[83] 5.67 (2.74-8.21)[87]
NAG	Tubule injury	Urine	Colorimetry	U/g	0.75-0.90 U/g[95] 1.06 ± 0.1 U/g (children)[90]

[1]Lower reference values are not presented due to the detection limit of 0.05 mg/L. NGAL: Neutrophil gelatinase-associated lipocalin; KIM-1: Kidney injury molecule-1; IL-18: Interleukin-18; L-FABP: Liver-type fatty acid binding protein; NAG: N-acetyl-β-D-glucosaminidase; ELISA: Enzyme-linked immunosorbent assay; U: Unit.

(GFR)[27]. Nonetheless SCr is primarily a marker of glomerular function, and SCr-based measurements may be inaccurate in detecting an abrupt decline in renal function, as the functional reserve of the remaining healthy nephrons prevents a significant rise in SCr until 50% of nephrons are lost[28,29]. Furthermore, the early phase of AKI is accompanied with few symptoms or may even be asymptomatic. Thus, it is critical to note that even if the SCr-based estimation of renal function is "normal", loss of renal reserve may already have begun.

Consequently, recent research has focused on novel biomarkers that are directly related to the underlying renal injury and may diagnose AKI more expeditiously and accurately, while concurrently predicting its severity[30,31]. Most perioperative studies on AKI have been performed in the setting of cardiac surgery. However, as the awareness of AKI is increasing, other surgical specialties are evaluating this adverse outcome as well[32,33]. Additional biomarkers of AKI to rely on would be preferable especially in urologic high-risk patients (*e.g.*, renal surgery, pre-existing CKD). In fact, several promising serum and urinary biomarkers are now available including serum and urinary Cystatin C (sCysC and uCysC), neutrophil gelatinase-associated lipocalin (sNGAL and uNGAL), and urinary Kidney Injury Molecule 1 (uKIM-1), Interleukin-18 (uIL-18), Liver-type fatty acid binding protein (uL-FABP) and N-acetyl-β-D-glucosaminidase (uNAG)[34]. However, these biomarkers are still under investigation: baseline values are often obtained from healthy volunteers and optimal cut-off values to define AKI need to be determined (Table 1). Some of these biomarkers already demonstrated additional prognostic value in the urologic surgery setting (Table 2), whereas others have yet to prove their clinical utility.

Novel biomarkers of acute kidney injury

Serum and urinary cystatin C: CysC is a low-molecular weight protein that is freely filtered across the glomerular membrane and in consequence less reliant on age, sex, race and muscle mass, compared to SCr[35]. Moreover, although CyC is not normally detected in the urine, it has been found in the urine of patients with tubular disease, suggesting its putative role as a marker of renal tubular damage[36]. Nephelometric measurements of CysC have upper reference values of 0.28 mg/L[37] in the urine and range between 0.53-0.95 mg/L in the serum of healthy individuals[38,39].

CysC has been proposed as a complementary or possibly marker of baseline renal function[35,40]. Although sCysC measurement is currently 10 times more expensive than SCr, it is implemented in routine renal function measurement of pediatric patients and used to monitor kidney transplant patients[41-43]. Furthermore, there is evidence suggesting that an elevation of sCysC predates minor decreases in GFR 1 to 2 d prior to symptoms, SCr elevation and/or renal function decline[40,44,45]. Early elevations of uCysC levels were significant predictors of AKI after elective cardiac surgery[46], and are correlated

Table 2 Biomarkers of acute kidney injury evaluated within urologic surgery settings

Ref.	Biomarker	Source	Cohort	Surgical setting	Outcome	Comparison	Time
Langetepe *et al*[65]	CysC, NGAL, KIM-1 Cr	Urine Serum	31 RCC patients	PN, RN	Increased values of CysC, NGAL, KIM-1 NGAL significant correlation to Cr No advantage for earlier detection of renal injury	Pre-/postoperative	24 h after surgery
Sprenkle *et al*[63]	NGAL	Urine	PN: 88 patients, RN: 32 patients, thoracic surgery: 42 patients	PN, RN (warm or cold ischemia)	No association between postoperative NGAL and any AKI AKI was not significantly associated with increased NGAL in PN patients No correlation with ischemia time Patients with eGFR < 60 mL/min per 1.73 m^2 had higher NGAL postoperatively than those with an eGFR > 60 mL/min per 1.73 m^2	PN/RN /thoracic surgery patients	4, 8, 12, 24 h post surgery
Parekh *et al*[62]	Cr, NGAL, CysC NGAL, LFABP, NAG, KIM-1, IL-18	Serum Urine, (renal biopsy)	20 patients with renal mass	PN (warm or cold ischemia)	Cr was significantly increased at 24 h CysC was not significantly changed at 2 or 24 h Significant increases serum NGAL at 2 and 24 h, increase of NGAL with increased ischemia time, no relation to peak Cr or morphology-score Early increases of L-FABP Early increase of NAG Increased NGAL at all times KIM-1maximally increased at 24 h IL-18 was increased at all time points	Correlation to renal biopsies (pre-, intra. postoperative)	2 or 24 h after surgery
Schmid *et al*[50]	Cr, CysC	Serum	31 RCC patients	PN, RN	Postoperative Cysc and Cr elevations similarly predict renal function deterioration CysC-based GFR appears superior to eGFR in "Cr-blind" area	Pre-/postoperative, 1 yr follow up	24 h, 1 yr after surgery
Xue *et al*[76]	Cr NGAL, KIM-1	Serum Urine	90 patients with obstructive uropathy	NA	KIM-1 and NGAL good accuracy for detecting AKI KIM-1 predicts the renal outcome 72 h postoperatively	Pre-/postoperative	4, 8, 12, 24, 48, 72 h after surgery
Cost *et al*[66]	NGAL	Urine (bladder and renal pelvis)	61 pediatric patients with ureteropelvic junction obstruction	Pyeloplasty	Significantly increased bladder NGAL Inverse correlation of bladder and renal pelvic NGAL levels with the differential renal function of the affected kidney	Healthy children	Intraoperative
Zekey *et al*[64]	Cr NGAL	Serum Urine	40 patients with kidney stones	SWL	No statistical Cr and urine NGAL levels	Before/after intervention	day 1, 2, 7 after intervention
Fahmy *et al*[74]	KIM-1, NAG	Urine	60 patients with kidney stones (50 SWL, 10 URS)	SWL, URS	KIM-1 values were increased in patients with kidney stones when compared with volunteers KIM-1 and NAG levels significantly increased post-SWL Poor kidney function was significantly associated with increased KIM-1 and NAG baseline and post-SWL No significant change in urinary KIM-1 and NAG concentrations before and after URS	Volunteers without kidney stones	2-3 h after intervention
Ng *et al*[82]	IL-18, NAG	Urine	206 patinets with renal stones	SWL	Increased IL-18 and NAG I slower shock wave delivery group	60 *vs* 120 shock waves/min	After intervention
Hatipoğlu *et al*[75]	KIM-1 (free radical production)	Urine	30 patients with kidney stones	SWL	Significant increase of KIM-1	Pre-/postoperative	2 h after intervention

PN: Partial nephrectomy; RN: Radical nephrectomy; NGAL: Neutrophil gelatinase-associated lipocalin; KIM-1: Kidney injury molecule-1; URS: Ureterorenoscopy; SWL: Shockwave lithotripsy; Cr: Creatinine; CysC: Cystatin C; LFABP: Liver fatty acid–binding protein; NAG: Nacetyl-b-D-glucosaminidase; eGFR: Estimated glomerular filtration rate; RCC: Renal cell carcinoma; NA: Not available.

with the need for renal replacement therapy in patients with acute tubular necrosis[47]. However, other studies were not able to corroborate these findings[37] and suggest that sCysC is unreliable in the context of

postrenal obstruction[48]. Yet, uCysC was shown to be independently associated with mortality in critically ill patients with AKI[49].

In patients undergoing partial or radical nephrectomy, elevations of both SCr and sCysC on postoperative day one predicted renal function deterioration one year after surgery, while sCysC correlated better to renal function estimates compared to SCr in the "SCr-blind" area[50].

Serum and urinary NGAL: Production of NGAL, a lipocalin protein involved in innate immunity by binding iron to limit bacterial growth[51], is upregulated following renal injury, and consequently detectable in serum and urine hours prior to functional changes[52,53]. sNGAL values in healthy individuals should be around 86.3 ng/mL in men and 88.9 ng/mL in women[39,54-56], but may increase > 10-fold in serum and > 100-fold in urine following an acute injury[57].

A meta-analysis of 19 observational studies including 2500 patients was performed to estimate the diagnostic and prognostic accuracy of NGAL for AKI detection and to establish the role of urinary and serum NGAL in the context of AKI[58]. Xin *et al*[59] showed that for patients undergoing cardiac surgery, an increase of sNGAL was not temporally different to the rise of SCr within 48 h after AKI, however uNGAL (and IL-18) significantly increased to a peak of 400 ng/mL within 2-4 h of AKI.

Induction of unilateral renal ischemia in animal models results in physiological changes of the ischemic and contralateral kidney, with a corresponding increase of uNGAL and decrease of renal function[60,61]. Parekh *et al*[62] studied the renal response to > 30 min of warm or cold clamp ischemia in patients undergoing partial nephrectomy and observed significant increases in sNGAL 2 and 24 h after surgery. While levels of all urinary biomarkers studied (NGAL, KIM-1, IL-18, NAG, L-FABP) increased 2 and/or 24 h after surgery, sCysC levels did not change significantly (SWL)[62]. Conversely, Sprenkle *et al*[63] did not observe increased uNGAL in partial nephrectomy patients within 24 h after surgery. Accordingly, no statistically significant change of uNGAL levels was observed in 40 nephrolithiasis patients treated with shock-wave lithotripsy[64]. Yet, our own data showed increased levels of uNGAL, KIM-1 and uCysC in 31 patients 24 h after partial or radical nephrectomy, but only uNGAL was correlated with SCr-based measurement of renal function[65]. Increased levels of uNGAL have also been obtained from bladder urine in children with ureteropelvic junction obstruction undergoing unilateral pyeloplasty[66]. Finally, uNGAL may serve as an early indicator for cisplatin nephrotoxicity[67], which may be useful for patients with muscle-invasive bladder undergoing neoadjuvant chemotherapy prior to radical cystectomy.

Urinary KIM-1: KIM-1 is a transmembrane glycoprotein undetectable in healthy kidney tissue, but it represents the most upregulated protein in proximal tubular cells after ischemic or nephrotoxic injury[68]. KIM-1 can be immediately detected in the urine following injury[69,70]. A strong correlation between immunohistochemical KIM-1 expression and tubular cell injury was shown in renal allograft biopsies of patients with active antibody-mediated transplant rejection[71], suggesting that KIM-1 is a reliable marker for tubular epithelial injury prior to elevated blood biochemical indexes and morphological changes. In addition, children with AKI following cardiac surgery demonstrated elevated uKIM-1 levels 12 h after surgery[72]. KIM-1 is measured in the urine by means of enzyme-linked immunosorbent assay, with normal values ranging between 59-2146 pg/mL in the healthy population[70,73].

A significant increase of uKIM-1 levels 2-3 h after SWL treatment[74,75] suggests direct ischemic damage and the release of free radicals. Both uKIM-1 and uNGAL demonstrated accuracy in detecting AKI among patients undergoing surgery for obstructive nephropathy; furthermore they might play a potential role in predicting postoperative renal recovery and long-term renal outcome[76,77].

Urinary IL-18: IL-18 is a pro-inflammatory cytokine that is activated in proximal tubule cells and excreted in the urine following a kidney injury. Increased expression of *IL-18* genes has been demonstrated after renal ischemic injury[78]. Animal models revealed that *IL-18* stimulates a positive feedback *via* IL-18 receptor during renal obstruction, which further stimulates IL-18 production and gene expression[79].

Initially described in the pediatric cardiac surgery setting, IL-18 the urine increased 6 h in after surgery, whereas SCr did not reveal AKI until 48-72 h after surgery[55]. Moreover, uIL-18 also increased significantly in adults and peaked at 600 pg/mL within 2-4 h after AKI[59]. Another study demonstrated an increase from 1.4 pg/mL to a peak of 234 pg/mL (about 25-fold) 12 h after cardiopulmonary bypass surgery in patients presenting AKI[55]. In patients with respiratory distress syndrome experiencing AKI, median uIL-18 was 104 pg/mL (range: 0 to 955 pg/mL), compared to 0 (range: 0 to 173 pg/mL) in control patients; IL-18 levels of > 100 pg/mL were associated with a 6.5-fold higher risk of AKI 24 h after hospitalization. Furthermore, higher level of uIL-18 (and serum IL-18) in ICU patients developing (dialysis-dependent) AKI was independently associated with mortality[80,81].

Finally, patients undergoing SWL showed a significant increase of uIL-18 (and uNAG) when treated with slower shock waves[82].

Urinary L-FABP: L-FABP is a 14-kDa protein expressed in proximal tubular epithelial cells. The urine of healthy individuals contains approximately 16 ng/mL L-FABP[83]. The gene responsible for L-FABP is associated with hypoxic stress. L-FABP binds unsaturated fatty acids and lipid peroxidation products during tissue injury from hypoxia[84]. Urinary excretion of L-FABP thus reflects stress within proximal tubular epithelial cells,

and its correlation with renal function deterioration has been reported with AKI following contrast nephropathy and cardiac surgery[83,85]. Although uL-FABP may be a promising biomarker for early detection of AKI, as demonstrated in an animal model of ischemia-reperfusion[86], or for prediction of the need for dialysis and in-hospital mortality[87], its value in urologic surgery warrants further investigation.

NAG: NAG, a tubular lysosomal brush border enzyme, is released into the urine following (reversible) renal proximal tubule injury. NAG is elevated in the urine of children with chronic renal obstruction[88], regardless of the grade of hydronephrosis[89], and following AKI[90,91]. Rat models with isolated blunt renal trauma showed increased uNAG in the early stage after injury[92]. However, the clinical utility of NAG remains limited as the urinary excretion of this enzyme is also increased in glomerular diseases such as diabetic nephropathy[93]. The combination of urinary L-FABP (high sensitivity) and uNAG or uNGAL (high specificity) may enhance the detection of early postoperative AKI in patients undergoing cardiac surgery[94,95].

CONCLUSION

A plethora of novel biomarkers for AKI have recently been described. Whereas sCysC, uCysC, sNGAL, uNGAL, uKIM-1 and uNAG have shown promise, we did not find convincing evidence for uIL-18 and uL-FABP. However, from a clinical perspective current use of these biomarkers in the urologic surgery setting is rare. Notable reasons behind this are the limited availability of assays, additional cost and the (currently) poor sensitivity and specificity demonstrated in urologic patients. Consequently, until now none of these biomarkers has been able to allow early detection of AKI in a way that would positively improve a patient's long-term outcomes and justify a regular implementation in specific urologic surgery settings. SCr remains the mainstay for evaluation of kidney function in urologic surgical patients. However, novel biomarkers may complement SCr measurement to indicate the need for urgent drainage or initiation of renoprotective measures. Moreover, it is likely that a combined use of these novel biomarkers will be needed to improve the diagnostic accuracy of AKI. Multiplex assays for simultaneous quantification of several biomarkers promise to overcome the flaws of single marker use and demonstrate the advantage of combinations reflecting different aspects of renal injury[96]. While these assays are currently more expensive compared to traditional SCr measurement, the hope is that the incremental diagnostic accuracy would offset costs by mitigating costly associated complications of AKI.

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REVIEW

Complement involvement in kidney diseases: From physiopathology to therapeutical targeting

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Abstract

Complement cascade is involved in several renal diseases and in renal transplantation. The different components of the complement cascade might represent an optimal target for innovative therapies. In the first section of the paper the authors review the physiopathology of complement involvement in renal diseases and transplantation. In some cases this led to a reclassification of renal diseases moving from a histopathological to a physiopathological classification. The principal issues afforded are: renal diseases with complement over activation, renal diseases with complement dysregulation, progression of renal diseases and renal transplantation. In the second section the authors discuss the several complement components that could represent a therapeutic target. Even if only the anti C5 monoclonal antibody is on the market, many targets as C1, C3, C5a and C5aR are the object of national or international trials. In addition, many molecules proved to be effective *in vitro* or in preclinical trials and are waiting to move to human trials in the future.

Key words: Complement cascade; Complement and glomerulopathies; Eculizumab; Targeting complement; Complement and renal transplantation

Core tip: Our therapeutical armamentarium is to date limited in many kidney diseases and in several aspects of renal transplantation. The findings that complement cascade is involved in many kidney diseases and in renal transplantation offer the availability of new therapeutical targets basing on the pathogenesis. The anti C5 monoclonal antibody, eculizumab, is now used to treat the atypical hemolytic uremic syndrome (aHUS), but 24 trials are ongoing in different renal diseases and in renal transplantation. Other targets as C1, C3, C5a, and C5aR are innovative treatments for diseases as aHUS, membranoproliferative glomerulonephritis, ischemia-reperfusion injury, and objects of ongoing trials.

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INTRODUCTION

The complement system serves as first line defense against invading pathogens and is a component of the innate immune system[1,2]. The complement system represents a link between the innate and adaptive immunity. In addition, several studies examined the cross-talk between complement and toll-like receptors, another component of the innate immune system and the complement system[3]. The complement system is composed of three distinct activation pathways: classic pathway (CP), alternative pathway (AP), and mannose-binding lectin pathway (LP). Any pathway activates the complement cascade generating C3-convertase which cleaves C3 into C3a and C3b[4]. In normal conditions a small amount of C3-convertase is activated by the AP and is necessary to have regulators to prevent complement attack on healthy self cells[5] (Figure 1). This regulation is provided by a combination of plasma and cell surface inhibitory proteins. Fluid phase regulators include C1-inhibitor (C1-INH) that prevents the auto activation of the initial complex of the CP, the decay-accelerating factor that binds to C4b, and acts as co-factor for factor I (CFI) cleavage of C4b opsonin. Another regulator for AP is factor H (CFH), which also acts as a co-factor for CFI in the inactivation of C3b. Other regulators are Clusterin and Vitronectin that inhibit the insertion of terminal complexes into the cell membranes. Finally, carboxypeptidase N acts as anaphylatoxin inhibitor.

Finally, cell surface regulatory proteins, including regulators complement receptor 1 (CR1) (C3b receptor), membrane co-factor protein (MCP, CD46), and decay-accelerating factor (DAF, CD55) act inhibiting the C3 and C5 convertases activity. Complement-mediated injury is the result of the prevalence of the activating factor over the complement regulators[6,7].

Independently from complement involvement, serum complements levels may are low or normal. The pathogenesis of hypocomplementemia is related to the high consumption rate due to immune deposits, but other factors are the presence of hereditary complement deficiency and the presence of circulating factors that promote complement activation and consumption. When the CP is activated both C3 and C4 may be low. C3 levels low alone may be expression of the activation of the AP.

In kidney diseases and in kidney transplantation, the complement cascade is frequently involved and might represent a first line therapeutic strategy.

RESEARCH

We have analyzed the available data on complement and renal diseases and renal transplantation by careful revision of the currently available data. Literature research was performed using PubMed (NCBI/NIH) under employment of the search terms "complement cascade", "complement and glomerulopathies", "dense deposit disease", "membranoproliferative glomerulonephritis", "C3 glomerulonephritis", "complement and renal transplantation", "targeting complement", "eculizumab". Studies currently under way were sought for in "clinicaltrials.gov" and the European EUDRACT register. The papers published in the last three years on international journals on transplantation and kidney disease were carefully examined. Almost 160 papers were selected for this review.

PHYSIOPATHOLOGY OF COMPLEMENT INVOLVEMENT IN KIDNEY DISEASES

There are 2 broad categories of kidney diseases in which the complement system has a pathogenic role. The first is associated with complement over activation, and the second with complement dysregulation. Moreover the complement system is frequently involved in the kidney injuries after kidney transplantation[8] and in the progression of kidney diseases. The principal complement abnormalities leading to renal diseases are summarized in Table 1[9].

OVERACTIVATION OF COMPLEMENT

Lupus nephritis

Deficiencies in the early components of the CP including C1q, C2 and C4 are associated with the development of systemic lupus erythematosus (SLE)[10]. Familial C1q deficiency has been found to be a relevant genetic risk factor for the development of SLE, indeed C1q deficiency results in impaired phagocytosis[11]. The apoptotic cells are not immunologically benign and the reduced phagocytic clearance of these cells increases the likelihood that auto antigens are presented to lymphocytes and induce the development of the autoimmunity. Thirty percent of patients with lupus nephritis have C1q antibodies[12]. The products of C5 metabolism may also contribute directly to glomerular injury and in studies of murine models of lupus nephritis, a monoclonal antibody that blocked C5 cleavage significantly ameliorated the glomerulonephritis and prolonged survival[13].

Anti glomerular basement membrane glomerulonephritis

The complement system is involved in anti glomerular basement membrane glomerulonephritis either through CP and enhancing the inflammatory response through C5a activation[14] and/or cell lysis effect of C5b-9[15].

Antineutrophil cytoplasmic antibody associated vasculitis

Several authors[16,17] documented the involvement of complement in antineutrophil cytoplasmic antibody (ANCA) glomerulonephritis. Chen *et al*[16] documented C3 deposits in the glomeruli of patients with high levels of proteinuria and poor renal function. C5-9, C3d and complement factor B (CFB) were also reported in

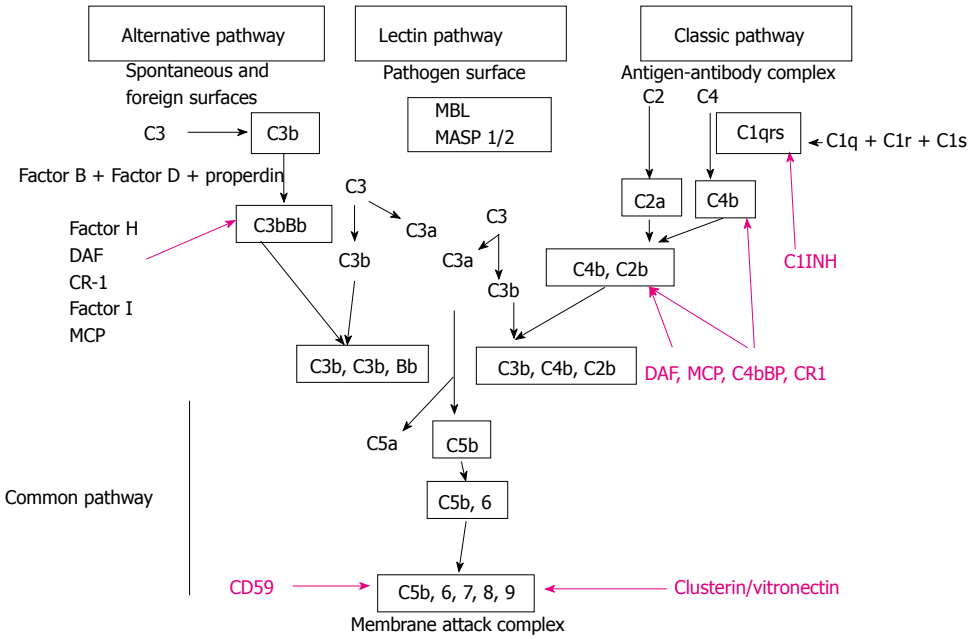

Figure 1 Representation of the classical, lectin and alternative pathways of complement activation, including regulatory molecules (purple). MBL: Mannose binding lectin; MASP 1/2: Mannan-binding lectin-associated serine protease-1; C1INH: C1 inhibitor; DAF: Decay accelerating factor; CR-1: Complement receptor 1; MCP: Membrane co-factor protein.

Table 1 Representative abnormalities in complement leading to renal disease

Components/related molecules	Diseases
Complement C3	C3 glomerulopathy (DDD), aHUS
Factor H	C3 glomerulopathy (DDD/C3GN), aHUS
Factor I	C3 glomerulopathy (C3GN), aHUS
MCP	aHUS
Factor B	aHUS
CFHR5	Familial C3 glomerulopathy (CFHR5 nepropathy)
CFHR3-1	Familial C3 glomerulopathy
CFHR1/3	IgA nephropathy, aHUS
Factor B autoantibody	C3 glomerulopathy (DDD)
Factor H autoantibody	C3 glomerulopathy (DDD/C3GN)
Bb (activated factor B)	HUS, ANCA-associated vasculitis
C3Nef	C3 glomerulopathy (DDD, C3GN)
Soluble C5b-9	HUS, TTP, ANCA-associated vasculitis
C3a	ANCA-associated vasculitis, TTP
C5a	ANCA-associated vasculitis
C1q/C1qR	C1q nephropathy
Properdin	TI injury due to massive proteinuria
C5	ANCA-associated vasculitis
Factor B	ANCA-associated vasculitis
CRaR	TI inflammation, IRI
C5aR	IRI
Factor H	IRI
C5b-9	IRI
CD59	IRI

DDD: Dense deposit disease; aHUS: Atypical hemolytic uremic syndrome; C3GN: C3 glomerulonephritis; MCP: Membrane co-factor protein; CFHR5: Complement factor H-related protein; ANCA: Anti neutrophil cytoplasmic antibody; C3Nef: C3 nephritic factor; TTP: Thrombotic thrombocytopenic purpura; TI: Tubulo-interstitial; IRI: Ischemia-reperfusion-injury.

biopsies from patients with myeloperoxidase (MPO)-ANCA-associated pauci-immune glomerulonephritis. Xing *et al*[17], from the same group, observed that C4d was negative in biopsies of patients with MPO-ANCA glomerulonephritis. These studies suggest that this model of glomerulonephritis requires the activation of the AP, not the CP or the LP. Further studies in patients with active ANCA associated vasculitis documented high levels of C3a, C5a, soluble C5b-9 and Bb[18]. Recently other authors documented[19] that the C5a specific receptor

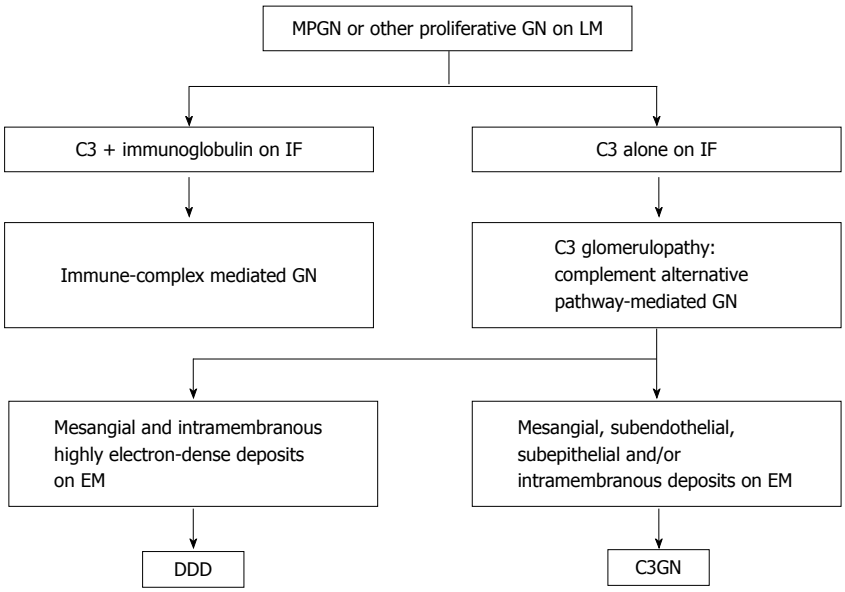

Figure 2 Reclassification of membrano-proliferative glomerulonephritis. C3GN: C3 glomerulonephritis; DDD: Dense deposit disease; EM: Electron microscopy; GN: Glomerulonephritis; IF: Immunofluorescence; LM: Light microscopy; MPGN: Membrano-proliferative glomerulonephritis.

(C5aR) expressed on neutrophils is involved in the pathogenesis of ANCA-induced glomerulonephritis (GN). Therefore targeting the C5a-C5aR receptor interaction in such patients might represent a therapeutical strategy[20]. A clinical trial to evaluate the safety and efficacy of an inhibitor of the C5a receptor (CCX168) is ongoing and an interim analysis reported promising results[21].

Membranous nephropathy

Several studies have identified that autologous antigens are the target of antibody response in idiopathic membranous nephropathy (MN). According to the latest studies[22] M-type phospholipase A2 receptor (PLA2R) located on podocytes has been identified as the target antigen in idiopathic MN. The predominant anti PLA2R IgG subclass activates the alternative or the mannose binding lectin (MBL) pathway[23]. This is confirmed by some studies documenting glomerular MBL and C4b deposition in MN[24,25].

In human secondary MN, C1q, C3, C4, CFB, MBL, and C5b-9 typically are present and co-deposited with IgG, suggesting that the LP and the AP could play the relevant role[26,27].

C1Q nephropathy

C1q nephropathy is characterized by the presence of conspicuous C1q immune deposits in glomeruli with no evidence of SLE MPGN type Ⅰ. C1q nephropathy is characterized by the C1q binding to poly-anionic substances (DNA, RNA, viral proteins) or to C1q receptors, according some authors C1q nephropathy has been thought to be a subgroup of primary focal segmental glomerular sclerosis[28].

IgA nephropathy

Two distinct mechanisms of complement activation are involved in IgA nephropathy. The AP is the key pathway in 75% of cases[29,30]. In 25% of biopsy specimens, the presence of glomerular IgA1 and C3 is associated with MBL and MBL-associated serine protease 1 (MASP-1) deposition. MBL binds to the abnormally galactosylated region of the IgA1 through its carbohydrate binding domain resulting in complement catabolism through the lectin binding pathway. The presence of MBL and MASP-1 is associated with disease severity and poor histological prognostic features[31].

Immune complexes-associated membranoproliferative glomerulonephritis

In the past on immunopathological basis the MPGN was classified, on the basis of immunopathological findings, into three subtypes: MPGN type Ⅰ, Ⅱ [also known as dense deposit disease (DDD), and Ⅲ]. Recently, the classification of MPGN has been completely reviewed by Bomback *et al*[32] on pathogenetic basis[33]. This led to a new understanding of the pathogenesis and to a reclassification of MPGN (Figure 2). The former MPGN type Ⅰ and Ⅲ belong to the chapter of complement over activation, while C3GN and the DDD will be described in the chapter of complement dysregulation.

In immune complex associated MPGN, the CP is activated by antibodies. Monoclonal antibodies or immune complexes precipitate catabolism, resulting in the chemo attraction of leukocytes and the direct cell injury by the MAC. The leukocyte generation of cytokines and proteases stimulate the mesangial cell proliferation and the matrix expansion[34].

DYSREGULATION OF COMPLEMENT

Atypical hemolytic uremic syndrome

The dysregulation of the AP cascade due to acquired or genetic factors leads to defective complement control that may cause a range of complement associated glomerulopathies (Figure 3). The better known among them is the atypical HUS (aHUS). aHUS classically is a triad of microangiopathic hemolytic anemia, acute kidney injury and thrombocytopenia caused by failure

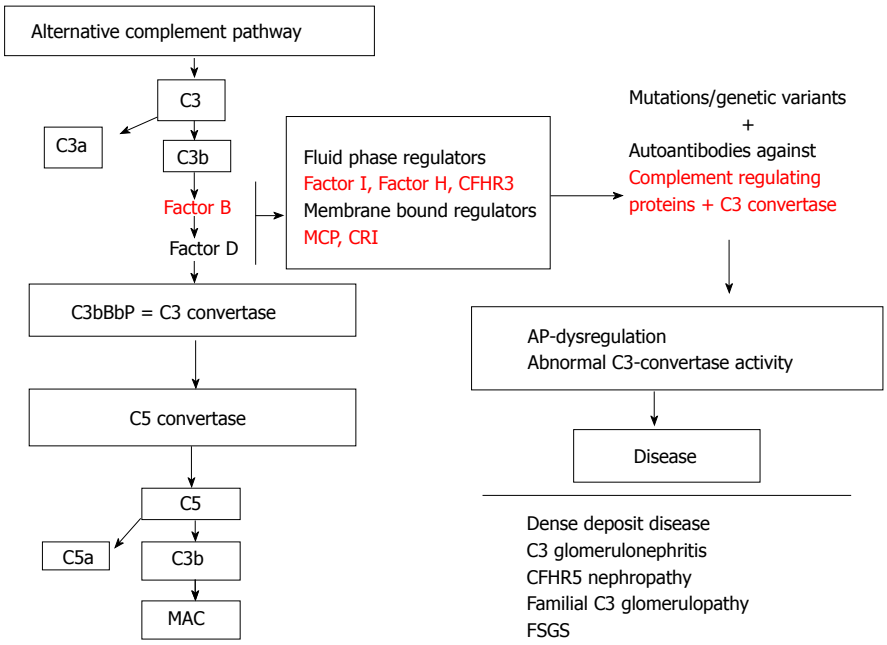

Figure 3 Dysregulation of the alternative complement cascade due to acquired or genetic factors leads to defective complement control causing a range of complement-associated glomerulopathies. CFHR3: Complement factor H-related protein 3; MCP: Membrane co-factor protein; CR1: Complement receptor 1; AP: Alternative pathway; CFHR5: Complement factor H-related protein 5; FSGS: Focal segments glomerular sclerosis; MAC: Membrane attack complex.

to regulate the alternative complement pathway. In over 60% of cases mutations have been identified in genes encoding complement regulatory proteins: CFH, CFI, MCP, thrombomodulin (THBD), and in genes encoding complement activators: CFB and C3. Complement cascade dysregulation causes a damage of endothelium leading to thrombosis and microangiopathic hemolytic anemia[35,36]. CFH mutations are observed in 25%-30% of patients with an aHUS[37]. Up to now, more than 80 mutations have been identified. Patients with aHUS and anti factor H antibodies have also been reported. These antibodies bind to short consensus repeats, thus reducing the CFH activity[38]. Reduction in MCP expression is reported in over 80% of cases with mutation in this gene[36,39]. Genetic disorders are rarely related to CFI[40]. *THBD* mutations with hyperactivity have been found in only 3%-5% of patients[41].

Recently, Noris *et al*[42] have documented that the classical HUS caused by Shiga toxin producing escherichia coli (STEC-HUS) and thrombotic thrombocytopenic purpura (TTP) are caused by inappropriate complement activation. Even if STEC-HUS, aHUS and TTP are all diseases of complement activation and recognize a common pathogenesis, we should remember that aHUS is linked to the complement dysregulation, while STEC-HUS and TTP are linked to the complement over activation and, on a pathogenetic basis, belong to the previous chapter.

In the HUS-SYNSORB Pk trial, children with STEC-HUS had increased plasma levels of Bb and C5-9 at the beginning of the study, which normalized after one month[43]. This suggests that patients with acute onset STEC-HUS have an activation of the AP in the acute phase of the disease, which normalizes within 1 mo. In the initial phases of STEC-HUS, the toxin triggers the endothelial complement deposition and interferes with the activity of the complement regulatory molecules[44]. Moreover, lack of the lectin-like domain of THBD, worsen STEC-HUS in mice[45].

Recent studies that further document the involvement of complement in STEC-HUS are those reporting the beneficial effect of Eculizumab (an anti C5 monoclonal antibody) in the outbreak of STEC-HUS induced by *E. Coli* 0104: H4 in Germany[46] and in the outbreak of STEC-HUS induced by the same strain in France[47].

Réti *et al*[48] recently reported increased levels of C3a and C5b-9 associated with decreased complement C3 levels during the acute phase of TTP. This fact indicates a complement consumption, which occurs in some TTP patients. To confirm complement involvement in TTP, in some patients refractory to treatment, eculizumab has been used with good results. These patients had severe TTP and a deficiency of disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13) due to high titers of anti ADAMTS13 antibodies[49].

C3 glomerulopathies

Isolated C3 deposition within the glomerulus is the defining histological criterion for C3 glomerulopathy. C3 glomerulopathy is a recently introduced pathological entity defined by a glomerular pathology characterized by C3 accumulation with absent or scanty immunoglobulin deposition. In August 2012, an invited group of experts met to discuss the C3 glomerulopathy in the first C3

glomerulopathy meeting[50]. According the conclusions of the meeting and the recent paper from Barbour *et al*[51] on the basis of histological and clinical features, C3 glomerulopathies may be distinguished into: (1) DDD; and (2) C3 glomerulonephritis (C3GN). A familial form of C3GN has been recently described: the CFHR5 nephropathy.

From the patho-physiological point of view, three different mechanisms may be operating in the different conditions:

Auto antibodies: C3 nephritic factor (C3NeF) is an autoantibody that binds to a neoepitope on the AP C3 convertase. C3NeF stabilizes convertase against the CFH-mediated decay resulting in an uncontrolled C3 activation[52]. C3NeF is common in DDD, less in C3GN and absent in CFHR5 nephropathy.

Other auto-antibodies have also been described. Two patients with DDD and auto antibodies targeting both CFB and C3b have been recently identified[53]. Auto antibodies anti CFH also occur in DDD and in C3GN[54,55].

Genetic sequence variations: On genetic basis, heterozygous mutations in the *CFH, CFI* and *MCP* genes have been documented in C3GN[56] and also in MPGN type I and DDD[57]. Some of these heterozygous mutations have been also observed in patients with aHUS[58] showing similarities between the two diseases.

Genetic structure variation: As aforementioned, CFHR5 nephropathy has been described as a familial form of C3GN[59]. A mutation in complement factor H-related protein 5 in patients with glomerulonephritis has been identified *via* a genome-wide linkage study. The mutant CFHR5 protein present in patient serum had reduced affinity for surface-bound complement. Genetic abnormalities in the *CFHR3* and *CFHR1* loci were also recently reported. Such patients develop an autosomal dominant complement-mediated GN similar to CFHR5 nephropathy[60].

As aforementioned, the complement cascade is involved also in other renal conditions as the progression of renal disease and renal transplantation.

COMPLEMENT AND PROGRESSION OF RENAL DISEASE

The progression of renal disease may be mediated by tubule interstitial inflammation. Several studies have confirmed this datum and the involvement of complement activation[61]. Complement activation by tubular cells is mediated by the properdin binding. This fact is principally relevant in the case of proteinuric renal disorders. Studies *in vitro* have documented that the complement system is activated by the AP[62,63].

Complement activation may occur also in non proteinuric renal diseases as documented by Bao *et al*[64]. In this condition the C3a receptor is involved in causing renal inflammation and fibrosis.

Another important factor is the "*in situ*" produced chemokines. Genomic studies performed by Bao *et al*[65] documented that in some murine models several pro inflammatory and pro fibrotic chemokine genes are up-regulated. This activation occurs upon complement activation.

According to the aforementioned studies, targeting complement might be a useful therapeutical approach for chronic kidney disease in the future. Further studies are necessary for a better understanding of the role of complement in mediating tubule interstitial damage and consequent fibrosis.

COMPLEMENT IN RENAL TRANSPLANTATION

Transplanted kidneys principally suffer from injuries such as ischemia reperfusion (I/R) injury and rejection. Complement may mediate all these conditions.

Ischemia-reperfusion injury

The short-term consequences injuries as I/R injury and hyper acute rejection are principally related to innate immunity, while later injuries such as the antibody mediated rejection (ABMR) and the cell mediated rejection (CMR) are related either to the innate and the adaptive immune system.

I/R causes in the transplant both a vascular and parenchyma cell injury. In I/R complement is principally activated through the AP as a consequence of *in situ* generation of C3[66]. Other studies suggest the activation of MBL[67]. The majority of transplanted kidneys are retrieved from cadaveric donors. In such kidneys C3 may be present in the organ before retrieval because of donor suffering. Damman *et al*[68] found higher gene expression of C3 and increased deposition of C3d in kidney biopsies obtained from deceased grafts. Now a large scale study in the United Kingdom is analyzing in renal from deceased donors, the soluble form of C3-1 as a protecting agent for IRI and to improve graft outcomes[69]. Going in molecular details, Simone *et al*[70] documented that in renal I/R injury complement activates nicotinamide adenine dinucleotide phosphate-oxidase (NAPDH oxidase) enzymes. During renal IRI an endothelial-to-mesenchymal transition (EndMT) may occur, mediated by complement activation. EndMT may have a critical role in generating renal fibrosis[71]. Curci *et al*[72] documented that, during I/R injury, an activation of the CP and LP of the complement system occurs primarily at the endothelial cell level. In a recent study, the same authors[73] analyzed in large mammals the role of complement in the induction of EndMT by using recombinant C1 inhibitor *in vivo*.

Their data documented that the activation of the serine/threonine-specific protein kinase (Akt) pathway was essential to induced EndMT *in vitro*. In accordance,

inhibition of complement *in vivo* abrogated the Akt signaling, with inhibition of EndMT and of tissue fibrosis.

Pratt *et al*[74] documented that C3 produced by a graft and by recruited immune cells is a two phases trigger that in the early period produces a post-perfusion injury, later may contribute to late rejection associated-allograft injury. Indeed a recent study[75] documented that I/R injury can affect the systemic immune response to antigens requiring a functional alternative pathway of complement. C3 split products, C3b and C3d, deposited on antigen presenting cells (APCs), can increase allo-antigens uptake and their presentation to the T cells. So doing, C3 positive APCs potentiate the T cell response *in vitro*[74]. The role of C3 in activating T cells is confirmed by studies documenting that the macrophages deficient for the C3 have impaired capability to stimulate the T cells[76,77]. The enhancement of the effector T cell expansion by complement should be ascribed to the limited antigen induced apoptosis[78]. In addition, other studies have documented the role of complement on the iTreg. Indeed, the iTreg-mediated tolerance to alloantigen in humans[79] might be mediated by the signaling through C5a receptor and C3a receptor.

As already mentioned, complement is involved both in the CMR and in the ABMR.

Cell-mediated rejection

The complement activation through any pathway generates C3a and C5a. These anaphylatoxins bind to both APCs and T cells to stimulate and activate T cells[80]. Li *et al*[81] demonstrated that the deficiency of the C5a receptor limited the adaptive response of recipient T cells to alloantigen. C1q appears to have a regulatory role in the threshold for the T cell activation by dendritic cells[82]. Moreover, in human kidney transplants with acute rejection, C5aR expression was increased in the renal tissue and in the cells infiltrating the tubular interstitium[83]. The same authors documented that in mice treated with a C5aR antagonist the infiltration of monocyte-macrophage was significantly attenuated, perhaps as a result of reduced levels of monocyte chemo attractant protein 1 and the intercellular adhesion molecule 1. However a murine model of kidney transplantation with C4 deficiency demonstrated that a CMR can occur in the absence of the CP or of the LP activation[84]. This suggests that the AP may play the key role in CMR.

Antibody-mediated rejection

The antibody-mediated renal allograft rejection often involves either donor specific antibodies (DSAs) and the CP of complement system activation. After binding to DSAs, complement is activated. C4d is a degradation product of C4 and, because it binds and remains covalently attached at the site of complement activation, represents a useful diagnostic tool[85]. Haidar *et al*[86] discovered that the deposition of C4d on erythrocytes was even more related to histological rejection signs, thus representing a possible not invasive diagnostic tool.

C3a and C5a likely act as potent chemo tactic factors promoting the infiltration of proinflammatory cells. In addition, the MAC might directly damage the allograft[85]. Expression of the membrane-bound regulator, CD55, is inversely related to C4d staining in biopsy specimens. Indeed CD 55 (also known as DAF), which accelerates the decay of C3 and C5 convertases, might modify the severity of the rejection. An increased CD55 expression is associated with an improved long-term kidney transplant outcomes in recipients without antibody-mediated rejection, suggesting a possible role for CD55 in the kidney protection[87].

In addition the kidney, after transplantation, may be involved in clinical conditions as recurrence of some renal diseases. Recently complement involvement has been documented also in chronic antibody mediated rejection.

Atypical hemolytic uremic syndrome is associated with a high rate of recurrence and poor outcomes after kidney transplantation. Acquired or inherited dysregulation of the alternative complement pathway, thought to be the driving force of the disease, is identified in most aHUS patients[88]. Recurrent thrombotic microangiopathy is very rare in patients who had developed end stage renal failure following HUS caused by Shiga-toxin producing STEC, whereas disease recurrence is common in patients with aHUS[89]. The recurrence rate[90] of C3 glomerulopathy on renal transplantation could be approximately estimated on about 60% as derived from two small case series of Servais *et al*[57] and Little *et al*[91] and confirmed in the recent paper of Zand *et al*[92]. In such conditions anticomplement therapy could be useful.

Moreover recent data document the complement involvement also in antibody mediated chronic rejection where the "bad" activity of antibodies can also be involved in previously considered "chronic" lesions (*i.e.,* transplant glomerulopathy[93,94].

TARGETING COMPLEMENT: THERAPEUTIC STRATEGIES

Complement is clearly involved in many kidney diseases as well as in kidney transplantation. Hence targeting complement cascade at different levels may represent a new therapeutic strategy directed against the pathogenetic mechanisms.

Since the end of 2000's several review papers reported the efficacy *in vitro* or in preclinical studies of anti complement molecules[95-97]. Unfortunately in the setting of renal diseases only an anti C5 monoclonal antibody is on the market. All others molecules are either on the market for diseases not involving the kidney or are still in preclinical phases (Table 2) or failed their efficacy.

Table 2 Some of the molecules aimed to target complement, phase of the trial and renal diseases related

Compound name	Complement target	Compound class	Phase/indication
C1 inhibitor (Berinert)	C1r, C1s, MASP1, MASP2	Regulator	P 1/2 transplant
Cp40, AMY 101			PC transplant , aHUS, DDD
sCR1, CDX-1135	C3 conv, C4b, C3b	Regulator	P 1 DDD
Mirococept, APT070	C3 conv, C4b, C3b	Regulator	P 1/2 transplant
Eculizumab	C5	Ab	P 4 aHUS, P 2/3 STEC-HUS, P 2 ANCA vasculitis, P 1 transplant
Mubodina	C5	Ab	PC aHUS, DDD
Ergidina	C5	Ab	PC transplant
CCX168	C5aR	Small molecule	P 2 ANCA vasculitis
ADC-1004	C5aR	Protein	PC transplant

MASP1: Mannan-binding lectin-associated serine protease; P: Phase; PC: Preclinical; aHUS: Atypical hemolytic uremic syndrome; DDD: Dense deposit disease; sCR1: Soluble complement receptor 1; Ab: Antibodies; STEC-HUS: Shiga toxin producing *Escherichia Coli*-hemolytic uremic syndrome.

TARGETING C5

The first available anti-complement therapy is eculizumab, a fully humanized monoclonal antibody that binds with high affinity to C5 and prevents the generation of MAC[98]. Eculizumab was first approved for the treatment of paroxysmal nocturnal hemoglobinuria (PNH)[99] and more recently, for the treatment of aHUS and other kidney diseases.

To date[100] 11 trials are ongoing for aHUS, 2 trials for STEC-HUS, 2 trials for MPGN and 1 trial for ANCA related diseases. Trials with eculizumab are also ongoing in the field of kidney transplantation, in particular 8 trials for the prevention and/or the treatment of acute or chronic ABMR, 3 trials for the prevention of delayed graft function (DGF), 1 trial for the prevention of I/R injury and 1 trial for the prevention of glomerular disease recurrence after transplantation.

After initial reports of the possible beneficial effects of eculizumab in treating patients with aHUS[101,102], more recently the beneficial effect of treating aHUS by eculizumab has been documented by two studies. One study[103] reported the data from 27 patients treated in off label studies. The other study[104] reported the data of 37 patients enrolled in 2 phase Ⅱ trials. The second of these phase Ⅱ trials was notable because 80% of subjects achieved thrombotic microangiopathy event-free status. These studies were the object of debate for the issues concerning the duration and the optimal dosing of therapy[105-108]. Indeed, although eculizumab revolutioned the treatment of aHUS, several unresolved issues remain, among which whether eculizumab should be always the first line therapy for aHUS and whether the drug should be considered as a life-long therapy also taking in account the treatment high cost. In addition, in PNH, but not by now in aHUS, patients have been described with unexplained eculizumab resistance. A recent study[109,110] documented that such resistance was due to C5 variants with mutations at Arg885.

As aforementioned also STEC-HUS and TTP are caused by inappropriate complement activation[42]. The eculizumab treatment has proved effective in these conditions. After the report of 3 cases[111], the eculizumab effectiveness has been documented in two STEC-HUS outbreaks occurring in Germany and in France[46,47]. The efficacy of eculizumab in treating the TTP has also been reported[49,112], even if others advocate rituximab as the best option in treating TTP[113].

The pathogenetic similarities between aHUS and some C3 glomerulopathies might imply that eculizumab treatment could fit well in treating all these diseases[103]. The new classification of C3 glomerulopathies (previously MPGN) have already been described[51]. Only DDD and C3GN have some similarities with aHUS and eculizumab could be beneficial for such patients. To date, in literature the eculizumab use for C3 glomerulopathies is limited to 6 case reports[114-119] and the results from a 1-year, open-label study of eculizumab therapy in 6 subjects[120,121]. The treatment results differ and indicate that eculizumab may be a non adequate treatment for a subgroup of patients with DDD and C3GN. Although some investigators suggested that aHUS should be considered part of a spectrum that includes DDD and C3GN, the underlying defect is not always the same. In aHUS the endothelial damage is often due to complement dysregulation at the level of cell membrane in the solid phase[36]. The solid phase dysregulation in aHUS translates to C5 convertase dysregulation being at least equal and often greater than C3 convertase dysregulation. Hence the blockade of C5 in such conditions is expected to yield improvement. In contrast, in some cases of C3 glomerulopathies a dysregulation of the fluid phase occurs and, as a consequence, C3 convertase dysregulation is greater than C5 dysregulation. These cases, because of a feed-back effect on the C3 convertase activity, could potentially be aggravated by C5 blockade[122]. Therefore, one of the major challenges in treating patients with C3 glomerulopathy with the anti complement therapy is how to distinguish the patients with primarily C3 convertase dysregulation from the patients with primarily C5 convertase dysregulation. Blockade at the level of C3 may be an alternative to eculizumab therapy, primarily in patients with C3 glomerulopathies

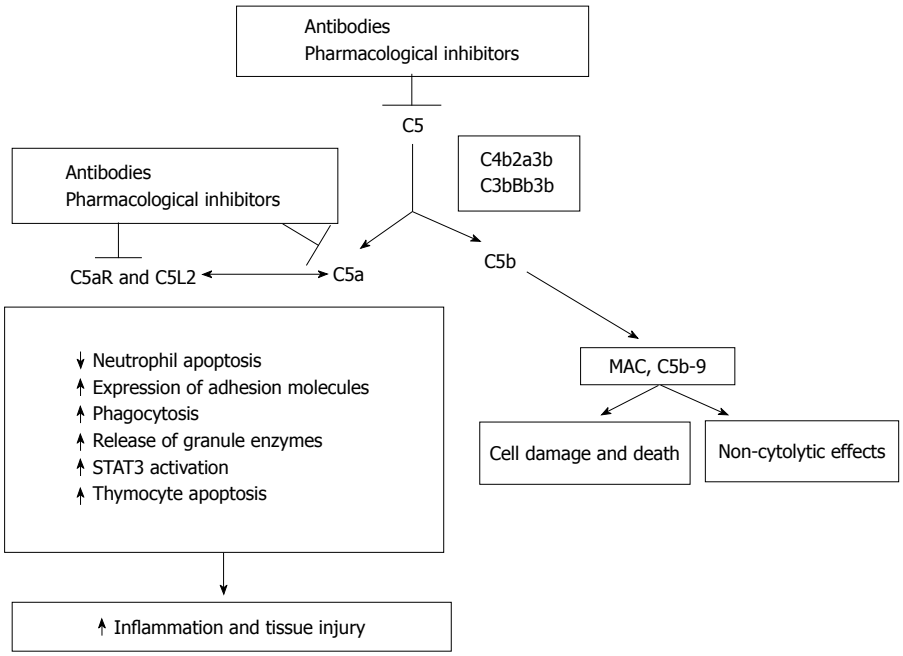

Figure 4 Significance of inhibiting the C5-C5a receptor axis. C5 convertases formed during activation of complement cascade cleave C5 into its active products. Inhibition is feasible pharmacologically or with neutralizing antibodies. MAC: Membrane attack complex; STAT3: Signal transducers and activators of transcription 3.

associated with a C3 convertase dysregulation greater than the C5 convertase dysregulation.

As mice deficient in C5 have demonstrated resistance against anti-MPO-mediated glomelulonephritis[123], an open label phase Ⅱ trial (NCT01275287), in which the patients with ANCA-associated glomerulonephritis were randomized to standard of care treatment *vs* standard of care plus eculizumab, was started. Unfortunately the trial was withdrawn because of lack of enrollment.

In the case of the recurrence after transplantation of a kidney disease susceptible to anti C5 therapy, eculizumab treatment is effective.

Zuber *et al*[124] successfully treated 22 renal transplant recipients with recurrence of aHUS.

McCaughan *et al*[117] reported a patient with DDD recurrence after kidney transplantation successfully treated by eculizumab. More recently Lonze *et al*[125] reported the cases of antiphospholipid antibody syndrome, two of them with the catastrophic variant, which were successfully treated at the time of transplantation with continuous systemic anticoagulation together with eculizumab prior to and following the live donor renal transplantation.

As aforementioned renal damage due to complement activation occurs in two phases after transplantation: during reperfusion after that the donor kidney has undergone a significant period of ischemia and during the acute rejection once the innate and adaptive immune system has recognized the donor antigens. In both conditions the complement may play a relevant role. Four clinical trials are now active aiming to control the ischemia-reperfusion injury and the consequent DGF. All these trials hypothesized that C5 cleavage is a key step in the pathogenesis of I/R injury following transplantation and its block could be an effective prophylactic tool to prevent acute kidney injury (NCT01919346, NCT01403389, NCT02145182, NCT01756508).

Eculizumab has also been successfully used in reducing antibodies in highly sensitized patients with positive cross-matches prior to transplantation[126-128]. In a larger case-control study the patients with DSAs were treated with eculizumab after transplantation and compared to the historical controls[129]. Eculizumab treatment was able in significantly lowering ABMR and in decreasing the 1-year transplant glomerulopathy incidence rate.

Table 3 summarizes all the trials ongoing with eculizumab in treating either glomerular disease and renal transplantation.

TARGETING C5a AND C5aR

C5a is a powerful anaphylatoxin that stimulates the cytokine production, enhances the T-cell activation and augments the leukocyte adhesion and the vascular permeability (Figure 4). There is an increased expression of the C5aR in transplanted kidneys with IRI or acute rejection[83,130]. Recently Cravedi *et al*[131] documented that pharmacological C5aR blockade in mice reduces the graft versus host disease, prolongs the survival and inhibits the T-cell responses. This provides the basis for future studies aimed to target C5aR. Several studies have documented that the activation of the C5-C5a receptor axis is involved in several human diseases[132]. In addition to eculizumab that to date is the only specific complement inhibitor approved for clinical use, several therapeutics targeting the C5a-C5aR axis are in different stages of clinical development ranging from preclinical studies to phase Ⅱ studies. These agents may target the axis at different levels, ranging from conversion of C5 to C5a and C5b, to inactivation of C5a, or to the inhibition of the two C5a receptors C5aR (CD88) and C5L2[132,133].

Table 3 Trials ongoing with eculizumab in renal diseases and in renal transplantation

Rank	Identifier	Status	Study name
1	NCT01221181	Active	Eculizumab therapy for dense deposit disease and C3 nephropathy
2	NCT02093533	Recruiting	Eculizumab in primary MPGN
3	NCT01275287	Active	Targeting complement activation in ANCA-vasculitis
4	NCT00844545	Completed	Open label controlled trial of eculizumab in adult patients with plasma therapy-resistant aHUS
5	NCT00844844	Completed	Open label controlled trial of eculizumab in adolescent patients with plasma therapy-resistant aHUS
6	NCT00844428	Unknown	Open label controlled trial of eculizumab in adolescent patients with plasma therapy-sensitive aHUS
7	NCT00838513	Unknown	Open label controlled trial of eculizumab in adult patients with plasma therapy-sensitive aHUS
8	NCT01194973	Unknown	An open-label, multi center clinical trial of eculizumab in adult patients with aHUS
9	NCT01193348	Unknown	An open label, Multi center clinical trial of eculizumab in pediatric patients with aHUS
10	NCT01755429	Unknown	The safety and efficacy of eculizumab in Japanese patients with aHUS
11	NCT01522170	Enrolling	aHUS observational long term follow up
12	NCT01522183	Recruiting	aHUS registry
13	NCT01770951	Completed	A retrospective, observational, non-interventional trial to assess eculizumab treatment effect in patients with aHUS
14	NCT02205541	Not yet recruiting	Eculizumab in shiga-toxin related hemolytic and uremic syndrome pediatric patients
15	NCT01410916	Completed	Safety and efficacy study of eculizumab in shiga-toxin producing *Escherichia coli* (STEC-HUS)
16	NCT01406288	Completed	Completed outbreak of HUS linked to *Escherichia coli* of serotype 0104:H4
17	NCT01756508	Recruiting	Eculizumab for prevention and treatment of kidney graft reperfusion injury
18	NCT01919346	Recruiting	Eculizumab for prevention of DGF in kidney transplantation
19	NCT01403389	Active	A study of the activity of eculizumab for prevention of DGF in deceased donor transplant
20	NCT02142182	Recruiting	A trial for prevention of DGF after kidney transplantation
21	NCT01567085	Active	Safety and efficacy of eculizumab in the prevention of AMR in sensitized recipients of a kidney transplant from a deceased donor
22	NCT02113891	Not yet recruiting	Eculizumab therapy for subclinical antibody-mediated rejection in kidney transplantation
23	NCT01095887	Active	Eculizumab added to conventional treatment in the prevention of antibody-mediated rejection in blood group incompatible living donor kidney transplantation
24	NCT01106027	Active	Dosing regimen of eculizumab added to conventional treatment in positive crossmatch deceased kidney yransplant
25	NCT01895127	Recruiting	Efficacy and safety of eculizumab for treatment of antibody-mediated rejection following renal transplantation
26	NCT00670774	Active	Dosing regimen of eculizumab added to conventional treatment in positive crossmatch living kidney transplant
27	NCT01399593	Active	Safety and efficacy of eculizumab to prevent AMR in living donor kidney transplant recipients receiving desensitization
28	NCT01327573	Active	Eculizumab therapy for chronic complement-mediated injury in kidney transplantation
29	NCT01029587	Recruiting	Eculizumab to enable renal transplantation in patients with history of catastrophic antiphospholipid antibody syndrome

MPGN: Membrano-proliferative glomerulonephritis; ANCA: Anti neutrophil cytoplasmic antibody; aHUS: Atypical hemolytic uremic syndrome; STEC-HUS: Shiga-toxin producing escherichia; DEGF: Delayed graft function; AMR: Antibody mediated rejection.

As aforementioned, after the findings that C5aR blockade protects against MPO-ANCA GN in mice[21] a clinical trial (NCT01363388) was started with a planned enrollment of 60 subjects affected by ANCA associated glomerulonephritis.

An important consideration, and a possible drawback, in blocking the C5a-C5aR axis is that the block itself might adversely affect the host defense and might counteract some of the useful recently identified functions of complement. Indeed, C5a may protect against neuron apoptosis[134], might act as an inhibitor of angiogenesis[135], and is essential for liver regeneration[136].

C1 INHIBITION

The beneficial effect of C1 inhibition on IRI has been widely studied by Castellano *et al*[71] and Curci *et al*[72]. These studies have been recently commented by Carney[73]. Purified or recombinant C1-INH is a host serine protease inhibitor that is able to block complement cascade acting either at level of classical and lectin pathway[137]. The first clinical indication of C1-INH has been hereditary angioedema. To date C1-INH has shown effects in several disease as myocardial ischemia and reperfusion injury[138], renal transplantation[139] and sepsis[140].

To date three clinical trials are ongoing in the field of kidney transplantation. The 2 first clinical trials (NCT01147302 and NCT01134510) have been made for the prevention of acute ABMR adding C1-INH to post-transplant treatment. The investigators observed that no patients developed ABMR during treatment with C1-INH and, in addition, noted a reduction in DGF due to I/R injury. As a consequence, recently a third trial with C1-INH was started (NCT02134314) to prevent DGF in patients receiving deceased donor kidney transplant.

TARGETING C3

In theory, the blockade at the level of C3 may be more effective than the anti C5 therapy, in particular for the C3 glomerulopathies when the C3 convertase

activation is prevalent over the C5 convertase. Soluble CR1 (sCR1) is among the proteins that regulate the C3 convertase. CR1 is a cell-surface glycoprotein expressed on several cells among which monocytes, APCs, T and B cells and podocytes. As a consequence sCR1 may modulate the complement cascade on all cells expressing on their surface CR1, follicular dendritic cells and a small T cells population[141-143]. In addition, CR1 is the only co-factor of factor I able to promote cleavage of inactive C3b and inactive C4b into their inactive protein fragments[144]. In normal condition only small quantities of sCR1 are in circulation. Lazar *et al*[145] and Li *et al*[146] administered high sCR1 in patients undergoing cardiac surgeries or cardiopulmonary by-pass to inhibit complement activity. These studies documented that sCR1 is effective and safe. sCR1 has been recently used in renal diseases and in renal transplantation.

Recently, Zhang *et al*[147] from Iowa University reported the results using sCR1 in mice deficient in factor H. sCR1 increases C3 serum levels and decreases C3 deposition. In the same report Zhang *et al*[147] reported the beneficial effect of treating by sCR1 a young patient affected by ESRD due to DDD. This group is currently enrolling patients for a small phase I trial of sCR1 (also called CDX-1135) in patients with DDD (NCT01791686).

C3 and C5 convertases decay is influenced by CR1. Treatment with sCR1 improved kidney transplant survival after a period of cold storage and when kidneys were transplanted across a complete major histocompatibility complex mismatch[148,149].

The effects of Mirococept (APT070) (sCR1) has been widely described by Sacks *et al*[69] and is currently the subject of a large scale study in kidney transplantation to test the superiority of Mirococept in the prevention of IRI in cadaveric renal allografts[150].

CONCLUSION

Emerging evidence has recently documented that the complement cascade as a common pathogenetic mechanism in many kidney diseases, in the chronic progression of the kidney diseases and in the kidney transplantation.

This finding led us to an improved understanding of the molecular mechanisms that are at the basis of the kidney diseases and, as a consequence allowed us to formulate a new classification of some renal diseases as the different kinds of hemolytic uremic syndromes and the membranoproliferative GN.

Among the new drugs aimed to target the complement in the renal diseases only the C5 monoclonal antibody, eculizumab, is to date on the market. Others are on clinical trials for the C3 glomerulopathies and the ANCA-mediated GN.

The use of eculizumab in treating the patients affected by aHUS has some limitations and is an example for the need of other drugs targeting the complement cascade. Indeed if eculizumab should be considered for all the patients with aHUS, because the dysregulation at C5 level is largely prevalent in this disease, the C3 glomerulopathies have variable degrees of the C5 convertase dysregulation.

In the cases were the C3 glomerulopathies are associated with a greater C3 convertase dysregulation, the blockade at the level of C3 should be the alternative to the eculizumab treatment.

The complement system is now recognized a pervasive, multifaceted mediator of transplant injury in animal models and in human transplant recipients[151]. The development of pharmacologic agents that block human complement components and receptors in the field of renal transplantation[152,153] now represents the basis of the concept that targeting complement in kidney transplant recipients will improve graft and patient survival rate.

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REVIEW

Pathogenesis of glomerular haematuria

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Abstract

Haematuria was known as a benign hallmark of some glomerular diseases, but over the last decade, new evidences pointed its negative implications on kidney disease progression. Cytotoxic effects of oxidative stress induced by hemoglobin, heme, or iron released from red blood cells may account for the tubular injury observed in human biopsy specimens. However, the precise mechanisms responsible for haematuria remain unclear. The presence of red blood cells (RBCs) with irregular contours and shape in the urine indicates RBCs egression from the glomerular capillary into the urinary space. Therefore glomerular haematuria may be a marker of glomerular filtration barrier dysfunction or damage. In this review we describe some key issues regarding epidemiology and pathogenesis of haematuric diseases as well as their renal morphological findings.

Key words: Haematuria; Pathogenesis; Glomerular filtration barrier; Dysmorphic red blood cells; Chronic kidney disease; Microscopic haematuria

Core tip: Recent advances suggest that glomerular haematuria may be a negative prognostic factor for renal function outcome. A more fragile and easily ruptured glomerular filtration barrier (GFB) may be responsible for glomerular bleeding. Several factors have been associated to this pathogenic process, including: (1) genetic alteration of GFB components, leading to a more fragile and easily ruptured GFB structure; (2) aberrant deposition of toxic molecules in the GFB; and (3) enhanced inflammatory response, as reported in autoimmune diseases, infections, or primary glomerulonephritis. In this review we fully describe these pathological mechanisms, with special interest in haematuric diseases and their renal morphological findings.

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INTRODUCTION

Haematuria is a common presenting feature of renal and urological diseases. It is described as the presence of more than 2 red blood cells (RBCs) per high-power field in the urine sediment. When the presence of RBCs in the urine is massive, the urine colour is red and is called macroscopic haematuria. Microscopic haematuria (MH) is detected by microscopical examination or dipstick, so its real incidence is unknown[1,2]. According with its origin haematuria can be glomerular or non-glomerular, however in this review we will focus exclusively on glomerular haematuria due to its implications in renal prognosis. The precise pathogenic mechanisms responsible of glomerular haematuria remain unclear. However, the identification of the specific molecular defect responsible of different genetic disorders commonly associated with haematuria has highlighted possible mechanisms. These genetic diseases originate glomerular filtration barrier (GFB) damage, leading to a more fragile and easily ruptured structure. Sometimes by directly alteration of the glomerular basement membrane (GBM) [as reported in Alport syndrome (AS), thin basement membrane nephropathy (TBMN) or hereditary angiopathy, nephropathy, aneurysms, and muscle cramps (HANAC) syndrome] or podocyte structure [Myosin heavy chain 9 (MYH9)-associated kidney disease], and others by aberrant deposition of toxic compounds like in storage disorders (Fibronectin glomerulopathy, Immunotactoid and Fibrillary glomerulonephritis). Inherit genetic mutations can also lead in an abnormal regulation of the complement alternative pathway and therefore C3 glomerular deposition [C3 glomerulopathies as complement factor H-related protein 5 (CFHR5) nephropathy or dense deposit disease], inducing a potent inflammatory response that results in phagocyte chemotaxis, with opsonization and lysis of cells which can easily explain haematuria. Haematuria can also be produced by inflammatory status as reported in autoimmune diseases [anti-neutrophil cytoplasmic antibodies (ANCA), Vasculitis, GBM disease, systemic lupus erithematosus or Cryoglobulinemia], infections (Endocapillary glomerulonephritis), or primary glomerulonephritis [IgA Nephropathy (IgAN), Membranoproliferative, Crescentic] (Table 1). In this review we will describe the molecular mechanisms responsible for histopathological findings in these diseases in order to explain the pathogenesis of haematuria and their relation with renal outcome.

PROGNOSTIC VALUE OF HAEMATURIA

Haematuria has traditionally been considered as a hallmark of some glomerular diseases, without repercussion on short and long-term kidney function[3]. However, over the last decade new evidences reported negative prognostic implications for both microscopic[4] and macroscopic haematuria[5] on the progression of renal disease. Thus, Vivante *et al*[4] reported that persistent asymptomatic isolated microscopic haematuria in 1 million young Israeli adults was significantly associated with increased risk of end stage renal disease (ESRD) after 22 years of follow up. Moreover, persistent glomerular hematuria in kidney donors has been associated with an increased risk of proteinuria and kidney disease progression at 2.3 years after donation[6].

Acute kidney injury (AKI) is a common complication of severe macroscopic haematuria, with an incidence of around 30% in IgAN patients with gross macrohaematuria bouts[7,8] and around 20% in warfarin-related nephropathy (WRN)[9]. Gutiérrez *et al*[5] reported that around 25% of IgAN patients did not recover baseline serum creatinine after cessation of macroscopic haematuria-associated AKI. In this study, duration of macroscopic bout was the more important prognostic factor determining incomplete recovery of renal function. Similarly in CFHR5-nephropathy almost all male patients who reached ESRD had episodes of macroscopic haematuria episodes after upper respiratory tract infections in childhood and adolescence[10].

The most important renal guidelines, Kidney Disease Outcomes Quality Initiative and Kidney Disease: Improving Global Outcome give a contradictory advice about haematuria management. These guidelines recommend to assess every chronic kidney disease (CKD) patient with dipstick[11,12], but haematuria is not recognized as a risk factor of CKD progression, and not recommend further monitoring or treatment in glomerulonephritis patients with isolated microscopic haematuria[13]. However they recognize that IgAN with haematuria and minimal proteinuria is a progressive disease[14], indicating that although clinical outcome for many haematuric patients is good, the lifetime risk for CKD patients may be elevated depending on the specific underlying disease.

CONSEQUENCES OF GLOMERULAR HAEMATURIA

Clinical data and basic research evidences suggest that haematuria induces renal damage. Acute tubular necrosis and intraluminal obstructive RBC casts are the most characteristic histologic findings in AKI during macroscopic hematuria. The principal mechanism of damage is the direct tubular toxicity of hemoglobin (Hb), heme, iron, or other molecules released from RBCs. It has been proposed that RBC passage throughout the GFB induces distortion of erythrocyte cytoskeleton, which is unable to maintain the cellular integrity, leading to RBC rupture. As consequence, the toxic molecules normally lock inside RBC's cytoplasm, such as Hb, heme, or iron, are released into the urinary space.

Hb is internalized into the epithelial tubular cell by the megalin/cubilin complex. Hb under the epithelial cell oxidant conditions dissociates into heme and globin. Heme oxigenase-1 (HO-1) catalyzes the conversion

Table 1 Classification of haematuric diseases by histopathological findings

Glomerular endothelial cell layer	GBM disorders	Mesangial deposits	Podocytary slit diaphragm disorders	Subendothelial/subepithelial deposit	Others
ANCA Endocapillary	Primary Alport TBMD HANAC ? LPHS Secondary Anti-GBM disease C3 glomerulopathy CFHR5 nephropathy	IgAN HSP	MYH9 disease Fabry disease	Primary GN MBP Endocapillary Crescentic Secondary GN SLE Cryoglobulinemia Fibrillar deposit Fibronectin Fibrillary Immunotactoid	WRN SCD

GBM: Glomerular basement membrane; ANCA: Antineutrophil cytoplasmic antibodies; CFHR5: Complement factor H-related 5 nephropathy; GN: Glomerulonephritis; HANAC: Hereditary angiopathy, nephropathy, aneurysms, and muscle cramps syndrome; IgAN: IgA nephropathy; LPHS: Loin back pain syndrome; MBP: Membranoproliferative; SCD: Sickle cell disease; HSP: Henoch-Schönlein purpura; SLE: Systemic lupus erythematous; TBMD: Thin basement membrane disease; WRN: Warfarin related nephropathy.

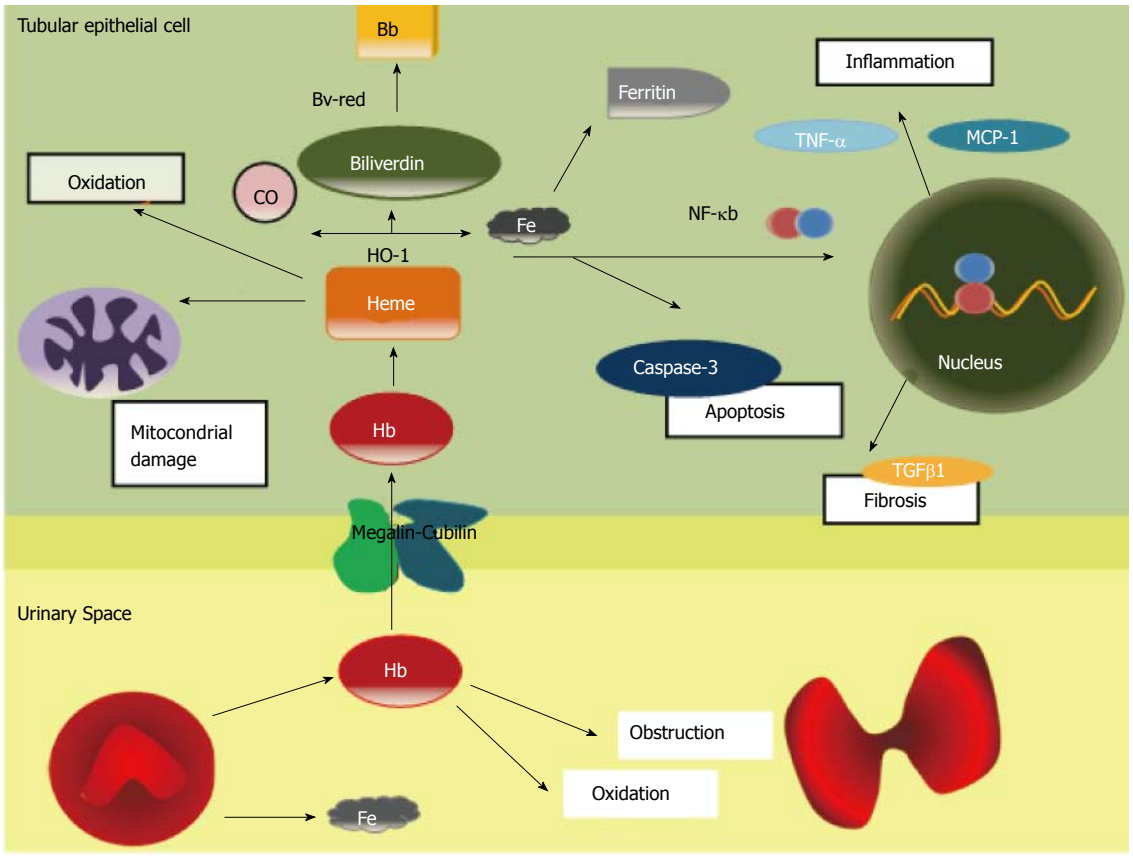

Figure 1 Haematuria-induced kidney injury in tubular cells. Hb: Haemoglobin; Bb: Bilirubin; Bv-red: Biliverdin reductase; CO: Carbon monoxide; Fe: Iron; HO-1: Heme oxygenase 1; MCP: Monocyte chemoattractant protein; NF-κb: Nuclear factor kappa b; TGF-β: Transforming growth factor beta; TNF-α: Tumour necrosis factor alpha.

of heme to biliverdin, iron and carbon monoxide[15]. At that time, the bilirubin reductase converts biliverdin in bilirubin and the iron is stored as Ferritin (Figure 1). HO-1 is now recognized as a protective molecule with anti-oxidant and anti-inflammatory properties against diverse insults in different tissues[16].

Free heme is also extremely toxic. In plasma and intracellular membranes, heme can oxidize lipids, denature proteins and perturb the cellular integrity[17]. In large amounts, heme may be a source of iron that drives oxidant injury after hypoxic and nephrotoxic insults[18]. Indirectly heme can also induce renal injury by

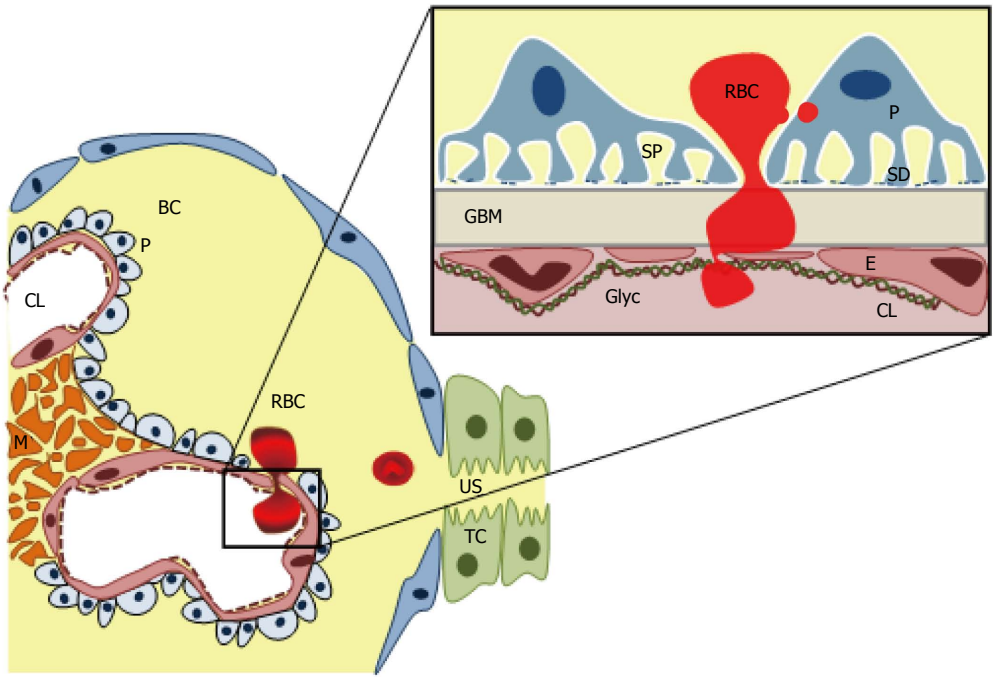

Figure 2 Glomerular filtration barrier structure and red blood cell egression leading to haematuria. CL: Capillary lumen; BC: Bowman's capsule; E: Endothelial cell; GBM: Glomerular basement membrane; Gly: Glycosaminoglicans; M: Mesangium; P: Podocyte; RBC: Red blood cell; SD: Slit diaphragm; SP: Subpodocyte space; TC: Tubular cell; US: Urinary space.

its proinflammatory effects, as inducing the chemokines such as monocyte chemoattractant protein-1 throughout the redox-sensitive transcription factor NF-KB[15]. The heme group of haemoglobin may also decrease nitric oxide availability, promoting intrarenal vasoconstriction and ischemia[19]. Finally, another possible mechanism involved in haematuria damage may be associated to a delayed dysmorphic RBC's elimination, which may explain the prolonged recovery period in patient with macrohaematuria induced AKI.

PATHOGENESIS OF HAEMATURIA

The presence of dysmorphic RBCs with irregular contours and shape in the urine is almost pathognomonic of glomerular haematuria[20] and indicates RBCs egression from the glomerular capillary into the urinary space. Therefore glomerular haematuria is a marker of the GFB dysfunction or damage[21].

GFB is an extremely complex and specialized structure[22,23], with different constituents and cell types, which allows a free permeability to water, small and midsized plasma solutes, but keeps a highly specialized selectivity for proteins and larger molecules according with size and molecular weight[24]. GFB has five major components: (1) from the vascular side, the endothelial surface layer, a complex glycosaminoglycan net which cover the endothelial layer as well as the fenestrations; (2) the endothelial cell; (3) the GBM; (4) podocytes with its interdigitating foot processes and specialized intercellular junctions, the "slit diaphragms"; and (5) finally on the urinary side, the subpodocyte space, an area delimited between the podocyte cell body and the foot processes (Figure 2). Furthermore, mesangial cell also indirectly contributes to GFB structure regulating and supporting the blood flow and the glomerular capillary structure, as well as controlling the mesangial matrix turnover (Figure 2). The GFB integrity is maintained by a complex interplay of signaling interactions between the three constituent cell types[24-26].

It has been thought that under physiological conditions, the endothelium with its fenestrations (50-100 nm) acts as molecular size sieve, self-sufficient to maintain the RBCs (6.2-8.2 μm) away from the GBM. However, haematuria in some diseases such as TBMN, Fibrillar deposit diseases or MYH9-associated kidney disease with typically intact endothelium, highlighted the key integrative role of GFB complex as a RBCs sieve. Therefore, how the RBCs, 100-fold bigger than the glomerular endothelium's pore, cross the GFB remains unclear. It is possible that a damaged GFB layer may release inflammatory or chemotactic signals promoting RBC passage throughout this layer, however the specific mechanisms have not yet found out.

According to its primary and histopathologic localization the haematuric disorders may be classified into: (1) Glomerular endothelial cell and surface layer injuries; (2) primary and secondary GBM disorders; (3) Diseases with mesangial deposition; (4) Diseases with subendothelial and subepithelial deposition; (5) Podocyte-associated disorders; and (6) Miscellaneous (Table 2).

Glomerular endothelial cell and surface layer injuries

In spite of the relatively big size of glomerular endothelial

Table 2 Possible pathogenic mechanisms of haematuria

Disease	Molecular defect	Prevalence	Main glomerular defect	Clinical expression		
				Haematuria	Proteinuria	CKD progression
Genetic disorder						
GFB structural damage						
Structural GBM damage						
ALPORT	X-linked: COL4A5 AR: COL4A3/COL4A4	1/50000	GBM	MH	Variable	100% approximately 20-30 yr
TBMD	COL4A3/COL4A4	1%	GBM	MH	Usually absent	20% CKD
HANAC	COL4A1	3 families	GBM	MH or gross	Not described	Variable
Structural podocyte damage						
MYH9	Non muscle myosin IIA heavy chain	1:100000	None	MH	Variable	ESRD by young adulthood
Storage disorders						
Fibronectine GN	Fibronectin	44 cases	Mesangial/ subendoth	60% MH	93% variable degree	ESRD at 20-60 yr
Fibrillary	10-30 nm fibrils	Rare	Mesangial /GBM	MH 47%-73% Gross 5%	Present 41%-55% nephrotic	50% ESRD in few years
Immunotactoid	> 30 nm fibrils	10-fold rarer than FGN	Mesangial/ subepith/subendoth	MH 80%	100%	17% ESRD in 3 yr
Fabry's disease	Lysosomal storage	1:3100- 1:1600	All the cells	MH	Usually nephrotic	ESRD after age 50 yr
Complement mediated						
C3 glomerulopathy	Alternative pathway	1-2 × 10^6	Mesangial/GBM	MH 87%	38%	Variable
Inflammatory disorders						
Autoimmune						
ANCA	Ab *vs* endothelium	10-20 × 10^6	Endothelium	MH	Variable	Variable
Anti GBM	Ab *vs* COL4	0.5-1 × 10^6/yr	GBM	MH	Variable	Variable
Infections (endocapillary)						
Primary GN (IgAN, membranoproliferative, crescentic)						
IgAN	Galactose-deficient IgA1	10%-16%	Mesangial	MH always 75% gross	Rare nephrotic Usual proteinuria	20% ESRD 20 yr after diagnosis
Miscellaneous						
WRN	Unknown	16.5% non-CKD 33% CKD	None	Usually MH	None	Accelerated CKD progression
LPHS	Unknown	Unknown	GBM (?)	MH or gross	Absent or minimal	GFR > 60

ANCA: Antineutrophil cytoplasmic antibodies; AR: Autosomal recesive; CKD: Chronic kidney disease; COL4A1: Alpha 1 chain of type Ⅳ collagen; COL4A3: Alpha 3 chain of type Ⅳ collagen; COL4A4: Alpha 4 chain of type Ⅳ collagen; COL4A1: Alpha 1 chain of type Ⅳ collagen; ESRD: End stage renal disease; GFB: Glomerular filtration barrier; GFR: Glomerular filtration rate; HANAC: Hereditary angiopathy, nephropathy, aneurysms, and muscle cramps syndrome; IgAN: IgA nephropathy; LPHS: Loin back pain haematuria syndrome; MH: Microscopic haematuria; TBMD: Thin basement membrane disease; WRN: Warfarin related nephropathy.

fenestrations, they play an important role in GFB perm selectivity due to its coating glycocalyx layer, composed principally by proteoglycans[27]. Glomerular endothelial cell glycocalyx and its associated surface layer retain more than 95% of the circulating proteins.

The glomerular endothelial layer is the main target of ANCAs, which attack small-vessel causing vasculitis, leading to necrotizing and crescentic glomerulonephritis. ANCA-vasculitis present an overall annual incidence of approximately 10-20 cases/million people, with an onset age peak of 65-74 years[28]. ANCA can induce the production and release of reactive oxygen species and lytic enzymes by infiltrated neutrophils[29], complement system *via* the alternative pathway[30] as well as endothelial cell as an amplification disease loop, resulting in endothelium lysis[31]. On early stages of ANCA-vasculitis the endothelial lesion could explain the onset of haematuria, although in advanced stages it could be explained by a severe GFB impairment usually involving all its layers. Although haematuria has been classically considered a marker of glomerular injury activity in ANCA, a recent report showed not repercussion of persistent haematuria (determined by dipstick) in GFR at 1 year[32]. However, in this study, persistent haematuria was associated to low baseline GFR and ANCA status.

Endothelial cell damage has been also reported in endocapillary glomerulonephritis (GN) and infection-associated GN. With a decreasing incidence over the last decades in developed countries[33], endocapillary GN is now more frequent in fragile patients, such as elder, alcoholics and intravenous drug users[34]. The typical presentation is nephritic syndrome or acute renal failure 15 d after an infection[35], in which haematuria is almost always present. Although the prognosis is excellent for children, in the 20%-74% of adults renal impairment persist[34-37]. The immune complexes produced *in situ* or deposited from circulation induce a severe inflammatory

response resulting in neutrophils chemotaxis and endocapillary hypercellularity, leading to haematuria. Endocapillary GN has been recently proposed as a C3 glomerulopathy, because the nephrogenic antigen triggers the activation of the complement alternative pathway.

GBM disorders

As previously reported, GBM has a key role on the glomerular filtration barrier permeability. GBM is composed of a dense gel-like meshwork of type Ⅳ collagen (COL4) and laminin, along with sulfated proteoglycans. The causes of GBM injury can be categorized on primary GBM disorders, as the collagen nephropathies, and secondary GBM diseases, including diseases with GBM as a target.

Primary GBM disorders: Collagen nephropathies are the main primary GBM disorders. Type Ⅳ collagen is the main component of GBM, so its mutations produce abnormal tights winding of the collagen triple helix. Type Ⅳ collagen-associated diseases are the most common hereditary disorders presenting with isolated microscopic haematuria resulting from mutations in genes for type Ⅳ collagen[38], especially on its alpha 3 (COL4A3) and 4 chains (COL4A4)[39].

AS was the first characterized GBM collagen-disorder. AS has a prevalence of 1 case/50000 live births[10]. The 85% of AS cases are due to X-linked mutations in α5 collagen chain (COL4A5), whereas the remaining 15% are due to autosomal recessive mutations in COL4A3 or COL4A4, although a minority of cases have been described as autosomal dominant sporadic mutations. X-linked AS is characterized by sensorineural hearing loss, ocular abnormalities and progressing nephropathy. These alterations are more severe in males. Autosomal reccesive Alport has the same clinical features than X-linked AS, with more aggressive and early CKD impairment (mean age at ESRD is 21 years[40]) without gender preference, and with typically asymptomatic parents genetically related. The electron microscopy show GBM thickening and thinning plus splitting and lamellation of lamina densa[41]. These alterations render in a persistent expression of foetal COL4A1 and COL4A2, being fragile and sensitive to proteases, allowing RBCs egression in the urinary space, and therefore persistent microhematuria. Persistent microhematuria is more frequent in children, often with macroscopic haematuria bouts, which suggest an exacerbating trigger factor over this chronically damaged GBM, although this promoter agent has not been yet identified. Importantly, renal function decreases progressively to ESRD before the fourth decade in 90% of patients[42,43].

Heterozygous mutations in the *COL4A3*, *COL4A4* or *COL4A5* genes produce TBMN. TBMN has an incidence of 1% and is characterized by a GBM < 150 nm. The slightly more compact GMB, due to a lack of non-collagenous molecules, is more fragile, which could explain the persistent isolated haematuria. TBMN's typical presentation include microhematuria and minimal or no proteinuria, with normal glomerular filtration rate and blood pressure. However, recent evidences showed a worse prognosis than it has been though[44], where microhematuria progress to proteinuria, to renal cysts[45] and to CKD in 26.6% of all patients, and in 48% of all patients > 50 years old[10,46].

HANAC syndrome is an extraordinary infrequent systemic basement-membrane disease, due to heterozygous mutation in *COL4A1*[47]. HANAC syndrome could present either with micro- or macroscopic bouts, related or not with impaired glomerular filtration rate and/or renal cysts. ESRD has not been yet described probably due to the low number of patients reported to date. Electron microscopy showed thickening and splitting on the basement membranes (including tubules, capillaries and GBM)[48]. The micro- and macroscopic haematuria bouts may be the result of the abnormal remodeling of the extracellular matrix and altered composition of all basement membranes[47].

Although loin pain haematuria syndrome (LPHS) is not a collagen nephropathy, we include it here due to its similarities with TBMN and AS histolopathologic features. More frequent in females (70%), LPHS presents with recurrent haematuria by the third decade of life. The electron microscopy showed abnormally thin or thick GBM[49]. It has been proposed that abnormalities on GBM allow RBCs leak into the urinary space causing intratubular obstruction and clots. The intratubular obstruction could induce interstitial edema and intraglomerular hypertension which originates further glomerular haemorrhage.

Secondary GBM disorders: Some disorders attack GBM, such as Anti-GBM disease and C3 glomerulopathy. Anti-GBM disease is characterized by autoantibodies against the alpha3 chain non-collagen 1 domain of type Ⅳ collagen. Anti-GBM disease shows an incidence of 0.5-1 case/million people per year[50]. It has been proposed that anti-GBM disease could be triggered over genetically predisposed patients (HLA-DRB1*1501 allele and genes of the FCGR and KLK families[51]) by environmental or cellular/humoral immunity factors. These auto-antibodies attack GBM disturbing its intrinsic structure, explaining the almost always present haematuria, with nephritic syndrome and crescentic glomerulonephritis.

On the other hand, the recently introduced C3 glomerulopathy, as a glomerular pathology with C3 accumulation with none significant immunoglobulin deposition[52]. C3 glomerulopathy clinically has been associated to haematuria, proteinuria and different degrees of renal dysfunction[53]. C3 glomerulopahty is secondary to an aberrant regulation of complement alternative pathway, either genetic or acquired. C3 glomerulopathies include dense deposit disease (DDD), C3 glomerulonephritis and complement factor H-related

(*CFHR*) genes mutations[54], such as hybrid *CFHR3-1* gene and an internal duplication within the *CFHR5* gene[55]. C3 glomerulopathy's incidence has been estimated in 1-2 cases/million people, independently of gender, although it has been reported an increased severity in males. DDD is characterized by linear, hyperosmiophilic, intramembranous dense deposit in lamina densa, restricted to both tubular and Bowman' s capsular basement membranes. Haematuria is observed in the 87% of the cases, mainly microscopic hematuria (68%)[53] and that persists during the follow up. Haematuria can be explained by the GBM impairment, although mesangial, subendothelial and subepithelial deposits has been also described[53]. Two evidences suggested the role of an infection triggering C3 glomerulopathies, firstly the concurrence of macroscopic bouts of haematuria with upper respiratory tract infections in CFHR5 nephropathy[54], and secondly the elevated antistreptolysin-O (a substance produced by group A Streptococcus bacteria) titters in C3 glomerulopathy[53]. Although proteinuria has pointed as the most important prognostic factor, in the Athanasiou cohort[56] all patients that reached ESRD presented macroscopic haematuria bouts associated with fever upper respiratory tract infections in the childhood and adolescence.

Mesangial deposit disorders

IgAN is the most common cause of glomerular haematuria. IgAN has an uncertain prevalence (10%-16%)[57] and is characterized by the presence of persistent isolated microscopic haematuria, with occasional macroscopical bouts associated to upper respiratory or gastrointestinal infections. Haematuria may be accompanied by proteinuria, sometimes in the nephrotic range. Although it has been consider benign, nearly 20% of patients develop ESRD within 20 years of diagnosis[58-61]. Mesangial hypercellulary is the usual histological finding, being the degree of interstitial fibrosis and tubular atrophy the strongest predictors of renal outcome[62]. However haematuria's role over IgAN outcome has not been properly addressed. Macroscopic haematuria bouts has negative implications on long-term prognosis[5] and although the prognosis of IgAN patients with isolated MH have been reported as good, almost 50% of the largest cohort presented spontaneous remission of MH during the follow up[63].

Even though mechanism of haematuria is unknown, during the episodes of macroscopic haematuria it has been detected an increase in circulating immune complexes composed of galactose-deficient IgA1 complexed with antiglycan antibodies[64]. These circulating immune complexes are deposited in the mesangium, inducing cell proliferation and secretion of several inflammatory mediators (including cytokines, growth factors and aldosterone/angiotensin) which can be released to the urinary space and induce both, podocytes and proximal tubular epithelial cells damage[65]. Therefore, these mediators could compromise GBM filtration-barrier function, allowing RBCs egression. The same pathological mechanism has been observed in Henoch-Schönlein purpura (HSP), a systemic disorder characterized by the coincidence of IgAN and leukocytoclastic vasculitis. In HSP patients, the plasma concentration of galactose-deficient IgA1 complexes are also increased and the subendothelial deposits, crescents as well as glomerular-tuft necrosis are even more frequent than in IgAN[65].

Subendothelial and subepithelial deposits diseases

Many nephropathies are characterized by the presence of deposits in subendothelial and subepithelial spaces, inducing a significant impairment in the GFB integrity and therefore haematuria.

Primary glomerulonephritis: Membranoproliferative, Endocapillary and Crescentic GN are the main primary GN associated with subendothelial and subepithelial deposits. It has been proposed that leucocytes and immune-complex can produce a severe inflammatory response activating glomerular cells and interfering with GBM structure, leading haematuria.

Fibril deposit disease: Fibronectin glomerulopathy (GFND) is a rare autosomal-dominant nephropathy due to a mutation in Fibronectin 1 (*FN1*) gene expressed[66]. FN1 is a dimeric glycoprotein constituent of the extracellular matrix. Its mutations altered the protein-dimers assembly into fibrils in the extracellular matrix and produce a disbalance between soluble and insoluble fibronectin, leading to its pathognomonic deposition in mesangium and subendothelial area[66,67]. In addition to fibronectine deposition, it has been reported IgA, C1q and fibrinogen deposits[68]. GFND may present at different ages, although mostly in adolescence or early adulthood. GFND is characterized by microhaematuria, proteinuria and hypertension. GFND patients progress to ESRD from second to sixth decade of life[66]. In these patients ESRD can recur after renal transplantation[69].

Fibrillary and Immunotactoid GN presented fibrils or microtubules deposition in mesangium, GBM or both. Immunotactoid GN can be differentiated from Fibrillary due to its typically wider fibrils with focal parallel alignment. The pathogenesis is unclear, however its response to immunosuppression pointed an underlying autoimmune condition[70]. Fibrillary GN presents deposits infiltrating both mesangium and lamina densa[71], which implied a severe impairment in the GFB allowing RBCs egression, explaining the pathogenesis of haematuria in these diseases.

Podocyte associated disorders

The podocytes are highly specialized epithelial cells, with interdigitating foot processes and specialized intercellular junctions term the "slit diaphragms", playing a key role in GFB integrity. Mutations in proteins involved

in the slit diaphragm and foot processes have been mainly associated with familial nephrotic syndrome. A previously kind of familial benign haematuria, MYH9-associated kidney disease, has been recently described as a genetic variation on *MYH9* gene. *MYH9* encode non-muscle myosin IIA heavy chain, a major protein of the actin-myosin's podocyte contractile apparatus, necessary to keep the capillary wall integrity[72]. Other autosomal-dominant syndromes as May-Hegglin anomaly and the Flechtner and Epstein syndromes also include abnormalities in *MYH9* gene, with a total incidence < 1:100000[38]. *MYH9* gene mutations presented variable degrees of sensorineural deafness and glomerulopathy[73], usually in African people. Haematuria and/or proteinuria are typically present since childhood, with a progression to ESRD by young adulthood[74]. Electron microscopy showed occasional focal thickening and splitting of the GBM[74,75]. *MYH9* mutations produce a more fragile podocyte and capillary wall which allow RBCs egression, explaining the presence of haematuria[75].

Fabry's disease also present haematuria. Fabry's disease is a lysosomal storage X-linked disorder, much more common than it has been though (1:3100[76]-1:1600[77]) and more frequent and aggressive in males. This lysosomal impairment leads to intracellular accumulations of globotriaosylceramide in almost all the human cells[78]. The globotriaosylceramide accumulation induce autophagy in podocytes and endothelial cells damage, resulting in focal and segmental sclerosis, as well as a significant impairment in the GFB, therefore leading proteinuria and haematuria[79].

Miscellaneous

There are several diseases associated to haematuria without any obvious histopathological finding to justify it. Warfarin coagulopathy (international normalized ratio > 3.0) may induce AKI, the so called WRN[80]. AKI could be caused by intratubular obstruction of RBC casts during glomerular haemorrhage, although atheroembolism[81], interstitial nephritis[82], and direct effects of warfarin on the glomerulus[83] have been also pointed. The real WRN incidence could be 16% in non-CKD and 37% in CKD patients[84]. There has been described several risk factors for WRN, including: (1) aspirin therapy; (2) drugs that increase glomerular hydrostatic pressure, such as dihydropyridine calcium channel blockers; (3) low serum albumin levels; and (4) concurrent congestive heart failure[85]. The correction of the warfarin coagulopathy with vitamin K prevents WRN, and could promote the recovery on animal models[86]. In WRN, 66% of the patients with macroscopic haematuria bouts show impairment of renal function. WRN is associated with an accelerated CKD progression and mortality rate, although this was related with the patienti's comorbidities[9]. It has been speculated that this warfarin iatrogenic coagulopathy may be observed in patients with permeable and previous "fragile" GFB (like subclinical GN or TBMD), allowing RBC egression.

Sickle cell disease (SCD) is a multisystemic disorder with homozygous or heterozygous inheritance of β-globin mutated gene, leading in the production of hemoglobin S (HbS), with a global incidence of 30/million people. HbS produce abnormally dense and rigid RBCs with tendency to sickle. The 3%-4% of SCD patients presented haematuria, although it is more frequent on heterozygotes with the sickle cell trait. The tortuous sickle RBCs can easily extravasate the glomerulus capillary wall, raising blood viscosity, and promoting microthrombi formation and ischemic necrosis in the vasa recta, and therefore inducing structural changes and haematuria[87]. Haematuria is mainly recurrent and macroscopic, and could be asymptomatic or painful due to passage of clots through the ureter. Furthermore, haemoglobinuria is also frequent is this patients due to its recurrent haemolytic anaemia crisis.

CONCLUSION

Recent findings suggest a pathogenic role for glomerular hematuria in kidney disease. Thus, the occurrence of macroscopic hematuria-associated AKI in IgAN nephropathy is associated with subsequent persistent impairment of renal function in a significant proportion of patients. An excessive anticoagulation, as a result of warfarin therapy, may also result in macroscopic hematuria-associated AKI and may compromise long-term renal function. Finally, persistent isolated microhematuria may also induce ESRD. The intrinsic pathogenical mechanism of glomerular haematuria remains unclear. Dysmorphic urinary RBCs pointed GFB dysfunction or damage as a possible alteration associated to this pathological process. Three possible pathological mechanisms may be implicated in GFB dysfunction and subsequent haematuria onset, including genetic alteration of GFB components, aberrant deposition of toxic molecules in the GFB, and an enhanced inflammatory response. However, although it has been identified some of the mechanisms involved in haematuria-associated renal damage, it is necessary to characterize new pathogenic effects of hematuria to identify new potential therapeutic targets. Future studies, in this line, will be of great interest.

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REVIEW

Renal dopaminergic system: Pathophysiological implications and clinical perspectives

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Abstract

Fluid homeostasis, blood pressure and redox balance in the kidney are regulated by an intricate interaction between local and systemic anti-natriuretic and natriuretic systems. Intrarenal dopamine plays a central role on this interactive network. By activating specific receptors, dopamine promotes sodium excretion and stimulates anti-oxidant and anti-inflammatory pathways. Different pathological scenarios where renal sodium excretion is dysregulated, as in nephrotic syndrome, hypertension and renal inflammation, can be associated with impaired action of renal dopamine including alteration in biosynthesis, dopamine receptor expression and signal transduction. Given its properties on the regulation of renal blood flow and sodium excretion, exogenous dopamine has been postulated as a potential therapeutic strategy to prevent renal failure in critically ill patients. The aim of this review is to update and discuss on the most recent findings about renal dopaminergic system and its role in several diseases involving the kidneys and the potential use of dopamine as a nephroprotective agent.

Key words: Dopamine; Hypertension; kidney; Na^+, K^+-ATPase; Sodium; Oxidative stress; D1 receptors; D2 receptors; Renal failure; Edema

Core tip: Renal dopaminergic system is a local and independent natriuretic system necessary to maintain the normal balance of sodium and water, blood pressure and renal redox steady state. Different findings from experimental and clinical studies highlight the participation

of renal dopamine in the pathophysiology of renal inflammation, hypertension, diabetic nephropathy and edema formation. Recent findings from experimental and clinical studies allow us to understand the complexity of this system as well as its possible contribution for future therapeutic strategies to prevent renal diseases.

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INTRODUCTION

Kidney has all the bioenzymatic machinery necessary to possess a local dopaminergic system. Renal dopamine production depends on the precursor L-dihydroxyphenylalanine and dopa decarboxylase activity. Although dopa decarboxylase is present in high concentrations in the proximal tubular cells, L-dopa uptake by sodium dependent and independent transporters represents the limiting step for intrarenal dopamine synthesis[1-4]. Intrarenal dopamine can leave the cell through the apical border by a diffusional process, whereas plasma dopamine can be uptaken through the basal cell border by a saturable process[5]. The organic cation transporters (OCTs and OCTNs) have been postulated as potential carriers for dopamine through the tubular cells[5-7]. Finally, dopamine can be eliminated with urine flow or degraded by methylation (*via* catechol-O-methyl transferase or COMT) to 3-methoxytyramine, and by deamination (*via* monoamine oxidase or MAO) to 3,4-dihydroxyphenylacetic acid[8].

Several organs and systems are involved in the regulation of blood pressure. In particular, the kidney plays an essential role in the etiology of hypertension, but also represents a target organ vulnerable to hypertensive tissue damage. Alterations in renal tubule transport may be linked to the onset of hypertension in which dopamine could play an important role by affecting sodium handling on the proximal tubule[9]. Dopamine, as a major regulator of proximal tubule salt and water reabsorption, exerts its physiological actions through two families of receptors located in the tubular cell surface: D1-like receptors (D1 and D5) and D2-like receptors (D2, D3 and D4)[10-14]. Through activation of D1-like receptors, locally produced dopamine acts as an autocrine/paracrine natriuretic hormone by inhibiting the activity of both apical (*e.g.*, Na^+/H^+ exchange, Cl^-/HCO_3^- exchange and Na^+/Pi cotransport) and basolateral (*e.g.*, Na^+, K^+-ATPase and Na^+/HCO_3^- cotransport) transporters[15-17]. The D1-like receptors, coupled to the stimulatory G proteins Gαs and Golf, are characterized by their capacity to activate adenylate cyclase, while D2-like receptors, coupled to the inhibitory G proteins Gαi and Go, are characterized by their capacity to inhibit adenylate cyclase and modulate ion channels[18,19]. The classical signaling pathway for D1-like receptors leads to activation of adenylate cyclase and increases cyclic adenosine 3′,5′-monophosphate (cAMP) levels and protein kinase A (PKA) activation. PKA may either directly phosphorylate a target protein, such as a sodium transporting protein, or initiate a cascade of phosphorylation events by phosphorylation and activation of dopamine and cAMP-regulated phosphoprotein DARPP32[1]. D1 receptor can also stimulate phospholipase Cβ1 in renal tubules[20]. On the other hand, D2-like receptors can suppress Akt (protein kinase B) signaling pathway[21]. Both types of dopamine receptors are also linked to mitogen-activated protein kinase activation through different pathways and can interact with each other, resulting in new signaling pathways. In renal cortical cells the interaction between D1 and D2 receptors increases phospholipase C stimulation[22].

At glomerular level, dopamine increases cAMP in mesangial and podocyte cells *via* D1-like receptor and inhibits angiotensin Ⅱ-mediated contraction in mesangial cells[23,24]. Through this mechanism, dopamine induces depolarization of the podocyte that may lead to its relaxation[25]. These data suggest that dopamine can augment natriuresis and diuresis by increasing directly water and sodium filtration at glomerular level. Besides these effects on sodium and water homeostasis, it has been demonstrated that dopamine could exert anti-inflammatory and anti-oxidants properties by activation of D1-like and D2-like receptors[26-29].

To date, several studies reported that an intact dopaminergic system is required to maintain renal hemodynamic, fluid and electrolyte balance, redox steady state and blood pressure within a normal range and to antagonize the renin-angiotensin system[30,31]. In this way, alterations in dopamine production and its receptor number, function and/or post-translational modification are associated with different pathological scenarios like oxidative stress, genesis and progression of renal dysfunction, edema formation and genetic or essential hypertension. In clinical practice, dopamine is used as a first line vasoactive agent in patients with hemodynamic instability unresponsive to fluid therapy[32]. However, despite its diuretic and natriuretic properties, its clinical use in patients with renal failure remains controversial.

EFFECTS OF INTRARENAL DOPAMINE ON OXIDATIVE STRESS AND RENAL INFLAMMATION

The redox state of cells represents a balance between the generation of free radical/highly reactive species and the presence of antioxidant mechanisms. Acting as cellular messengers, reactive oxygen species (ROS) are

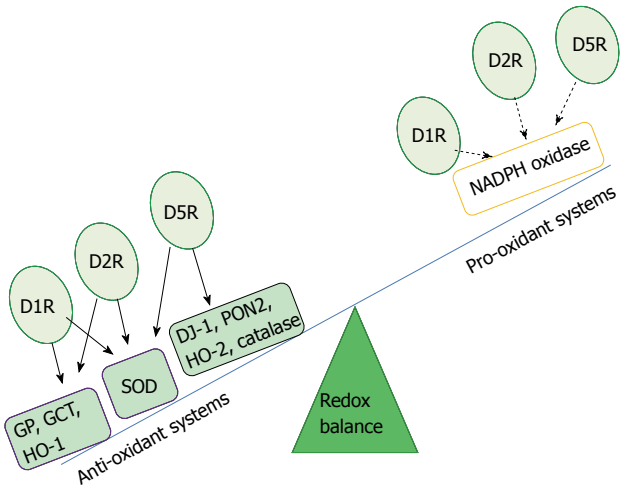

Figure 1 Dopamine receptors and regulation of redox state. Full line: Stimulation; Dotted line: Inhibition. D1R: Dopamine receptor subtype 1; D2R: Dopamine receptor subtype 2; D5R: Dopamine receptor subtype 5; NADPH: Nicotinamide adenine dinucleotide reduced form; SOD: Superoxide dismutase; HO-1: Heme oxygenase 1; HO-2: Heme oxygenase 2; PON2: Paraoxonase 2; DJ-1: Parkinson protein 7; GP: Glutathione peroxidase; GCT: Glutamyl cysteine transferase.

implicated in the destruction of invading pathogens. Pathological situations involving overproduction of free radicals (*e.g.*, atherosclerosis, hypertension, *etc.*) can lead to an increase in oxidative stress status, disrupting the normal cellular signaling mechanisms by alteration of the normal redox state of cells[33-36].

Oxidative stress and infiltration of inflammatory cells in the kidney are involved in the development of renal injury and hypertension[37]. Renal tubule cells produce both pro- and anti-inflammatory cytokines and chemokines, which are secreted across their apical and basolateral membranes, and are implicated in the development and progression of glomerular and tubular injury[38,39].

Several enzymes and receptors are involved in the regulation of the redox balance, including nicotinamide adenine dinucleotide reduced form (NADPH) oxidase and dopamine receptors (D1-like and D2-like receptors)[30]. Renal dopaminergic system represents a negative regulator of ROS. In this sense, D1, D2, and D5 receptors can exert antioxidant effects through direct and indirect inhibition of pro-oxidant enzymes, specifically, NADPH oxidase, and through stimulation of antioxidant enzymes such as superoxide dismutase (SOD) and heme-oxygenase (HO) among others, which can also indirectly inhibit NADPH oxidase activity[30,40]. Particularly, stimulation of D2 receptors in the kidney increases the expression of endogenous anti-oxidants, such as Parkinson protein 7 (PARK7 or DJ-1), paraoxonase 2, and HO-2, all of which can inhibit NADPH oxidase activity. By inhibition of phospholipase D2, the D5 receptor reduces NADPH oxidase activity. This receptor subtype also increases the expression of another antioxidant enzyme, HO-1. Finally, D1 receptor inhibits NADPH oxidase activity *via* PKA and PKC cross-talk and stimulates SOD, glutathione peroxidase, and glutamyl cysteine transferase activities (Figure 1)[30,40].

Additionally, it has been demonstrated that dopamine regulates the immune response and the inflammatory reaction by inhibiting the release of interferon γ (IFNγ), interleukin 2 (IL-2), and IL-4 and the lipopolysaccharide-stimulated production of IL-12p40 in immune cells[29,41]. Other authors showed that mice with intrarenal dopamine deficiency have increased oxidative stress and inflammatory cells infiltration; and that reduction in intrarenal dopamine synthesis is associated with increased detrimental effects of angiotensin Ⅱ on renal injury[42,43].

Experimental studies demonstrated that mice lacking D2 receptor (-/-) have increased levels of blood pressure as well as renal expression of inflammatory factors and renal injury[44,45]. To clarify if decreased D2 receptor function increases the vulnerability to renal inflammation, independently of blood pressure, Zhang *et al*[46] carried out experiments with D2 receptor (-/-) mice, and demonstrated that the treatment with apocynin (an inhibitor of NADPH oxidase) normalized blood pressure levels and decreased oxidative stress, without affecting the expression of inflammatory factors. In support of this evidence, it was reported that short-term D2 receptor silencing in one kidney (leaving the other kidney intact) in mice, induced the overexpression of inflammatory factors and markers of renal injury in the treated kidney, without increasing blood pressure levels[46]. Altogether, these studies indicate that D2 receptor impairment may cause renal inflammation as a primary effect, contributing to the subsequent development of hypertension. Polymorphisms of the human D2 receptor gene may be of clinical relevance since reduction in D2 receptor expression and function may lead to renal damage and oxidative stress[45,46].

Although these evidences indicate that alteration of the renal dopaminergic system may be associated with increased blood pressure *via* oxidative stress, this seems not to be the only mechanism by which impairment of renal dopaminergic system could lead to hypertension.

ROLE OF RENAL DOPAMINERGIC SYSTEM IN THE PATHOPHYSIOLOGY OF HYPERTENSION

Essential hypertension affects 25% of the adult population and constitutes a major risk factor for stroke, myocardial infarction, and heart and kidney failure[47-50]. The etiology of hypertension is complex and involves both genetic and environmental factors[51]. Genome-wide association studies have been able to identify 2% of genetic factors believed to influence blood pressure[52-58]. However, the studies were not designed to identify predisposing genes engaged in a complex network of gene-gene and gene-

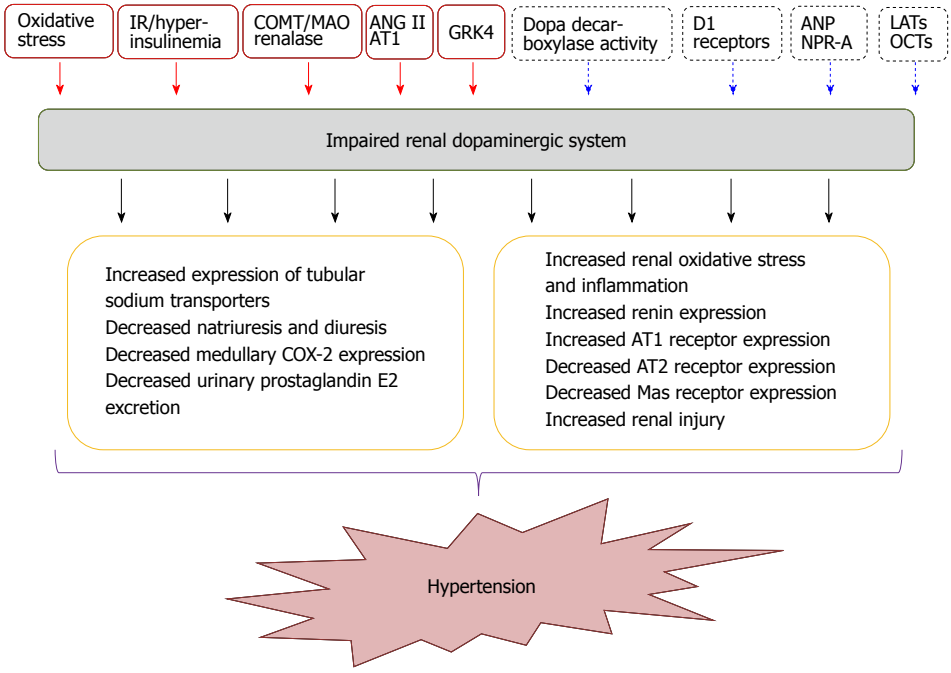

Figure 2 Impaired renal dopaminergic system and its association with hypertension. Red full squares and arrows indicate those factors that promote the impairment of renal dopamine; blue dotted squares and arrows indicate those factors that enhance renal dopaminergic system. IR: Insulin-resistance; COMT: Catechol-O-methyl-transferase; MAO: Monoamine-oxidase: AT1: Angiotensin II receptor subtype 1; AT2: Angiotensin II receptor subtype 2; COX-2: Cyclooxygenase type 2; ANP: Atrial natriuretic peptide; NPR-A: Natriuretic peptide receptor type A; LATs: L-aminoacids transporters; OCTs: Organic cationic transporters; GRK4: G-protein receptor kinase 4.

environment interactions[59]. Many genes have been proposed to cause hypertension and more than one gene is undoubtedly involved. On the other hand, the impairment of renal dopaminergic system functionality in hypertension has been extensively studied in patients as well in experimental animals, and strong evidences indicate that the alteration of this system plays a pathophysiological role in the development of different types of hypertension[30,60] (Figure 2). Several animal experimental models of hypertension exhibit one or more defects related to the renal dopaminergic system, and vice versa, different models with impairment of this system are associated with the development of hypertension (Table 1).

The interaction between renal dopamine and angiotensin Ⅱ can take place at receptors level[18]. In this way, AT2 and D1 receptors cooperatively oppose the vasoconstrictor and antinatriuretic functions elicited by angiotensin Ⅱ at AT1 receptor. It has been demonstrated that *in vivo* administration of fenoldopam (a highly selective D1-like receptor agonist) in sodium loaded Sprague Dawley rats induces the translocation of AT2 receptors from intracellular compartment to the apical plasma membranes[71]. This effect was confirmed by the fact that fenoldopam-induced natriuretic response was completely inhibited by the intrarenal co-infusion of the AT2 receptor antagonist PD123390[72]. Therefore, the alterations in D1 receptor-dependent translocation of AT2 receptor must be considered as a contributor factor for the initiation and progression of disease processes including hypertension. Blood pressure levels increase with aging, and alterations in both D1 and AT1 receptor functions are closely associated with the development of age-related hypertension[68,73-75]. Aging is a process associated with increase in oxidative stress and dysfunction of renal D1 and AT1 receptors[76-79]. Both receptors influence the activity of tubular Na^+, K^+-ATPase and contribute to maintain sodium homeostasis and blood pressure[77,79]. It has been reported in spontaneously hypertensive and obese Zucker rats an increase in oxidative stress and altered renal D1 and AT1 receptor functions[80-83].

Another possible mechanism involved in the impaired natriuretic effect in spontaneously hypertensive rats (SHRs) could be related to impaired endothelin B and D3 receptor interaction[84]. The endothelin B and dopamine receptors can interact to regulate renal function and blood pressure[85]. It has been demonstrated that activation of renal D3 receptor induces natriuresis and diuresis, but this effect is reduced in the presence of an endothelin B receptor antagonist, demonstrating that dopamine effects depends partially on endothelin B receptors. Moreover, stimulation of endothelin B receptor increases D3 receptor protein expression and vice versa in renal proximal tubule from Wistar Kyoto rats but not from SHRs[84,86]. Another study indicates that D3 receptors physically interact with proximal tubule endothelin B receptors and that the blunted natriuretic effect of dopamine in SHRs may be explained, in part, by abnormal D3/endothelin B

Table 1 Renal dopaminergic system impairment in experimental hypertension

Animal experimental model	Renal dopaminergic system impairment	Principal findings	Ref.
Spontaneously hypertensive rats	D1-like receptor function impairment caused by a defective coupling of the receptor with AC	Increased sodium reabsorption as a mechanism of hypertension	Ohbu *et al*[61]
Dahl salt-sensitive rats	D1-like receptor function impairment caused by a defective coupling of the receptor with AC	Prehypertensive Dahl salt-sensitive rats exhibit a blunted natriuretic response to dopamine compared with Dahl salt-resistant rats	Nishi *et al*[62]
DOCA salt-sensitive rats	Decreased renal dopamine production	Renal dopaminergic system is dominantly supressed in this model of hypertension	Iimura *et al*[63]
Dopamine receptor knockout mice	Defective D1-D2 like receptor/signal transduction	Impaired D1 and D2-like receptor signal pathway associated with development of hypertension	Banday *et al*[64] Zeng *et al*[65] Albrecht *et al*[66]
C57BL/6 mice	D1-like receptor function impairment associated with increased expression of GRK4 upon salt loading	Impaired ability to excrete a salt load with a resultant increase in blood pressure levels	Escano *et al*[67]
Mice with selective proximal tubule AADC deletion	Deletion of the kidney's ability to generate dopamine is associated with unbuffered response to angiotensin II that leads to hypertension and decreased longevity in mice	Increased expression of tubular sodium transporters, decreased natriuresis and diuresis in response to L-Dopa, decreased medullary COX-2 expression and urinary prostaglandin E2 excretion, increased renin and AT1 receptor expression, decreased AT2 and Mas receptor expression, and finally salt-sensitive hypertension.	Zhang *et al*[42]
Old FBN rats	Reduction of G-protein coupling in response to D1R activation associated with exaggerated AT1 receptor activity	Increase of oxidative stress	Chugh *et al*[68]
Renalase knockout mice	Alteration of urinary dopamine concentration in luminal fluid and proximal tubular transport	Impaired sodium excretion with increased blood pressure	Desir[18]
3/4 nephrectomized (3/4nx) rats	Decrease in urinary levels of dopamine and in renal AADC activity	A reduction in the natriuretic response to volume expansion with a time-dependent increase in both systolic and diastolic blood pressure	Moreira-Rodrigues *et al*[69]
Obese Zucker rats	Decrease in D1-like dopamine receptor binding sites and diminished activation of G proteins	Overproduction of ROS	Hussain *et al*[70]

GRK4: G protein receptor kinase 4; D1R: Dopamine receptor subtype 1; COX-2: Cyclooxygenase type 2; AT1: Angiotensin II receptor subtype 1; AT2: Angiotensin II receptor subtype 2; ROS: Reactive oxygen species; AC: Adenylate cyclase; FBN: Fischer 344 x Brown Norway F1.

receptor heterodimerization[85].

The interaction between prostanoids and renal dopamine on sodium and water excretion must also be considered. It has been demonstrated that the natriuretic response to dopamine was lower in Dahl salt-sensitive rats but this effect was reversed when chromosome 5 was transfered into these rats, leading to an increase of the renal expression of CYP4A protein and the production of 20-HETE[87]. Moreover, the inhibition of Na^+, K^+-ATPase activity by dopamine in the proximal tubule may be the result of the synergism between 20-HETE and the D1 signaling pathway[88]. In addition, other metabolites of arachidonic acid produced in the proximal tubule are epoxyeicosatrienoic acids and dihydroxyeicosatrienoic acids. As 20-HETE, epoxyeicosatrienoic acids can also regulate Na^+, K^+-ATPase activity and serve as second messengers for the natriuretic effects of dopamine. Since renal production of cytochrome P450 metabolites of arachidonic acid is altered in hypertension, a lower prostanoid synthesis may be involved in the impaired response to dopamine in this context[89].

Another protein that could be involved in the pathophysiology of hypertension is the novel amine oxidase, renalase[18]. Renalase is synthesized in the kidney with high expression in the proximal tubule, and then secreted into plasma and urine[19]. Renalase specifically degrades catecholamines, including dopamine. Recent findings indicate that renalase deficiency is associated with increased blood pressure and elevated circulating catecholamines[90,91]. Renalase expression depends on salt intake, and recombinant renalase exhibits a potent and prolonged hypotensive effect on blood pressure in Dahl salt-sensitive rats and rats with chronic kidney disease[92,93]. Urinary renalase metabolizes urinary catecholamines and it has been hypothesized that it might regulate dopamine concentration in the luminal fluid. However, the mechanisms of hypertension in animals with renalase deficiency and its relationship with the renal dopaminergic system are still unclear and deserve to be investigated in more detail.

Given its participation on sodium and water excretion and blood pressure regulation as well as its antioxidant properties in the kidney, alteration in renal dopaminergic system should also be considered in the pathophysiology of other diseases associated with

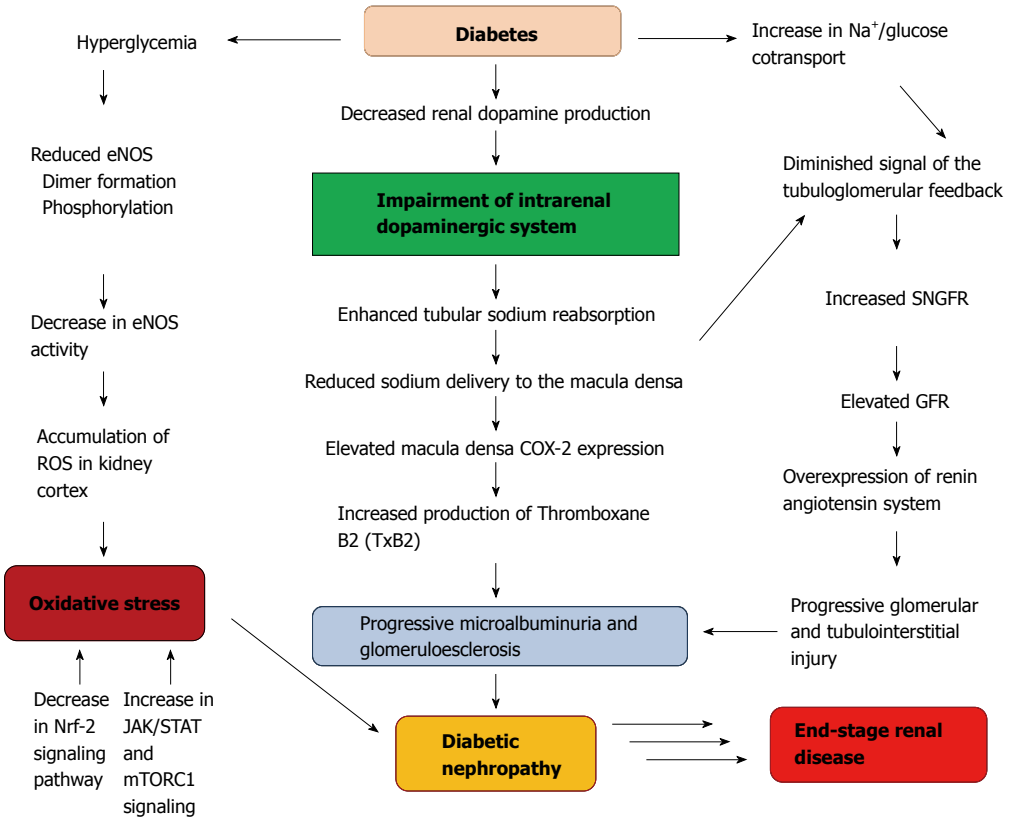

Figure 3 Association between diabetes and renal dopaminergic system in the pathophysiology of diabetic nephropathy. COX-2: Cyclooxygenase-2; GFR: Glomerular filtration rate; SNGFR: Single nephron glomerular filtration; eNOS: Endothelial nitric oxide synthase; ROS: Reactive oxygen species.

kidney damage such as diabetic nephropathy.

RENAL DOPAMINE, HYPERINSULINEMIA AND PATHOPHYSIOLOGY OF DIABETIC NEPHROPATHY

Diabetes in cursive is the most prevalent cause of end-stage kidney disease and its incidence has increased by more than 50% in the last 10 years[94]. Diabetic nephropathy is associated with elevated glomerular filtration rate, enhanced tubular sodium reabsorption, reduced sodium delivery to the macula densa and also with progressive glomerular and tubulointerstitial injuries[95,96]. Diabetic nephropathy is a major cause of mortality in both types diabetes. Adults with type 1 and 2 diabetes demonstrate insulin resistance, which is associated with diabetic nephropathy[97,98]. Experimental studies of diabetic nephropathy helped to understand the pathophysiological mechanisms underlying the disease process and allowed to identify potential molecular targets for future pharmacological treatment. In this way, it has been demonstrated a decrease in endothelial nitric oxide synthase (eNOS) activity and renal dopamine production and an increase in Nrf-2, JAK/STAT and mTORC1 signaling as contributor factors in the development of diabetic nephropathy[99]. Alteration of mechanisms involving dopamine handling and signaling by the proximal tubule cells could lead to a progressive damage in diabetic nephropathy. In addition to the renin angiotensin system, alterations of renal cyclooxygenase-2 (COX-2) function are also involved in renal hemodynamic changes and structural abnormalities observed in diabetic nephropathy[100-102]. Previous findings showed that renal dopamine inhibits COX-2 expression in the macula densa, suggesting that the impairment of intrarenal dopaminergic system observed in diabetes, may contribute to reduce the luminal offer of sodium to this area, resulting in an elevated macula densa COX-2 expression[17,103,104] (Figure 3). Another experimental study carried out in mouse models of type 1 diabetes demonstrated that enhanced proximal tubule dopamine levels by deletion of $COMT^{-/-}$ gen was associated with substantial amelioration of early hyperfiltration, decreased macula densa COX-2 expression, decreased albuminuria and glomerulopathy, and inhibition of inflammation markers, oxidative stress, and fibrosis[31,99]. Conversely, depletion of proximal tubule dopamine levels by deletion of dopa decarboxylase gen in diabetic mice developed a marked increase in albuminuria as well as increment of mesangial expansion, renal macrophage infiltration, and renal nitrotyrosine levels[31]. These findings contribute to confirm the major role played by the intrarenal dopaminergic system on the development and progression of kidney injury caused by diabetes mellitus.

In renal proximal tubule cells, insulin and dopamine

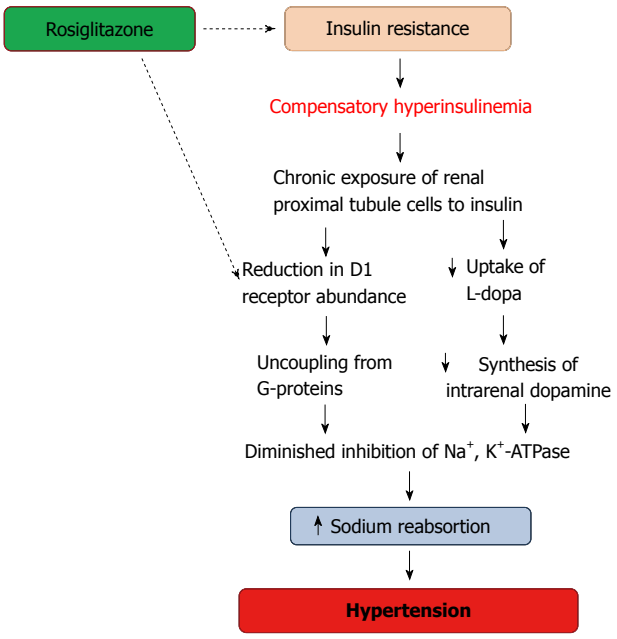

Figure 4 Association between insulin resistance and impairment of renal dopaminergic system. Full lines: Stimulation; stripped lines: Inhibition.

counter-regulate each other by opposing their effects on Na^+, K^+-ATPase activity[70]. Although insulin has been reported to enhance renal proximal tubule uptake of L-dopa in normal fed control rats, this regulatory mechanism is absent in cells isolated from animals fed with fructose, a model of insulin resistance. In addition, the chronic exposure of renal proximal tubule cells to insulin causes a reduction in D1 receptor abundance and uncoupling from G-proteins, which results in the impairment of the inhibitory effects of dopamine on Na^+, K^+-ATPase[70,105]. Hyperinsulinemic animals and patients with type 2 diabetes present a defective renal dopaminergic system[106]. In obese Zucker rats, a model of type 2 diabetes or in insulin-induced hypertension, renal D1 receptors are down-regulated and dopamine fails to produce diuresis and natriuresis[70]. Moreover, when these animals were treated with an insulin sensitizer (rosiglitazone), plasma insulin levels decreased and D1 receptor function were restored[107,108] (Figure 4). Based on these evidences, it is possible that the regulatory mechanisms of the renal dopaminergic system are impaired in diabetic nephropathy due to insulin resistance.

Previous studies have shown that in both type 1 and type 2 diabetes, expression of renal D1 receptor gene was reduced above 50% through a down-regulation mechanism that involves the extracellular cAMP-adenosine signaling pathway[70,109]. An increase in intrarenal dopamine synthesis and the subsequent stimulation of vascular D1 receptors appear to prevent early glomerular hyperfiltration in diabetic rats[110]. Conversely to the D1 like receptors, selective antagonism of D2 like receptors was demonstrated to reverse glomerular hyperfiltration induced by experimental diabetic hyperglycemia[111]. Additionally, activation of the D3 receptor in rats caused diuresis, natriuresis and increased glomerular filtration[112]. To demonstrate the renoprotective effect of a D3 receptor antagonist (A-437203), hypertensive type 2 diabetic (SHR/N-cp) rats were used to evaluate the renal effects of D3 antagonism on glomerulosclerosis damage index, glomerular volume, desmin expression as marker of podocyte damage, and urinary albumin excretion[113]. The results of this study suggest that D3 receptor antagonism has a beneficial effect on renal morphology and albuminuria, which is comparable in magnitude to that of angiotensin-converting enzyme inhibitor treatment as the gold standard[113].

Despite its role in the pathophysiology of diabetic nephropathy, the potential clinical use of dopamine in this context is still matter of basic research. Nonetheless, the therapeutic use of dopamine is restricted to its dose-dependent actions on the cardiovascular system.

DOPAMINE AS NEPHROPROTECTIVE AGENT? EXPERIMENTAL AND CLINICAL EVIDENCES IN RENAL DYSFUNCTION

Dopamine represents an essential drug in intensive care units and is still used as a first line vasopressor agent especially in hypotensive adult patients refractory to fluid resuscitation[32]. Because of its interaction with different catecholamine receptors, the pharmacological profile of dopamine is dose dependent[114]. At low doses (0.3-5 μg/kg per minute), dopamine stimulates D1 and D2 receptors inducing natriuresis, diuresis and enhances renal blood flow by renal vasodilation. At higher doses, when adrenergic stimulation prevails, dopamine increases renal blood flow through stimulation of cardiac output[32,114,115]. In healthy adult volunteers, the administration of a low dose dopamine increases renal blood flow and induces natriuresis and diuresis[116,117]. For these reasons, a low-dose dopamine represents a therapeutic option to limit or prevent renal failure in critically ill patients by increasing renal blood flow[118]. Although a low dose dopamine appears to be able to increase urinary output in critically ill adult patients at risk of renal failure, a high number of clinical studies indicate that the administration of a low dose dopamine might not be able to exert any protective effect to prevent the onset or improve the course of an established acute renal failure, but on the contrary, its use may increase its risk[32,114,119-122]. Taken all together, the nephroprotective action of a low dose dopamine in critical ill patients remains to date controversial (Table 2).

Although these evidences attempt to the clinical use of dopamine, some other findings support the clinical benefit of its use in different scenarios like cardio-renal syndrome, cardiopulmonary bypass and acute decompensated heart failure under treatment with atrial natriuretic peptide (ANP)[119,128,129]. Renal dysfunction is

Table 2 Clinical studies providing evidence against/in support of clinical use of low dose dopamine

	Study design	Results	Ref.
Against clinical use of low dose dopamine	The Australian and New Zealand Intensive Care Society (ANZICS): multicenter, randomized, double-blind, placebo-controlled	324 patients with at least two criteria for the systemic inflammatory response syndrome and clinical evidence of early renal dysfunction: continuous intravenous infusion of low-dose dopamine (2 μg/kg per minute) did not attenuate the peak serum creatinine compared with placebo. There was no statistical difference in mortality between dopamine and placebo arms	Bellomo *et al*[119]
	Meta-analysis study: 17 studies were randomized clinical trials (n = 854)	Low dose dopamine administration did not prevent mortality or the onset of acute renal failure, or the need for haemodialysis in clinically ill patients	Kellum and M Decker[120]
	Meta-analysis study: 15 randomized controlled studies	Dopamine administration did not present beneficial results in terms of serum creatinine changes and incidence of acute renal failure in clinically ill patients	Marik[121]
	Sepsis Occurrence in Acutely Ill Patients (SOAP): Cohort, multiple-center, observational study	Dopamine administration in shock patients, compared to patients who did not receive it, was associated with 20% increase in ICU and hospital mortality rates	Sakr *et al*[122]
	Renal Optimization Strategies Evaluation (ROSE) study: multicenter, double-blind, placebo-controlled randomized clinical trial	Low dose dopamine (2 μg/kg per minute) did not enhance decongestion or improved renal function when added to diuretic therapy in 360 patients with acute heart failure and renal dysfunction	Chen *et al*[123]
In support of clinical use of low dose dopamine	Dopamine in Acute Decompensated Heart Failure (DAD-HF) Trial: randomized clinical trial	The addition of low-dose dopamine (5 μg/kg per minute) to low-dose furosemide (5 mg/h) was associated with improvement in renal function profile and potassium homeostasis at 24 h and it was equally effective as high-dose furosemide (20 mg/h) alone on subjective perception of dyspnoea in 60 patients with acute decompensated heart failure	Giamouzis *et al*[124]
	Retrospective clinical study	Continuous infusion of furosemide in addition to low-dose dopamine compared to intermittent boluses of furosemide was less nephrotoxic and carried a lower readmission rate at 30 d in 116 patients with acute decompensated heart failure	Aziz *et al*[125]
	A prospective single-center randomized double-blind placebo controlled trial	The treatment with high-dose fenoldopam at 1 μg/kg per minute (short-acting D1 agonist) during cardiopulmonary bypass in 80 pediatric patients undergoing cardiac surgery for congenital heart disease significantly decreased urinary biomarkers of acute kidney injury (urinary neutrophil gelatinase-associated lipocaline and cystatin C levels) and also reduced the incidence of acute kidney injury in the postoperative period and the use of diuretics and vasodilators	Ricci *et al*[126]
	Clinical case finding	Low doses of ANP (0.0125 μg/kg per minute) with low dose dopamine (1.0 μg/kg per minute) in acute decompensated heart failure increased urine output, decreased heart rate, improved congestion with a reduced brain natriuretic peptide level, reduced serum creatinine and the levels of urinary liver-type fatty acid binding protein -a novel reno-tubular stress marker- and 8-hydroxydeoxyguanosine -an oxidative stress marker	Kamiya[127]
	Prospective randomized clinical study	Low dose dopamine infusion reduces renal tubular injury following cardiopulmonary bypass in 48 patients with normal or near normal baseline renal function	Sumeray *et al*[128]

one of the most important co-morbidities in heart failure, being a potent predictor of cardiovascular complications and mortality[130]. This relationship is commonly termed cardio-renal syndrome, where both, cardiac and renal dysfunctions, share similar pathophysiology such as activation of the renin-angiotensin-aldosterone and sympathetic nervous systems, imbalance between nitric oxide and reactive oxygen species, and inflammation[131]. Clinical guidelines recommend the treatment of heart failure or renal failure separately without consensus about how managing patients with cardio-renal syndromes[129]. Its specific treatment points out to ameliorate decreased urine output and glomerular filtration rate, increased serum creatinine, and to prevent weight loss[132]. Recent studies in this clinical setting have focused on newer therapies, including renal protective dopamine[124,125,129] (Table 2).

Acute kidney injury after cardiac operations with cardiopulmonary bypass is a life-threatening complication, with a reported incidence of up to 36%[133]. To prevent this situation diuretics have been the mainstay to promote renal function and urine flow after pediatric cardiopulmonary bypass[134,135]. Fenoldopam mesylate is a short-acting D1 agonist that

appears to improve renal function in clinical situations of reduced blood flow by enhancing renal blood flow[126,136]. Then, co-administration of both agents could be a reasonable therapeutic strategy to preserve renal function in this context (Table 2).

A primary therapeutic goal for acute heart failure is to achieve decongestion to relief symptoms without inducing renal dysfunction[137,138]. However, adult patients with acute heart failure and moderate or severe renal dysfunction are at risk for inadequate decongestion and enhanced renal dysfunction, both condition associated with worse prognosis[139]. Renal adjuvant therapies like dopamine could enhance decongestion and preserve renal function during treatment of acute heart failure. In this setting, combined therapy with ANP and dopamine might be useful to improve the management of acute decompensated heart failure without renal injury in patients who do not respond to ANP alone[127]. This beneficial interaction between both agents could be related to previous experimental findings that demonstrated that ANP stimulates dopamine uptake by tubular cells, reduces its catabolism and diminishes the turnover[140,141]. These effects may favor renal biodisponibility of dopamine in tubular cells and enhance overinhibition of renal Na^+, K^+-ATPase activity[141]. Nonetheless, future prospective studies are needed to affirm this suggestion (Table 2).

As it has been demonstrated in patients with heart failure, the interaction between two natriuretic hormones, such as ANP and dopamine, can also be present in other situations with increased extracellular fluid, such as nephrotic syndrome.

RENAL DOPAMINE IN THE PATHOGENESIS OF EDEMA FORMATION

Nephrotic syndrome exhibits increased proteinuria and enhanced sodium retention that contribute to edema and ascites formation[142]. Although reduced plasma volume and serum albumin concentration contribute to sodium retention in nephrotic syndrome, a primary intrarenal sodium handling abnormality could also be implicated in this clinical scenario[143]. In this way, experiments carried out in rats with puromycin aminonucleoside (PAN) induced nephrotic syndrome showed a blunted activity of the renal dopaminergic system evidenced by decreased urine dopamine, decreased availability of D1 receptor in renal proximal tubules and reduced dopa decarboxylase activity[144]. These findings were associated with an increase in Na^+, K^+-ATPase activity in renal proximal tubules[144]. On the other hand, renal dopamine and ANP are known to interact with each other in order to regulate sodium homeostasis[145]. The complex interaction between these two natriuretic systems is evidenced by the fact that ANP stimulates proximal tubular dopamine uptake through natriuretic peptide receptor type A (NPR-A), guanylate cyclase stimulation and protein kinase G (PKG) activation[4]. ANP also recruits silent D1 receptor from the interior of the renal tubular cells towards the plasma membrane where they become functionally active[146]. A recent work of Fernandes Cerqueira *et al*[147] demonstrated in rats with PAN-nephrotic syndrome that the increase of natriuresis and urinary cGMP excretion evoked by an acute volume expansion were blunted, despite the increased levels of circulating ANP, suggesting the unresponsiveness to ANP in this pathology. Treatment with a phosphodiesterase type 5 inhibitor (zaprinast) restored the excretion of cGMP and the natriuresis to similar levels of control rats, and increased the expression of D1 receptors in tubular cells[147]. This evidence indicates that D1 receptors are involved in ANP unresponsiveness observed in PAN-nephrotic syndrome, where the alteration of renal dopaminergic system represents a contributor factor for the edema and ascites formation (Figure 5).

Beyond its renal actions, dopamine effects on fluid homeostasis can also be exerted in other tissues. In this way, recent findings support a possible use of dopamine in edema resolution of pulmonary pathologies[148,149]. Acute lung injury and its severe form, the acute respiratory distress syndrome are prevalent causes of morbidity and mortality[150]. The outcome of patients with acute hypoxemic respiratory failure improves when lung epithelial function is restored and pulmonary edema resolves[151,152]. Pulmonary edema is cleared from the alveoli by active sodium transport, in which sodium enters into the cell *via* apical amiloride-sensitive sodium channels and pumped out from the cell *via* the basolaterally located Na^+, K^+-ATPase. Water follows the sodium gradients, resulting in alveolar fluid reabsorption[153]. Although dopamine inhibits Na^+, K^+-ATPase in the kidney and promotes natriuresis and diuresis, in alveolar cells dopamine increases, in a dose-dependent manner, lung edema clearance in rats by 40%-70% above the control clearance levels[154-156]. Experimental studies using models of lung injury have demonstrated that alveolar fluid clearance is impaired in parallel with decreased Na^+, K^+-ATPase function. In these lung injury models, dopamine (10^{-5} M) instilled into airspace increased alveolar fluid reabsorption by translocating preformed Na^+, K^+-ATPase pumps from intracellular pools (*i.e.*, late endosomal compartment) to the cell plasma membrane in alveolar epithelial-type Ⅱ cells[150,157,158]. This effect is produced through short-term and long-term fashion mechanisms. The short-term mechanism depends on the activation of D1 receptors since fenoldopam reproduces dopamine actions, meanwhile the long-term mechanism implies D2 receptors[159,160]. Accumulation of protein-rich alveolar edema fluid in acute lung injury is the result of an increased microvascular permeability[161-164]. Many experimental and human studies support the hypothesis that vascular endothelial growth factor (VEGF) plays a critical role in shaping the vascular barrier function in acute lung injury[165-169]. Although, D1

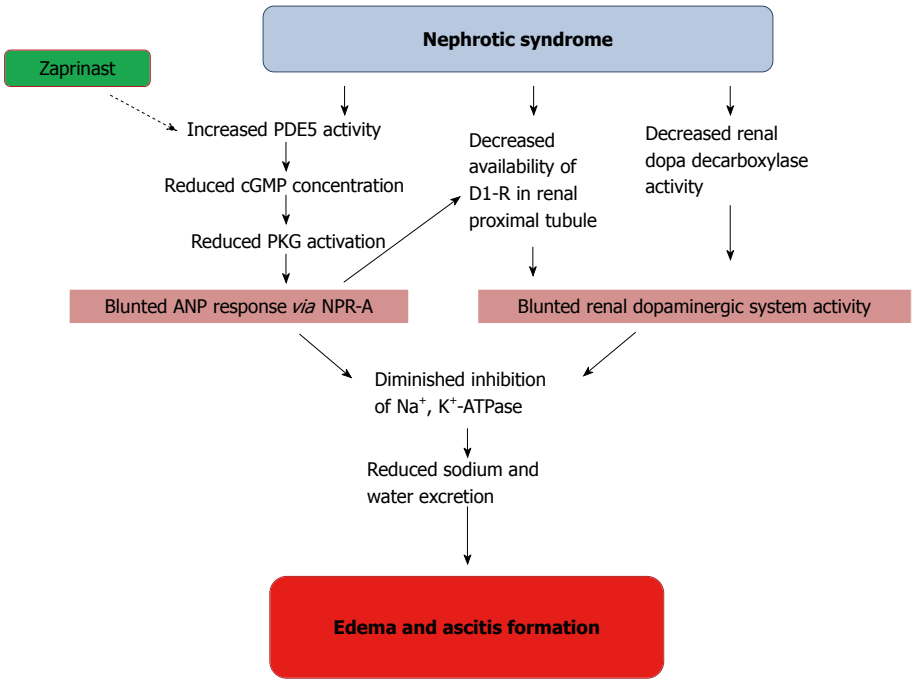

Figure 5 Impaired interactions between ANP and renal dopamine in nephrotic syndrome. Full lines: Stimulation; stripped lines: Inhibition. PDE5: Phosphodiesterase type 5.

and D2 receptors are implicated in the synthesis and trafficking of Na^+, K^+-ATPase, the D2 receptor is also implicated in the regulation of VEGF-induced vascular permeability as well as angiogenesis[159,160,170-172]. This was confirmed by the fact that a D2 receptor agonist failed to reduce pulmonary edema in D2 receptor (-/-) mice, suggesting that dopamine acts through D2 receptor to inhibit pulmonary edema-associated vascular permeability, which is mediated through VEGF-VEGFR2 signaling and conveys protective effects in an acute lung injury model[148]. Although D1 and D2 receptors subtypes seem to be beneficial to reduce edema formation, D3 receptor appears to exert an opposite effect. Several clinical findings reported that pramipexole, a potent non ergoline agent with high affinity to D3 receptors and used for Parkinson's disease, restless legs syndrome, resistant depression and bipolar depression, is associated with the development of pedal and chronic lower limb edema (with a frequency that ranges from 5% to 22.5%)[173-176]. This adverse effect disappears after the discontinuation of the drug[173-176]. Since dopamine is an important regulator of the sympathetic nervous system, aldosterone secretion, as well as adenosine triphosphate-mediated sodium/potassium channels, the peripheral effects of pramipexole at these levels could have a role[177,178]. However the relationship between D3 receptor agonism and edema formation remains unclear.

FUTURE PERSPECTIVES

An intact renal dopaminergic tonus is required for the maintenance of sodium homeostasis and normal blood pressure. By its anti-oxidative and anti-inflammatory properties, intrarenal dopamine plays a major role as a nephroprotective agent to prevent or ameliorate renal dysfunction. Oxidative stress or hyperinsulinemic states may decrease the number of functional dopaminergic receptors in the proximal tubules. In this way, it is worthwhile to test the effect of antioxidant drugs to enhance or restore the biodisponibility of these receptors. A recent observation that dopamine receptors availability in the plasma membrane may be regulated by other hormones, like ANP, could open up a possible therapeutic approach[179].

It has been emphasized the importance of endogenous dopamine and renal D1 receptor on the regulation of sodium and body fluid homeostasis. Although there is evidence that a defective renal dopaminergic system contributes to the development and maintenance of hypertension, it is still not clear what triggering factors cause the selective defects in the renal dopaminergic system. Some of these triggering factors could be an excess of sodium intake that could lead to an activation of intrarenal angiotensin Ⅱ and increase in ROS, an increase in carbohydrate intake and a high fat diet, both factors that promotes an insulin resistance state. Furthermore, the renal dopaminergic system is sensitized by a high salt intake and volume expansion, which opens the question about how intrarenal sodium sensors may influence on renal dopamine bioavailability. This approach may lead to the development of new pharmacological strategies in conditions of salt retention and hypertension. Moreover, identification of abnormalities in different steps of crucial importance for the regulation of the renal dopaminergic tonus should provide additional molecular biological tools for the early diagnosis and treatment of pre-hypertensive patients.

The fact that dopamine exerts nonselective actions upon multiple dopaminergic and adrenergic receptors must be considered, and this, can limit its therapeutic

use in renal diseases. The potential therapeutic use of exogenous dopamine and D1-like receptor agonists is limited to special conditions like critical ill patients who are at risk of kidney failure. However, given the controversial results from clinical studies the use of dopamine in this context must be examined more closely.

At last, further clinical studies must be carried out to confirm the participation of renal dopaminergic system in pathological contexts involving impaired sodium excretion as nephrotic syndrome or insulin resistance states.

CONCLUSION

Intrarenal dopamine represents a local natriuretic system with beneficial actions on blood pressure, oxidative stress and inflammation. Dopamine secreted into the tubular lumen acts *via* D1-like and D2-like receptors in an autocrine/paracrine manner to inhibit different tubular ion transporters and to regulate the production of reactive oxygen species and the inflammatory response. These renoprotective effects can be affected by situations that impair its integrity and functionality. The comprehension of the mechanisms by which renal dopaminergic system is involved in the pathogenesis and development of renal diseases may contribute to improve the diagnosis, evolution, prognosis and treatment of renal pathologies.

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REVIEW

Oxidative stress as a potential causal factor for autoimmune hemolytic anemia and systemic lupus erythematosus

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Abstract

The kidneys and the blood system mutually exert influence in maintaining homeostasis in the body. Because the kidneys control erythropoiesis by producing erythropoietin and by supporting hematopoiesis, anemia is associated with kidney diseases. Anemia is the most prevalent genetic disorder, and it is caused by a deficiency of glucose 6-phosphate dehydrogenase (G6PD), for which sulfhydryl oxidation due to an insufficient supply of NADPH is a likely direct cause. Elevated reactive oxygen species (ROS) result in the sulfhydryl oxidation and hence are another potential cause for anemia. ROS are elevated in red blood cells (RBCs) under superoxide dismutase (SOD1) deficiency in C57BL/6 mice. SOD1 deficient mice exhibit characteristics similar to autoimmune hemolytic anemia (AIHA) and systemic lupus erythematosus (SLE) at the gerontic stage. An examination of AIHA-prone New Zealand Black (NZB) mice, which have normal *SOD1* and *G6PD* genes, indicated that ROS levels in RBCs are originally high and further elevated during aging. Transgenic overexpression of human SOD1 in erythroid cells effectively suppresses ROS elevation and ameliorates AIHA symptoms such as elevated anti-RBC antibodies and premature death in NZB mice. These results support the hypothesis that names oxidative stress as a risk factor for AIHA and other autoimmune diseases such as SLE. Herein we discuss the association between oxidative stress and SLE pathogenesis based mainly on the genetic and phenotypic characteristics of NZB and New Zealand white mice and provide insight into the mechanism of SLE pathogenesis.

Key words: Autoimmune hemolytic anemia; Systemic lupus erythematosus; Red blood cells; New Zealand black mice; New Zealand white mice

Core tip: Superoxide dismutase (SOD1) deficient C57BL/6 mice exhibit characteristics similar to autoimmune hemolytic anemia (AIHA) and systemic lupus erythematosus (SLE) at the gerontic stage. An examination of AIHA-prone New Zealand Black (NZB) mice indicated that reactive oxygen species (ROS) levels in red blood cells are originally high and

further elevated during aging. Transgenic overexpression of human SOD1 in erythroid cells effectively suppresses ROS elevation and ameliorates AIHA symptoms in NZB mice. Herein we discuss the association between oxidative stress and SLE pathogenesis based mainly on the genetic and phenotypic characteristics of NZB and New Zealand white mice.

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INTRODUCTION

The kidney has multiple functions that include maintaining the functions of homeostasis such as the excretion of waste, maintenance of the electrolyte balance of body fluids, and endocrine secretion. As an endocrine organ, the kidney plays an essential role in erythropoiesis by producing erythropoietin that supports hematopoiesis in bone marrow[1]. Chronic kidney disease causes renal anemia by reducing erythropoietin production, and, hence, exogenous erythropoietin is widely used as a potent medicine for the treatment of patients with renal anemia[2]. Defected iron metabolism due to chronic inflammation and cytokine imbalance is also involved in chronic kidney disease-induced anemia[3].

A variety of contributing factors including defected hematopoiesis and accelerated hemolysis are involved in anemic pathogenesis. Glucose 6-phosphate dehydrogenase (G6PD) deficiency, which is the most common genetic defect in the human population[4], causes an insufficient supply of NADPH in RBCs and results in anemia. Although the actual mechanism of anemia due to the G6PD deficiency is not totally understood, the involvement of sulfhydryl oxidation is suspected to be a contributing factor.

Aberrant immune responses in some autoimmune diseases also cause anemia. Autoimmune hemolytic anemia (AIHA) is the pathological condition whereby antibodies attack RBCs, and it often precedes a diagnosis of systemic lupus erythematosus (SLE)[5,6]. Both genetic and environmental factors are involved in the etiology of AIHA and SLE, but molecular mechanisms for a majority of the diseases are largely ambiguous. Reactive oxygen species (ROS) are elevated and appear to be a likely underlying mechanism for these pathological conditions[7-9]. In this review article we discuss recent advances in the research on AIHA and SLE from the viewpoint of oxidative stress using animal models.

G6PD deficiency and oxidative stress

ROS are produced under various conditions such as inflammation and hypoxia-reperfusion injury, and they are involved in a variety of diseases including anemia and renal failure[10]. While reduction-oxidation (redox) reactions play essential roles in metabolic reactions, which includes oxidative phosphorylation that consumes respired oxygen, ROS are simultaneously produced as byproducts. Meanwhile, hemoglobin (Hb), which constitutes a major protein (5 mmol/L) in RBCs and contains Fe(Ⅱ)-heme. When hemoglobin is oxygenated ($Hb\text{-}O_2$), a part of $Hb\text{-}O_2$ suffers autoxidation to methemoglobin (MetHb), which possesses Fe(Ⅲ)-heme and is unable to bind oxygen, and releases superoxide[11,12]. Calculation has shown that the rate for the autoxidation of hemoglobin is 2%-3% (in humans) and 4% (in mice) of total hemoglobin per day. Thus, RBCs are under oxidative stress constitutively, and cellular components face the risk of oxidative damage (Figure 1). NADPH is the principle electron donor for most redox systems that include antioxidation by glutathione peroxidase-glutathione reductase and peroxiredoxin (Prdx)-thioredoxin reductase axes[13] and reductive carbonyl detoxification by the aldo-keto reductase family. Under healthy conditions, the resultant methemoglobin is reduced back by methemoglobin reductase in a NADPH-dependent manner and kept at low levels.

Elimination of the resultant ROS and maintaining the redox potential within cells are prerequisites for the survival for RBCs, so that antioxidative enzymes, such as superoxide dismutase (SOD), catalase, glutathione peroxidase, and Prdx, have crucial roles in keeping RBCs healthy. Antioxidants with a small molecular weight, notably glutathione and vitamin C (ascorbic acid), also play roles in redox homeostasis. Oxidative stress induced by SOD and Prdx deficiencies participate in the pathogenesis of anemia, as described below.

Approximately 60 years have passed since the discovery of G6PD deficiency, but the actual mechanism of G6PD deficiency-triggered anemia remains undefined[4]. Because G6PD is the rate-determining enzyme in the pentose phosphate pathway and is involved in the production of NADPH, a G6PD deficiency shifts the cellular redox balance to an oxidized state[14]. Meanwhile, most redox proteins, excluding the ones possessing electron-accepting prosthetic groups, consist of reactive sulfhydryl residues, which are also highly susceptible to oxidative modification. Oxidized or aged proteins undergo proteolytic degradation, and RBCs lack the cellular organelle and protein synthesis machinery that is necessary for their renewal. Thus, oxidative stress appears to cause selective decreases in redox-sensitive proteins. The production of NADPH is elevated by activated G6PD in response to oxidative stress[15], and it supports the reductive detoxification of ROS and oxidized molecules, although its activation mechanism is still unclear.

Mouse models developing anemia, AIHA, and SLE

There are animal models that are applicable to research

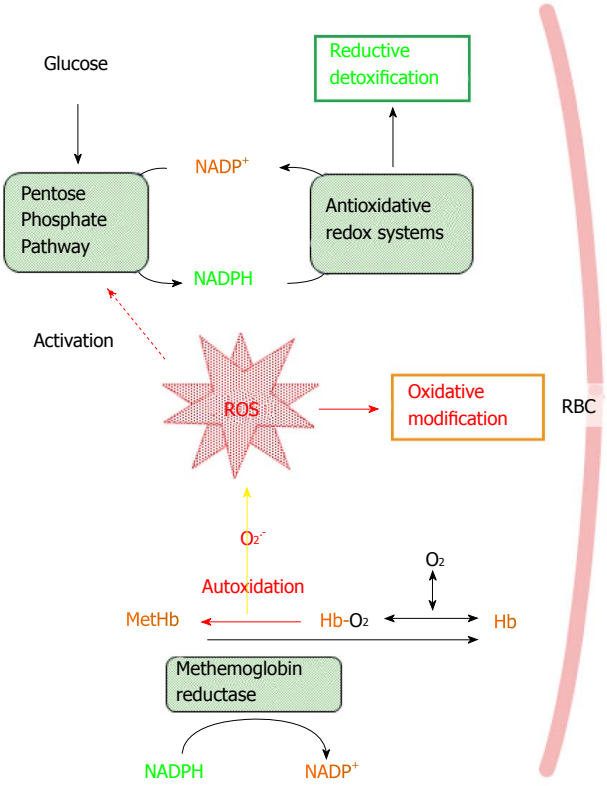

Figure 1 Oxidative stress and antioxidative/redox systems in red blood cells. A part of oxygenized hemoglobin (Hb-O_2) is autoxidized to methemoglobin (MetHb) and releases superoxide ($O_2^{\cdot-}$), which may cause oxidative damage to RBCs. MetHb can be reduced back to Hb by methemoglobin reductase in NADPH-dependent manner. G6PD, a rate-determining enzyme in the pentose phosphate pathway, is involved in the production of NADPH that supports antioxid/redox systems as well as methemoglobin reduction by donating redox potential. G6PD deficiency affects entire antioxidative/redox systems, which can consequently accelerate the destruction of RBCs and lead to anemia. RBCs: Red blood cells. NADPH: Triphosphopyridine nucleotide.

into the etiology of anemia. For example, a direct cause for iron deficiency anemia is defective hemoglobin synthesis due to insufficient heme supply. The involvement of oxidative stress has been implicated in the pathogenesis of some types of chemically induced anemia, such as that induced by pheynylhydrazine[16].

Several strains of animals that spontaneously develop anemia have been used for pathophysiological examinations. New Zealand Black (NZB) mice constitute a strain that develops AIHA during late middle age, at around 40-50 wk. IgG bound to RBCs increases from about 3 mo of age and induces anemia from about 6 mo of age onward[5]. AIHA is exacerbated by an aberrant immune system with notably impaired $CD4^+CD25^+$ regulatory T cells[17] and a Th1 and Th2 cytokine imbalance[18]. Peripheral B-1 cells appear to be a source of autoantibody-producing cells[19]. A dominant T-cell epitope in AIHA is a major glycosylated membrane protein of RBCs, which is also known as an anion transporter band 3[20]. When the *AE1* gene encoding band 3 is deleted, the congenic NZB mice still produce autoantibodies against another glycoprotein, glycophorin, and develop AIHA[21]. Thus, a defect in these glycoproteins is not a primary cause, but other latent abnormalities remain.

Defected genes have been identified in pathological model animals for SLE, MRL/*lpr* and MRL/*gld* mice[22]. Mutations in Fas and Fas ligand genes cause SLE in MRL/*lpr* and MRL/*gld* mice, respectively, *via* malfunctioning apoptotic removal of self-recognizing preB cells at an infant stage[23]. Although mutations in FAS/APO-1 and Fas ligand are found in human SLE patients[24-26], the incidence is not high. Thus, causal factors for SLE are still largely unknown in the human population. (NZB x NZW) F1 mice are another SLE model animal and show characteristics similar to human SLE[27]. While NZB mice spontaneously develop AIHA symptoms that are limited to the blood system, (NZB x NZW) F1 mice exhibit symptoms in a systemic fashion that include lupus nephritis and cardiovascular abnormalities[28-30]. Although NZW mice possess a larval defect in the immune system, they show virtually normal phenotypes and survival times. Genetic analysis of NZW mice has advanced in the past decade, and the latent factor responsible for the onset of SLE has been unveiled.

Anemia observed in antioxidative enzyme gene-modified mice

Because antioxidation plays an essential role in maintaining RBC function, a deficiency of antioxidative enzymes occasionally exerts severe damage to RBCs. Anemia is caused by a deficiency of antioxidative enzymes SOD1[31], SOD2[32], Prdx1[33], and Prdx2[34], but not by deficiencies of glutathione peroxidase 1[35] or catalase[36]. Phenotypic characteristics regarding anemia differ in genetically modified mice, as follows.

SOD1 DEFICIENCY

Among three SOD isozymes present in mammals, SOD1 is a sole superoxide-scavenging enzyme in mature RBCs, and its deficiency causes anemia[31]. Hemoglobin is a major protein in RBCs, and suffers autoxidation, which results in the production of superoxide[11,12]. Without SOD1, the radical chain reaction initiated by superoxide oxidatively damages RBCs, and ultimately accelerates their destruction. Thus, SOD1-deficient RBCs show a shortened life span that is approximately 60%-70% that of the RBCs of wild-type mice[31].

SOD1 deficiency accelerates hemolysis in the blood and phagocytotic removal of RBCs by liver Kupffer cells[37]. An elevation of ROS levels in RBCs, oxidation of RBC components, and augmented production of autoantibodies in RBCs have been observed in SOD1 deficient C57BL/6 (B6) mice[31]. Elevated production of antibodies against lipid peroxidation products, 4-hydroxynonenal and acrolein, occurs[15]. A general antioxidant, *N*-acetyl cysteine (NAC), ameliorates these phenotypes and suppresses anemia and AIHA development. Restricted expression of human *SOD1* in erythroid cells suppresses oxidative stress in RBCs,

which rescues aberrant phenotypes related to anemia and autoimmune responses in *SOD1*-deficient B6 mice. This substantial amount of evidence supports the notion that overproduced ROS due to *SOD1* deficiency can trigger anemia.

Superoxide is continuously produced from oxygenized hemoglobin[11], and hence it is regarded as one of the sources for ROS. Based on theoretical calculation[12], an approximate 200-fold elevation in superoxide results from *SOD1* deficiency. Superoxide would conversely result in the conversion of hemoglobin to methemoglobin and enhance the oxidative modification of RBCs. A marked reduction in glutathione peroxidase 1 protein and its activity is seen in SOD1 deficiency[38], which is caused by an irreversible inactivation *via* conversion of the catalytic selenocysteine to dihydroalanine by elevated ROS[39]. However, the contribution of this low glutathione peroxidase 1 activity to anemia is ambiguous because a deficiency of either glutathione peroxidase 1 or catalase does not cause hematological abnormalities in mice[35,36]. Because thioredoxin reductase is also a selenoenzyme[40], inactivation by the elevated ROS due to a SOD1 deficiency may have a role in the destruction of RBCs.

SOD2 DEFICIENCY

Mice lacking SOD2, a mitochondria-specific isoform, in the whole body show dilated cardiomyopathy, hepatic lipid accumulation and early neonatal death[41]. Hematopoietic chimeras in which all blood cells are derived from the fetal liver stem cells of *SOD2*-deficient mice are employed to examine the effect of SOD2 deficiency on hematopoiesis. The chimera mice are persistently anemic and characteristically similar to the human disorder sideroblastic anemia[32]. Enhanced protein oxidation and altered membrane deformation appear to reduce the life span of RBCs[42,43]. SOD2-deficient reticulocytes reveal up-regulated transferrin receptors[44] and mitochondrial proliferation and mitochondrial membrane thickening[45]. It is noteworthy that mature RBCs, which do not possess mitochondria, show an elevated production of ROS, abundant iron-stainable granules, and oxidatively damaged proteins. These observations imply that the life-span of the resultant RBCs is reduced due to oxidative damage that is experienced before final maturation of the erythroid cells.

PRDX DEFICIENCY

Among 6 Prdx family members, deficiency of either *Prdx1*[33] or *Prdx2*[34] causes hemolytic anemia. *Prdx1*-deficient mice show increased ROS, hemoglobin instability, Heinz body formation, and a decreased erythrocyte life span[33]. Cancers develop in some organs of *Prdx1*-deficient mice, but a causal connection to anemia is unknown. Prdx2 is a predominant form of Prdx family members in RBCs[46] and its function in RBCs has been thoroughly characterized. Prdx2 exists as either a stable dimer or a hyperoxidized form in RBCs[47]. Prdx2 functions in a dimer form with a head-to-tail arrangement. During the peroxidase reaction two pairs of disulfide bonds between the catalytic Cys at the N-terminus and the resolving Cys at the C-terminus in the two subunits are formed as an intermediate[13]. However, Prdx2 appears to function as a non-catalytic scavenger of peroxides in RBCs due to an insufficient thioredoxin-thioredoxin reductase system[48,49]. Sulfenic acid is a physiological intermediate of sulfhydryl groups in the catalytic Cys, but excessively produced hydrogen peroxide hyperoxidizes it to sulfinic acid and then sulfonic acid during the reaction cycle of Prdx, which results in a loss of peroxidase activity[13]. The slow turnover rate of Prdx2 increases the chance for hyperoxidization by hydrogen peroxide in RBCs. Sulfinic acid in Prdx can be converted back to sulfhydryl by sulfiredoxin in an ATP-dependent manner in many cells[50,51]. However, because of an insufficient amount of sulfiredoxin in RBCs, hyperoxidized Prdx2 would proceed to proteolytic removal. Although cyclic changes of the hyperoxidized Prdx has been shown in cultured RBCs[52], this phenomenon cannot be explained by virtue of sulfiredoxin but may be caused by the proteolytic removal of hyperoxidized Prdx2. Because Prdx2 is involved in maintaining hemoglobin stability[53], hemolytic anemia found in Prdx2-deficient mice may be related to the decrease in the life-span of hemoglobin.

Oxidative stress as a potential cause for anemia and autoimmune responses in NZB mice

SOD1-deficient mice produce anti-RBC autoantibody and ultimately develop lupus nephritis-like symptoms in the gerontic stage[15,31], so that we hypothesized that oxidative stress is one of the causal factors for some autoimmune diseases such as SLE and AIHA in C57BL/6 mice. However *SOD1* deficiency is far from a physiologic condition, and we have tried to validate this hypothesis based on physiological conditions using NZB mice.

NZB mice[54] and (NZB × NZW)F1 mice[55] are the established model animals that spontaneously develop AIHA and SLE, respectively, at around 40-50 wk of age. Abnormal proteolytic cleavage of the membrane proteins of RBCs has been proposed as a likely cause because the cleaved membrane proteins, such as band 3, are highly antigenic[56-58]. However, elevated proteolytic activity in the RBCs of AIHA patients or AIHA-prone mice is unknown. Despite extensive investigation on the etiology of the mice, it remains unclear what actually triggers the autoantibody production in the NZB mice[5].

We first recognized that the ROS levels are originally high at a young age (4 wk) and increase as NZB mice age compared to control mice[38]. Increases in the autoantibodies against RBCs show a correlation with the elevated levels of ROS in RBCs. Antioxidants such as NAC suppress autoantibody production in the mice, supporting the oxidative stress theory of AIHA in mice. The onset of AIHA occurs prematurely and

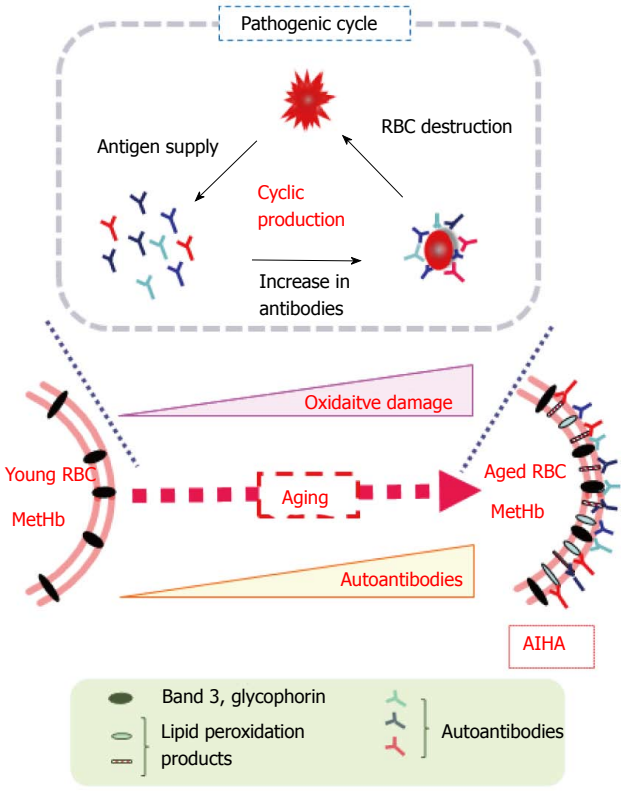

Figure 2 Hypothetical role of oxidative stress in triggering the autoimmune reaction against red blood cells in New Zealand Black mice. Elevated ROS trigger oxidative modification of RBC components and result in the production of oxidatively modified compounds such as 4-hydroxy 2-nonenal and acrolein that are highly antigenic. During aging, the oxidation of susceptible molecules and the production of antibodies recognizing them occurs repeatedly, which results in an accumulation of epitopes and autoantibodies. The elevated levels of autoantibodies ultimately trigger AIHA in aged NZB mice. RBCs: Red blood cells; AIHA: Autoimmune hemolytic anemia; MetHb: Methemoglobin; NZB: New Zealand Black; ROS: Reactive oxygen species.

mortality increases in the *SOD1*-deficient congenic NZB mice compared with control NZB mice[59]. The transgenic expression of human SOD1 in RBCs reduces oxidative stress to RBCs and oxidative modification of lipids and proteins and consequently rescues the AIHA phenotypes in NZB mice. Figure 2 provides a schematic mechanism for the onset of AIHA based on our hypothesis regarding the oxidative damage of RBCs. Because oxidative modification elevates during aging and oxidized molecules are highly antigenic, oxidative stress would elevate the autoantibodies by increasing the autoantigens, and would ultimately cause AIHA onset.

Either suppression of the antioxidative/redox system or activation of ROS generation causes the elevated ROS in RBCs. As mentioned above, the mechanism of G6PD deficiency-induced anemia is attributed to a short supply of NADPH, which triggers the oxidation of sulfhydryls in RBCs[4]. However, no report has shown abnormalities in glucose G6PD in the RBCs of NZB mice. Regarding the antioxidative enzymes catalase, SOD, glutathione peroxidase, and glutathione reductase, nonsynonymous nucleotide polymorphisms have been identified in the genes in 10 inbred mouse strains, including NZB mice[60]. Thus, the origin of oxidative stress in NZB mice is unclear as of this writing.

Oxidative stress as a potential cause for SLE

Superoxide anion diffuses across the RBC membrane *via* the anion channel band 3 protein[61], which is a potent antigenic molecule in RBCs[56-58]. ROS appear to derive from inside the RBCs because lipid peroxides are high in the RBCs but about the same in plasma among the congenic mouse groups. Based on theoretical calculation, more than 100 mol/L of superoxide is released daily from hemoglobin autoxidation[12].

Involvement of oxidative stress has been implied in the pathogenesis of human SLE. For example, lipid peroxidation product 4-hydroxy 2-nonenal may modify Ro60, which is the 60-kDa autoantigen of autoimmunity in both SLE Sjögren syndrome, and differentially participate in Sjögren syndrome or SLE[62,63]. Children with SLE carry increased levels of 4-hydroxy 2-nonenal-modified proteins in plasma[64]. Plasma concentrations of 4-hydroxy 2-nonenal as well as malondialdehyde and oxidized glutathione increase during aging in human plasma and RBCs[65]. Both mitochondrial electron transport chain activity at complex I and oxygen consumption are increased in the lymphocytes of SLE patients[66]. On the contrary, NAC suppresses oxygen consumption and hydrogen peroxide levels. Other studies have shown the beneficial effects that antioxidants such as vitamin E, all-trans-retinoic acid, fish oil, and cystamine has on (NZB X NZW)F1 mice[67-69].

Oxidative modification is caused by relatively large amounts of ROS and generally causes oxidation in a non-specific manner. While lymphocytes are defective, and aberrant immune responses occur in AIHA and SLE, it is unclear how they are stimulated to produce autoantibodies. Because oxidized cells are efficiently phagocytosed by macrophages, there is more chance for the immune system to recognize the resultant oxidized molecules as antigens[70,71]. In fact, oxidatively modified albumin is well recognized by the antibodies from SLE patients[72], and oxidatively modified lipids are identified as epitopes for innate immunity and are responsible for diseases such as atherogenesis[73-75]. Lipid peroxidation products, such as 4-hydroxy 2-nonenal and acrolein, have been identified as bona fide epitopes for autoantibodies on RBC membranes[15]. Thus, oxidative stress participates in the formation of novel epitopes by oxidizing proteins and lipids. It is also noteworthy that anti-DNA antibodies, which are typically elevated in SLE patients, also recognize 4-hydroxy 2-nonenal-bound proteins[76,77].

Hypothetical mechanism for SLE onset in (NZB x NZW)F1 mice

An early genetic study suggests that three genes, one from NZB and two from NZW mice, are involved in

the development of SLE in the (NZB x NZW) F1 mice and that the gene from NZB mice should function dominantly[78]. Recent genetic studies have indicated several candidate genes for AIHA and/or SLE in model mice[79]. Three major genomic intervals (Sle1, Sle2, and Sle3) have been identified on the New Zealand mouse strains and regarded as systemic autoimmune disease susceptibility loci in NZM2410 mice, which is an acute lupus-prone strain derived from a cross between NZB and NZW[28,30]. High titers of IgG autoantibodies against nuclear proteins and DNA are produced by B6 mice congenic for the *Sle1* locus[80]. T cells specific for histone are present[81], implicating *Sle1* in the loss of tolerance that leads to the development of antinuclear antibodies. The *Sle1b* sublocus contains the SLAM (signaling lymphocyte activating molecule) family (Slamf) genes derived from the lupus-prone NZW mice[82,83].

Several candidate genes for autoimmune diseases in humans have also been screened out by genome-wide association studies[84]. Those genes include *HLA*, *STAT4*, and *PTPN22*. Among them, an allelic variant of protein tyrosine phosphatase nonreceptor 22 (*PTPN22*) shows the most promise because it has been associated with multiple human autoimmune diseases, such as type 1 diabetes, rheumatoid arthritis, and SLE. *PTPN22* encodes lymphoid tyrosine phosphatase (Lyp) which participates in the negative regulation of T-cell receptor (TCR) proximal signaling[85,86]. Lyp is also referred to as PEST domain-enriched tyrosine phosphatase (Pep), and it suppresses the activity of the Src family protein tyrosine kinases and inhibits T-cell activity[87]. Because PEST domain, which is rich in proline (P)-glutamate (E)-serine (S)-threonine (T), undergoes rapid degradation, Lyp is vulnerable to proteolytic cleavage. Lyp reportedly negatively regulates T cell receptor signaling[88,89], and the decreased activity would conversely activate the signaling pathway.

In the past two decades, the signaling function of ROS has attracted much attention in the research field of oxidative stress. In this aspect, ROS specifically inactivates susceptible molecules, *e.g.*, phosphotyrosine phosphatase (PTP) families such as PTP1B, Cdc25, SHP1 and SHP2[90,91]. PTP has reactive cys-SH at its catalytic center, which is a preferred target of locally produced ROS. Multiple reports have indicated that PTP variants are linked to human hereditary disorders[92], which indicate that PTP activities play pivotal roles and hence oxidative inactivation affects a variety of cells including lymphocytes.

Because Lyp/Pep is a member of the PTP superfamily and easily oxidized by ROS such as hydrogen peroxide, it may play a role in the sustained activation of lymphocytes, and, hence, it would also play a role in the autoimmune response. A Pep variant (Pep-R619W; Rep with substitution of arginine-619 to Tryptophane-619) protein linked to autoimmune disease is more rapidly degraded and shows greater association with, and *in vitro* cleavage by, calpain 1 than normal allele Pep-R619[93]. Conversely, Pep overexpression in T cells attenuates autoimmune diabetes in NOD mice by preferentially modulating TCR signaling-mediated functions in diabetogenic T cells but not in regulatory T cells[94]. Lyp-R620W is also involved in the breakdown of peripheral tolerance and in the entry of autoreactive B cells into the naive B cell compartment. Moreover, lymphocytes with a variant of Pep-R619W, corresponding to human Lyp-R620W, are hyper-responsive to antigen-receptor engagement. Thus, Pep-R619W uniquely modulates T and B cell homeostasis, leading to a loss in tolerance[95].

Elevated ROS would cause inactivation of Lyp/Pep by oxidizing catalytic Cys and may accelerate its degradation *via* the PEST domain. If ROS inactivates Lyp/Pep, the incidence of autoimmune response would be elevated. This oxidative stress-triggered SLE onset is only hypothetical at this moment and hence requires direct demonstration. The crystallographic analysis of Lyp shows a unique disulfide bond that may play a role in protecting the enzyme from irreversible oxidation[96], and hydrogen peroxide actually inactivates the Lyp phosphatase to a lesser extent compared with CD45 phosphatase[97]. Based on the literature and our own observations, we can propose a hypothetical model to explain SLE onset in (NZB x NZW)F1 mice (Figure 3). Because the F1 mice inherit a SLAM variant from NZW mice and high levels of ROS from NZB mice, which may oxidatively inactivate the Lyp/Pep, lymphocytes are hyper-activated, leading to SLE onset in aged mice. Low CD45 phosphotyrosine phosphatase activities that have been reported by two groups[98,99] may support our hypothesis.

Potential roles of oxidative stress in lupus nephritis

Lupus nephritis is a serious pathological condition of SLE. The incidence of SLE in women is nine times greater than in men[100], while the sex difference is not observed for the autoantibody production in SOD1-deficient mice[31]. Immune complex formation and complement activation are major causes, but other pathogenic factor is involved in lupus nephritis[101]. Despite deficiency of the gamma chain of the Fc receptor in F1 mice, ameliorated glomerulonephritis, immune complex deposition still occurs[102]. Thus, glomerular deposition of C1q as immune complexes, complement activation, and Fc gamma receptor activation together appear to be required for the renal damage[103]. As discussed above, oxidative stress is a potent risk factor for the autoantibody production by affecting immune system and hence would be involved in the kidney damage by increasing the immune complex deposition. However, since kidney is the organ considerably susceptible to oxidative damage[104], elevated ROS may directly affect the renal function and be an independent risk factor for lupus nephritis in SLE.

Perspectives

In addition to the results of studies on the supplementary administration of antioxidative compounds,

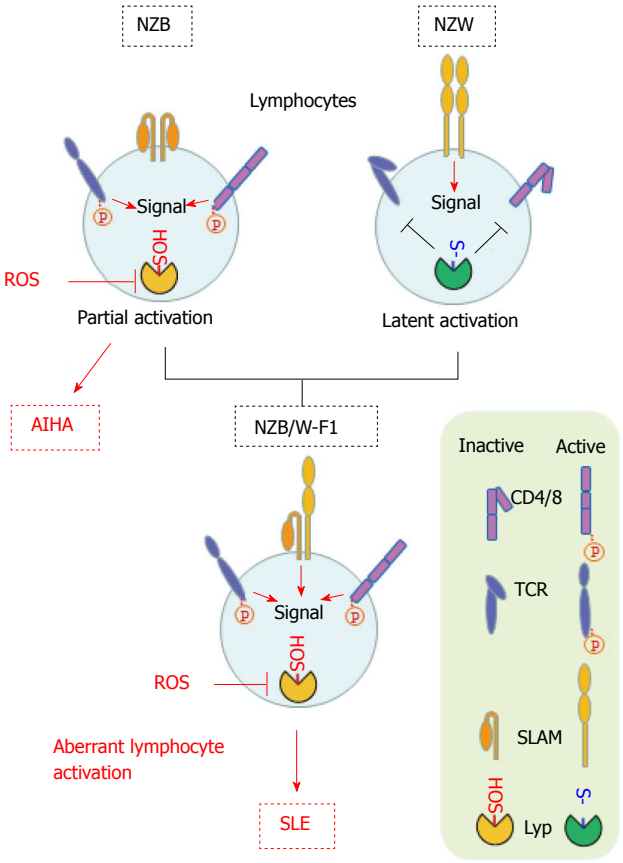

Figure 3 Hypothetical model for triggering systemic lupus erythematosus in (NZB x NZW) F1 mice. Lyp/pep suppressively regulates lymphocyte receptors such as CD4/8 and TCR. Production of ROS are originally high in the cells derived from NZB mice and oxidize the sulfhydryl group in the catalytic Cys to sulfenic acid (-SOH). So that Lyp/pep proteins in lymphocytes in the F1 mice are prone to oxidative inactivation. On the other hand, lymphocytes in NZW mice possess the variant form of SLAM that would be responsible for sustained activation of the lymphocytes. (NZB x NZW) F1 mice inherit this potentially pathogenic nature from parental strains. SLE: Systemic lupus erythematosus; ROS: Reactive oxygen species; NZB: New Zealand black; TCR: T-cell receptor; AIHA: Autoimmune hemolytic anemia.

observations from pathological models and genetically modified mice support the view that ROS are one of the underlying mechanisms for AIHA and/or SLE. ROS cause opposing responses; they trigger cell growth arrest and accelerate cellular senescence, but stimulate the cellular proliferation on the other hand[90,91]. In the latter case, transient elevation in ROS levels occurs when cells are stimulated by growth factor and is involved in sustaining the signal transduction. Antioxidant therapy appears to be effective, but may be potentially adverse because of a possible impairment of the ROS signaling during proliferation of hematopoietic cells. Elucidation of target molecules by oxidative modification and pathogenesis could lead to safer forms of preventive and therapeutic treatment.

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Impact of obesity on kidney function and blood pressure in children

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Abstract

In recent years, obesity has become an increasingly important epidemic health problem in children and adolescents. The prevalence of the overweight status in children grew from 5% to 11% from 1960s to 1990s. The epidemic of obesity has been paralleled by an increase in the incidence of chronic kidney disease (CKD) and hypertension. Results of several studies have demonstrated that obesity and metabolic syndrome were independent predictors of renal injury. The pathophysiology of obesity related hypertension is complex, including activation of sympathetic nervous system, renin angiotensin aldosterone system, hyperinsulinemia and inflammation. These same mechanisms likely contribute to the development of increased blood pressure in children. This review summarizes the recent epidemiologic data linking obesity with CKD and hypertension in children, as well as the potential mechanisms.

Key words: Obesity; Chronic kidney disease; End-stage renal failure; Hypertension; Blood pressure

Core tip: Excess weight gain appears to be a major risk factor for chronic kidney disease and hypertension. The potential mechanisms involve insulin resistance, inflammation, renal renin-angiotensin-aldosterone hyperactivity, and sympathetic nervous system hyperactivity. Increased awareness is needed in children for early diagnosis and implementation of prevention and treatment measures.

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INTRODUCTION

Throughout the world, the increasing rate of childhood obesity has been steadily on the rise over the past

decades. In the first decade of this century, up to 28% of school and 12% of preschool children were determined to be overweight or obese in developed countries, and the international obesity task force addressed childhood obesity as a global "public health crisis"[1]. The impact of obesity on metabolic disease has been well demonstrated, and recently there is increasing evidence that obesity appears to be an independent risk factor for chronic kidney disease (CKD). Baseline body mass index (BMI) has been suggested as an independent predictor of CKD progression[2]. Obesity is strongly associated with the two most common causes of end-stage renal disease (ESRD), namely hypertension and diabetes. In addition, the metabolic syndrome, a major consequence of obesity, also seems to be an independent risk factor for ESRD[3]. Recent evidence also supports the hypothesis that reduced insulin sensitivity and hyperinsulinemia are among the most important factors leading to renal injury[4]. In concert with the increasing prevalence of obesity in children, hypertension has also made an epidemiological shift. Hypertension is a common feature present in a large proportion of obese and overweight individuals. It is correlated with the degree of obesity and significantly increased the risk of coronary artery, stroke and peripheral artery diseases. Moreover, the burden of hypertension attributable to obesity is very high[5]. This review focuses on the impact of obesity on the kidney and blood pressure in children as well as the mechanisms linking obesity to CKD and hypertension.

IMPACT OF OBESITY ON THE KIDNEY FUNCTION

Epidemiology of obesity

The BMI has been used to define obesity based on associated health risk factors in adult individuals. The National Institute of Health (NIH) in the United States determined an adult with a BMI of < 18.5 as underweight, 18.5-24.9 as normal, 25-29.9 as overweight and > 30 as obese. However, the criteria used to define children who are overweight or obese has varied. Most studies concerning childhood obesity or overweight in the United States are based on the Centers for Disease Control and Prevention (CDC) growth charts in 2000. The CDC defined children with > 85th percentile BMI to be overweight and BMI > 95th percentile to be obese[6]. The National Health and Nutrition Examination Survey (NHANES) data demonstrated almost doubling in prevalence of children with BMI > 85th percentile from 1999 to 2004. Recently, the NHANES showed stable prevalence of high BMI in children < 19 years old, with 10% of infants and toddlers < 2 years old with a weight-for-height $\geqslant$ 95th percentile, 17% of children aged 2-19 years old $\geqslant$ 95th percentile, and 32% $\geqslant$ 85th percentile of BMI for age[7,8].

Obesity and the risk factors for CKD

Childhood obesity is fast becoming a worldwide epidemic, and the state of being overweight/obese continues to persist into adolescence. Clustering of cardiovascular risk factors has been shown in obese children with the highest degree of insulin resistance, and these children are likely to develop obesity related kidney damage. In fact, there is a rapidly increasing prevalence of overweight and obese patients with CKD[9]. Reports in a Californian cohort of 330252 persons suggested a strong dose-response relationship between the baseline BMI and the risk of CKD. According to recent studies, obesity also appears to be an independent risk factor for CKD in children. Pediatric nephrology patients had consistently markedly higher BMI z-scores than the normal population at a tertiary center in Canada over a period of two decades. In another study of children with renal transplants, kidneys obtained from obese donors (BMI > 30 kg/m^2) had a lower glomerular filtration rate (GFR) and higher allograft dysfunction rate than kidneys obtained from lean individuals (BMI < 25 kg/m^2)[10-13]. Furthermore, Pantoja Zuzuárregui *et al*[14] demonstrated that obese children have larger kidneys than those of normal weight patients.

Role of obesity in CKD initiation

This question still remains whether obesity and obesity-related metabolic syndrome could directly induce renal injury. Though the theory needs to be confirmed by cause-and effect studies, more and more epidemiologic studies and clinical observations suggested that the obesity metabolic syndrome played a key role in the development of CKD[15]. Recently, several researches indicated that CKD is temporarily related to obesity independently of hypertension. Bonnet *et al*[16] demonstrated that excessive body weight was considered to be a new independent risk factor for clinical and pathological progression in IgA nephritis. Obesity was also shown to independently affect the process of CKD, for instance in patients with unilateral renal agenesis[17] or after unilateral nephrectomy[18]. In addition, kidneys that were obtained from obese individuals (BMI > 30 kg/m^2) were more likely to correlate with a lower GFR and a higher rate of renal allograft dysfunction than kidneys that were obtained from lean donors[12]. These results indicated that obesity could contribute to or even initiate the development of CKD. Kincaid-Smith challenged the long-held notion that hypertension accounts for > 30% of cases of ESRD in the United States and suggested that insulin resistance may be the real culprits in the development of glomerulosclerosis[19]. This notion is supported by the fact that there are no pathologic studies or large clinical studies to provide strong evidence of a relation between

hypertension and ESRD[18].

Obesity related glomerulopathy

Obesity is associated with glomerular hyperfiltration and hypertension. Obesity related glomerulopathy (ORG) is clinically characterized by moderate proteinuria, minimal edema, lower serum cholesterol and higher serum albumin[20]. ORG has been described as a secondary form of focal segmental glomerulosclerosis (FSGS) occurring in obese patients. The first research between obesity and renal injury was reported in 1974[21]. One year later, Cohen also described the presence of significant glomerular enlargement, variable widening of mesangial regions and mild hypercellularity in obese patients, and these features also were found even in children as young as 3 years old[22]. Obese children have larger kidneys and increased renal blood flow than normal weight individuals of similar age. Recently, some reports of improvement in ORG with reduction in body weight were demonstrated. In a recent clinical report, a 17-year-old girl with ORG and nephrotic-range proteinuria, one year after bariatric surgery, her renal function was normal and had no proteinuria[23]. However, the improvement in proteinuria might not correlate with histological change. The pathology of ORG may be biased by the fact that most of the kidney samples were obtained in patients with proteinuria. It suggested that ORG could not be the histopathological feature in nonproteinuric obese individuals with renal dysfunction.

Metabolic syndrome, inflammation and renal injury

The metabolic syndrome or insulin resistance syndrome represents a clustering of CKD risk factors. According to Bogalusa Heart study, metabolic syndrome was characterized as having four of the aforementioned components at or above the 75th percentile for age and gender in children[24]. The primary cause of the metabolic syndrome seems to be obesity. In the NHANES Ⅲ study, the prevalence of metabolic syndrome was 28.7% in overweight adolescents, compared with 0.1% in those with normal BMI and 6.1% in adolescents at risk of being overweight[25]. Up to 90% of overweight individuals had at least one component of the syndrome, and about 56% had two components of the syndrome. There is a plausible association between metabolic syndrome and obesity. One of the important features of metabolic syndrome is insulin resistance. Insulin resistance may lead to a proinflammatory state in obese children. Plasma concentrations of some inflammatory mediators such as tumor necrosis factor (TNF-α), C-reactive protein (CRP) and interleukin (IL)-6 were increased in patients with metabolic syndrome[26]. These results suggest that inflammation is a key risk factor for obesity and inflammation has been strongly associated with the metabolic syndrome. Recent evidence shows that inflammation is linked to obesity in CKD patients. Beddhu *et al*[27] found that in the NHANES III cohort, the metabolic syndrome was associated with greater odds for inflammation at various levels of creatinine clearance. Wu *et al*[28] showed that lipid metabolism related genes and inflammatory cytokines were increased in glomeruli of patients with ORG compared with gender and age matched glomeruli of control kidney samples. Ramkumar *et al*[29] also demonstrated a strong relationship between high BMI and inflammation characterized by a CRP level > 3 mg/dL in patients with CKD. These findings strengthen the notion that inflammatory risk factors and lipid byproducts play a key role in the progress of renal dysfunction in obese patients. Strong evidence shows that obesity, in particular central body fat distribution, has been implicated in kidney dysfunction. In fact, obesity and overweight are associated with many other risk factors, *i.e.,* hyperinsulinemia, hypertension, impaired glucose metabolism and hyperlipidemia, renin-angiotensin-aldosterone (RAAS) activity, oxidative stress and proinflammatory cytokines. Above all, reduced insulin sensitivity presents the most important relationship between obesity and other metabolic complications (Figure 1), which leads to CKD[30,31].

IMPACT OF OBESITY ON BLOOD PRESSURE

Epidemiology

Hypertension is a common feature in a large proportion of obese and overweight individuals. It is correlated with the degree of obesity and significantly exaggerated the risk of stroke, coronary and kidney disease. The association between obesity and hypertension in children has been reported in many studies. Rosner *et al*[32] collected data from 8 US epidemiological studies including over 47000 children and the results demonstrate that blood pressures differ between white and black children in relation to their body size. They found the risk of increased blood pressure was markedly higher in the upper compared with the lower decile of BMI irrespective of race, age and gender. Freedman *et al*[33] showed that overweight children were 4.5 and 2.4 times as likely to have increased systolic and diastolic blood pressure, respectively, than normal children. Sorof *et al*[34] recently demonstrated that there was a 3 times prevalence of hypertension in obese compared with non-obese adolescents in a school based hypertension and obesity screening study.

Obesity as a major cause of hypertension

More recent evidence shows that excess weight gain is one of the best predictors of the development of obesity. In addition, blood pressure is closely correlated with BMI and other biochemical and anthropometric indices of obesity, such as serum insulin, leptin and waist to hip ratio[5,35]. The strong relationship between obesity and hypertension cannot be attributed to

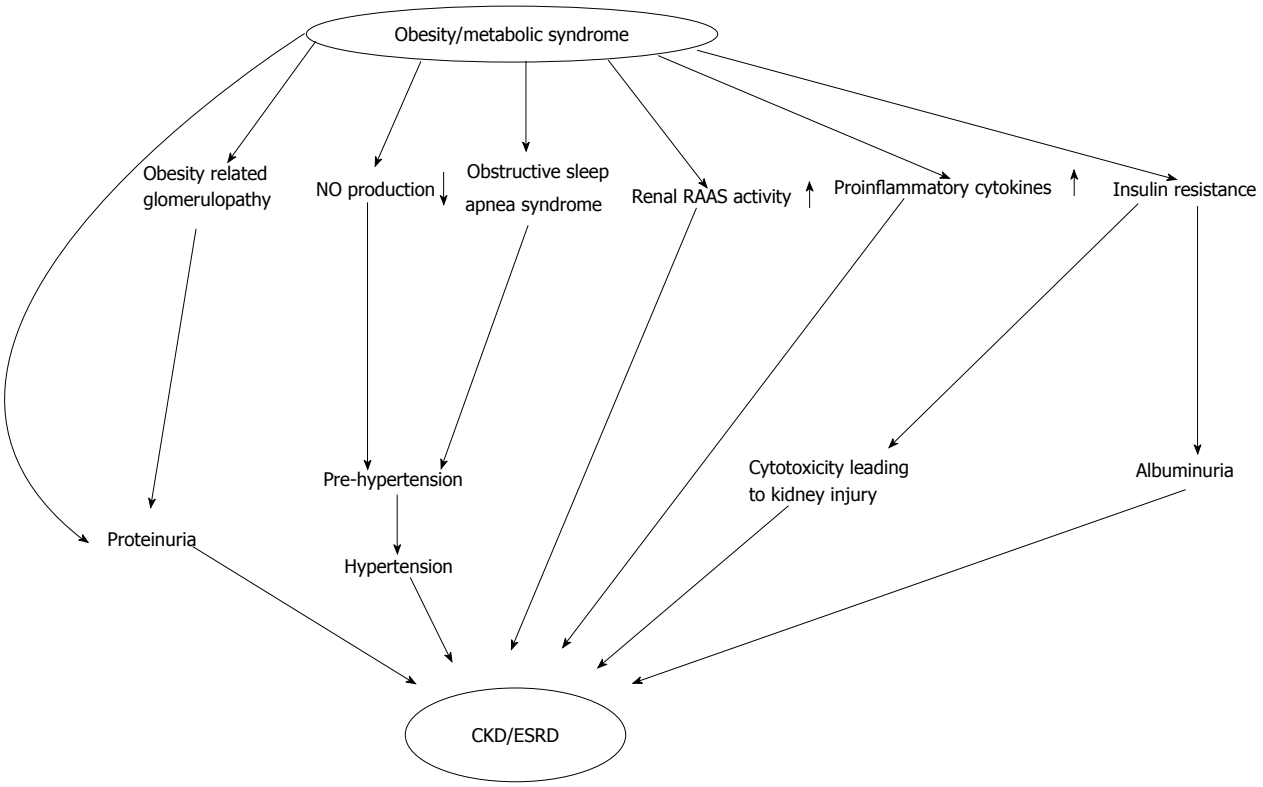

Figure 1 Obesity leads to progression of chronic kidney disease through various pathways. NO: Nitric oxide; RAAS: Renal angiotensin aldosterone system; CKD: Chronic kidney disease; ESRD: End-stage renal disease.

genetic factors, because the association between obesity and hypertension has been observed in diverse populations throughout the world. Although the precise contribution of excess weight to hypertension has not been clearly established, Garrison *et al*[5] reported that about 78% hypertension in men and 65% in women may be directly attributed to excess body mass. Moreover, this association between obesity and hypertension can be modified by factors, such as the duration of obesity and the distribution of body fat. Clinical research has also demonstrated the therapeutic role of weight loss for reducing blood pressure. Even weight loss in "normotensive" overweight individuals can decrease the blood pressure. Experimental research of dietary-induced or genetic animal models of obesity has permitted mechanistic insights into these factors that link hypertension and obesity. Dobrian *et al*[36] showed that weight gain induced by long-term high-fat diets consistently increased blood pressure in a rat model[37]. In addition, renal and metabolic changes observed in animal models of diet-induced obesity seem to mimic very closely the findings in obese humans.

Mechanisms of hypertension in obesity

Obesity-associated hypertension is a complex multi-factorial disease, including activation of RAAS, altered vascular function and increased sympathetic nervous system (SNS)[38]. The potential relationship among these mechanisms is shown in Figure 2. Insulin resistance alone, or in combination with hyperleptinemia, activates the SNS, which cause vasoconstriction and reduced renal blood flow, leading in turn to activation of RAAS and water and sodium retention[39]. The serum level of leptin has a strong association with increased blood pressure, and eventually activated SNS. In addition, recent reports show that other mechanisms may be involved in the pathogenesis of hypertension in obese children, such as proinflammatory cytokines and oxidative stress pathway. These signaling pathways likely contribute to increased arterial stiffness and endothelial dysfunction (Figure 2)[40]. Moreover, sleep apnea syndrome or poor sleep quality often increase the risk of the development of hypertension in obese children[41]. The potential mechanisms for sleep apnea or poor sleep quality may be triggered by intermittent hypoxia and increased inflammatory cytokines, and may eventually exacerbate the progression of hypertension in obese individuals. Pacifico *et al*[42] demonstrated that low serum $25(OH)_2D_3$ levels were associated with metabolic syndrome and hypertension in Caucasian children and adolescents. This suggests that low vitamin D level often observed in obese children, may have a strong association with hypertension and metabolic syndrome. The uric acid may be also involved in obesity-induced hypertension. A high fructose diet can lead to hyperuricaemia owing to increased uric acid production by adipose tissue in obese individuals[43]. Several researches have demonstrated a strong relationship between uric acid and hypertension in children and adolescents. The Moscow Children's hypertension study showed hyperuricaemia (> 8.0 mg/dL) only in 9.5% of children with normal blood

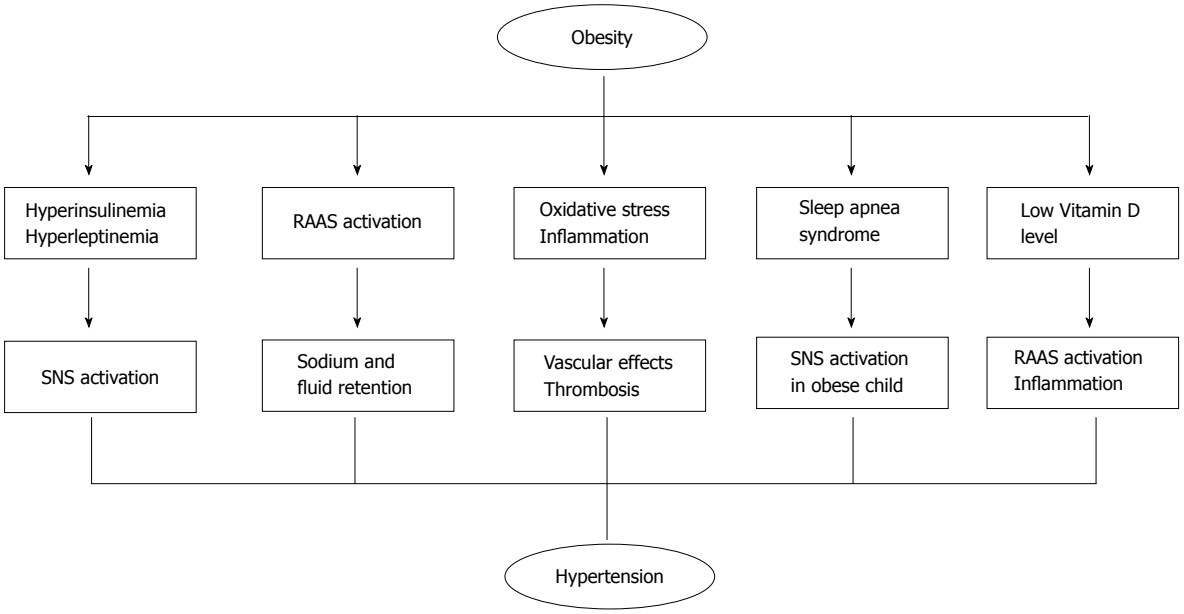

Figure 2 Mechanisms of hypertension in obesity. RAAS: Renal angiotensin aldosterone system; SNS: Sympathetic nervous system.

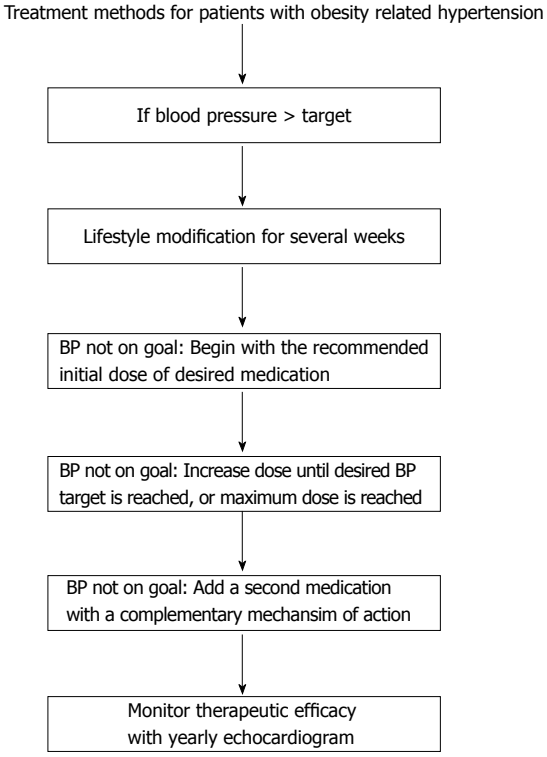

Figure 3 Stepped-care approach to antihypertensive therapy in obese children. BP: Blood pressure.

pressure, but as high as 49% in children with borderline hypertension, and up to 73% of children with moderate and severe hypertension[44]. These findings also need to be confirmed by large-scale epidemiological studies.

Therapeutic approaches for obesity related hypertension

Lifestyle interventions were recommended for all the obese children with hypertension. These include increased physical activity, low sodium diet and other healthy dietary choices for weight loss[45]. The effects of high sodium intake may have an important role of elevated blood pressure in overweight and obese adolescents compared with the general individuals. In addition, decreasing sodium intake may have a beneficial effect on blood pressure in obese individuals[46]. Pharmacological and surgical options were limited for the treatment of obese children. Calcium channel blockers and angiotensin converting enzyme (ACE) inhibitors are the most frequently prescribed drugs for primary hypertension in children and adolescents[47]. Because of the important role of RAAS and SNS activation in obesity related hypertension, ACE inhibitors are considered a very good choice for the treatment of hypertension[48]. Moreover, ACE inhibitors and angiotensin receptor blockers may have additional reno-protective role in obese patients[45]. Beta blockers may impair lipid and glucose metabolism and they are not preferably the first choice therapy in obese hypertensive individuals[49,50].

The current approach for obesity-related hypertension in children is summarized in Figure 3[51]. Lastly, obesity related hypertension should be considered a chronic medical condition and likely requires long-term treatment.

CONCLUSION

Obesity has reached epidemic proportions and continues to be a growing problem worldwide. Excess weight gain appears to be a major risk factor for CKD and hypertension. The potential mechanisms involve insulin resistance, inflammation, renal RAAS hyperactivity, SNS hyperactivity, and perhaps other unknown mechanisms. Obesity related renal injury and hypertension is already well recognized in the adult population. Increased awareness is needed in children for early diagnosis and

implementation of prevention and treatment measures.

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Strategies to optimize shock wave lithotripsy outcome: Patient selection and treatment parameters

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Abstract

Shock wave lithotripsy (SWL) was introduced in 1980, modernizing the treatment of upper urinary tract stones, and quickly became the most commonly utilized technique to treat kidney stones. Over the past 5-10 years, however, use of SWL has been declining because it is not as reliably effective as more modern technology. SWL success rates vary considerably and there is abundant literature predicting outcome based on patient- and stone-specific parameters. Herein we discuss the ways to optimize SWL outcomes by reviewing proper patient selection utilizing stone characteristics and patient features. Stone size, number, location, density, composition, and patient body habitus and renal anatomy are all discussed. We also review the technical parameters during SWL that can be controlled to improve results further, including type of anesthesia, coupling, shock wave rate, focal zones, pressures, and active monitoring. Following these basic principles and selection criteria will help maximize success rate.

Key words: Shock wave lithotripsy; Kidney stones; Nephrolithiasis; Treatment outcome; Optimization

Core tip: Shock wave lithotripsy is a commonly utilized technology for kidney stone treatment that has declining efficacy over the past decade. The paper outlines how to optimize outcomes with proper patient selection and control of treatment parameters.

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INTRODUCTION

Shock wave lithotripsy (SWL) was introduced in 1980, modernizing the treatment of upper urinary tract stones. Prior to the SWL era, proximal ureteral and renal calculi required major operations with a prolonged recovery time. Because SWL is a non-invasive surgical procedure with a low complication rate allowing same day discharges, it has been the most commonly utilized treatment of kidney stones

Table 1 Stone criteria for shock wave lithotripsy

Sub-optimal features suggesting alternate therapy
Stone size > 2 cm
Multiple stones
Lower pole stone
Hounsfield unit > 1000
History of cystine, calcium oxalate monohydrate, matrix stones

over the past 3 decades[1-3]. Over the past 5-10 years, however, use of SWL has been declining and just recently, a group in Canada showed ureteroscopy has surpassed it as the most common treatment of nephrolithiasis[1-4]. While ureteroscopy is more invasive than SWL, it is still minimally invasive, with a low morbidity profile, and it is more reliably definitive than SWL requiring fewer subsequent procedures to establish stone-free status[5]. As SWL technology has transformed to a more convenient and easier process, success rates have declined. SWL outcomes, however, can be optimized with careful patient selection and control of specific treatment parameters. Herein, we review how to maximize the success rate of SWL and reduce failures by defining the appropriate range of uses and outlining what technical factors can be controlled to improve efficacy.

PATIENT SELECTION

Success rate of SWL varies considerably. This variability is a direct result of well-established stone-specific and patient-specific features. While the American Urological Association guidelines for management of ureteral calculi cite SWL as a primary treatment option if intervention is needed, and the technology could theoretically be used on any urinary stone, selectivity is crucial to maximize efficacy[6] .

Stones have varying responsiveness to SWL depending on several aspects. Stone size and number, location, density, and composition all affect the stone-free rate following SWL (Table 1). The American Urological Association Guideline on the management of staghorn calculi recommends against SWL as monotherapy because of poor outcomes, with only 54% overall stone-free rate, and increased complications (pain, obstruction, infection, bleeding, loss of kidney)[7]. SWL may be appropriate as an adjunctive procedure following percutaneous nephrolithotomy for staghorn calculi if there is a small residual stone. In general, it is still recommended that nephroscopy be the final procedure performed to confirm stone clearance in this setting[7]. If SWL is used as monotherapy for staghorn calculi, then a stent or nephrostomy tube should be placed prior to intervention, though the drainage mostly helps to prevent complications, and does not necessarily improve outcome. Multiple procedures are generally required for this scenario.

While staghorn is the extreme of large stone size, any stone over 2 cm is associated with an inferior outcome when treated with SWL[8-11]. Larger stones usually require more procedures and have increased complications such as obstruction from steinstrasse or larger fragment passage. If a stone is larger than 2 cm, then an alternate treatment may be best. In addition to stone size, total stone burden should be considered when electing treatment. If there are several stones throughout the kidney or bilateral stones amenable to single stage ureteroscopy *vs* multi-stage SWL then the patient should be counseled that stone-free rate may be higher with fewer procedures with the former option.

In addition to stone burden dispersed throughout the kidney making SWL less ideal, different stone locations affect success rates of the procedure. Specifically, there is an abundance of literature showing a lower stone-free rate for kidney calculi located in the lower pole treated with SWL with highest success rates in renal pelvic, upper pole and ureteropelvic junction stones[12-15]. Lower pole 1, a prospective, multicenter, randomized controlled trial evaluating treatment outcome for lower pole kidney stones, illustrated a 37% *vs* 95% stone-free rate for SWL *vs* percutaneous nephrolithotomy[12]. Outcome worsened further for lower pole kidney stones larger than 2 cm when treated with SWL (stone free rate 14%)[12]. This inferior outcome is directly related to the infundibulopelvic angle and lack of fragment clearance, rather than actual successful fragmentation. Success rates can be further delineated with measurements of infundibular width and length. One research group evaluated these anatomical features using intravenous pyelogram measurements and better stone clearance with SWL was achieved in kidneys with a wide infundibulopelvic angle or a short length and a broad width[15].

In addition to kidney stone locations, ureteral stone location affects outcome as well. Lower stone free rates are seen with distal ureteral stones, particularly stones greater than 1 cm, and SWL is not recommended as the primary treatment option but is an acceptable secondary alternative[6]. In general, SWL of the pelvis (distal ureteral stones) is avoided in women of childbearing age due to the theoretical risk of adjacent adnexal injury[6,16].

Both how hard a stone is and its composition also affect outcome of SWL. Density alone is a great predictor of successful fragmentation. Several groups have found that Hounsfield unit (HU) measurement of the stone on computed tomography imaging is associated with stone-free rate[17-19]. One group reported treatment failure in close to 50% of patients for stones great than 1000 HU[19]. Another study found at least 3 SWL sessions were required 70% of the time if HU was more than 750, and stone-free rate was still only 65%[18]. Specific stones compositions are more dense than others, and therefore have well-established resistance to SWL. Brushite, cystine, and

Table 2 Patient criteria for shock wave lithotripsy

Sub-optimal features suggesting alternate therapy
Obesity - skin to stone distance > 10 cm
Pelvic kidney
Horseshoe kidney
Calyceal diverticulum

Table 3 Absolute contraindications to shock wave lithotripsy

Anticoagulation
Bleeding diathesis
Pregnancy
Severe skeletal malformations
Distal obstruction
Infection associated with obstruction

calcium oxalate monohydrate are well-known to have very poor responses to SWL[7,20-24]. If suspicious for these stone compositions based on prior history or crystal presence on urinalysis, SWL is best avoided and another treatment selected. Matrix stones, while not dense, are made of organic matter and do not break with SWL[25]. Ureteroscopy or percutaneous nephrolithotomy should be used to treat this rare stone type if known.

Once the checklist for SWL has been reviewed for ideal stone characteristics, patient-specific features need to be evaluated. Body habitus and renal anatomy both affect SWL outcome (Table 2). Obesity, specifically skin to stone distance (SSD) measured on axial imaging, predicts outcome, with greater than 9 or 10 cm having a poor result[26-28]. This is because the shock wave fired loses energy as it travels through excess body fat in a patient with an elevated body mass index[29]. Pelvic kidneys and horseshoe kidneys also have a lower stone-free rate with a greater number of SWL sessions needed to achieve success[30,31]. SWL is generally not recommended in patients with a calculus in a calyceal diverticulum. While some patients may have symptomatic relief with stone fragmentation, stone-free rate is only 21% because the diverticular neck does not allow for stone passage[32]. If the ostium of the diverticulum is well-visualized, the stone is small, and the diverticula fills with contrast, success rates have been shown to be improved[33]. Hydronephrosis and renal insufficiency are also associated with lower success rates but the mechanism for this is unknown[34]. Anticoagulation, bleeding disorders, pregnancy, severe skeletal malformations, distal obstruction, and infection associated with obstruction are all absolute contraindications to SWL (Table 3)[6,35].

While some patients may still choose SWL despite not satisfying all criteria, keeping these general principles in mind regarding stone-specific characteristics and patient features when electing SWL will improve the procedure success rate.

Table 4 Technical factors that optimize shock wave lithotripsy outcome

General anesthesia
Optimal coupling
Low shock wave rate (60 shocks per minute)
Wider focal zone
Active intraoperative monitoring

TREATMENT PARAMETERS

Once SWL is selected as the procedure for definitive management based on the above criteria, several technical parameters during the procedure can be controlled to also optimize outcomes (Table 4).

The first way to improve outcome begins before the procedure even starts when selecting anesthesia. With more modern lithotripters having a narrow focal zone, unforeseen movements may shift the location of the stone out of the treatment zone, thus delivering shocks to surrounding tissue instead of the desired target. One way to minimize movement is to administer general anesthesia, as the anesthesiologist can control respirations with adjustments of rate and volume as needed, thus providing more control over kidney and stone motion. Several studies have shown improved SWL outcomes with higher stone free rates using general anesthesia *vs* sedation[36,37].

The next way to improve outcome is during the preparation. The original lithotripter in 1980 immersed patients completely in a bathtub and therefore used water as the medium to couple the shock wave to the patient. This was the optimal coupler as there was no air present to dissipate any energy. With miniaturization of the technology, most lithotripter machines now have a dry treatment head and use gel or oil for coupling. This has negatively impacted the outcome as air bubbles that form within the medium dampen the energy and reduce the impact on the stone. Efficacy can be reduced by as much as 40% with the presence of as few as 2% of air pockets[38]. Avoiding patient movement or repositioning during the procedure will lessen the impact of this effect minimizing the number of air pockets created. Additionally, medium application as a large volume mound directly from the stock container has been shown to minimize air bubble creation far more than dispensing from a squirt bottle or applying with the hand[39].

Once ready to initiate SWL several settings can be adjusted as well to optimize outcome. Shock wave rate can be set prior to initiating treatment and a slow rate of 60 shocks per minute has been shown to not only reduce tissue injury but also have a superior stone free rates[40-45]. This optimal rate has been confirmed by several studies including a meta-analysis of randomized controlled trials[46]. If the lithotripter being used, allows for control of focal zone size and

pressures, a wider zone with lower pressures have been shown to have the best outcomes while reducing tissue injury[47-50]. Another setting recommendation for SWL is pre-treating the stone at a low energy for 100-200 shock waves and then pausing for several minutes prior to going to a higher energy[50,51]. While this does not necessarily improve efficacy of SWL it does improve outcome by decreasing injury to the kidney[52-54]. Once the procedure begins, active monitoring of the stone location with continuous ultrasound or spot fluoroscopy every couple of minutes or every 100-200 shocks, will confirm that the target is still appropriately positioned within the treatment zone.

Following these general guidelines for control of technical parameters during SWL will help to optimize outcome and improve stone free rates while minimizing tissue injury.

CONCLUSION

SWL is an excellent treatment modality for upper urinary tract treatment stones however success rate has decreased in the recent years secondary to changes in the machine design. Careful patient and stone selection and control of technical parameters improves stone free rates and will more likely result in a successful outcome.

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Primary and secondary hyperoxaluria: Understanding the enigma

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Abstract

Hyperoxaluria is characterized by an increased urinary excretion of oxalate. Primary and secondary hyperoxaluria are two distinct clinical expressions of hyperoxaluria. Primary hyperoxaluria is an inherited error of metabolism due to defective enzyme activity. In contrast, secondary hyperoxaluria is caused by increased dietary ingestion of oxalate, precursors of oxalate or alteration in intestinal microflora. The disease spectrum extends from recurrent kidney stones, nephrocalcinosis and urinary tract infections to chronic kidney disease and end stage renal disease. When calcium oxalate burden exceeds the renal excretory ability, calcium oxalate starts to deposit in various organ systems in a process called systemic oxalosis. Increased urinary oxalate levels help to make the diagnosis while plasma oxalate levels are likely to be more accurate when patients develop chronic kidney disease. Definitive diagnosis of primary hyperoxaluria is achieved by genetic studies and if genetic studies prove inconclusive, liver biopsy is undertaken to establish diagnosis. Diagnostic clues pointing towards secondary hyperoxaluria are a supportive dietary history and tests to detect increased intestinal absorption of oxalate. Conservative treatment for both types of hyperoxaluria includes vigorous hydration and crystallization inhibitors to decrease calcium oxalate precipitation. Pyridoxine is also found to be helpful in approximately 30% patients with primary hyperoxaluria type 1. Liver-kidney and isolated kidney transplantation are the treatment of choice in primary hyperoxaluria type 1 and type 2 respectively. Data is scarce on role of transplantation in primary hyperoxaluria type 3 where there are no reports of end stage renal disease so far. There are ongoing investigations into newer modalities of diagnosis and treatment of hyperoxaluria. Clinical differentiation between primary and secondary hyperoxaluria and further between the types of primary hyperoxaluria is very important because of implications in treatment and diagnosis. Hyperoxaluria continues to be a challenging disease and a high index of clinical suspicion is often the first step on the path to accurate diagnosis and management.

Key words: Primary hyperoxaluria; Transplantation; Renal stones; Secondary hyperoxaluria; Renal failure

Core tip: Hyperoxaluria is a disorder characterized by increased urinary oxalate excretion. Primary hyperoxaluria is an inherited defect of oxalate metabolism while secondary hyperoxaluria is seen in states of increased ingestion of oxalate, its precursors or altered gut flora. These disorders can lead to recurrent renal stones, nephrocalcinosis and eventually end stage renal disease. Despite these common features, the sub types of hyperoxaluria differ in their pathogenesis, severity of clinical presentation and treatment plan. Prompt clinical recognition and distinction between these disorders is essential not only for timely intervention but also impacts prognosis in patients with hyperoxaluria.

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INTRODUCTION

Oxalate is the ionic form of oxalic acid and is derived from various animal and plant sources. Oxalate is excreted mainly through the kidneys. Hyperoxaluria is a state of disordered metabolism characterized by an increased urinary excretion of oxalate. The normal daily oxalate excretion in healthy individuals ranges between 10-40 mg per 24 h. Concentrations exceeding 40-45 mg per 24 h are considered as clinical hyperoxaluria[1-3]. This may result from increased endogenous production of oxalate in primary hyperoxaluria (PH) or from increased intestinal absorption or increased intake of oxalate precursors in secondary hyperoxaluria (SH).

Hyperoxaluria has the potential to cause devastating consequences which can present as early as infancy or in the sixth decade of life and if not addressed appropriately, can cause significant morbidity and mortality including End Stage Renal Disease (ESRD)[4]. Elevated plasma oxalate levels lead to oxalate deposition in various organ systems. Systemic oxalosis should be prevented but the diagnosis is often delayed in more than 40% of patients. In a survey by Hoppe *et al*[5], 30% of the patients were diagnosed only when they had already reached ESRD. In some cases, the diagnosis may first be made when the disease recurs following renal transplant[6]. Hyperoxaluria continues to be a challenging disease and appropriate treatment requires a high index of suspicion and a timely diagnosis.

This review highlights the mechanisms underlying both primary and secondary hyperoxaluria, clinical manifestations, important elements in screening and diagnosis, and our current knowledge of modalities of treatment.

SOURCES OF OXALATE

Oxalate is obtained from exogenous sources as well as endogenous synthesis. Oxalate is abundantly found in plant and animal sources. Dietary sources richest in oxalate include nuts, plums, chocolate, beetroot, strawberries, rhubarb, tofu and spinach[1,7]. Juicing is a recent popular trend where a diet based mainly on fruits and vegetable juices is consumed and may supply a very high amount of daily oxalate[8,9]. Studies have demonstrated that as the dietary intake of oxalate increases, so does the urinary concentration of oxalate[10]. Endogenous synthesis of oxalate occurs in the liver[11] through a pathway that generates glyoxalate as an intermediate molecule[12]. Glyoxalate is synthesized from oxidation of glycolate through enzymatic action of glycolate oxidase or from metabolism of hydroxyproline which is found in collagen or dietary sources. Increased glyoxalate is converted to oxalate by action of lactate dehydrogenase in the absence of enzymatic activity as is seen in the various types of PH[12,13]. This pathway is depicted in Figure 1.

RENAL HANDLING OF OXALATE

Renal oxalate handling comprises glomerular filtration, tubular secretion and tubular reabsorption[14,15]. Glomerular filtration depends on the plasma oxalate levels while tubular transport is mediated by SLC26 family of transport proteins. SLC26A1 mediates oxalate uptake into the cell across the basolateral membrane in exchange for sulfate[16,17]. On the apical side of the tubular cells, SLC26A6 is the dominant chloride-oxalate exchanger which promotes chloride reabsorption in exchange for oxalate secretion and has been implicated in the development of renal stones. This exchanger also mediates intestinal secretion of oxalate and loss of this exchanger has been shown to promote increased intestinal absorption of oxalate in the small intestine[18,19]. In rat kidney, tubular reabsorption has been demonstrated in the S1 and S2 segments of the proximal tubule[14] which may help decrease the tendency for calcium oxalate supersaturation in the earlier parts of the nephron[3].

Overall, the contribution of tubular secretion in addition to glomerular filtration is critical in regulating plasma oxalate levels as a strong correlation has been demonstrated between high plasma oxalate levels and oxalate secretion[20]. It has also been noted that tubular oxalate secretion is increased in PH patients possibly in an attempt to mitigate the life threatening consequences of systemic oxalosis[21]. Increased tubular secretion has also been noted in patients with hyperoxaluria following intestinal bypass[22].

GENETIC AND BIOCHEMICAL BASIS OF DISEASE

Primary hyperoxaluria

Primary hyperoxaluria type 1 (PH1) is the most common and severe form of PH. It accounts for approximately 80% of the cases of PH and is caused by defect in the

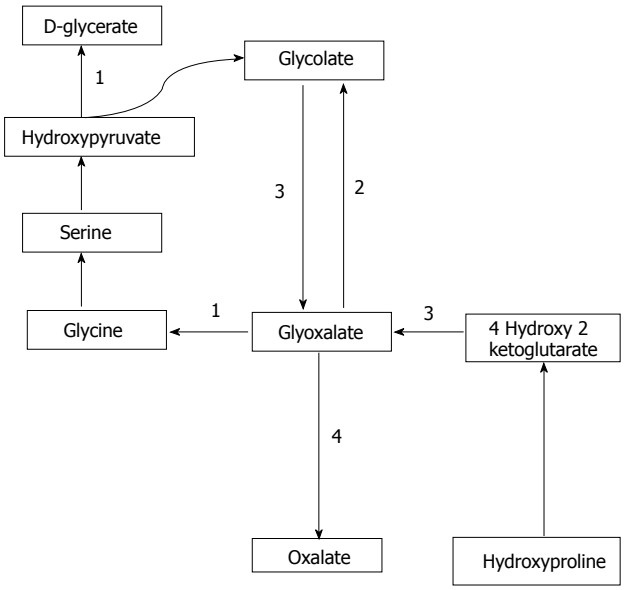

Figure 1 Pathway of oxalate synthesis and enzymatic defects in PH. A: PH1, alanine glyoxalate aminotransferase; B: PH 2, glycolate reductase hydroxy pyruvate reductase; C: PH 3, 4-hydroxy 2-ketoglutarate aldolase; D: Lactate dehydrogenase.

Vitamin B6 dependent hepatic peroxisomal enzyme, Alanine Glyoxalate Aminotransferase (AGT). This enzyme catalyzes the transamination of L-alanine and glyoxalate to pyruvate and glycine. The enzyme defect has been attributed to a mutation in the *AGXT* gene located on chromosome 2[23,24].

Primary hyperoxaluria type 2 (PH2) represents about 10% of the patients with PH. Dysfunction of the enzyme glyoxalate/hydroxypyruvate reductase (GRHPR) occurs secondary to a mutation in the *GRHPR* gene located on chromosome 10[25-27]. Consequently, there is increased urinary excretion of L-glyceric acid and oxalate.

Primary hyperoxaluria type 3 (PH 3) is a recently described entity and it occurs in 10% PH cases. The genetic defect in PH3 has been localized to the *HOGA1* gene located on chromosome 9 which codes for the mitochondrial 4-hydroxy 2-oxoglutarate aldolase[28]. This enzyme breaks down 4-hydroxy 2-oxoglutarate into pyruvate and glyoxalate which in turn is converted into oxalate.

SECONDARY HYPEROXALURIA

The causes of SH are increase in dietary and intestinal absorption (enteric hyperoxaluria), excessive intake of oxalate precursors and alteration in intestinal microflora.

Increased dietary intake of oxalate

Oxalate rich dietary sources include rhubarb and spinach and daily intake may be in excess of 1000 mg/d[29]. Increased dietary absorption may occur in "juicing" which is being propagated as a health fad for clearing toxins from the body and also for weight loss. Previously dietary oxalate was thought to make only a minimal (10%-20%) contribution to the amount of oxalate excreted in urine but studies have shown that this is not correct. In a study by Holmes *et al*[10], dietary intake contributed to about 50% of the oxalate secretion proving that dietary ingestion is an important determinant in total oxalate excretion. Bioavailability of oxalate from food and, thus, urinary oxalate, is also influenced by the forms of oxalate in the food, techniques of food processing and cooking and other constituents in the meal[30]. Dietary ingestion of oxalate is reduced by concurrent ingestion of calcium or magnesium which complex with oxalate and form insoluble salts[10,31].

Hyperoxaluria associated with fat malabsorption

Fat malabsorption increases the intestinal absorption of oxalate due to increased intestinal permeability to oxalate and formation of calcium and fatty acid complexes leading to increased amounts of soluble oxalate. An intact colon is required for increased oxalate absorption *via* this mechanism[32]. This form of hyperoxaluria is seen in partial gastrectomy, bariatric surgery, jejunoileal bypass, and inflammatory bowel disease[7,33].

Role of oxalobacter formigenes

Oxalobacter formigenes (*O. formigenes*) is an aerobic gram negative bacterium that uses oxalate as its energy source and decreases intestinal absorption of oxalate and thus reduces urinary oxalate excretion[34,35]. This has been well documented in both human and animal experiments[36,37]. Loss of this bacterium occurs after the use of antibiotics[38] and its restoration may have a role in treatment of hyperoxaluria.

Excess intake of oxalate precursors

Ascorbic acid (Vitamin C) is a precursor of oxalate and intake of excessive quantities of vitamin C may result in precipitation of calcium oxalate[39,40]. Oxalate is a product of ethylene glycol causing calcium oxalate deposition and renal failure[41,42]. Hyperoxaluria has also been reported following renal transplantation due to mobilization of oxalate and deposition within the renal allograft[43]. Increased intestinal absorption of oxalate and tubular secretion has also been reported in patients with cystic fibrosis leading to hyperoxaluria[3,44,45].

"Juicing" deserves a special mention as it supplies a high amount of daily oxalate. The increased amount of fluid intake in the juices increases the paracellular absorption of oxalate in the intestines. This may overwhelm the ability of the kidney to excrete the increased dietary load especially in patients with chronic kidney disease. Oxalate is ingested in the fruits and vegetables used to make the juices such as kiwi, spinach and beetroot. Low calcium intake and ingestion of excess of vitamin C is also noted which

together with the oxalate intake heighten the risk of acute kidney injury[8,9].

CLINICAL PRESENTATION

The prevalence of PH1 is approximately 1-3 cases per million population[46,47]. At least 1% of the ESRD seen in the pediatric population is attributable to PH1 in European and Japanese studies[48,49]. It is more frequently seen in Kuwaiti and Tunisian populations where consanguineous marriages are practiced[50,51]. PH1 is the most severe type of PH although there is significant variability in its clinical presentation. Patients may present early in life during infancy with life threatening oxalosis and failure to thrive or in adulthood after passing an occasional stone. Overall, the disease is characterized by recurrent nephrolithiasis and progressive nephrocalcinosis leading to renal damage and as a result, the majority of the patients reach ESRD during 3rd-5th decade of life[52,53].

PH2 is a less aggressive form of PH with better preservation of renal function and lower incidence of end stage renal disease and less severe nephrocalcinosis compared to PH1. The differences are accounted for by the higher oxalate excretion in PH1 and altered urine composition with reduced urinary levels of citrate and magnesium in PH1 compared to PH2[54].

PH3 generally presents with recurrent nephrolithiasis in the early decades of life. It is also characterized by the increase in urinary calcium levels and genetic defects in the *HOGA1* gene have also been implicated in cases of idiopathic calcium oxalate urolithiasis[55]. The disease course is more benign compared to other forms and although limited clinical data is available, no cases of ESRD have been reported to date with PH3[56,57].

Patients with secondary hyperoxaluria have a predisposition to developing recurrent calcium oxalate stones due to the underlying disorder. This leads to worsening renal damage and progression to ESRD. Systemic oxalosis is less common in secondary hyperoxaluria but reported in some severe cases of Crohn's disease[58].

SYSTEMIC OXALOSIS

Calcium oxalate salts are poorly soluble in body fluids. Calcium oxalate deposits within renal tissue as nephrocalcinosis and also forms renal stones (nephrolithiasis). This leads to progressive renal injury and inflammation and tubular obstruction leading to interstitial fibrosis, declining renal function and eventually ESRD[52,59].

When glomerular filtration rate (GFR) drops below 30-40 mL/min per 1.73 m^2, renal capacity to excrete calcium oxalate is significantly impaired. At this stage, calcium oxalate starts to deposit in extra renal tissues in a process called systemic oxalosis. Calcium oxalate deposits have been reported in the myocardium, cardiac conduction system, kidneys, bones and bone marrow. This leads to cardiomyopathy, heart block and other cardiac conduction defects, vascular disease, retinopathy, synovitis, oxalate osteopathy and anemia that is noted to be resistant to treatment[52,60,61].

SCREENING FOR HYPEROXALURIA

Screening for hyperoxaluria must be undertaken in every child with the first episode of renal stone and all adults who present with recurrent calcium oxalate stones. Screening should also be done at first presentation of nephrocalcinosis or family history of stone disease at any age. Furthermore, screening must be offered to relatives of an index case. PH1 should be strongly considered in the differential in any patient with renal failure of unknown etiology, particularly when there is nephrocalcinosis with reduced renal function or a high occurrence of renal stones. Presence of monohydrate calcium oxalate crystals in biological fluids or tissues is also a strong pointer towards primary hyperoxaluria and should be followed up with additional testing[62].

DIAGNOSIS

Diagnosis of hyperoxaluria is established using a combination of clinical, radiological, biochemical, histopathological and genetic studies in primary hyperoxaluria. Precise diagnosis is of paramount importance for prognostic and treatment implications and also for prenatal screening in appropriate cases where PH is suspected.

In patients with a clinical suspicion for hyperoxaluria, the diagnostic workup should begin with ultrasound or other radiological imaging of the kidneys and the rest of the urinary tract to confirm the presence of nephrocalcinosis and urolithiasis[2,53]. Stone analysis should be done and may yield the initial diagnostic clues for PH. Stones in PH are composed of monohydrate calcium oxalate (whewellite) which assume a dumbbell shaped form[63].

The initial biochemical tests include urinary oxalate excretion preferably measured in 24 h urine collection and adjustment of the oxalate excretion per 1.73 m^2 of the body surface area is recommended[2]. Urinary oxalate: urinary creatinine ratios can be used but age specific normal values must be known. These values however should be interpreted with caution as the ratios decline in early life and are also subject to variability based on nutritional intake. Oxaluria must be confirmed using two urine samples. PH is characterized by urinary oxalate excretion > 1.0 mmol/1.73 m^2 per 24 h in majority and in some cases may exceed 2.0 mmol/1.73 m^2/ 24 h in contrast to the normal urinary excretion which is typically < 0.45 mmol/1.73 m^2 per 24 h. In patients with hyperoxaluria > 0.8 mmol/1.73 m^2 per 24 h, urinary glycolate and glycerate levels should be measured. About two thirds of PH1 patients have elevated urinary glycolate levels but it is important to remember that normal glycolate levels do not exclude

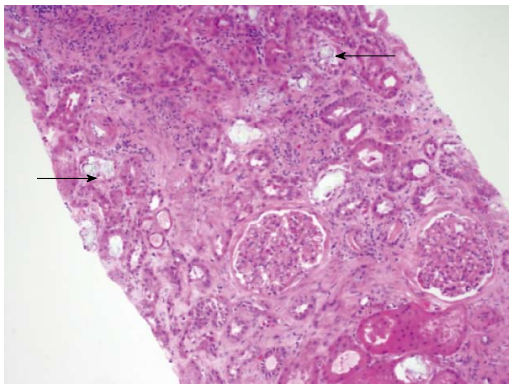

Figure 2 Calcium oxalate deposition in the renal tubules (black arrows).

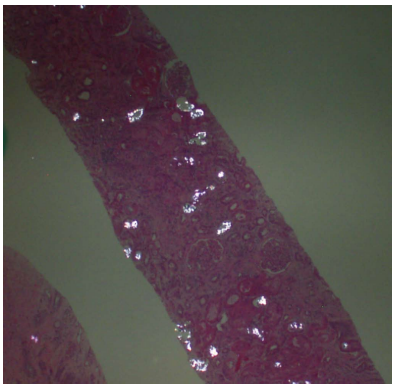

Figure 3 Examination of renal biopsy specimen under polarized light. Calcium oxalate crystals depict a characteristic birefringence.

the diagnosis. Urinary glycerate levels are noted to be high in PH2 patients[2,53].

As GFR declines, urinary excretion of oxalate decreases and the urinary oxalate estimation may no longer be accurate. Plasma oxalate should be measured in these circumstances. In PH patients with ESRD, plasma oxalate levels are typically higher than 80 μmol/L while in non PH hyperoxaluric patients, the plasma oxalate level may range between 30-80 μmol/L[64-66]. This is in contrast to plasma oxalate levels of 1-5 μmol/L in normal subjects[1].

Non-invasive, definitive diagnosis of PH is provided by testing of *AGXT*, *GRHPR* and *HOGA1* genes. There are 150 known mutations for *AGXT*[67], 16 for *GRHP*[26] and 15 for *HOGA1*[28,55-57,68]. Williams *et al*[69] showed that targeted analysis of the three most common mutations in *AGXT* (c.33_34insC, c.508G>A, and c.731T>C) provides the diagnosis in 34.5% PH1 patients while exon sequencing of exon 1, 4 and 7 increases the yield and allows diagnosis in 50% PH1 patients. Prenatal diagnosis can be done by testing chorionic villi. In patients with one or no known mutation, intragenic and extragenic linkage analysis is recommended for diagnosis[70,71] .When DNA screening is non diagnostic but clinical suspicion is high, liver biopsy is undertaken for establishing the diagnosis. However, this is an invasive method and carries a high risk of complications like bleeding[53].

In SH, stones are usually mixed (whewellite and weddellite) in contrast to PH. The excretion of urinary oxalate is increased in SH and may be > 0.7 mmol/1.73 m^2 per 24 h but in some cases may exceed 1.0 mmol/1.73 m^2 per 24 h[2,72,73]. Other available diagnostic tests include use of PCR in stool samples to identify *oxalobacter formigenes*[74,75]. Also, Increased intestinal oxalate absorption can be assessed by an absorption test using (^{13}C2) oxalate[76]. This test can help identify hyperabsorbers who would benefit from dietary interventions focusing on lowering oxalate and increasing calcium in the diet. This diagnostic test also helps to differentiate between primary and secondary forms of hyperoxaluria[33].

Radiological imaging may aid in diagnosis of multisystem involvement. Renal involvement, apart from urolithiasis, may show two distinct patterns: medullary nephrocalcinosis which is evaluated well on ultrasound while CT scan is a better modality for diagnosis of cortical nephrocalcinosis. CT may also be helpful in detection of calcium oxalate deposition in various other organ systems like bowel wall, muscle and arteries. The effects on the heart can be evaluated by electrocardiography and echocardiography. Skin biopsy may be necessary for skin lesions secondary to calcium oxalate deposition which can resemble the lesions of calciphylaxis[62]. On histopathological examination, calcium oxalate crystals demonstrate a characteristic birefringence when examined under polarized light. Figures 2 and 3 demonstrate calcium oxalate deposition in renal tissue.

TREATMENT

Conservative measures

Conservative measures are recommended soon after the diagnosis is made. High fluid intake is vital in preventing stone formation[77]. Patients with hyperoxaluria should be advised to increase their fluid intake to 3-4 L/d[53,60]. In infants and children, a gastrostomy tube may have to be placed to achieve this and special attention should be given to fluid intake in states of fluids losses like vomiting and diarrhea[57,62].

Dietary interventions do not play a major role in the management of primary hyperoxaluria as absorption of oxalate from the intestine is very small. In a study by Sikora *et al*[78], intestinal absorption of oxalate in patients with PH was noted to be less than 7%. This was attributed to less absorption and translocation of the SLC26A6 transporters favoring oxalate secretion over absorption. On the other hand, diet modification is a very important element in the treatment of secondary hyperoxaluria where efforts should be made to reduce oxalate intake in the diet. Calcium intake should not be restricted as it complexes with oxalate and prevents its absorption[10]. However, excessive intake of Vitamin C should be avoided.

Role of pyridoxine

Pyridoxine supplementation has been shown to be

beneficial in patients with PH1. Pyridoxine functions as a cofactor for the enzyme AGT which is defective in PH1. Administration of supraphysiological doses of pyridoxine may stabilize this enzyme and also enhance its enzymatic activity[57]. The recommended initial dose of pyridoxine is 5 mg/kg with a maximum dose of 20 mg/kg[79]. Pyridoxine has been demonstrated to be effective in only 30% of the patients[80,81] and therapeutic success is noted by a approximately 30% reduction in urine oxalate excretion after 3 mo of pyridoxine supplementation at the maximal dose[53,60]. Certain genotypes (508G>A (Gly170Arg) and 454T>A (Phe153Ile) are known to be more responsive to pyridoxine treatment than others[82,83] although pyridoxine therapy should be tested in all patients with PH1. Early initiation of pyridoxine treatment and compliance with the treatment regimen in pyridoxine responsive patients may help to prevent renal failure in PH1[57].

Urinary alkalinization

Alkalinization of the urine is well known to prevent stone formation as citrate complexes with calcium and thus decreases the amount of calcium oxalate available for precipitation. This same principle can be used in patients with hyperoxaluria. Potassium citrate can be used at a dose of 0.1-0.15 g/kg body weight[84]. Urinary pH must be maintained between 6.2 and 6.8[7]. In patients with renal failure, potassium salt can be replaced by sodium citrate[85]. Other inhibitors of crystallization are orthophosphate[86] and magnesium[7] though there is no conclusive evidence that magnesium therapy alone inhibits stone formation.

Probiotics (O. formigenes)

Despite our knowledge of *O. formigenes* and its use of oxalate as an energy source, the use of probiotics to reduce urinary oxalate excretion has not been demonstrated in human studies[57,87]. The results in animal studies however have been encouraging[88,89].

Management of renal stones

For management of renal stones, endoscopy is currently the procedure of choice as it allows direct visualization of the stones. Extracorporeal shock wave lithotripsy (ESWL) has been the standard of treatment for many years. However, with use of this technique, the shock waves may be mistakenly used on areas of nephrocalcinosis instead of stones due to lack of direct visual assessment which is achieved with endoscopy[90]. Further, gravel in the urinary tract following the ESWL procedure may form a nidus for calcium oxalate deposition and recurrent stone formation in patients with hyperoxaluria. In contrast, endoscopy allows complete retrieval of stones and their fragments and yields excellent results[62].

Renal replacement therapy

Patients reaching ESRD need optimization of renal replacement therapy to ensure adequate oxalate removal. Oxalate deposition occurs when the oxalate levels reach the threshold for supersaturation which is estimated to be 30-45 μmol/L. Hemodialysis (HD) removes oxalate more efficiently than peritoneal dialysis (PD)[66]. However, there is significant oxalate rebound following hemodialysis and levels can reach 80% of the pre-hemodialysis levels[91]. The weekly removal of oxalate by hemodialysis or peritoneal dialysis has been calculated to be 6-10 mmol/1.73 m^2[92,93] which leaves patients in a positive oxalate balance and at high risk for systemic deposition. Illies *et al*[94] studied 6 patients with PH1 who were on dialysis and awaiting liver transplant. Based on their observations, they made recommendations for improvement of the dialysis prescription. Dialysis should be initiated early (around GFR of 20-30 mL/min per 1.73 m^2) before ESRD is reached. Dialysis should be done with high flux dialyzers and maximum possible blood flow rate. To improve efficiency of oxalate removal by HD, additional sessions per week are preferable as compared to more time per session. Combination of HD and PD may be used to further enhance oxalate elimination. The timing of HD and PD should be coordinated as PD may be more efficient in removing oxalate in the later phases of the interdialytic period when rebound is much higher than in the earlier interdialytic phase. Efforts should be made to keep the oxalate level below 50 μmol/L[94]. The intensification of dialysis may pose a burden on the patient and family and it is important to keep this in mind while designing an individualized dialysis plan.

Transplantation

Transplantation must be planned when GFR falls between 15-30 mL/min per 1.73 m^2. As the defective enzyme is liver specific in PH1, these patients require preemptive liver, sequential liver- kidney, or combined liver-kidney transplantation. Transplantation strategy is decided based on individual presentation and clinical course as disease expression may vary among patients with PH1. Preemptive liver transplantation can be considered in patients who have progressive renal disease and approach a GFR of 50 mL/min per 1.73 m^2. Sequential liver-kidney transplantation can be performed in children who are small for a combined liver-kidney transplant[95]. In contrast, combined liver-kidney transplant is best suited for patients who are on chronic renal replacement therapy and not responsive to pyridoxine[57]. Isolated kidney transplantation may be the procedure of choice for adult patients who are sensitive to pyridoxine[96]. However, in isolated renal transplant, allograft survival rates have been reported to be inferior in patients with primary hyperoxaluria compared to patients who received renal transplant for a non PH1 cause of ESRD[48]. Thus, caution should be exercised while advocating this approach.

For patients with PH2, isolated kidney transplantation

Table 1 Comparison between primary and secondary hyperoxaluria

Clinical feature	Primary hyperoxaluria	Secondary hyperoxaluria
Etiology	Inborn error of metabolism with specific enzymatic defects PH 1: Alanine glyoxalate aminotransferase PH 2: Glyoxalate/hydroxypyruvate reductase PH 3: 4-hydroxy 2-oxoglutarate aldolase	Increased dietary intake of oxalate or precursors Increased intestinal absorption Altered intestinal microflora
Clinical presentation	PH 1: Recurrent stones, nephrocalcinosis, ESRD common Clinical heterogeneity in presentation, varies from an infantile to an adult onset form PH 2: Recurrent stones, nephrocalcinosis less common, ESRD has been reported (approximately 20% cases) PH 3: Hypercalciuria with hyperoxaluria is reported, no reports to date of ESRD	Recurrent renal stones, nephrocalcinosis, CKD and ESRD
Systemic oxalosis	Frequent part of the presentation	Less common but may occur in severe cases of inflammatory bowel disease or short bowel syndrome
Diagnosis:		
History	Family history is often suggestive with other affected relatives	Dietary history may be an important pointer towards the diagnosis
Urinary excretion	> 1.0 mmol/1.73 m^2 BSA	Usually < 1.0 mmol/1.73 m^2 BSA but in some cases of enteric hyperoxaluria may extend into the primary range
Composition of renal stones	95% calcium oxalate monohydrate (whewellite)	Mixed stones (whewellite and weddellite)
Other diagnostic points	Plasma oxalate levels in ESRD are > 60-80 mmol/L as compared from non-PH causes of ESRD	^{14}C test can be used to assess for increased intestinal absorption
Treatment:		
General measures:	Daily fluid intake > 3.0 L/d Pyridoxine in PH1 Urinary alkalinization Thiazides for PH3 Renal replacement therapy when ESRD occurs	Hydration and urinary alkalinization Renal replacement therapy when ESRD occurs
Specific measures: Dietary management	No role as dietary absorption is < 5%	Important role as dietary absorption is > 40%
O. formigenes	No role in management	No role demonstrated in human studies
Transplantation	PH1: Liver kidney transplant (combined or sequential) Isolated kidney transplant in pyridoxine sensitive adult patients PH2: Isolated kidney transplant PH3: No role of kidney transplant	Limited data available regarding transplants for treatment of SH

PH: Primary hyperoxaluria; SH: Secondary hyperoxaluria; CKD: Chronic kidney disease; ESRD: End stage renal disease; BSA: Body surface area.

Table 2 Additional resources for information on hyperoxaluria

Resource	Web address
Oxalosis and Hyperoxaluria Foundation	http://www.ohf.org/
Rare Disease Initiative of the Renal Association	http://rarerenal.org/
Rare Diseases Clinical Research Network (links to the Rare Kidney Stone Consortium)	http://www.rarediseasesnetwork.org/
Children Living with Inherited Metabolic Diseases	http://www.climb.org.uk/
Genetics Home Reference	http://ghr.nlm.nih.gov/
Office of Rare Diseases Research	http://rarediseases.info.nih.gov/
National Organization for Rare Disorders	http://www.rarediseases.org/

is the preferred treatment of choice[53,57] as the defective enzyme is found in various body tissues[97]. For patients with PH3, there are no reports of ESRD to date and as a result, no recommendations for renal transplantation have been made in this subset of PH patients[57].

In secondary hyperoxaluria, there is a paucity of data regarding renal transplantation in those who develop ESRD. There is an increased risk for allograft dysfunction by the rapid release of oxalate from systemic deposits leading to recurrent nephrocalcinosis. Ceulemans *et al*[98] performed combined intestinal and kidney transplants in a patient with hyperoxaluria due to short bowel syndrome which may be a promising approach in patients with enteric hyperoxaluria but this needs to be evaluated in larger studies. The differences between primary and secondary hyperoxaluria are depicted in Table 1.

Future directions

Gene therapy, chaperone treatment, liver cell transplantation and proteomic analysis of urine for diagnosis are amongst the new approaches being evaluated for

management of patients with primary hyperoxaluria[7,57,62].

Additional resources

There are numerous online resources for physicians and patients to obtain more information about hyperoxaluria. The resources and their web address are outlined in Table 2.

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MINIREVIEWS

Treatment of hypogonadotropic male hypogonadism: Case-based scenarios

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Abstract

The aim of this study is to review four case-based scenarios regarding the treatment of symptomatic hypogonadism in men. The article is designed as a review of published literature. We conducted a PubMed literature search for the time period of 1989-2014, concentrating on 26 studies investigating the efficacy of various therapeutic options on semen analysis, pregnancy outcomes, time to recovery of spermatogenesis, as well as serum and intratesticular testosterone levels. Our results demonstrated that exogenous testosterone suppresses intratesticular testosterone production, which is an absolute prerequisite for normal spermatogenesis. Cessation of exogenous testosterone should be recommended for men desiring to maintain their fertility. Therapies that protect the testis involve human chorionic gonadotropin (hCG) therapy or selective estrogen receptor modulators (SERMs), but may also include low dose hCG with exogenous testosterone. Off-label use of SERMs, such as clomiphene citrate, are effective for maintaining testosterone production long-term and offer the convenience of representing a safe, oral therapy. At present, routine use of aromatase inhibitors is not recommended based on a lack of long-term data. We concluded that exogenous testosterone supplementation decreases sperm production. It was determined that clomiphene citrate is a safe and effective therapy for men who desire to maintain fertility. Although less frequently used in the general population, hCG therapy with or without testosterone supplementation represents an alternative treatment.

Key words: Hypogonadism; Selective estrogen receptor modulator; Male fertility; Clomiphene; Human chorionic gonadotropin

Core tip: Symptomatic hypogonadism is both a common and growing health issue. Our four case-based scenarios assess different treatment options for hypogonadotropic male hypogonadism such as clomiphene citrate, human chorionic gonadotropin, and anastrozole. Furthermore, we provide clinical recommendations that can help physicians when confronted with situations such as the ones presented in this article.

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INTRODUCTION

According to a recent study by Mulligan *et al*[1], symptomatic hypogonadism affects approximately 40% of men aged 45 years or older. With the maturation of the Baby Boomer population, it is anticipated that there may be a significant increase in men desiring children at an older age. Testosterone therapies have been increasingly used in aging men, as well as men of reproductive age. A study by Samplaski *et al*[2] showed that 88.4% of men were azoospermic while on exogenous testosterone. In addition, this study demonstrated that cessation of therapy led to recovery of spermatogenesis in most infertile males. More startlingly, an estimated 6.5 million men in the United States will have hypogonadism by 2025[3]. Out of concern for this growing epidemic, this article will review four case-based scenarios concerning the treatment of hypogonadism in men. These case studies will include an assessment of the efficacy of potential treatment options as well as provide clinical recommendations for physicians.

CASE STUDIES

Case 1: The infertile male who presents while on testosterone therapy

In our first case-based scenario, a 38-year-old hypogonadal male patient has severely abnormal semen analyses as a result of use of testosterone therapy. His baseline Testosterone (T) level is 260 ng/dL. This male's baseline semen analysis prior to T therapy is 50 million/mL at 60% motility and now presents with 8 million sperm/mL with motility of 40%. This patient has been on a topical T therapy for 1 year and his luteinizing hormone (LH) is 4 mIU/L and follicle-stimulating hormone (FSH) level is 4 mIU/mL. This male commonly presents with infertility after being treated with testosterone therapy for symptomatic hypogonadism. Severe oligozoospermia and azoospermia be seen. Often times, the prescribing physician has not asked the patient about interest in fertility or has expressed unawareness of the detrimental effects of testosterone therapy on spermatogenesis.

Commentary

Exogenous testosterone is detrimental for spermatogenesis: Exogenous testosterone's mechanism creates a negative feedback on the hypothalamic-pituitary axis. This effectively decreases the production of gonadotropin and gonadotropin-releasing hormone. Consequently, the secretion of FSH and LH are also inhibited. These impairments on hormones result in overall decreases in intratesticular testosterone levels (ITT) as well as testosterone production. Typically, ITT concentrations are roughly fifty to one hundred times serum levels. Exogenous testosterone treatment can suppress ITT production to such an extent that spermatogenesis can be dramatically compromised at ITT concentrations to less than 20 ng/mL[4]. ITT is an absolute requirement for normal spermatogenesis. Without ITT, one can develop azoospermia[5,6]. However, the rates of success in recovering spermatogenesis after use of exogenous T are generally quite favorable.

Contraceptive studies using testosterone demonstrate that spermatogenesis may return after cessation of testosterone therapy: In one investigation, Gu *et al*[7] from China administered 500 mg of testosterone undecanoate monthly for 30 mo. The study used a primary outcome of pregnancy rate. In more than 1500 person-years of exposure in the 24-mo efficacy phase, only nine pregnancies were reported (855 men) resulting in a failure rate of 1.1 per 100 men. Forty-three men (4.8%) did not attain azoospermia or severe oligozoospermia ($< 1 \times 10^6$ sperm/mL). One hundred and eight days was the median time to the onset of azoospermia or severe oligozoospermia. Only two participants did not return to a normal fertility range of spermatogenesis. Spermatogenesis recovered at a median time of 196 d which was calculated from the beginning of the recovery phase. Recovery of sperm concentrations to baseline values was 182 d and to normal sperm output ($> 20 \times 10^6$/mL) was 230 d. Most notably, all but 17 participants who completed the 12-mo recovery period returned to normal levels of spermatogenesis. Furthermore, 15 of the 17 patients who did not recover returned to normal reference levels after an additional 3-mo follow-up. Although this study had a follow-up period of 2.5 years, it is important to note that longer-term data are not available in the published literature.

Recovery of spermatogenesis may be prolonged for some men, and may vary based on ethnicity: In a separate investigation, Liu *et al*[8] performed an integrated, multivariate analysis of 30 studies published between 1990-2005, in which semen analyses were recorded each month until recovery to a threshold of 20 million/mL. One thousand five hundred and forty-nine healthy eugonadal men aged between 18 and 51 years were treated with either androgens or androgens plus progestagens. The strength of this large meta-analysis was > 1200 man-years of treatment and > 700 man-years of post-treatment recovery. It required median times of 3.4, 3.0 and 2.5 mo for sperm to recover to thresholds of 20, 10, and 3 million per mL, respectively. Shorter treatment duration, shorter-acting testosterone preparations, older age, higher sperm concentrations at baseline, Asian origin, faster suppression of spermatogenesis, and lower LH levels at baseline identified with higher rates of recovery. As this

contraceptive trial was performed in men of Chinese ethnicity, similar trials with men of other ethnicities might not be reliable.

It should be advised that recovery of spermatogenesis might be prolonged for a small number of men, which may be of a larger concern with advanced maternal age. This study concluded that hormonal male contraceptive regimens demonstrate complete reversibility within an anticipated time course. However, the absence of pregnancy outcome data is a noteworthy limitation of the published literature. In addition, it is critical to stress that none of the literature measures time to fecundity and pregnancy outcomes do not correlate with semen analysis data.

Low-dose human chorionic gonadotropin maintains ITT in normal men with testosterone-induced hypogonadism: Low dose human chorionic gonadotropin (hCG) with intramuscular testosterone enanthate (200 mg/wk) can also maintain ITT and serum testosterone levels[9]. Some men are reluctant to stop testosterone therapy due to the symptomatic benefit, despite understanding the fertility risk. Use of hCG with testosterone may be a viable alternative for this select group of men. The use of hCG with intramuscular testosterone was initially studied for the development of a male contraceptive agent. Coviello *et al*[9] administered low doses of hCG (0, 125, 250, or 500 IU every other day) to normal men during this 3 wk study and measured serum and ITT levels. While the administration of testosterone alone resulted in profound decreases in ITT concentrations (94% from baseline in the TE and placebo hCG group), the addition of low dose hCG resulted in maintenance of the ITT levels. Although serum T increased from baseline in all groups, ITT remained significantly higher than serum T in all four groups after treatment. Despite supraphysiologic doses of exogenous testosterone, high levels of ITT can be maintained with the low-dose hCG.

Prevention of azoospermia and maintenance of fertility in hypogonadal men on TRT with low dose hCG: Hsieh *et al*[10] also studied the effect of hCG administration with testosterone replacement therapy on spermatogenesis. In this small series, ten men received short-acting testosterone preparations in addition to low doses of hCG. The key finding of this study was that spermatogenesis was maintained. Although there was a relatively small decrease in sperm density, no men became azoospermic.

Clinical recommendation

Discontinuing testosterone therapy alone may be adequate to return spermatogenesis to baseline levels as suggested by the hormonal contraception trials. However, men studied in these contraceptive trials most commonly had normal baseline T levels and semen analyses, and may not reflect men presenting with low T or infertility. In our experience symptomatic hypogonadal men may often be reluctant as their symptoms return. These men would especially benefit from medical therapy.

Case 2: The subfertile male who has been prescribed testosterone therapy to improve sperm production and fertility

The second case-based scenario is one of a 33-year-old subfertile male who has been treated with testosterone therapy to improve fertility potential. He had a sperm concentration of 12 million sperm/mL and motility of 45% prior to T therapy. His baseline T level was 400 ng/dL. His baseline serum T level was 270 ng/dL (normal is 300-800 ng/dL). The LH level was low normal at 3 mIU/L and the serum FSH was 5 mIU/mL. indicative of mid-normal range. Upon presentation, this male has seen his physician for infertility and has been found to have impaired semen quality. His physician prescribed an intramuscular T preparation 6 mo ago, not recognized the potential for a detrimental effect on spermatogenesis. At present, his sperm density is 2 million sperm/mL and the motility is 40%. The serum T level on therapy is 600 ng/dL. Upon physical examination, the testes are normal in size and there is no sign of varicocele. Otherwise, this patient is healthy and does not use any illicit drugs. His prolactin and estradiol was normal. This gentleman has an abnormal semen analysis and low T with an unspecified cause.

Commentary

Testosterone therapy does not improve spermatogenesis and should not used by men of reproductive age: This practice is not unusual. Ko *et al*[11] conducted a recent survey of United States urologists. The survey observed that up to 25% of these urologists have used testosterone therapy in an effort to improve spermatogenesis. However, this thought process is incorrect. As discussed before, testosterone therapy results in a mechanism that impairs spermatogenesis. Furthermore, in a recent study, testosterone use has increased greatly from 2000 to 2011 in the United States, specifically since 2008. This can be attributed to novel types of testosterone therapy, a greater awareness of symptoms associated with below normal testosterone levels, and consumer marketing by pharmaceutical companies. This investigation revealed that 12.4% of men in the United States who began testosterone therapy were between 18 and 39 years of age with 74% being between 40 and 64 years of age[12]. This is of upmost concern since it suggests that testosterone is being given to men that could be in their reproductive years.

Clomiphene citrate may improve serum testosterone levels: Clomiphene citrate (CC) can be a fairly effective treatment option in increasing serum testosterone levels[13,14]. Clomiphene is a selective

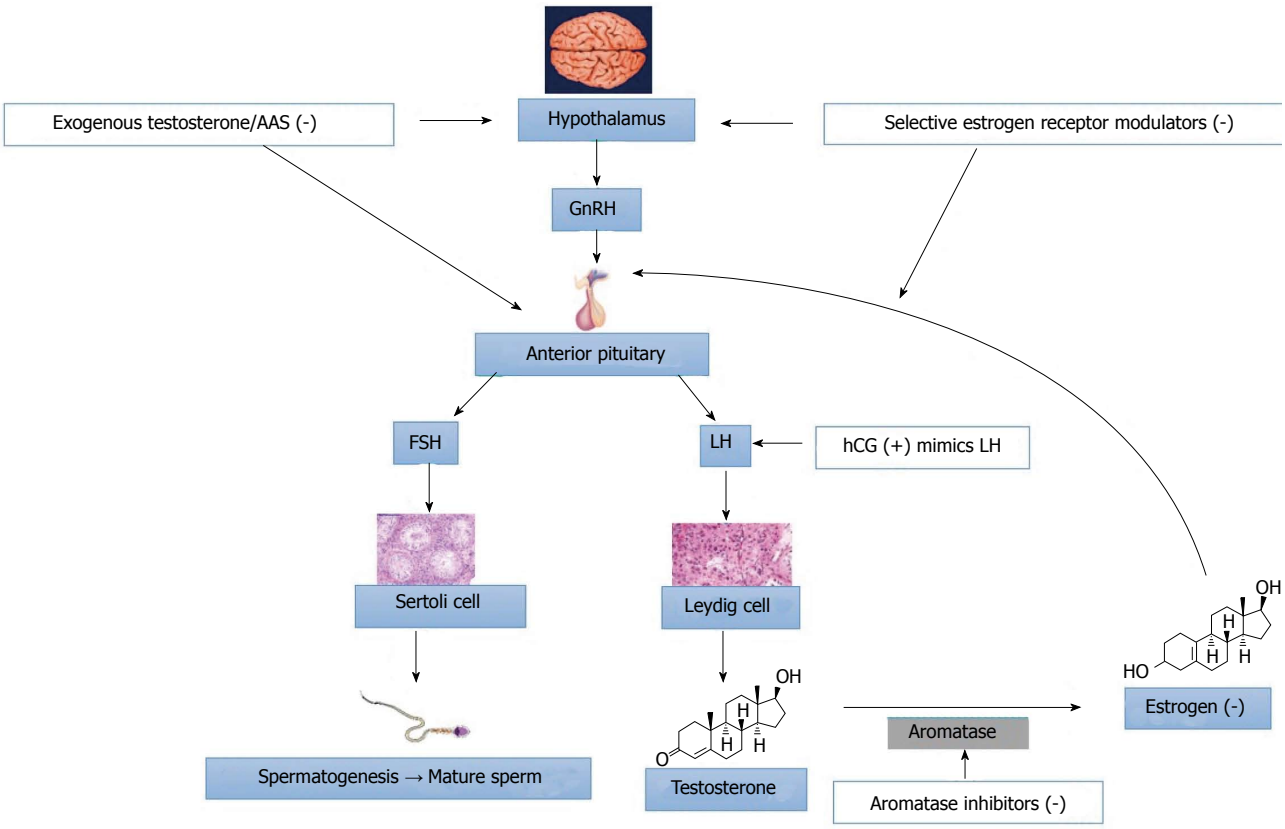

Figure 1 Hypogonadism therapeutic hormones and mechanism of action. Exogenous T and anabolic steroids negatively affect the HPG axis. SERMs such as CC stop the negative feedback of estrogen on the HPG axis. hCG stimulates Leydig cells. Aromatase inhibitors inhibit the conversion of T to E. LH: Luteinizing hormone; FSH: Follicle-stimulating hormone; AAS: Anabolic-Androgenic steroid; hCG: Human chorionic gonadotropin; GnRH: Gonadotropin-releasing hormone.

estrogen receptor modulator (SERM)[15]. This class of medications competitively binds to estrogen receptors on the hypothalamus and the pituitary gland (Figure 1). Accordingly, the pituitary gland recognizes less estrogen and creates more LH, which escalates overall testosterone production by the testes. CC is administrated orally with a common dosing that starts at 25 mg every other day with an upward titration to 50 mg daily, as needed. When LH and FSH levels are already high, it is not as effective in raising serum testosterone levels. Currently, use of hormonal dynamic testings, such as clomiphene or hCG stimulation testing, are not well-defined or commonly used. Potential side effects include gynecomastia, hypertension, cataracts, weight gain, and acne.

Clomiphene citrate as a treatment option for patients with hypogonadism: In a recent study by Da Ros *et al*[16], clomiphene citrate was tested for effectiveness in restoring endogenous testosterone production. In these trials, 125 men with an average age of 62 years were given clomiphene citrate (25 mg daily). Before treatment, all men had either below normal or low normal testosterone levels. Moreover, all patients complained about decreases in libido. The average follow up was 6 mo. Post-treatment testosterone levels increased by an average of 115%. The study concluded that clomiphene citrate should be considered as a therapy for male patients with hypogonadism.

Clomiphene citrate is an effective and less expensive treatment option: Taylor *et al*[17] conducted a study in which CC gave rise to significant increases in testosterone levels from baseline values. This was similar to increases made in testosterone gel replacement therapy (TGRT). One hundred and four men began CC (50 mg every other day) or TGRT (5 gm of 1% gel). The average follow up was 23 mo for CC or 46 mo for TGRT. Average post-treatment testosterone levels were 573 ng/dL (average baseline 277 ng/dL) in the CC group and 553 ng/dL (average baseline 221 ng/dL) in the TGRT group. The authors observed that the cost per month of CC was about $190 less than the cost of Testim® 1% (5 gm daily) at $270 and Androgel® 1% (5 gm daily) at $265. Compared with TGRT, CC demonstrates a less expensive option for men with hypogonadism, representing efficacy with minor side effects.

Long-term use of Clomiphene citrate is a safe way to improve serum testosterone levels: A similar study on clomiphene was performed by Moskovic *et al*[18] where forty-six hypogonadal males with an average age of 44 years were treated with clomiphene citrate for more than 12 mo. The main outcome measures were long-term results of clomiphene treatment on

hypogonadal males and predictors of response. Average baseline serum T levels were 228 ng/dL. Post-treatment serum T levels were 612 ng/dL, 562 ng/dL, and 582 ng/dL after 1, 2, and 3 years, respectively. Patients were also given the Androgen Deficiency in Aging Males (ADAM) questionnaire. The average pre-treatment ADAM score was 7 as compared to an ADAM score of 3 after 1 year of treatment. This investigation concluded that CC is effective as a long-term therapy for men with symptomatic hypogonadism. In addition, CC can improve many of the ADAM symptoms.

In an earlier study from the same institution, Katz *et al*[3] concluded that long-term use of CC improved serum testosterone levels to normal in a safe and effective manner. In this analysis, eighty-six men between 22 and 37 years old with hypogonadism (T levels < 300 ng/dL) were assessed and treated for an average of 19 mo. The participating men started with 25 mg of CC every other day. They were then titrated to 50 mg every other day. 550 ng/dL was the goal testosterone level. Once preferred testosterone levels were reached, testosterone/onadotropin levels were measured biannually. With regards to questions on the Androgen Deficiency in Aging Males (ADAM) questionnaire, advances were noted in each area excluding loss of height. Five of the ten variables saw significant improvement including feeling sad/grumpy, lack of energy, decreased life enjoyment, decreased libido, and decreased sports performance. This study demonstrates that CC is both an effective and safe testosterone therapy substitute in hypogonadal men.

A randomized, prospective trial of CC for men with hypogonadism with normal semen parameters is vital to confirm the recommendation for the use of SERMs for fertility preservation. It is essential that this study show that semen profiles are not negatively affected. A purified androgenic isomer of generic clomiphene is presently completing phase Ⅲ clinical trials in the United States[19]. The anticipated patient is the overweight, hypogonadal male interested in maintaining fertility potential.

Oral enclomiphene citrate initiates production of serum testosterone and sperm in men with low testosterone: Androxal®, or enclomiphene citrate, is the trans-isomer of clomiphene citrate[20]. Enclomiphene citrate is currently completing phase Ⅲ clinical trials in the United States and may in the future be another alternative treatment to testosterone therapies. In a randomized study by Kaminetsky *et al*[21], the investigators compared levels of testosterone, FSH, and LH after hypogonadal males used either oral enclomiphene citrate or testosterone gel. Twelve male subjects were assigned to either of the two treatments. At baseline, the average testosterone level for all patients was 165 ± 66 pg/dL. Treatment with both enclomiphene and testosterone gel raised serum testosterone levels back to the normal range. Both groups had about the same serum T levels after 3 mo and 6 mo. After 6 mo, serum T levels were 525 ± 256 pg/dL for enclomiphene and 545 ± 268 pg/dL for testosterone gel. The distinguishing factors between these two treatments are their FSH and LH levels as well as their sperm counts. Only enclomiphene citrate was associated with rises in FSH and LH as well as sperm counts. All of the enclomiphene citrate subjects had sperm counts above 75 million/mL, with an average sperm count of 176 million/mL. In contrast, the testosterone gel subjects did not surpass sperm counts of more than 12 million/mL. These findings were also evident throughout the follow-up period. This study suggests that enclomiphene citrate may prove to be a superior treatment as it is effective in increasing testosterone as well as sperm counts. The rise in FSH and LH levels could also point towards a shift back to normal endogenous testosterone production.

Wiehle *et al*[22] carried out another study for enclomiphene citrate. This randomized study also compared the effects of enclomiphene (Androxal®) *vs* AndroGel®, a transdermal testosterone. Enclomiphene citrate was given in three different doses: 6.25 mg, 12.5 mg and 25 mg Androxal®. Forty-four men with testosterone levels less than 350 ng/dL at baseline were included in the study. Their average age was 53 years. After six weeks of treatment, patients who took 25 mg enclomiphene had an average testosterone level of 604 ± 160 ng/dL while patients on the transdermal testosterone had an average testosterone level of 500 ± 278 ng/dL. While these results were almost equivalent, AndroGel® patients saw a decrease in FSH and LH levels whereas enclomiphene patients saw an increase. These outcomes correlate with the results of the aforementioned study. This study concluded that enclomiphene citrate was capable of increasing serum T and LH levels.

Repros Therapeutics Inc[19] observed the effect of 12 d of use of clomiphene citrate, enclomiphene, and zuclomiphene in baboons. All of the animal subjects were administered 1.5 mg of one treatment per day. Zuclomiphene did have much of a significant effect on increasing testosterone levels from baseline levels of 170 ng/dL. Enclomiphene had a much greater effect (8-fold increase to 1144 ng/dL) than clomiphene citrate (5-fold increase to 559 ng/dL). However, neither clomiphene nor enclomiphene demonstrated any effect on FSH or LH levels. This could be due to a flaw in the study.

Clinical recommendation

Similar to the first case study, testosterone (T) therapy should be stopped, and treatment with clomiphene should begin. Cessation of T therapy should be the first treatment concern for nearly all men who are interested in preserving their fertility. Longer durations of T therapy are likely to have more significant effect on the return of testosterone but undoubtedly the amount of T would be expected to have an effect on return of spermatogenesis. Clomiphene would only be expected

to benefit men with secondary hypogonadism based on its mechanism of action. It is important to assess serum LH levels prior to therapy to determine that these levels are low or normal.

Case 3: The symptomatic hypogonadal male desiring to preserve his fertility

In the third case-based scenario, a 42-year-old male patient with symptomatic hypogonadism has a desire to father children at an unspecified future time. Upon presentation, this male has symptomatic hypogonadism without a specific underlying cause. While he knows he wants to have children in the future, he does not have a clear idea regarding timeframe. He is not married and does not have any children. This male's baseline T is 220 ng/dL. His LH is 4 mIU/L and FSH level is 4 mIU/mL. Semen analysis is 26 million sperm/mL with motility of 70%. He is healthy, has a normal physical exam and is currently not on any therapy.

Commentary

Clomiphene citrate results in similar satisfaction and efficacy to testosterone therapy: There has been concern that clomiphene citrate may not result in as much symptomatic improvement compared to testosterone therapies. There are no prospective, controlled trials to confirm or refute this concern. In a recent retrospective, age-matched comparison, Ramasamy *et al*[23] assessed their results using the ADAM questionnaire and serum T levels in 31 men on topical testosterone, 31 men on injectable testosterone and 31 men on clomiphene. Clomiphene-treated men had similar total testosterone levels to topical testosterone-treated males. Men on injectable testosterone had the highest serum T levels. Similar ADAM questionnaire satisfaction was noted between treatment groups. The authors concluded that testosterone supplementation regimens and clomiphene citrate are efficacious for improving serum total testosterone. No difference in overall hypogonadal symptoms was noted among men on any testosterone supplementation therapy. Despite lower serum total testosterone, men on clomiphene citrate and testosterone gels reported satisfaction similar to that of men treated with testosterone injections.

Exogenous hCG increases serum testosterone levels thus increasing ITT concentrations: Although most men taking testosterone for contraceptive use trials recover their baseline spermatogenesis, this recovery could take up to 18 mo and may not always happen. Use of clomiphene is generally effective, but off-label. High quality safety studies of greater than 18 to 24 mo of use are lacking. Another treatment option could be hCG.

hCG is an LH analog that stimulates Leydig cell to produce more testosterone. hCG stimulates testosterone production by the Leydig cells by functioning as an LH analogue. hCG can be extracted from urine as well as other recombinant sources. Exogenous hCG increases serum testosterone levels and ITT concentrations. hCG alone can only maintain spermatogenesis for a short period of time. In a small case series directed by Depenbusch *et al*[24], thirteen azoospermic men with hypogonadotropic hypogonadism were initially administered hCG and human menopausal gonadotropin (hMG) to induce spermatogenesis. hCG was then administered 500-2500 IU hCG subcutaneously biweekly alone for up to two years (range 3-24 mo). After 12 mo of treatment, sperm counts decreased gradually but remained present in all patients, except for one who became azoospermic. The declining sperm counts demonstrate that FSH is crucial for the continuation of normal spermatogenesis.

Low levels of hCG increase ITT concentrations and serum testosterone levels: Treating patients with a high dose of hCG is not necessary with regards to the upkeep of spermatogenesis. In a study conducted by Roth *et al*[25], 37 normal patients became experimentally gonadotropin deficient through the use of GnRH antagonists. The patients were then randomized and treated with 0, 15, 60, or 125 IU SC hCG every other day or 7.5 g testosterone gel daily for a duration of 10 d. Steroid measurements at baseline and endpoint were taken after obtaining testicular fluid by percutaneous aspiration. ITT concentrations increased proportionally to the dose from 77 nmol/L to 923 nmol/L in the 0- and 125-IU treatment groups, respectively ($P < 0.001$). Furthermore, significant correlation ($P < 0.01$) existed between serum hCG and both ITT and serum T levels. The study established that low dose hCG treatment dose-dependently increases ITT concentrations in normal men brought about with gonadotropin deficiency. Even though hCG can be advantageous in increasing serum T levels, this treatment can be very costly. In addition, the fact that this medication is applied through an injection can deter potential users.

Aromatase inhibitors as a treatment option: Another therapeutic target is the aromatase enzyme, which converts testosterone to estradiol. It is mainly located in the testes, brain, adipose tissue, and liver. Estradiol inhibits the secretion of gonadotropins, which may affect the production of ITT. The purpose of aromatase inhibitors is to inhibit the process of converting androgens to estrogen, effectively increasing T, LH, and FSH levels and having effects similar to those of anti-estrogens. This type of treatment has been employed to stimulate spermatogenesis and bring about improvements to male fertility. Aromatase inhibitors may be more beneficial than antiestrogens in male patients with low serum T to estradiol ratios (< 10) and who are obese.

Typically, aromatase inhibitors have been classified into two types: steroidal or non-steroidal. Anastrozole

Table 1 Treatment options for hypogonadism in men desiring to preserve their fertility

Name of medication	Dosing and administration	Side effects	Anticipated results
Clomiphene	Oral: 25 mg every other day with max of 50 mg daily	Gynecomastia, weight gain, hypertension, cataracts, and acne	Recovery of spermatogenetic function, increases in testosterone levels
hCG	Intramuscular injection; 125-500 IU every other day	Headache, restlessness, tiredness, swelling of the ankles/feet, mental/mood changes, pain/swelling of the breast	Recovery of spermatogenetic function, increases in testosterone levels
hMG	Intramuscular injection; 75 IU three times a week	Gynecomastia, dizziness, fainting, headache, loss of appetite, irregular heartbeat	Recovery of spermatogenetic function
Anastrozole	Oral; either 0.5 or 1 mg	Blood pressure increase, anorexia, malaise, rash, peripheral edema, aches glossitis, paresthesias vomiting/nausea	Improvements in T/E2 ratio, increases in sperm concentration

IU: International units; hMG: Human menopausal gonadotropin; T/E2 ratio: Testosterone/estradiol ratio; hCG: Human chorionic gonadotropin.

and letrozole are examples of non-steroidal aromatase inhibitors. Typically, adrenal steroid supplementation is not required. Although aromatase inhibition by these two medications is almost 100%, their administration does not completely suppress estradiol levels in men and actually decreases the plasma T to estradiol ratio by 77%. This incomplete suppression may be linked to the high levels of circulating testosterone in men and may provide an advantage by limiting the adverse side effect profile.

Men with conditions including idiopathic male infertility, primarily men with lower serum testosterone to estradiol ratios (< 10), and men with hypogonadism, often related to obesity have been treated with aromatase inhibitors. They have also been used in men with Klinefelter's syndrome in order to stabilize serum T levels prior to testicular sperm extraction.

Testolactone or anastrozole may increase sperm quality and concentrations: Raman *et al*[26] conducted a study in order to detect a difference between treatment with testolactone or anastrozole. The male patients in this study had T/E2 ratios that were less than 10. The patients were treated with either testolactone or anastrozole. Both treatments resulted in significant improvements in sperm concentrations, morphology, motility, and T/E2 ratios. With regards to the small group of 25 infertile, oligospermic men treated with anastrozole, sperm concentration increased substantially from 5.5 to 15.6 million per mL, and the total motile sperm concentration per ejaculate increased from 833 to 2931 million ($P < 0.005$). The study reported no changes in the azoospermic group treated with anastrozole. In addition, pregnancy rates were not reported for any of the patients regardless of improvements in the semen parameter.

Clinical recommendation

Options for therapy include long-term CC with or without drug holidays, testosterone with low dose hCG, testosterone with or without drug holidays, or alternating combinations of the prior options (Table 1). Katz *et al*[3] conducted a study demonstrating the effectiveness of treating 86 hypogonadal men with low-dose clomiphene over the course of 19 mo. No major side effects were reported and improvements were made in more than three items on the ADAM questionnaire for 60% of the patients. Long term studies with anastrozole are lacking.

Case 4: The anabolic steroid abuser presents with symptomatic hypogonadism

Lastly, the fourth case-based scenario is centered on a 30-year-old male with symptomatic hypogonadism due to chronic anabolic steroid abuse. He completed 3 cycles of nandrolone over the last two years. These men are typically using anabolic steroids to improve muscle mass and body image. Long-term use in cycles are commonly observed. Sophisticated hormonal regimens that are self-prescribed have evolved. Severe impairments in spermatogenesis may be seen and may be permanent depending on the duration and potency of agents used. He has been off anabolic steroids for 3 mo. At present time his T level is 170 ng/dL and his LH and FSH are low at 1.5 (UNITS). His semen analysis is 1 million sperm/mL with 10% motility. Upon physical examination, the testes have mild atrophy and his overall physique is still muscular.

Commentary

Chronic anabolic steroid use is detrimental to spermatogenesis: Anabolic-androgenic steroids use is currently widespread as they are now readily available in over-the-counter medicines. According to recent evidence, illegal use of anabolic steroids may be the most prevalent cause of symptomatic hypogonadism in young men[27]. Other side effects of anabolic steroid use include hepatotoxicity, cholestasis, renal failure, gynecomastia, and infertility[28]. Furthermore, a meta-analysis study comprised of 197 studies was done on the epidemiology of anabolic steroids use[29]. The study resulted in a global lifetime prevalence rate of 3.3%. This data demonstrates the prevalence of this problem, which is connected to symptomatic hypogonadism.

Men using anabolic steroids may be deficient in ITT concentrations required to sustain spermatogenesis even though they may have serum androgen concentrations that are between normal and high. Many males who use anabolic steroids acquire hypogonadotropic hypogonadism along with an ensuing atrophy of the testes.

The user of anabolic steroids often has azoospermia or oligozoospermia as well as irregularities of sperm morphology and motility[30,31]. For men with hypogonadotropic hypogonadism from anabolic steroid abuse, administration of intramuscular injections of hCG at doses of 2000 to 3000 units 2 to 3 times per week for 4 mo can initiate spermatogenesis[32,33].

Cessation of anabolic steroid abuse may reverse suppression of spermatogenesis: Mills *et al*[34] tested the recovery of spermatogenesis after exogenous testosterone administration in 26 men with a recent history of anabolic steroid use. In this relatively small study, all men discontinued exogenous testosterone usage and began treatment with human chorionic gonadotropin (hCG) 3000 units IM every other day. The treatment lasted for at least 3 mo. In regards to the two men who remained azoospermic, one had insufficient follow-up and the other was suspected of continued anabolic steroid use. Men who were using intramuscular testosterone (hCG) at the time of presentation recovered spermatogenesis in an average of 3.1 mo. However, men receiving transdermal testosterone supplementation at the time of presentation took an average of 7.4 mo to recover. Mills *et al*[34] concluded that impairment of fertility following testosterone replacement therapy suppression is reversible and that the rate of sperm may be related to the delivery system.

Clinical recommendation

hCG with or without human menopausal gonadotropin has been used most commonly to restore fertility. hCG may be considered, but it requires frequent injections and can produce side effects (Table 1). Occasionally, hCG can exacerbate depression and irritability in hypogonadal men. Cessation of the anabolic steroids with use of clomiphene may be the most beneficial. Non-responders to hCG will require the addition of human menopausal gonadotropin with a daily injection of 75 IU[34].

CONCLUSION

Men wishing for future fertility should refrain from utilizing exogenous testosterone due to the potential for long-term detrimental effects on spermatogenesis. In a minority of cases, spermatogenesis is not recovered, although it is difficult to say whether this is due to testosterone treatment or to the natural evolution of the condition. Clomiphene citrate, an oral selective estrogen receptor modulator, is an off-label yet innocuous and potent therapy for men who wish to retain future potential fertility. hCG therapy, although less used, with or without testosterone supplementation represents an alternative treatment. Currently, it is not recommended to repeatedly use aromatase inhibitors due to a paucity of long-standing data.

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Is there a role for systemic targeted therapy after surgical treatment for metastases of renal cell carcinoma?

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Abstract

Metastatic renal cell carcinoma (mRCC) is a challenging disease. Despite the new targeted therapies, complete remissions occur only in 1%-3% of the cases, and the most effective first-line treatment drugs have reached a ceiling in overall survival (ranging from 9 to 49 mo). Metastasectomy remains to be the only curative option in most patients with mRCC. Prognostic nomograms have been recently published, so we have tools to classify patients in risk groups, allowing us to detect the cases with the higher risk of recurrence after metastasectomy. Although sparse, there is some evidence of effectiveness of neoadjuvant targeted therapy before metastasectomy; but with an increase in surgical complications due to the effects of these new drugs in tissue healing. We have aimed to answer the question: Is there a role for systemic targeted therapy after surgical treatment for metastases of renal cell carcinoma? We have made a search in Pubmed database. As far as we know, evidence is low and it's based in case reports and small series of patients treated with adjuvant drugs after neoadjuvant therapy plus metastasectomy in cases of partial response to initial systemic treatment. Despite the limitations and high risk of bias, promising results and cases with long-term survival with this approach have been described. Two ongoing clinical trials may answer the question that concerns us.

Key words: Metastatic renal cell carcinoma; Targeted therapy; Metastasectomy; Surgery; Adjuvant treatment

Core tip: We have made a search in Pubmed database looking for evidence to support adjuvant systemic therapy after metastasectomy in metastatic renal cell carcinoma. As far as we know, evidence is low and it's based in case reports and small series of patients. Despite the limitations and high risk of bias, promising results and cases with long-term survival with this approach have been described. Two ongoing clinical trials may answer the question that concerns us.

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INTRODUCTION

Renal cell carcinoma (RCC) represents 2%-3% of all cancers[1]. We know that the last two decades there has been 2% increase per year in its incidence worldwide[2].

According to largest published series, approximately 20%-30% of patients with renal cell carcinoma present metastasis at time of diagnosis. Besides, another 20%-40% of patients with localized disease who have had a surgical treatment, either partial or radical nephrectomy, will have progression during follow-up[3].

The more frequently affected organs are lungs, lymph nodes, liver and bone[4]. Nowadays, there are six targeted therapies approved for mRCC treatment. These new agents have completely changed the treatment and prognostic of patients with mRCC, but the cure is rare with medical treatment alone. Metastasectomy when feasible remains a curative option in some patients[5].

These are some of the reasons why metastatic renal cell carcinoma (mRCC) is a challenging disease. The present review aims to clarify if there is an evidence to support combination of metastasectomy and adjuvant systemic targeted therapy in mRCC.

LITERATURE STUDY

We have made a search in Pubmed database, using the key words: "renal cell carcinoma", "metastatic renal cell carcinoma", "renal cell carcinoma metastasis", "metastasectomy", "neoadjuvant treatment", "adjuvant treatment", "local treatment", "surgery"; in all languages and no date restrictions.

We included in the review all the studies that underwent the inclusion criteria: surgical treatment of metastatic renal cell carcinoma (yes/no), with emphasis on those that focused on neoadjuvant/adjuvant systemic therapy and mestastasectomy.

RESULTS

Epidemiology of mRCC treated with metastasectomy

As mentioned above, around 20%-30% of patients with RCC have metastases when diagnosed, and 20%-40% of those with localized advanced disease will progress to metastatic disease.

The most commonly affected organ in mRCC is the lungs. Lymph nodes, liver, bone, adrenal glands and brain are other typical sites; but there are reported metastases in rare organs, like pancreas, skin, bladder, *etc.*

In a recent publication, the distribution according to different organs was: 45.2% in lungs, 29.5% in bone, 21.8% in lymph nodes and 20.3% in liver. It was observed that in patients with multiple metastatic sites, 16% and 49%, brain and bone were affected, respectively[6].

Without treatment, survival of RCC is lower than 10% at 5 years[7].

Other specific characteristic of RCC is the existence of documented late metastases (> 20 years from the primary diagnosis).

The first evidence of long survival after resection of a solitary lung lesion was published in 1939[8]. Since then, several retrospective series have confirmed the effectiveness of metastasectomy. However, there are no randomized or prospective studies available.

Some authors have reported 37.2%-42% 5-year survival rates in cases of mRCC with complete resection, in observational studies[9,10].

The best response has been found in resection of solitary lung metastases, with 56% 5-year survival compared to 28% for skin, 20% for visceral organs, 18% for peripheral bone, 13% brain and 9% for axial bone metastases[11].

General prognostic factors: Knowledge of prognostic factors is important for a correct selection of patients candidates to surgery.

A retrospective study of 278 cases treated with nephrectomy and a solitary metastasis treated with surgery found that the factors associated with favourable outcome were: solitary site and single metastatasis, complete resection, a long disease-free interval and metachronous presentation[12].

In a large series of clear cell mRCC from de Mayo Clinic (Rochester, MN, United States) of 727 cases, prognostic factors of poor survival were: constitutional symptoms at nephrectomy, metastases to the bone or liver, multiple metastases, metastases at time of nephrectomy or in the 2 year thereafter, caval thrombus, Fuhrman grade 4 and coagulative tumour necrosis. In this study, complete resection of metastatic sites improved survival significantly[13].

Eggener *et al*[14] have published that in mRCC patients, the risk score classification according to Motzer classical factors and metastasectomy were independent factors of good outcome. The best survival was observed in patients with favourable risk and metastasectomy (71% 5-year survival) compared to that with high risk, with no survival at 5 years, independently of metastasectomy.

Recently, Tosco *et al*[15] have published a predictive model based on the following independent prognostic factors: primary tumour T stage $\geqslant$ 3, primary tumour Fuhrman grade $\geqslant$ 3, nonpulmonary metastases, disease-free interval $\leqslant$ 12 mo and multiorgan metastases. The Leuven-Udine (LU) prognostic groups are: (1) Group A (0-1 risk factors) with 5-year cancer specific survival (CSS) of 83.1%; (2) Group B (2 risk factors) with 5-year CSS of 56.4%; (3) Group C (3 risk factors) with 5-year CSS of 32.6%; and (4) Grupo D (4-5 risk factors) with 5-year CSS of 0%.

Another multiinstitutional study of 556 patients with mRCC who underwent metastasectomy in 48 Japanese hospitals found four adverse prognostic factors: incomplete resection of metastases, brain metastases, C-reactive protein > 1.0 mg/dL and high grade[16].

In conclusion, the prognostic factors of poor survival in patients with mRCC treated with metastasectomy are: (1) Primary tumour T stage ≥ 3; (2) Primary tumour Fuhrman grade ≥ 3 or high grade according to Japanese classification (nuclei of tumour cells larger than nuclei of normal tubular cells); (3) Nonpulmonary metastases; (4) Disease-free interval ≤ 12 mo; (5) Multiorgan metastases; (6) Incomplete resection of metastases; (7) Brain metastases; (8) C-reactive protein > 1.0 mg/dL; and (9) Motzer Classification risk score for mRCC (MSKCC risk score).

There are studies that evaluate the role of metachronous multiple metastasectomies.

In 2010, Szendröi *et al*[17] reported a case of a patient with 11-year survival after multiple and successive metastatasectomies.

In a study of 141 cases[12], 5-year survival after complete resection of second and third metastases did not differ from patients with first complete metastasectomy [43% overall survival (OS) in first resection, 46% in second and 44% in third].

In a large cohort of 887 mRCC cases, 125 underwent complete resection of multiple metastases. 5-year OS of cases with complete resection of multiple non-lung-only metastases was 32.5% compared to 12.4% of those with incomplete resection[18].

Organ-specific surgery: Lung metastases resection has demonstrated to prolong survival, with 5-year OS of 37%-55%[10,18-20].

Some prognostic factors have been described in these studies: (1) Incomplete resection has a poorer outcome (0-22% 5-year OS); (2) The presence of multiple metastases. More then 7 pulmonary metastases had worse 5-year OS (14.5% *vs* 46.8% of those with less than 7 lesions)[21]; (3) The presence of mediastinic node plus lung metastases impacted in survival (19 mo of median survival *vs* 102 mo)[22]; (4) Short disease-free interval after nephrectomy[23]; (5) Synchronous lung metastases (0% 5-year OS in patients treated with nephrectomy and metastasectomy)[24]; and (6) Size of metastases, with 0.5 cm as the established limit[25].

A prognostic model has been created based in a study of 200 cases of a single institution[26]. In multivariate analysis, size more than 3 cm, N^+ at diagnosis, pleural invasion, synchronous metastases, tumor-infiltrated hiliar, incomplete resection (R1 or R2) and mediastinal nodes were independent prognostic factors. Munich score classified patients in three groups of low, intermediate and high risk, with different median OS (90, 31 and 14 mo respectively): (1) Munich Ⅰ (low): R0, no risk factor; (2) Munich Ⅱ (intermediate): R0, ≥ risk factor; and (3) Munich Ⅲ (high): R1 or R2.

Bone metastases are often symptomatic. The indications for surgical treatment are prolongation of survival and alleviation of pain or stabilization of the extremity.

In a retrospective series of 99 cases surgically treated, the factors of good outcome were: single metastasis, wide resection and cytoreductive nephrectomy[27].

One study included a literature review, with 5-year OS of 35.8%-55%, with the best outcome in cases of peripheral skeletal location and histological subtype clear cell[28].

In a large series of M.D. Anderson Cancer Center[29] of 295 patients with 368 metastases treated, the OS rates were: 47% 1st year, 30% 2nd year and 11% 5th year. Patients with solitary metastasis showed better results, with a 5-year OS of 35%.

Patients with liver metastases have a poor prognosis due to that only 5% of the cases have a solitary metachronous lesion[30].

A series of 31 cases showed that negative resection-margin was an independent prognostic factor in multivariate analysis. The 5-year OS was 38.9%[31].

The largest retrospective series (88 patients with only liver metastases) found that those patients with synchronous metastases and a high grade RCC did not show benefit from surgery. The morbidity was 20.1%[32].

Most of the cases of brain metastases (80%) are diagnosed by symptoms. Without treatment the prognostic is poor, with a survival of less of a few months. Treatment options are surgery and stereotactic radiosurgery.

In a series of 50 cases, resection of lung metastases and supratentorial (*vs* infratentorial) localization were good prognostic factors. Adjuvant radiotherapy showed no survival advantage[33].

A series of 69 cases published in 2003, with 146 lesions treated with radiosurgery achieved good local control. OS was 6 mo from treatment. Age, neurologic status and radiosurgery dose had an impact in OS[34].

A study of 46 cases with 99 brain lesions treated with radiosurgery achieved local control in 84.7% of patients. Median OS was 10 mo, but reached 18 mo when > 75% volume decrease[35].

There have been reported 411 patients with pancreatic metastases of RCC in 170 publications[36]. Of 411 cases, 321 were surgically treated; with 65.3% of solitary lesions in surgery group. The 5-year OS was 72.6%, and disease specific survival was 57%. In-hospital mortality was 2.8%, 35.8% of patients underwent pancreaticoduodenectomy and 19.9% total pancreatectomy.

There are reports of RCC metastases in other organs, like adrenal, bladder, vagina, thyroid gland, paranasal sinuses. These publications are case reports and no clear prognosis knowledge can be made.

The panel of European Association of Urology Guidelines has made a systematic review in accordance with Cochrane review methodology[37]. They concluded that all the studies were retrospective with a high risk of bias, but with the exception of brain and possibly bone metastases, surgery remains to be by default the best treatment for most sites.

In the last actualization of the Guidelines, the conclusion is that "no general recommendations can be made and the decision of metastasectomy has to be taken for each site, and on a case-by-case basis: performance status, risk profiles, patient preference and alternative techniques must be considered".

Rationale of multimode therapy in mRCC

mRCC is a complex entity that can be treated with cytokine treatment, sequential targeted therapies and metastasectomy.

The correct moment and sequence of each treatment is not clear, but we have some evidence that combination of surgery and systemic therapies can achieved excellent outcomes.

Cytoreductive nephrectomy: It is known that nephrectomy is curative if surgery can excise all tumour deposits.

In a metaanalysis of two randomized trials comparing immunotherapy only *vs* nephrectomy and immunotherapy, a long-term survival was reported in cases treated with nephrectomy and immunotherapy in patients with good performance status[38].

In a retrospective study[39], the previous advantage of cytoreductive nephrectomy was confirmed in patients treated with vascular endothelial growth factor-targeted therapy (VEGF-targeted therapy).

However, the value of cytoreductive nephrectomy followed by VEGF-targeted therapy has to be confirmed by ongoing trials.

Adjuvant therapy after nephrectomy in localized RCC: Adjuvant tumour vaccination might improve duration of PFS in patients with T3 RCC, but has not effect in OS. Adjuvant therapy with cytokines does not improve survival[40,41].

There are several ongoing phase Ⅲ trials of adjuvant sunitinib, sorafenib, pazopanib, axitinib and everolimus.

Presurgical treatment for locally advanced RCC: Neoadjuvant treatment could be used with the following objectives: (1) Decrease tumour size and facilitate surgery; (2) Allow the performance of nephronsparing surgery; (3) Improve survival acting against micrometastases; (4) Reduce morbidity of surgery by decreasing size and vascularisation of tumour; (5) Knowledge of response to systemic therapy before surgery; and (6) Future research.

Targeted therapies have been used in neoadjuvant/preoperatory settings in cases of locally advanced RCC (huge tumours, cases with large nodes near hilium and inferior vena cava thrombus) and in cases of T2 RCC with the aim to perform a nephronsparing procedure.

Sunitinib, sorafenib, axitinib, everolimus and temsirolimus are the 5 neoadjuvant therapies that have been used for locally advanced RCC treatment and before nephronsparing surgery.

First evidence of radiological downstaging effect of kinase inhibitors was reported in phase 2 and 3 trials[42,43].

In 2009, a complete histologic remission after sunitinib neoadjuvant therapy was reported in a case of T3b renal cell carcinoma[44].

The response of renal tumours to targeted therapies has been reported in small retrospective series and case reports. The most important series are summarized in Table 1[45-54].

Powles *et al*[55] reported that in cases of mRCC treated with sunitinib prior to nephrectomy, progression prior to planned nephrectomy, high Fuhrman grade and MSKCC poor risk at diagnosis were independent prognostic factors.

Another group made a systematic review[56] and concluded that downsizing of primary tumours with neoadjuvant sunitinib or sorafenib was related to size at presentation, being the major effect in tumours sized 5 to 7 cm.

Due to the high rate of surgical complications in IVC thrombus RCC, reduction of size of tumour thrombus with neoadjuvant sunitinib, sorafenib, axitinib and temsirolimus has been reported.

The majority of the published information are case reports[57-60]. The largest series reported 25 patients, 7 of which had level 3 or 4 IVC thrombus. 12% reduced thrombus size (only after sunitinib treatment), but the reduction (Median 1.5 cm) didn't have any impact on the surgery approach[61].

In 2010, Bex *et al*[62] reported two cases of IVC thrombi progression during neoadjuvant treatment with sunitinib.

Recently, Bigot *et al*[63], in a retrospective series of 14 cases treated with sunitinib or sorafenib, found that 43% of the patients had a measurable decrease while 14% had an increase in thrombus size. Only 1 case downstaged thrombus level. However, 50% of renal tumours experienced a significant reduction in size. They concluded that neoadjuvant therapy had limited impact on IVC thrombi RCC surgical management.

In conclusion, the response of primary tumour to targeted therapies is unpredictable, although 42%-100% cases show tumour shrinkage. The major effect reported was after sunitinib treatment and in smaller tumours (5 to 7 cm). Morbidity of these novel agents should be taken into account.

There are a few studies focused on the concept of neoadjuvant systemic therapy prior to metastasectomy.

Rini *et al*[64] described 2 patients with long-term response who were treated with adjuvant sunitinib and metastasectomy.

Thomas *et al*[65] reported 19 cases treated with surgery after targeted therapy, 3 of them with metastasectomy with partial response and good outcome.

In 2009, Daliani *et al*[66] reported 38 patients with mRCC, treated with targeted therapy and a partial response/stable disease who underwent metastasectomy

Table 1 Main series of neoadjuvant therapy in locally advanced renal cell carcinoma

Ref.	No.	Therapy	Median size	% median reduction	Partial response	< 30% reduction	% cases tumour shrinkage	Toxicity Garde 3-4
Thomas *et al*[45]	19	Sunitinib	10.5	24%	3	8	42%	37%
Hellenthal *et al*[46]	20	Sunitinib	7	27.90%	2	15	85%	30%
Silberstein *et al*[47]	14	Sunitinib	7	21%	4	10	100%	3 urine leaks
Kondo *et al*[48]	9	Sunitinib/sorafenib	-	9%-30%	3	6	100%	2 major surgery complications
Rini *et al*[49]	28	Sunitinib	-	22%	-	-	-	-
Powles *et al*[50]	52	Sunitinib	-	-	-	-	73%	27%
Bex *et al*[51]	10	Sunitinib	-	14%			60%	
Kats-Ugurlu *et al*[52]	10	Sorafenib	7.5	-	-	-	-	-
Cowey *et al*[53]	30	Sorafenib	8.7	9.60%	2	23	80%	-
Karam *et al*[54]	24	Axitinib	-	28.30%	11	-	100%	41.70%

(84% only one organ site). Ten percent of patients suffered complications. Twenty-one percent of patients were remained of disease. Absence of histological viable tumour in metastasectomy specimens and lung metastases had an OS of 5.6 years compared with those who did not (1.4 years).

In 2012, Karam *et al*[67] reported 22 cases with mRCC who received neoadjuvant treatment prior to metastasectomy with one of the following targeted therapies: sunitinib, sorafenib, bavacizumab, everolimus, pazopanib, Interleukin-2, ABT-510. 4 cases had multiple mestastases and 6 suffered complications. At 109 weeks, only one patient died from RCC. 11 (50%) cases experimented no recurrence.

Another study of 2012[68], reported 11 patients treated surgically after $\geqslant$ 3 mo of stable partial remission with sunitinib, bevacizumab or sunitinib plus temisrolimus. Seven cases had node retroperitoneal disease. Only 1 complication was reported. 5 cases showed no recurrence after a median follow-up of 12 mo.

In a series of 143 patients with mRCC treated with systemic therapy, those who were treated with metastasectomy too (n = 42) had a better OS (18.8 mo *vs* 15 mo, P = 0.07)[69].

A group of Japanese authors described two cases of large adrenal mestastases with liver and pancreas invasion that were successfully treated with sunitinib prior to surgery with a good outcome[70,71].

Johannsen *et al*[72] studied the discontinuation of targeted therapy after complete response to sunitinb. 12 cases were identified, 50% (6 cases) treated with sunitinib and consolidative metastasectomy (lungs, bone, skin and thyroid). No adjuvant treatment was prescribed. Only 5 of 11 patients experienced recurrence, with effective rescue after targeted therapy in all cases. In a recent actualization of the series, with 36 cases, 33.3% remained free of recurrence during follow-up. Factors that correlate with outcome, including metastasectomy, could not be identified[73].

Adjuvant targeted therapy after metastasectomy

It is known that neoadjuvant targeted therapy can be related with surgical complications, as mentioned above. Systemic treatment can obliterate normal tissues planes and make surgery more difficult and risky[74]. A recent review concluded that no general recommendations can be made about use of targeted therapy in preoperatory setting[75].

Based on evidence of effectiveness of multimodal treatment in different moments of mRCC, we try to answer the question of the title: Is there a role for systemic targeted therapy after surgical treatment for metastases of renal cell carcinoma?

In 2007, Kwak *et al*[76] reported 93 patients with mRCC treated with metastasectomy with or without adjuvant immunotherapy. Overall survival of group treated with surgery plus immunotherapy was 56.1 mo *vs* 21.3 mo in the only-surgery group. But when patients were stratified by time of metastases, no differences were found. In multivariate analysis only multiplicity of metastases and metastases sites were independent prognostic factors. Authors concluded that metastasectomy plus adjuvant immunotherapy did not render a higher overall survival.

Jacobsohn *et al*[77] reported no effect of adjuvant Interferon after lung metastasectomy.

Since then, some case reports suggest that adjuvant targeted therapy could be effective after metastasetomy.

In 2010, a study with 88 cases with liver metastases of RCC was published. Sixty-eight were treated with surgery and 78% of cases received adjuvant treatment in both groups (metastatasectomy yes/no). The 5-year overall survival rate after metastasectomy was 62.2% with a median survival of 142 mo compared with 29.3% and 27 mo in the control group. High-grade RCC as well as patients with synchronous metastases did not benefit from surgery[32].

A case report of a man with mRCC who was treated with metastasectomy for multiple organs deposits and adjuvant pazopanib showed 8-year survival[78].

In 2012, Gardini *et al*[79] described 8 cases of pancreatic metastases of RCC treated surgically and with adjuvant therapy (mostly immunotherapy), with disease free survival after 3 years of 30%.

In most of previous papers of neoadjuvant treatment after metastasectomy, adjuvant systemic therapies

are also used. For instance, in Karam *et al*[67] study, 9 of 22 patients received at least one adjuvant targeted therapy. Effect of this intervention in survival was not assessed. Daliani *et al*[66] also gave consolidative adjuvant systemic therapy.

A study of 106 cases with mRCC and brain metastases used combination of targeted therapy and local treatments. The patients were treated with sunitinib (n = 77), sorafenib (n = 23), bevacizumab (n = 5), and temsirolimus (n = 1). Local disease treatment included whole brain radiotherapy (81%), stereotactic radiosurgery (25%), and neurosurgery (25%). On multivariable analysis, surgery or radiosurgery failed to demonstrate to increase OS[80].

Two ongoing clinical trials published in Pubmed are studying adjuvant therapy after metastasectomy: (1) RESORT protocol[81]: a randomized, open-label, multicenter phase Ⅱ study to evaluate efficacy of sorafenib in patients with mRCC after complete metastasectomy. One hundred and thirty-two patients will be randomized to receive sorafenib or best supportive care, with a follow-up of 36 mo; and (2) SMAT-AN 20/04 of the Working Group of Urological Oncology (AUO)[82]: a prospective randomized multicenter phase Ⅱ study on resection of lung metastases in clear cell carcinoma ± adjuvant sunitinib over 1 year.

CONCLUSION

mRCC is a challenging disease. Despite the new targeted therapies, complete remissions occur only in 1%-3% of the cases, and the most effective first-line treatment drugs have reached a ceiling in OS (ranging from 9 to 49 mo)[5].

Metastasectomy remains to be the only curative option in most patients with mRCC. Prognostic models for general[15,16] and lung metastases[26] have been recently published, so we have tools to classify patients in risk groups, allowing us to detect the cases with the higher risk of recurrence after metastasectomy.

Although sparse, there is some evidence of effectiveness of neoadjuvant targeted therapy before metastasectomy; but with an increase in surgical complications due to the effects of these new drugs in tissue healing.

In 2007, Jacobsohn *et al*[77] concluded that metastasectomy plus adjuvant immunotherapy did not result in a higher overall survival and published a paper titled: "No role of adjuvant therapy after complete metastasectomy in metastatic renal cell carcinoma?"

Since then, mRCC treatment has dramatically changed after the approval of new drugs. We have aimed to answer the question: Is there a role for systemic targeted therapy after surgical treatment for metastases of renal cell carcinoma? As far as we know, evidence is low and it's based in case reports and small series of patients treated with adjuvant drugs after neoadjuvant therapy plus metastasectomy in cases of partial response to initial systemic treatment. Despite the limitations and high risk of bias, promising results and cases with long-term survival with this approach have been described[32,66,67,78-80].

Two ongoing clinical trials[81,82] may answer the question that concerns us. While we wait for the results, the recommendations of European Association of Urology Guidelines[37] are a rationale tool: "the decision of metastasectomy has to be taken for each site, and on a case-by-case basis: Performance status, risk profiles, patient preference and alternative techniques must be considered". From our point of view, adjuvant targeted therapy after metastasectomy combined or not with neoadjuvant treatment could be an effective multimodal approach in the future.

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Primary glomerular diseases in the elderly

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Abstract

Primary glomerular diseases in the elderly population are a frustrating topic due to difficulties in both the diagnosis and decision making about treatment. The most frequent type of primary glomerular disease in elderly is membranous nephropathy; while its counterpart in younger population is IgA nephropathy. The most frequent cause of nephrotic syndrome in the elderly is also membranous nephropathy. Pauci-immune crescentic glomerulonephritis (GN) rate increases both in elderly and very elderly population. Pauci-immune crescentic GNs should be regarded as urgencies in elderly patients as in their younger counterparts due to potential for causing end-stage renal disease in case of delayed diagnosis and treatment, and also causing mortality due to alveolar hemorrhage in patients with pulmonary involvement. Renal biopsy is the inevitable diagnostic method in the elderly as in all other age groups. Renal biopsy prevents unnecessary treatments and provides prognostic data. So advanced age should not be the sole contraindication for renal biopsy. The course of primary glomerular diseases may differ in the elderly population. Acute kidney injury is more frequent in the course and renal functions may be worse at presentation. These patients are more prone to be hypertensive. The decision about adding immune suppressive therapies to conservative methods should be made considering many factors like co-morbidities, drug side effects and potential drug interactions, risk of infection, patient preference, life expectancy and renal functions at the time of diagnosis.

Key words: Elderly; Membranous nephropathy; Renal biopsy; Pauci-immune crescentic glomerulonephritis; Primary glomerular disease

Core tip: Primary glomerular diseases in the elderly population are a frustrating topic due to difficulties in both diagnosis and treatment. The most frequent type of primary glomerular disease and the most frequent cause of nephrotic syndrome is membranous nephropathy. The frequency of pauci-immune glomerulonephritides increases considerably in the very elderly population. Renal biopsy is the inevitable diagnostic method in the elderly as in all other age groups. The decision about adding immune suppressive therapies to conservative methods should be made considering many factors like co-morbidities, drug side effects, patient preference, life expectancy and renal functions at the time of diagnosis.

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INTRODUCTION

Primary glomerular diseases (PGD) in the elderly deserves mention under a heading separate from PGDs in young adults due to differences in epidemiological and clinical characteristics, and difficulties in diagnosis and decision making about diagnosis. Co-morbidities, shorter life expectancy, complications of renal biopsy and immunosuppressive medications are among the factors that challenge the clinicians about diagnosis and treatment. Paucity of clinical studies and so insufficiency of evidence and guidelines are other problems in the elderly population that increases progressively due to increased mean life expectancy[1]. First, general epidemiological and clinical characteristics of PGDs in the elderly will be mentioned followed by details about specific diseases.

The frequency of PGDs in the elderly may change in countries. Ethnic predisposition, different approaches about biopsy indications and differences in the methods and design of epidemiological studies are among the causes of this variability. We learn about epidemiological data about PGDs in the elderly, from glomerulonephritis or biopsy registries of countries. These studies may be classified as involving "elderly" (> 60-65 years) and "very elderly" (> 80-85 years) patients.

Some of the registries that you can gain information about epidemiological data in the elderly population in Europe are those of Italy, Spain, Czech Republic and Turkey[2-5]. Membranous nephropathy (MN) was reported in these studies as the most frequent PGD and the most frequent cause of nephrotic syndrome in patient older than 65 years. The PGD in the second order changes in different countries. The evaluation of pauci-immune crescentic glomerulonephritis (pauci-immune crescentic GN) within PGDs in some studies while within secondary glomerular diseases in the others leads to difficulties in evaluation of epidemiological studies. The most frequent biopsy indication is nephrotic syndrome as expected whether accompanied by acute kidney injury (AKI) or not. The manuscript by Yokoyama *et al*[6] who presented data of Japan Renal Biopsy Registry has a special place in the literature due the highest number of patients. Data of 2802 patients aged > 65 (group A) and 276 patients aged > 80 years (group B) were presented in this study. Forty-five percent of cases were PGDs. The most frequent PGDs in group A and B were MN, IgA nephropathy (IgAN) and minimal change disease (MCD) in order, while the most frequent diagnoses in elderly patients who had renal biopsy due to nephrotic syndrome were MN, MCD and focal segmental glomerulosclerosis (FSGS) with decreasing order. The most frequent biopsy indication was nephrotic syndrome in both groups, while rapidly progressive glomerulonephritis (RPGN) was the second most frequent cause in group B. When compared with patients aged less than 65 years, pauci-immune crescentic GN, MN, type 1 and 3 membranoproliferative glomerulonephritis (MPGN) were more frequent and IgAN was significantly less frequent in patients aged more than 65 years. The ratio of renal biopsies performed due to RPGN was higher in the elderly population compared to younger counterparts. There are also current studies presenting epidemiological data of elderly patients in a single center besides registry studies[7-10]. MN was again the most frequent diagnosis in these studies except in the study by Brown *et al*[10] in which pauci-immune crescentic GN was the most frequent PGD. Recent studies about the epidemiology of PGD in elderly are summarized in Table 1.

Recent articles have been published about epidemiological data of very elderly patients although the age limit is variable[11-15]. Although all cases are not PGDs in these studies, they provide important information about PGDs in the very elderly population. The most frequent PGD in studies originating from United States[11,14] was pauci-immune crescentic GN, while it was MN in other studies from European and Asian countries. Biopsy indications in these studies follow the same order, and provide clues about behavior regarding biopsy indication in this special age group in these countries. The most frequent biopsy indication is AKI in United States, while it is nephrotic syndrome in other European and Asian countries. Studies performed with very elderly patients are summarized in Table 2.

Although renal biopsy is the inevitable diagnostic method in glomerular diseases, it is not performed in some of the patients due to various factors including co-existing systemic diseases, shorter life expectancy, reluctance of the clinicians about biopsy and immunosuppressive treatment and patient preference. There are studies in the literature reporting that bleeding risk after renal biopsy in elderly patients is not different from other age groups[16,17]. But, the possibility that clinicians would have performed renal biopsy in elderly patients with lower risk in these studies in which data of biopsy series are presented, should be kept in mind. As well known, the most important predictor of bleeding complication is serum creatinine level[17]. This complication is more common in patients with renal failure compared to patients without. The concern of clinicians about this complication is not undue considering physiological changes related to age, co-existing systemic diseases (hypertension, atherosclerosis, diabetes mellitus, amyloidosis), and overestimation of glomerular filtration rate with creatinine level due to decreased muscle mass. When possible complications of immunosuppressive treatment add on these concerns, some clinicians prefer conservative methods without performing renal biopsy. Some other clinicians on the other hand try empiric

Table 1 Recent epidemiological studies in the elderly

Country	Ref.	Date	Number of cases	Age	The most frequent PGDs
Italy[1]	Vendemia *et al*[2]	2001	280	> 65	1. MN 2. Pauci-immune GN 3. MPGN
Turkey	Ozturk *et al*[5]	2014	150	> 60	1. MN 2. Pauci-immune GN 3. FSGS
Japan	Yokoyama *et al*[6]	2012	2802	> 65	1. MN 2. IgAN 3. MCD
Brasil	Carmo *et al*[7]	2010	113	> 60	1. MN 2. FSGS 3. MCD
South Africa	Okpechi *et al*[8]	2013	111	> 60	1. MN 2. IgAN 3. Pauci-immune GN
China	Jin *et al*[9]	2014	851	> 65	1. MN 2. IgAN 3. MCD
Ireland	Brown *et al*[10]	2012	236	> 65	1. Pauci-immune GN 2. MN 3. IgAN

[1]Only patients with PGDs were included in this study, while other studies included patients with secondary glomerular diseases also. FSGS: Focal segmental glomerulosclerosis; GN: Glomerulonephritis; IgAN: IgA nephropathy; MCD: Minimal change disease; MN: Membranous nephropathy; MPGN: Membranoproliferative glomerulonephritis; PGD: Primary glomerular disease.

immunosuppressive treatment without biopsy. Yoon *et al*[18] evaluated this subject in their study. They evaluated renal and patient survival rates of 99 patients (age > 60 years) presenting with nephrotic syndrome who were grouped as those who had renal biopsy (n = 64) and those who did not (n = 35). The major defect of this study was the lower mean age and better renal functions in the group who had renal biopsy. Although complete remission was more frequent (45% *vs* 26%, P = 0.013) in the biopsy group in which statistically significantly more patients had immunosuppressive therapy (P < 0.005), renal survival rates were similar. Patient survival was lower in the group without biopsy which was not a surprise considering significantly higher mean age.

On the other hand, there are factors that lead the clinician towards biopsy like need of urgent diagnosis for optimum treatment of pauci-immune glomerulonephritides presenting as RPGN; the risk of not giving specific treatment considering more susceptibility of elderly to infective and thrombotic complications of nephrotic syndrome[19,20]; prevention of unnecessary treatments by renal biopsy; and provision of prognostic data. Studies with very elderly patients revealed that therapeutic approach may change 40%-67% with renal biopsy[11,14]. So, advanced age should not be the sole contraindication for renal biopsy. The clinician has to decide respecting the preference of the patient within this multifactorial equation.

Renal biopsy in elderly has the potential to be problematic for pathologists as well as clinicians. Varying degrees of "background" glomerulosclerosis, tubular atrophy, arteriolar hyalinosis that may be seen as a result of both senility and co-morbidities may superimpose primary and secondary glomerular diseases[21].

Primary glomerular diseases in the elderly present as nephrotic syndrome, nephritic syndrome, RPGN, asymptomatic urine abnormalities or chronic glomerulonephritis as in other age groups. But nephrotic syndrome and acute nephritic syndrome including RPGN comprises most of the cases as can be understood from biopsy indications in reported by biopsy series. PGDs causing nephrotic syndrome are MN, FSGS and MDH, while MPGN, IgAN and pauci-immune crescentic GNs comprise the major causes of nephritic syndrome. But different and complex forms of presentation are not rare. As an example, AKI superimposed on nephrotic syndrome is more frequent in elderly population. Some of the authors consider AKI on the basis of nephrotic syndrome as idiopathic if there is no clear reason as drug use, exposure to radio contrast agent or interstitial nephritis[22].

The treatment of PGDs in the elderly causes difficulties as the diagnosis. Co-morbidities, the number of pills that the patients take, potential drug interactions, risk of infection, patient preference, expected life expectancy, renal functions at the time of diagnosis, increased drug toxicity risk due to age related decreased in drug metabolism and excretion[23,24] are some of the factors effective on the decision of the clinician about treatment. Moreover, disease specific secondary causes should be searched for promptly as well as

Table 2 Recent epidemiological studies in the very elderly population

Country	Ref.	Date	Number of cases	Age	The most frequent PGD
Japonya	Yokoyama *et al*[6]	2012	276	> 80	1. MN 2. IgAN 3. MCD
United States	Moutzouris *et al*[11]	2009	235	> 80	1. Pauci-immune GN 2. MN 3. IgAN
Italy	Rollino *et al*[12]	2014	131	> 75	1. MN 2. Pauci-immune GN 3. IgAN
Japan	Omokawa *et al*[13]	2012	73	> 80	1. MN 2. MCD
United States	Nair *et al*[14]	2004	100	> 80	1. Pauci-immune GN 2. MN
Spain	Verde *et al*[15]	2012	71	> 85	1. MN 2. Pauci-immune GN 3. IgAN

GN: Glomerulonephritis; IgAN: IgA nephropathy; MCD: Minimal change disease; MN: Membranous nephropathy; PGD: Primary glomerular disease.

any contraindication for treatment and screening for malignancy appropriate for the age group should be performed.

Conservative methods are the sine qua non of treatment of patients with nephrotic syndrome in this age group. Salt restriction, smoking cessation, diuretics, renin-angiotensin-aldosterone system blockers, statins, anticoagulant agents and pneumococcal vaccination are the components of conservative treatment[25,26]. Anticoagulation is recommended in patients with serum albumin level below 2 g/dL and co-existing risk factors if bleeding risk not high. But treatment decision should be individualized as in all cases. An article reporting the importance of forming a scaling system for thrombosis and bleeding before decision about anticoagulant use has been published recently[27].

Immunosuppressive therapy should be considered in cases with nephrotic proteinuria in spite of conservative methods, progressively declining renal functions, life threatening complications of nephrotic syndrome like thrombosis, and patients with RPGN. No guideline has been developed up to now for glomerulonephritides in the elderly. "Kidney Disease Improving Global Outcomes (KDIGO) Clinical Practice Guideline for Glomerulonephritis" published in 2012 helps the clinicians caring elderly patients. But it is difficult to adopt all recommendations to old patients. The clinician has to choose the correct treatment method considering both positive and negative sides together. All medications should be used in doses appropriate for the renal function of the patient.

MEMBRANOUS NEPHROPATHY

The most frequent PGD in adult population all over the world is IgAN as is well known[28,29]. But MN with its frequency increasing with aging, is the most common PGD and the most common reason of nephrotic syndrome in elderly. AKI is more frequent in the course of disease compared to other PGDs. Advanced age has been reported to be a risk factor for AKI in patients with MN[30]. Moreover, hypertension and worse renal functions at the time of presentation are expected to be more prevalent in elderly patients. There are studies reporting increased risk of thrombotic[31] and infectious[32] complications compared to adult patients.

The PGD that is most associated with malignancies is MN, and it is speculated that it accompanies tumors in 10% of cases[33,34]. M type anti phospholipase A2 antibodies that started a new era protects from unnecessary interventional investigations[35,36]. There is a tendency to screen patients with M type anti phospholipase A2 antibodies in accordance with age; while more complicated screening is necessary in those without anti phospholipase A2 antibody[37]. Another difference is in the subtypes of IgG on immune fluorescent microscopy, although not routinely studied. IgG4 predominates in primary MN, while IgG1 and/or IgG2 staining is expected to be positive in MN associated with malignancies[36]. Malignancies are usually clinically evident at the time of diagnosis of nephrotic syndrome. However, there are reported cases with malignancies reported late in the course. Some authors think that screening for cancer should be repeated within 5-10 years in cases with histological and serological testing resembling secondary MN[38,39]. History of medications, screening for infection (hepatitis B and malaria) and evaluation for systemic lupus erythematosus should not be forgotten. Nonsteroidal anti-inflammatory drugs (NSAID) are in the first order among drugs related with MN. NSAIDs may cause MN and MDH as well as non-glomerular diseases[40,41].

It has been shown that corticosteroid therapy alone in elderly patients with MN is not enough and actually, it is related with more complication[42,43]. Ponticelli

protocol (in which steroids are used in combination with either chlorambucil or cyclophosphamide) can be tried[44]. KDIGO guideline proposes immunosuppressive treatment in patients with severe life-threatening symptoms and findings, proteinuria more than 4 g/d in spite of conservative methods, or at least 30% increase in serum creatinine level within the last 6-12 mo[26]. However, there are no up-to-date randomized controlled trials about side effect profile and efficacy of steroid treatment in old patients. Besides, studies about the role of cyclosporine plus low dose steroid, and mycophenolate mofetil are not enough also. We can mention a study in which mizoribin was used in a few old patients. But the number of patients is not enough, and mizoribin group was not compared with patients receiving only steroid treatment[45].

MINIMAL CHANGE DISEASE

Minimal change disease which is one of the important causes of nephrotic syndrome in elderly presents withy hypertension and AKI more compared with younger population. Some authors believe that AKI superimposed on nephrotic syndrome in elderly is commonly associated with MCD, and elderly patients are more prone to acute tubular necrosis[46,47]. Relapses are rarer in patients older than 40 years compared to patients younger than 40 years[47,48]. All immunosuppressive medications used in the treatment of glomerulonephritis have been tried with considerable success, although steroids remain to be the mainstay of treatment[26].

FOCAL SEGMENTAL GLOMERULOSCLEROSIS

We do not have enough data in the literature about the clinical characteristics and treatment of FSGF in the elderly. Tip variant of FSGS has been reported to be the histologic type presenting with sudden onset of severe nephrotic syndrome and also the type which is the most sensitive to steroid treatment. Tip lesions ten de be more prevalent in older patients[49,50]. Important predictors of renal prognosis are the magnitude of proteinuria, the level of kidney function, and the amount of tubulointerstitial injury[51]. Corticosteroids are the first line treatment in appropriate patients while second line treatment with cyclosporine plus low dose steroid may be preferred in cases for which there is considerable risk for corticosteroid side effects[26]. Evaluation for secondary causes of FSGS should not be omitted. Interferons[52] and intravenous use of bisphosphonates (especially pamidronate)[53] which are commonly prescribed in this population are examples for causes of secondary FSGS. American Society of Clinical Oncology published an update for use of bisphosphonates in multiple myeloma including knowledge about dose reduction in case of decreased renal function[54].

IGA NEPHROPATHY

IgA nephropathy is associated with more severe renal manifestations at presentation in the elderly. It has been reported in Spanish Registry of Glomerulonephritis that 27.8% of patients with IgAN older than 65 years presented as AKI[3]. This ratio reached to 53% in another study with the emphasis that tubular injury is more prominent than glomerular damage in these patients[55]. Advanced age has been determines as a risk factor for progression to end-stage renal disease (ESRD) which was found to be 1.95 times more common compared to young adults[56]. An article has been published recently reporting that 70% of patients reach ESRD within 20 years[57]. The only immunosuppressive medication proved to be effective in IgAN is corticosteroids. Although persistent proteinuria in spite of conservative measures is an indication for corticosteroid treatment according to KDIGO guideline, it may not be wise to give corticosteroid treatment to elderly patients with normal renal functions, blood pressure and non-nephrotic range proteinuria, especially in the presence of comorbidities. However, IgAN presenting as crescentic glomerulonephritis should be treated as pauci-immune crescentic glomerulonephritis[26].

MEMBRANOPROLIFERATIVE GLOMERULONEPHRITIS

Primary MGPN is a rare disease. So, secondary causes, especially monoclonal gammopathies and hepatitis C infection, should be ruled out case of pathological diagnosis of MPGN[58,59]. Although usually not responsive, corticosteroid + mycophenolate mofetil or corticosteroid + oral cyclophosphamide may be tried in patients with MPGN type Ⅰ presenting with nephrotic syndrome and/or rapid increase in creatinine levels[26,60]. But patients and relatives should be informed thoroughly about the low response rates before deciding for immunosuppressive treatment.

PAUCI-IMMUNE CRESCENTIC GLOMERULONEPHRITIS

Pauci-immune crescentic GN is disease group with increased rate both in elderly and very elderly population[61]. This group represents renal involvement in anti-glomerular basement membrane disease and anti-neutrophil cytoplasmic autoantibody associated vasculitides. Renopulmonary syndrome is the more frequent type of presentation although isolated renal involvement may also be seen. The first explanation for increased frequency is the peak that the systemic vasculitides show between ages 65-74 years[62]. Moreover, presentation with RPGN increases the probability of performing renal biopsy in these patients for whom

the clinicians may prefer to remain conservative otherwise. Pauci-immune crescentic GNs should be regarded as urgencies in elderly patients as in their younger counterparts due to potential for causing ESRD in case of delayed diagnosis and treatment, and also causing mortality due to alveolar hemorrhage in patients with pulmonary involvement[63]. Renal biopsy should be scheduled immediately and serum samples should be taken for determination of anti-neutrophilic cytoplasmic antibody, anti-glomerular basal membrane antibody and then immunosuppressive treatment should be started as soon as possible. In the absence of absolute contraindications, pulse corticosteroid and cyclophosphamide treatment should be started together with plasma exchange in the presence of alveolar hemorrhage or rapid decline in renal functions[26]. In case of vasculitides limited to kidney, decision about treatment and its duration should be made regarding comorbidities and activity/chronicity of lesions on renal biopsy. Renal survival in anti-glomerular basal membrane disease is related with creatinine levels at the time of admission[64]. So, early diagnosis and treatment have prime importance. Independent determinants of mortality in anti neutrophil cytoplasmic autoantibody-associated vasculitides have been found to be advanced age and pulmonary infections[65]. KDIGO guideline recommends crescentic forms of any PGDs to be considered as pauci-immune GN and treated so[26].

As a conclusion, PGDs in elderly are a group of diseases that challenges the clinicians in both diagnosis and treatment. Although MN is the most common PGD in this age group, crescentic glomerulonephritides should always be considered due to irretrievable results.

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Effect of urinary stone disease and its treatment on renal function

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Abstract

Urolithiasis is a common disease that affects urinary tract in all age groups. Both in adults and in children, stone size, location, renal anatomy, and other factors, can influence the success of treatment modalities. Recently, there has been a great advancement in technology for minimally invasive management of urinary stones. The epoch of open treatment modalities has passed and currently there are much less invasive treatment approaches, such as percutaneous nephrolithotomy, ureteroscopy, shockwave lithotripsy, and retrograde internal Surgery. Furthermore, advancement in imaging technics ensures substantial knowledge that permit physician to decide the most convenient treatment method for the patient. Thus, effective and rapid treatment of urinary tract stones is substantial for the preservation of the renal function. In this review, the effects of the treatment options for urinary stones on renal function have been reviewed.

Key words: Kidney stones; Chronic kidney disease; Estimated glomerular filtration rate; Renal function; Urinary stone disease

Core tip: In this article, urinary stone disease, treatment options and its effects on renal function are examined. Moreover, in the light of recent publications, effect of treatment options on functional state of the kidney in patients with renal impairment is investigated.

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INTRODUCTION

Urolithiasis is a widespread disease that affects the urinary system and a considerable, high-priced reason of morbidity. Urinary stone disease influences all age groups. The reported prevalence rate of stone disease is 5%-12% in men, 4%-7% in women[1]. Stone

formation is affected by gender, age and geography. Men's possibility of forming stones is more than women's. However, the ratio has decreased from a 3:1-male to female predominance to less than 1.3:1[2]. Recent studies indicate a raise in the prevalence of urinary stones, and this raise comprised in all gender, racial and ethnic ensemble in the United States[3].

Eating habits and environmental conditions also have a major act in the formation of urinary stones. Diabetes mellitus (DM), gout, and obesity are closely associated with urinary stone formation[4-6].

Children represent about 1% of all patients with urolithiasis, who have a almost 100% risk for recurrent stone formation. Both in adults and in children, stone size and location, other factors, including stone composition, patient factors, and renal anatomy, can influence the success of specific treatment modalities[7-9].

Over the years, there has been a great advancement in technology for minimally invasive treatment of urinary stones. The epoch of open pyelolithotomy has supplanted and currently there are much less invasive interventions, for instance, percutaneous nephrolithotomy (PCNL), ureteroscopy, shockwave lithotripsy (SWL), and RIRS (retrograde internal surgery). However, recurrent stone formation is still a major issue among patients with urolithiasis[10,11].

Chronic kidney disease (CKD) is a significant health issue that affects 13% of the adults in the United States. The potential reasons of renal failure in patients with urolithiasis are multifactorial and enclose hydronephrosis, infection, DM, hypertension (HT), repeated stone surgeries, eating habits, environmental, and genetic factors[12]. There are many studies that associate stone disease with varying degrees of renal insufficiency, patients with CKD present 0.8% to 17.5% of those presenting with urinary stone disease[13]. DM and HT, well-defined risk factors, were considerably related with renal impairment in patients with urinary stone[4,12].

PATHOGENESIS

The pathogenesis of renal calculi is a complicated process and similarly based on stone phenotype. Despite many investigations, the events that lead to kidney stone formation is as yet unknown. Several chemical theory has been proposed to explain the formation of stones[14]. These theories are supersaturation, nucleation and crystal formation, crystal retention, and effect of inhibitors and promoters on crystal growth.

In respect to the underlying reasons of kidney failure, previous researches have demonstrated that stone formation is related with the sedimentation of fragments in the peritubular field and in the medullary interstitium. A sedimentation like this can create inflammation and aid the advancement of fibrosis, which follows in tubular damage and detriment of kidney function. Moreover, fragment transition itself results in temporary occlusion, and occlusion is a well-defined risk factor for renal detriment in respect to the effect of fragments on kidney function, minor studies have contrasted the influences of the diverse stone types on kidney function. Two of the most widespread types of non-calcium stones were uric acid and struvite. The existence of struvite stones in some patients can reason the improvement of kidney insufficiency on account of recrudescent urinary tract infection (UTI). Patients with uric acid stone are mostly related with diseases which end up with renal insufficiency such as diabetes and gout[12].

DIAGNOSIS

Urinary stone formers generally complain with lumbar pain, vomitting, and occasionally fever, however, may not have any symptom as well. Routine assessment consists of an exhaustive history and physical examination. The preliminary diagnosis should be promoted by proper radiological technic. A wide variety of imaging methods are recently present to assist in the detection of urinary stones.

Imaging allows for the rapid and definitive diagnosis of stones, ensures significant knowledge that enables physicians to decide the most proper intervention for the patient. This knowledge contains the location and size of stone, and situation of kidney and collecting system[15]. Early on, abdominal plain films kidneys-ureters-bladder (KUB) and intravenous urography (IVU) were accepted as gold standards for the diagnosis of urolithiasis. However, with the advent of technologies such as ultrasound (US), noncontrast computed tomography (NCCT), a much larger assortment of imaging studies are now available to physicians appraising patients for stone disease.

Urinary stones can be categorized according to images on radiogram. Stones which contain calcium should be observable on radiogram (radiopaque). However, uric acid, ammonium urate, xanthine, and drug stones are not directly-visible (radiolucent). The declared sensitivity and specificity of radiogram in the determine of stone in patients with renal colic and no history of urolithiasis is limited. Further disadvantages of abdominal plain films in the detection of calculi include impaired image quality in obese patients and difficulty in differentiating pelvic vascular calcifications (phleboliths) from stones in the pelvic ureter. In addition, KUB generally will not generate useful information regarding the presence and/or degree of urinary tract obstruction[16].

Ultrasound is commonly performed during the evaluation of urolithiasis. The main advantage of ultrasound has over other imaging modalities such as NCCT or IVU is that is implemented without any radiation exposure. US can specify stones placed in the pelvis, calices, and proximal and distal ureter, as well as in patients with hydronephrosis[17].

Before the improvement of NCCT, IVU was considered the standard imaging technique for the assessment of

Table 1 Effects of shock wave lithotripsy on renal functions and glomerular filtration rate levels

Ref.	No. of patients	Patients' feature	Follow-up	Pre-SWL mean ± SD GFR (mL/min)	Post-SWL mean ± SD GFR (mL/min)	*P* value
Eassa *et al*[25]	108	Solitary kidney	3. 8 ± 3.5/yr	84.6 ± 24.7	82.5 ± 26.5	0.33
Fayad *et al*[27]	100	Children	6 mo	113.13 ± 4.51	113.01 ± 4.27	0.46

Difference is considered statistically significant at $P < 0.05$ and highly significant at $P < 0.01$. SWL: Shock wave lithotripsy; GFR: Glomerular filtration rate.

urinary stones. NCCT has higher sensitivity and specificity for detection of stones in urinary tract than IVU[18]. Uric acid and xanthine stones (radiolucent) can be determined by NCCT. Nonetheless, indinavir stones (radiolucent) cannot be specified by NCCT[19]. NCCT can define density and internal formation of the stone and the distance from skin to stone. IVU can provide information about renal function and whether a kidney is obstructed. Delayed images can be useful in evaluating ureteral anatomy for filling defects or strictures. It also provides detailed pelvicalyceal anatomy, which can be useful in planning surgical interventions, especially in those individuals with urinary tract anomalies. Therefore, IVU has largely been replaced by computed tomography (CT) with intravenous contrast or CT urograms. Low-dose NCCT (30 mAs) provides information close to those of standard NCCT (180 mAs) in demonstrating ureteral stone > 3 mm in patients with a BMI < 30[20]. It has been declared that low-dose NCCT provides significant information for the assessment of renal colic in pregnant patients[21].

TREATMENT OPTIONS AND EFFECTS ON RENAL FUNCTIONS

Extracorporeal SWL

Since its introduction, SWL has been a cornerstone for the management of the stone disease. SWL is the most common first line treatment for the majority of renal stones. Several studies have demonstrated stone-free rates as follows: renal pelvis 76%, upper calyx 69%, middle calyx 68%, and lower calyx 59%. Stone free rates were dependent on stone burden, with stones < 10 mm allowing excellent stone-free rates[2,22]. SWL success depends on many determinants, such as stone burden, position, composition of the stones, habitus of patient, and the efficacy of the lithotripter. SWL is a non-invasive treatment modality, nevertheless it might be related with some complications, for instance tissue injury, bleeding, adjacent organ injury, urinary tract obstruction, post treatment obstruction, and urinary tract infections, in early period. Clinically significant subcapsular and perirenal hematomas occur infrequently, with reported rates between 0.24% and 4.1%. Comorbidities for instance, HT, DM, obesity, coronary artery disease increase the risk of complication.

Moreover, new onset hypertension or diabetes mellitus after SWL treatment is controversial and previous studies are incoherent[23-25]. It has been investigated whether the influence of SWL on kidney function in long-term period. Cass reported that there was an average decline in eGFR of 22% in more than 24 mo of follow up. In contrast, it is stated that serum creatinine levels were not markedly affected after SWL in patients with a solitary kidney in approximately 4 years follow up[26]. In addition, it is stated that SWL treatment was not associated with a significant impact on kidney function or subsequent renal scaring, regardless of stone size or number of SWL seances in children[27]. The results of these two studies are shown in (Table 1).

SWL is an influential, proper, and noninvasive intervention in patients with urinary stones. Although the acute effects of SWL are well-known, it is accepted that treatment of renal stones with SWL does not affect kidney functions in the long term.

PERCUTANEOUS NEPHROLITHOTOMY

One of the most important factors in selecting the optimal surgical procedure for the patient with nephrolithiasis is stone burden because it has been shown to strongly influence stone-free rate, need for auxillary procedures, and complication rate for some treatment modalities[28]. PCNL is recommended for the treatment of all stones greater than or equal to 2 cm[29]. Deem *et al*[30] randomized 32 patients with moderate sized (1-2 cm) upper or middle calyceal or renal pelvis stones to PCNL or SWL and evaluated them at 3 mo with NCCT. PCNL stone-free rate was superior to SWL (85% *vs* 33%, respectively) and none of the PCNL patients required a secondary procedure, whereas 77% of the SWL patients required at least one other procedure and 17% required more than one[30].

The influence of PCNL on kidney function was evaluated in 81 patients with a solitary kidney. Mean eGFR increased from approximately 45 preoperatively to 52, 1 year after intervention[31]. However, achievement and complication rates of PCNL are different in patient with a solitary kidney. The goal of stone eradication in these patients is aimed at preserving nephrons, preventing stone-related complications, chronic renal failure, and dialysis. A recent global study recommends that the stone-free rate for PCNL in solitary kidneys is lower than in patients with bilateral

Table 2 Effects of percutaneous nephrolithotomy on estimated glomerular filtration rate

Ref.	*n*	Baseline eGFR (mL/min per 1.73 m²)	Postoperative eGFR	1 yr eGFR
Ozden *et al*[33]	67	37.9 ± 14.05	45.1 ± 16.8	51.3 ± 19.31
Canes *et al*[31]	81	44.9 ± 19.2	42.7 ± 18.0	51.7 ± 23.1
Bilen *et al*[36]	185	42.4	48.4	-

eGFR: Estimated glomerular filtration rate.

kidneys. Furthermore, it is concluded that kidney function deterioration and transfusion rates are greater in patients with a solitary kidney[32].

Besides, the existence of urinary stones in patient with CKD necessitates exclusive consideration. Hydronephrosis and infection are independent parameters for renal impairment in the patients with urinary stones. The alterations in the renal parenchyma created by infection become more evident with concomitant hydronephrosis. The duration of disease, repeated interventions, and stone recurrence also have unfavorable influences on renal function. Hence, the treatment of urinary stone disease in patients with CKD acts a significant role in improving renal function[33]. Kukreja *et al*[34] reviewed the influence of PCNL on renal function in 84 CKD patients with renal stone disease. They stated an overall improvement in renal function in 39%, stable function in 29%, and decreased function in 32% of patients. However, serum creatinine has been used instead of eGFR in this study. Factors predicting impairment in kidney function were proteinuria > 300 mg/d, cortical atrophy, recurrent UTI, stone burden > 1500 mm², time passed after surgical intervention < 15 years, and pediatric age group[34]. In another study, Kurien *et al*[35] studied 91 adult patients with serum creatinine level greater than 1.5 mg/dL who performed PCNL. Most patients had stage 3 or 4 CKD and most showed improvement or stabilization in renal function after PCNL. Postoperative complications and peak eGFR were the main factors predicting deterioration of kidney function during follow-up[35].

In another study, Bilen *et al*[36] evaluated 185 subjects with eGFR < 60 mL/min per 1.73 m² undergoing PCNL and found the average preoperative eGFR substantially improved from 42.4 to 48.4 at three months follow-up. None of the patients required dialysis during that relatively shorter follow-up. They also found that nearly all stage 5, half of stage 4, and one quarter of stage 3 subjects had some benefit from surgery. Renal function recovery was minimum in stage 2 subjects. They hypothesized that the effect of the calculi itself in a severely affected kidney is greater than the effect of PCNL and the opposite is probably true for moderately affected kidneys in which the detriment of surgery may be more significant, particularly if associated with UTI[36].

In our study, we analyzed 67 subjects, retrospectively. The eGFR was less than 60 mL/min per 1.73 m². The mean follow up was approximately 46 mo. The mean eGFR before and after PNL was approximately 38 and 46. DM and UTI were independent parameters for renal impairment at 1 year. Of the 67 subjects, 47% had downstaging. On the other hand, 3% of subjects had upstaging at the first year. During the 5-year study period, 1 of the subjects progressed to end-stage CKD. However, 6% of subjects with Stage 5 evolved to Stage 4[33]. The effects of PCNL on eGFR have shown in (Table 2).

Another important factor that has been proposed to affect renal function is the number of tracts. Animal and human studies have demonstrated that renal damage from nephrostomy tracts is minimal based on nuclear renography and has no effect on systemic renal function[37,38].

In summary, PCNL remains the primary modality for treatment of complex stones in patients with CKD. Nevertheless, it has important complications which are very important for in patients with CKD. The most common are hypotermia, bleeding, metabolic acidosis, serum electrolytes disturbances, urosepsis, and rarely death[39]. Anemia and underlying platelet dysfunction in patients with CKD may play important role in the high rate of transfusion[35,36].

RETROGRADE INTRARENAL SURGERY

Retrograde intrarenal surgery for the treatment of kidney stones has been more preferred approach owing to technological innovation, such as new model flexible ureteroscopies, laser fibers and baskets. The declared stone free rate of RIRS is about 97%, complication rates are lower than in other initiatives[40]. RIRS can be considered a proper alternative to PCNL in cases with significant comorbidity (anticoagulation, cardiopulmonary disease, advanced age)[41], and in cases with additional adverse anatomical factors such as obesity and renal malformations[42].

Giusti *et al*[43,44] stated that results of RIRS for stones up to 2 cm in diameter in 29 patients with solitary kidney. The primary stone free rate (SFR) was 72.4%, the secondary SFR was 93.1%. The mean number of applications per patient was 1.24. Median follow up time was 35.7 ± 19.3 (12-72) mo. Serum creatinine level, not eGFR, was used to evaluate effect of procedure on renal function and reported that there was no deterioration in kidney function[43]. Although RIRS has been related with repeated operations for the treatment of renal stones greater than 2 cm in diameter, it contributes to preservation of renal parenchyma, which might be substantial for patient with renal insufficiency[44,45].

Consequently, more comprehensive studies are needed for evaluating the effect of RIRS on renal function.

CONCLUSION

Urinary stone disease in patients with renal insufficiency can be caused to diverse clinical conditions. Patients with CKD can be a sufferer from other medical comorbidities. Urinary tract stone disease is the direct reason of ESRD in 3.2% of patients on dialysis. Coronary heart disease risk factors, such as obesity, hypertension, hyperuricemia, dyslipidemia and CKD, were related with urinary stone disease. Of these risk factors, hypertension and hyperuricemia demonstrated the most potential relation with urinary stones[46,47]. Therefore, to prevent morbidity and mortality of CKD, patients with urinary stone disease should be evaluated substantially and treated by an appropriate method.

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Management of hepatorenal syndrome

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Abstract

Hepatorenal syndrome (HRS) is defined as development of renal dysfunction in patients with chronic liver diseases due to decreased effective arterial blood volume. It is the most severe complication of cirrhosis because of its very poor prognosis. In spite of several hypotheses and research, the pathogenesis of HRS is still poorly understood. The onset of HRS is a progressive process rather than a suddenly arising phenomenon. Since there are no specific tests for HRS diagnosis, it is diagnosed by the exclusion of other causes of acute kidney injury in cirrhotic patients. There are two types of HRS with different characteristics and prognostics. Type 1 HRS is characterized by a sudden onset acute renal failure and a rapid deterioration of other organ functions. It may develop spontaneously or be due to some precipitating factors. Type 2 HRS is characterized by slow and progressive worsening of renal functions due to cirrhosis and portal hypertension and it is accompanied by refractory ascites. The only definitive treatment for both Type 1 and Type 2 HRS is liver transplantation. The most suitable bridge treatment or treatment for patients who are not eligible for transplantation is a combination of terlipressin and albumin. For the same purpose, it is possible to try hemodialysis or renal replacement therapies in the form of continuous veno-venous hemofiltration. Artificial hepatic support systems are important for patients who do not respond to medical treatment. Transjugular intrahepatic portosystemic shunt may be considered as a treatment modality for unresponsive patients to medical treatment. The main goal of clinical surveillance in a cirrhotic patient is prevention of HRS before it develops. The aim of this article is to provide an updated review about the physiopathology of HRS and its treatment.

Key words: Hepatorenal syndrome; Cirrhosis; Renal failure; Vasoconstrictors; Transplantation

Core tip: Hepatorenal syndrome (HRS) is a severe complication of chronic liver diseases and is usually associated with a poor prognosis. It is not a renal disease but a renal dysfunction that develops as a result of a systemic condition associated with liver failure. To prevent HRS by taking some preventive measures is possible and although the definitive treatment is liver transplantation, a rapid diagnosis and prompt initiation of the treatment leads to an important improvement in the prognosis. In this review, we cover the physiopathology, diagnosis and treatment options of HRS.

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INTRODUCTİON

Hepatorenal syndrome (HRS) is defined as unexplainable progressively increasing serum creatinine in a patient with advanced liver disease. HRS is representative of the end stage of a process associated with progressive decrease in renal blood flow and glomerular filtration rate (GFR). Diagnosis is by exclusion of other causes of renal failure since there is no specific diagnostic test. In 1956, a special type of acute renal failure associated with low urinary output and very low urinary sodium excretion without proteinuria was defined[1]. In postmortem examination of these patients, it was observed that the kidney's histological structure was preserved. In 1969, the kidney taken from cadaveric donors with HRS functioned normally[2]. So, it is possible to conclude that HRS is not a renal disease but a renal dysfunction that occurs as a result of a systemic condition. The definitive treatment is liver transplantation (LT). HRS is an important risk factor since it increases the waiting list mortality and incidence of complications after LT[3] and renal function before LT is a predictor of survival[4]. Vasoconstrictive agents constitute the main part of pharmacological treatment, providing a bridge to LT. Hemodialysis, renal replacement therapies and artificial liver support systems may also be used as bridge treatment. The goal of treatment in HRS should be early diagnosis, effective and quick treatment and, most important of all, to take preventive measures. Despite all treatment options, likelihood of failure is still high.

DEFINITION

HRS is one of the most severe complications of cirrhosis and is defined as renal insufficiency emerging in chronic liver disease patients when all the other causes of renal failure are excluded[5]. Renal vasoconstriction, a result of progressive liver failure, is the main underlying reason for renal failure in HRS.

HRS was first classified into two groups, Type 1 and Type 2, by the International Ascites Club in 1994. According to this classification, Type 1 HRS is associated with doubling of initial serum creatinine to a level of more than 2.5 mg/dL or reduction in creatinine clearance because of a decreased glomerular filtration rate to a level less than 20 mL/min in a time period shorter than 2 wk[5-7]. Type 1 HRS usually occurs following a precipitating factor such as infectious conditions, particularly spontaneous bacterial peritonitis (SBP) which is considered the most important factor for HRS[8-11]. Type 2 HRS is a moderate and steady type of renal failure and serum creatinine level is higher than 1.5 mg/dL and often associated with sodium retention[5,7]. Type 2 HRS usually arises spontaneously as a result of refractory ascites[5].

In addition to this data, it is important to consider that the creatinine levels are not always increased in cases of renal failure in decompensated cirrhosis[12,13]. It is possible to say that even milder degrees of renal failure may be associated with a poor prognosis in cirrhotic patients[1,14]. According to the RIFLE (Risk, Injury, Failure, Loss, End stage renal disease) classification, it has been shown that even a small increase in serum creatinine level may be associated with clinically significant outcomes in patients with cirrhosis[15-17]. In accordance with this, the International Ascites Club and the Acute Dialysis Quality Initiative suggested a new definition for acute kidney injury. This new definition includes an increase in serum creatinine level to 0.3 mg/dL or more in a period less than 48 h or a 50% increase in serum creatinine level compared to the baseline levels recorded in previous 6 mo period, regardless of final serum creatinine levels[18].

PATHOPHYSIOLOGY

HRS is a sort of renal dysfunction which is generally reversible and occurs because of advanced liver disease. Although it is not completely ravelled, the most characteristic reason underlying renal dysfunction in HRS is renal vasoconstriction[19].

The four major factors considered to be responsible are: (1) decreased circulating blood volume and, as a result, decreased mean arterial blood pressure because of splanchnic vasodilatation; (2) renal vasoconstriction as a result of the activated renin-angiotensin-aldosterone system since the sympathetic nervous system has been activated; (3) cardiac dysfunction due to cirrhosis; and (4) release of several cytokines and vasoactive mediators which may affect blood flow to the kidneys and glomerular vascular bed[20,21].

The main pathophysiological mechanism in HRS is reduction of circulating blood volume due to increased resistance to blood flow in the cirrhotic liver, resulting in splanchnic blood pooling, which is in fact a multifactorial process[1]. Decreased circulating blood flow which means decreased mean arterial blood pressure causes stimulation of baroreceptors in the carotid body and consequently activation of the sympathetic nervous system. This is followed by activation of the renin-angiotensin-aldosterone system and nonosmotic release of vasopressin which causes a further decrease in systemic vascular resistance, hypotension and vasoconstriction in the renal vessels and glomerular vascular bed[22]. This vasoconstriction cannot be only explained by increased activity of endogenous vasoconstrictor systems. Because of the extreme hemodynamic changes in advanced liver diseases, renal vasodilatator systems become insufficient, creating a vicious cycle which contributes more and more to renal vasoconstriction[22-24].

Factors contributing to the persistence of renal vasoconstriction in spite of the vasodilatation of the

peripheral vasculature have been investigated in several studies. Iwao *et al*[25] investigated the contributing factors of hyperdynamic circulation in cirrhotic patients and found that mesenteric blood flow decreases as liver disease worsens. They concluded that splanchnic arterial vasodilatation plays an important role in the pathogenesis of decreased systemic vascular resistance in cirrhotic patients[25]. In accordance with this data, in some other human and animal studies, it has been shown that splanchnic circulation is the main vascular bed responsible for peripheral vasodilatation[26-29].

Advanced liver disease due to portal hypertension is characterized by a state of hyperdynamic circulation which is accompanied by increased cardiac output[30]. It is hard to understand how cardiac output is increased while myocardial function is usually impaired in cirrhotic patients. The heart in cirrhotic patients usually has several structural and functional abnormalities associated with alterations in ventricular wall size, systolic and diastolic function[31,32]. Although the reasons for these alterations are not known clearly, neurohumoral factors and continous mechanical stress may be responsible[33]. Ventricular function is inhibited due to circulating cytokines, such as tumor necrosis factor-alpha, and nitric oxide in cirrhotic patients. One of the contributing factors to the ventricular dysfunction is reduced beta adrenergic receptor signal transduction in the myocardium[34,35].

Whatever the cause, ventricular wall thickness is increased slightly and the diastolic function deteriorates, especially increasing with physical stress and with the presence of ascites and systolic dysfunction[30,34,36].

Sympathetic nervous system activity is shown to be increased in cases of portal hypertension as a result of the hepatorenal reflex[37,38]. Hepatorenal reflex activation occurs due to decreased sinusoidal blood flow or increased sinusoidal pressure in the liver, as shown in several animal models[38,39]. Increased renal sympathetic nervous system tone is held to be responsible for renal vasoconstriction together with thromboxanes, endotoxins, endothelins and neurotransmitters. Together with the activation of the sympathetic nervous system because of a low effective circulating volume stimulating baroreceptors in the carotid body and aortic arch, activation of the renin-angiotensin-aldosterone system and nonosmotic release of antidiuretic hormone occurs. Although all of these compensatory mechanisms help to provide an effective circulating volume and relatively normalize the mean arterial blood pressure, they also have important effects on renal function.

Vasoactive mediators and cytokines are the other actors in HRS, agents that affect both the systemic and renal circulation. The major ones studied include prostaglandins, endothelins, endotoxins, glucagon, nitric oxide and tumor necrosis factor-α. Among these, nitric oxide has a special role. Primary arterial vasodilatation in the splanchnic circulation, a result of portal hypertension, is the mainstay in explaining the development of renal insufficiency in cirrhotic patients[20]. The major cause of this arterial vasodilatation in the splanchnic circulation is increased synthesis and activity of nitric oxide and some other vasoactive agents[20]. The correlation between increased levels of nitric oxide and high plasma renin-angiotensin-aldosterone system activity and antidiuretic hormone levels accompanied by low urinary Na excretion in cirrhotic patients, especially with ascites, is remarkable[40,41]. It is thought that in the maintenance of hyperdynamic circulation, the hallmark of HRS, nitric oxide may be the primary factor[42]. However, increased nitric oxide levels are not able to prevent renal vasoconstriction. In the early stages of cirrhosis, renal perfusion is provided by increased synthesis and activity of renal vasodilatators, especially prostaglandins and kallikreins[43,44]. Vasodilating prostaglandins are the major actors in supplying glomerular blood flow at the beginning[45], but as the liver disease progresses, vasoconstrictor systems are further activated and synthesis and activity of renal vasodilating factors progressively decrease. The prostaglandin level in the urine of cirrhotic patients is high when compared with that of patients with HRS[46]. The reason for decreased prostaglandin production in HRS is a mystery but it is known that it is not the only factor in the development of HRS.

DIAGNOSIS

HRS is an important risk factor for renal failure in cirrhotic patients. In a prospective study, it was estimated that the 1 year probability of HRS in cirrhotic patients is 18% and the 5 year probability is 39%[47]. HRS was observed in 28% of alcoholic hepatitis cases without identifiable cirrhosis[48]. Major factors precipitating HRS are hyponatremia, high plasma renin-angiotensin-aldosterone system activity, gastrointestinal bleeding, bacterial infections, spontaneous bacterial peritonitis, large volume paracentesis without albumin infusion, some drugs, such as diuretics, aminoglycosides, non-steroid anti-inflammatory drugs, angiotensin converting enzyme inhibitors, surgical interventions and cholestasis[47,49,50]. Also, Doppler ultrasonography may be helpful to detect increased renal resistive index indicating renal vasoconstriction[19].

In chronic liver diseases, it may be difficult to diagnose renal failure since reduction in GFR is usually masked. This may be because urea and creatinine production is decreased due to chronic liver disease, the muscle mass is decreased due to chronic disease and the protein intake is decreased due to the loss of desire to eat. There are no specific diagnostic criteria for diagnosing HRS. The diagnosis is by exclusion of other causes of renal failure in cirrhotic patients. The major symptoms of HRS are decreased GFR (< 40 mL/min) and increased serum creatinine (> 1.5 mg/dL). Other symptoms defining functional characteristics

of HRS are decreased Na excretion (< 10 mmol/L), higher urine osmolality compared to plasma osmolality, hyponatremia (< 130 mmol/L) and decreased diuresis (< 500 mL).

The most widely accepted diagnostic criteria were developed in 1996 by the International Ascites Club when the major and minor criteria were defined. According to this diagnostic criteria, diagnosis of HRS requires the inclusion of all major criteria and the presence of minor criteria are thought to be suggestive for the diagnosis of HRS. Various new concepts have arisen since the first publication of the criteria for the diagnosis of HRS in 1996. These were modified in 2007 by the International Ascites Club[6]. According to current diagnostic criteria, minor criteria are omitted and concurrent bacterial infection is now not a factor that should be excluded in the diagnosis of HRS. Another important alteration is using albumin instead of 0.09% NaCl solution for plasma volume expansion.

The new diagnostic criteria defined by the International Ascites Club in 2007 are listed in Table 1.

Creatinine clearance is the most important diagnostic tool. It is important to exclude other causes of renal failure before the diagnosis of HRS. These include hypovolemia, parenchymal renal diseases, use of nephrotoxic drugs and shock. In cases of hematuria, severe proteinuria and increased renal size on USG, renal parenchymal disease should be considered in the differential diagnosis[51]. In such cases, renal biopsy is required so that the potential need for combined liver and kidney transplantation can be defined[51]. If there is an organic cause of renal insufficiency, urine analysis to see the Na concentration is clinically important. Since muscle mass and production of creatinine in the liver is decreased in chronic liver diseases, serum creatinine levels are not very reliable to evaluate renal function in liver diseases. Creatinine monitoring blood urea level is also insufficient in reflecting the GFR in cases of chronic liver disease[5,6]. So, investigations should be conducted to find more sensitive and specific markers. Some of these markers are cystatin-C, symmetric dimethylarginine, kidney injury molecule-1 and neutrophil gelatinase-associated lipocalin. Cystatin-C has been found to be more sensitive than creatinine in defining decreased GFR in cirrhotic patients[52,53]. Symmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, has been shown to be increased in cases of HRS when compared with cirrhotic patients with normal kidney functions[54]. The investigations regarding kidney injury molecule-1 and neutrophil gelatinase-associated lipocalin which are very susceptible to ischemia and indicators of renal tubular injury are ongoing[55,56]. There are few studies about renal tubular damage markers. β2 microglobulin is one of them. It is especially increased in cases of aminoglycoside nephrotoxicity[57]. To make a differential diagnosis of HRS from acute tubular necrosis, gamma glutamyl transpeptidase, transaminase, neutrophil gelatinase-associated lipocalin, IL 8, liver type fatty acid binding protein and hepatitis virus cell receptor Type 1 are other markers which are of interest nowadays but their significance has not yet been evaluated[58].

TREATMENT

The main principle in the treatment of HRS is to bring back renal function until the patients undergo LT. So, all the therapeutic interventions for HRS are a sort of bridge therapy. During the treatment of HRS, etiology oriented treatment of liver diseases such as antiviral drug treatment should not be impeded. The choice of medical treatment depends upon several factors, including the availability of drugs, which is variable according to country and even region, whether the patient is admitted to an intensive care unit and if the patient is a candidate for LT. Cirrhotic patients with gastrointestinal system (GIS) bleeding, ascites, infections, arterial hypotension and dilutional hyponatremia should be monitored closely because of the increased risk of HRS.

Complications like GIS bleeding and spontaneous bacterial peritonitis should be prevented and urgently treated. Large volume paracentesis with plasma volume expansion decreases the incidence of HRS. Diuretic treatment may trigger HRS because of intravascular volume depletion so diuretic treatment should be stopped and electrolyte imbalances such as hyponatremia and hypocalcemia should be corrected. NSAIDs should also be stopped and appropriate infection treatment should be planned.

Effective circulating volume should be increased. Infusion of 0.9% NaCl and synthetic plasma expanders, even by monitoring central venous pressure, has not found to be helpful. It is proved that albumin is the most useful of all volume expanders. After albumin use, incidence of Type 1 HRS has been shown to be decreased. When albumin is used concomitantly with other agents, it has been observed that the effectiveness of these agents was also increased[23,51].

Prostaglandins, dopamine and endothelin receptor blockers were the first renal vasodilatators used in HRS treatment. Oral prostaglandin-E_1 analogue misoprostol or IV prostaglandin infusion did not provide a significant improvement in HRS[59,60]. Intravenous dopamine infusion has also been investigated in several studies but no improvements have been observed in renal function[61,62].

There is no specific vasoconstrictive agent used to increase systemic vascular resistance. Several vasoconstrictive agents such as norepinephrine, angiotensin 2 and vasopressin have been used for this purpose but alone they were not found to be effective. Vasoconstrictive agents, especially when used together with plasma expanders, are the most helpful pharmacological agents in the management of HRS[63,64]. Development of synthetic vasopressin analogues provides an important progression in HRS treatment. Ornipressin and terlipressin are vasoconstrictive agents that are effective on mesenteric circulation rather than renal and other vascular systems. Ornipressin is not

Table 1 Criteria for diagnosis of hepatorenal syndrome in cirrhosis

Cirrhosis with ascites
Serum creatinine > 1.5 mg/dL (133 μmol/L)
Absence of shock
Absence of hypovolemia as defined by no sustained improvement of renal function (creatinine decreasing to < 133 μmol/L) following at least 2 d of diuretic withdrawal (if on diuretics) and volume expansion with albumin at 1 g/kg per day up to a maximum of 100 g/d
No current or recent treatment with nephrotoxic drugs
Absence of parenchymal renal disease as defined by proteinuria < 0.5 g/d, no microhematuria (< 50 red cells/high powered field) and normal renal ultrasonography

being used because of its severe ischemic side effects.

Terlipressin and albumin infusion are the most important choices of treatment in Type 1 HRS[65]. It has been observed that terlipressin is effective in 40%-60% of patients with Type 1 HRS. Clinical response to terlipressin treatment is slow but the reduction in serum creatinine level is continuous[65,66]. To reverse HRS may take a long time and it has been observed that it recurred in 50% of patients. In cases of recurrence, the same treatment regimen is usually found to be successful[7]. When terlipressin and albumin treatment is successful, arterial blood pressure, urine amount and serum Na level increase. Systemic circulation improves and plasma renin and norepinephrine levels decrease significantly. Time required for recovery usually changes depending on the initial serum creatinine level but mean recovery time is 7 d. If the initial serum creatinine level is low, the recovery will be faster[23,51,67,68]. Terlipressin therapy is suggested to be used in combination with albumin. Terlipressin therapy may be given as an IV bolus (0.5-1 mg/4-6 h) or IV continuous infusion with an initial dose of 2 mg/d. During the follow-up period, if a 25% decrease is not observed in serum creatinine level, the IV bolus dose may be increased up to 2 mg/4 h or the IV continuous infusion dose may be increased up to a maximum of 12 mg/d. Monitoring CVP is essential and albumin infusion is required to retain CVP at a level of 10-15 cm H_2O. Albumin is given for 2 d in the form of IV bolus therapy with an initial dose of 1 g/kg (maximum 100 g/d) and the maintenance albumin dose should be 25-50 g/d until terlipressin therapy is ceased and serum creatinine level becomes normal[69]. There are some studies that showed nearly 75% improvement in HRS patients by using a continuous IV infusion of terlipressin. In these studies, how terlipressin is given was also found to be important[70-72]. The information about the treatment of Type 2 HRS by albumin and vasoconstrictive agents is limited. When albumin and vasoconstrictive agents are used in Type 2 HRS treatment, improvement in renal function has been observed but there is a 50% recurrence rate after the cessation of therapy[23,73,74]. The most common side effects of terlipressin treatment are cardiovascular and ischemic and are reported in nearly 12% of patients treated[20,75]. According to the 2012 Cochrane meta-analysis, GIS and infectious side effects did not increase significantly during terlipressin therapy, whereas cardiovascular side effects increased remarkably[76].

Other vasoconstrictive agents currently being used in HRS treatment are somatostatin analogues (octreotide), α-adrenergic agonists, midodrine and norepinephrine. Their effectiveness has been studied in several studies; some found that they are less effective than terlipressin[23,77,78] and some found that their effectiveness is similar to terlipressin[79,80]. Midodrine is an orally available α-adrenergic agonist. Its effect is systemic vasoconstriction. When it is used in combination with octreotide and albumin, systemic and renal hemodynamic status is improved[81]. Midodrine is given orally with an initial dose of 7.5 mg/8 h (maximum: 15 g/8 h) and octreotide may be given either as continuous infusion with a dose of 50 mcg/h or subcutaneously with a dose of 100-200 mcg/8 h. In combination with midodrine and octreotide, albumin is given as an IV bolus with an initial dose of 1 g/kg (maximum: 100 g) and a maintenance dose of 25-50 g/d. Using midodrine and octreotide in combination has been shown to decrease mortality[82]. Nevertheless, the number of patients reported using this therapy is not enough[78,83] so more trials are required for a more accurate conclusion.

Norepinephrine is a vasoconstrictive agent generally used in intensive care units since it is not convenient to use in general medical wards. It is given as an intravenous continuous infusion with a dose of 0.5-3 mg/h. The effectiveness of norepinephrine and terlipressin were shown to be similar, while norepinephrine was cheaper[80,84].

Since dopamine is known to decrease renal vascular resistance and increase renal blood flow, low doses of dopamine were tried in the past. Its clinical effectiveness could not be proved either alone or in combination with ornipressin and the results are controversial[23,78].

According to several studies, increasing mean arterial blood pressure is suggested to have favorable effects on the treatment process. The most used predictors of favorable treatment response are a serum bilirubin concentration of < 10 mg/dL and an increase in mean arterial blood pressure ⩾ 5 mmHg on the third day of treatment[68].

The principle is that the earlier the treatment has been started, the better the results are. If serum creatinine level is < 5 mg/dL when the treatment is started, the probability of a favorable response is increased.

In a study by Nazar *et al*[68] in patients with both decreased bilirubin level and increased mean arterial

blood pressure, treatment success was 100%. In patients with only a decreased bilirubin level, the success rate was found to be 53% and it was 25% in patients with only an increased mean arterial blood pressure. In the patient group, bilirubin levels did not decrease, mean arterial blood pressures did not increase and the success rate was 10%[68]. If there is treatment unresponsiveness, underlying renal disease other than HRS should be considered.

The goal of all vasopressor treatments is to achieve a 10-15 mmHg increase in the mean arterial blood pressure. Increased mean arterial blood pressure is usually associated with decreased serum creatinine levels[85].

For patients with HRS who are not admitted to an intensive care unit, combination therapy with terlipressin and albumin is suggested. If terlipressin is not available, combination therapy with midodrine, octreotide and albumin should be used in patients who are not in the intensive care unit. After two weeks of medical treatment, if there are no improvements in renal function, medical treatment is considered to be useless.

Transjugular intrahepatic portosystemic shunts (TIPS) have been reported to improve renal function in patients with Type 1 HRS[86-88] and it has also been used for the treatment of refractory ascites in patients with Type 2 HRS[89-91]. However, TIPS therapy is possible under limited circumstances because of its contraindications and complications. Major complications associated with TIPS are hepatic encephalopathy which is a common and treatable condition, worsening of hepatic function, bleeding due to the procedure and acute kidney injury because of intravenous contrast injection during the procedure[92]. The underlying mechanism explaining how renal function is improved after TIPS is not known completely. TIPS provides portal decompression in cirrhotic patients, portal pressure decreases and blood pooling in the splanchnic vascular bed returns to the systemic circulation. As a result of this, RAAS and SNS activity is suppressed and renal vasoconstriction improves. In a study investigating renal function after TIPS in seven patients with Type 1 HRS, a significant decrease in serum creatinine level and increase in urine volume was observed in six of the patients in a one month period. This was accompanied by significant improvement in renal blood flow and GFR[86]. However, amelioration of renal function may take as long as six months in some cases after TIPS[89]. Also, the effect of TIPS on survival in Type 1 HRS patients is appreciable. HRS improved in nearly 50% of patients and survival increased more than three months after TIPS[78,87]. In cases of Type 2 HRS when TIPS is applied to control ascites, it was found to be successful and 70% of patients survived the following year[87,93]. The average survival after TIPS was approximately five months which was longer than the expected survival for such patients[86]. Unfortunately, mostly it is too late for patients with HRS to undergo TIPS and so it is suggested as a choice of treatment for only a selected group of patients. Before the decision to undergo TIPS, the high incidence of complications and especially encephalopathy should be considered.

Although LT is the most effective and definitive treatment of liver failure and HRS, supportive treatment modalities are required until LT is carried out. Non biological liver support systems have been developed for this purpose. The mechanism of action is provided by detoxification through a semi-permeable membrane in these non biological support systems. During the course of liver failure, according to the dominant clinical presentation (HRS, hepatopulmonary syndrome, hyperbilirubinemia), the most appropriate type of support system, each with different prominent features, will be chosen. Whereas in HRS venovenous hemodiafiltration is the first choise of treatment in cases of treatment unresponsiveness, a molecular adsorbents recirculating system should be considered. High-flux dialysis provides effective elimination of water soluble substances such as ammonia and lactate but it is insufficient in eliminating substances binding to proteins such as bile acids. Plasma exchange is no longer being used as it is too risky since large volumes are required to be exchanged in this procedure. For that reason, nowadays continuous venovenous hemodiafiltration may be useful in patients with HRS if there is a reversible precipitating factor such as infection[71]. Hemodialysis or continuous hemofiltration is used in the treatment of acute renal failure in cirrhotic patients[94,95]. In a study by Witzke *et al*[96], thirty-day survival was reported as 50% after renal replacement therapy but it is obvious that long term survival is usually poor. According to the Acute Dialysis Quality Initiative Group, renal support therapies should be suggested for patients who are candidates for LT[71]. There are several studies reporting that albumin dialysis has been beneficial in HRS[23,97]. In a randomized controlled trial, a molecular adsorbents recirculating system was observed to be more effective and safer when compared to standard medical treatment in the management of type 3-4 hepatic encephalopathy[23,97]. However, the data about this subject in the literature is limited.

Renal transplantation is the best treatment choice for both Type 1 and Type 2 HRS patients[98]. If liver transplantation is performed after the HRS is improved, posttransplantation morbidity and mortality is decreased. Three year survival after liver transplantation in patients with HRS is 60%, while it is 70%-80% if it is performed before HRS has developed[98,99].

PREVENTION

Prevention of HRS is important since it develops with a constant frequency in cases of SBP and alcoholic hepatitis. It is possible to prevent HRS if SBP is urgently diagnosed and treated. Albumin infusion may help to prevent HRS when SBP develops. Albumin infusion is started together with antibiotherapy with an initial dose of 1.5 g/kg at the time of diagnosis of infection

and albumin infusion is repeated after 48 h with a dose of 1 g/kg[23,100]. The incidence of renal dysfunction is decreased when compared to patients who are not treated with albumin (8% *vs* 31%) and mortality is also decreased (16% *vs* 35%)[100]. Norfloxacin is recommended in selected patients with cirrhosis and ascites. Four hundred mg/day dose of oral norfloxacin in a one year time period was found to decrease SBP development (7% *vs* 61%), decrease HRS development (28% *vs* 41%) and improve survival at three months (94% *vs* 62%) and one year (60% *vs* 48%)[100,101]. In a study investigating whether pentoxifylline is beneficial or not, significant benefit with 1200 mg/d pentoxifylline was observed when compared with placebo[102] but a meta-analysis revealed that pentoxifylline has no benefit in HRS[103].

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MINIREVIEWS

Evidence-based medicine: An update on treatments for peritoneal dialysis-related peritonitis

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Abstract

Peritonitis continues to be a major complication of peritoneal dialysis (PD), and adequate treatment is crucial for a favorable outcome. There is no consensus regarding the optimal therapeutic regimen, and few prospective controlled studies have been published. The objective of this manuscript is to review the results of PD peritonitis treatment reported in narrative reviews, systematic reviews, and proportional meta-analyses. Two narrative reviews, the only existing systematic review and its update published between 1991 and 2014 were included. In addition, we reported the results of a proportional meta-analysis published by our group. Results from systematic reviews of randomized control trials (RCT) and quasi-RCT were not able to identify any optimal antimicrobial treatment, but glycopeptide regimens were more likely to achieve a complete cure than a first generation cephalosporin. Compared to urokinase, simultaneous catheter removal and replacement resulted in better outcomes. Continuous and intermittent IP antibiotic use had similar outcomes. Intraperitoneal antibiotics were superior to intravenous antibiotics in reducing treatment failure. In the proportional meta-analysis of RCTs and the case series, the resolution rate (86%) of ceftazidime plus glycopeptide as initial treatment was significantly higher than first generation cephalosporin plus aminoglycosides (66%) and glycopeptides plus aminoglycosides (75%). Other comparisons of regimens used for either initial treatment or treatment of gram-positive rods or gram-negative rods did not show statistically significant differences. The superiority of a combination of a glycopeptide and a third generation cephalosporin was also reported by a narrative review study published in 1991, which reported an 88% resolution rate.

Key words: Peritonitis; Peritoneal dialysis; Antibiotic; Treatment

Core tip: This manuscript revised the data from narrative and systematic review, as well as those from a proportional meta-analysis study, regarding comparisons between antibiotic regimens used to peritoneal dialysis-related treatment, empathizing protocols for initial treatment.

There is no consensus on the best treatment and the only published systematic review and its recent update have failed to find superiority of any regimen. This type of analysis, commonly excludes several studies, some of them with a great number of cases. Therefore, this review intends to contribute in this issue analyzing the results from different types of reviews.

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INTRODUCTION

Since the introduction of peritoneal dialysis (PD) in routine clinical practice, peritonitis has been the main complication influencing patient mortality. Peritonitis continues to be the most frequent cause of technique failure[1], despite technological improvement. The choice of initial treatment for PD-related peritonitis remains a challenge to nephrologists who perform PD, particularly because of the lack of evidence to indicate the best therapeutic protocols, beyond temporal changes in the bacterial antibiotic susceptibility profile.

Coagulase negative staphylococci (CNS) are the most common etiological agents of PD-related peritonitis. In most PD centers[2], these microorganisms cause approximately one-third of the episodes. Over the last two decades, *Staphylococcus aureus* has lost its status as a PD-related peritonitis etiology, possibly because of technological advances in connection systems and the routine use of antibiotic prophylaxis at the catheter exit site[3]. However, the proportion of cases due to gram-negative bacilli has increased in several centers[4]. In addition, a gradual increase in the frequency of methicillin-resistant CNS and gram-negative species resistant to commonly used antibiotics has been reported[5,6].

Historically, the choice of initial antimicrobial regimen for PD-related peritonitis has been based on the recommendations of the International Society for Peritoneal Dialysis (ISPD), which published six documents between 1989 and 2010[7-12]. According to these guidelines, the initial treatment of peritonitis (prior to the results of microbiological tests) should be based on a combination of drugs for coverage of gram-positive cocci and gram-negative bacilli. The recommendations regarding the class of antimicrobials have varied over time. In general, for coverage of gram-positive cocci, the use of a first generation cephalosporin or vancomycin has been proposed, while for gam-negative bacilli an aminoglycoside or ceftazidime has been recommended. However, based on the available literature there is no consensus regarding the best antimicrobial therapy for the initial treatment of these infections, and few prospective and controlled studies have been published.

This manuscript intends to review the results from evidence-based medicine, comparing different treatment protocols for PD-related peritonitis in narrative reviews, systematic reviews and proportional meta-analysis.

NARRATIVE REVIEWS

Since the introduction of ambulatory PD as a modality of renal substitutive therapy as part of the clinical routine, several reviews have been published discussing general and specific aspects of this therapy, including peritonitis and its management; however, few of these articles have focused on comparing the therapeutic regimens.

In 1991, Millikin *et al*[13] published the first robust review compiling existing data on antimicrobial treatment of PD-related peritonitis. That study reported on studies of antimicrobial treatment for peritonitis published in the medical literature before January 1990. According to the review, the regimens most frequently used for empirical therapy were a combination of two antimicrobial drugs; the majority of the regimens involved an aminoglycoside associated with an antibiotic to gram positive organism coverage. An aminoglycoside with a first-generation cephalosporin was used in 165 episodes, with an overall resolution rate of 83%, while the combination of an aminoglycoside with a glycopeptide resulted in a clinical response in 88% of 286 cases. When a glycopeptide associated with a third generation cephalosporin was used, the resolution rate reached 93% as reported by three studies in a total of 197 peritonitis episodes.

The efficacy of drugs used for treatment of infections due to gram positive cocci was proven in 413 peritonitis episodes. The resolution rate was 90% for a first generation cephalosporin, used in 164 episodes. A similar clinical response was observed whether intraperitoneal (IP) cefazolin was prescribed for intermittent or continuous administration. However, the results from second-generation cephalosporins, used for treatment in 29 episodes, showed a resolution rate of 76%. In turn, the prescription of a glycopeptide, particularly vancomycin, resulted in a resolution rate of 94% in 220 cases.

For gram negative peritonitis episodes, aminoglycoside monotherapy produced a clinical response in 48% of the 58 episodes, while a monobactam (aztreonam) resolved 22 of 27 cases (81%), and a quinolone resolved 13 of 17 cases (76.4%). In 97% of cases involving *pseudomonas* peritonitis, an aminoglycoside was used either as monotherapy or in combination with anti-*pseudomonas* penicillin. When the peritonitis episode was at the exit site or was catheter related (n = 47), the response rate was only 32%. *Pseudomonas* peritonitis that was not associated with catheter infection, however, responded to these agents in 73% of 44 cases.

In 2000, our group published a literature review analyzing the therapeutic response from the empirical antimicrobial regimen proposed in the first, second, and third report of the Ad Hoc Committee on Peritonitis

Management of the International Society of Nephrology ("ISPD guidelines"), published between 1985 and 2000[14].

From 1985 to 1990, covering the period from the first report by The Ad Hoc Committee on Peritonitis Management[7], a total of six publications with 204 peritonitis episodes, a resolution rate higher than 80% was observed with the combination of a first generation cephalosporin and an aminoglycoside. In 1993, the second report by The Ad Hoc Committee on Peritonitis Management[8] recommended the initial use of vancomycin plus an aminoglycoside, both by an intermittent IP route, or IP injection of vancomycin combined with a third generation cephalosporin.

Results from the empirical prescription of vancomycin plus an aminoglycoside were reported in 23 publications between 1985 and 2000, corresponding to more than 1300 peritonitis episodes. A clinical response above 80% was reported in almost all of the series. In the series with the largest number of consecutive episodes (241 cases), the authors observed a resolution rate of 86%.

Vancomycin associated with ceftazidime was used in four studies, with a total of 302 episodes, resulting in a resolution rate above 90%. In the study with the largest number of cases (102 episodes) a cure rate of 92% was reported[15].

The third report of The Ad Hoc Committee on Peritonitis Management was published in 1996[9]. Based on the emergence of vancomycin-resistant enterococci and the possibility of gene transfer or resistance to *Staphylococcus aureus*, that document recommended the non-use of vancomycin in the empirical treatment of peritonitis. The combination of a first generation cephalosporin with an aminoglycoside again became the recommended empirical treatment for PD-related peritonitis.

Between the publication of the third report of The Ad Hoc Committee on Peritonitis Management and its fourth version in 2000[10], the results obtained with this protocol were reported in six publications[14]. In five of these reports, the resolution rate was over 75%. In our center, a study reporting 34 peritonitis episodes demonstrated complete cure in only 55% of the cases[16].

SYSTEMATIC REVIEWS

Wiggins *et al*[17] published a systematic review of randomized controlled trials (RCTs) on PD-related peritonitis in 2007. The study included 36 trials published from 1985 to 2006. The results indicated that there was no superior antimicrobial agent or regimen, although glycopeptide-based regimens achieved a significantly higher complete cure rate (three studies, 370 episodes) than first-generation cephalosporin-based regimens. Vancomycin and teicoplanin resulted in similar treatment failure and relapse rates (two trials, 178 participants). Equivalent treatment failure rates and risk of relapse were observed between IP intermittent or continuous antibiotic administration (four trials, 338 participants), while one trial with 75 patients showed an advantages of IP antibiotics over intravenous therapy. Based on one trial with 37 patients with relapsing or persistent peritonitis, simultaneous catheter removal/replacement was demonstrated to be superior to urokinase at reducing treatment failure rates. Catheter removal was not decreased by urokinase treatment compared with placebo (two trials, 168 participants). Based on one trial with 36 patients, there was no statistically significant difference in clinical response within a 24-h period of peritoneal lavage when compared to non-lavage.

Recently, Ballinger *et al*[18], from the same group of investigators, published an update of this systematic review. The authors included RCTs and quasi-RCTs to assess the treatment of peritonitis in adults and children. In total, there were 42 studies published up to March 5 2014, with 3013 episodes of peritonitis. Their results were similar to the previous analysis; the authors did not identify any optimal antibiotic agent or combination of agents. The advantages of a glycopeptide-based regimen over those based on a first generation cephalosporin regarding complete cure rate were demonstrated (three studies, 370 participants). However, no differences between these regimens have been found when the endpoints were primary treatment failure (two studies, 305 participants), relapse (3 studies, 350 participants), catheter removal (two studies, 305 participants), and microbiological eradication (one study, 45 participants). Similarities between vancomycin and teicoplanin in the treatment failure and relapse were shown, although the authors provided new information, showing that the primary treatment failure rate was lower with teicoplanin than vancomycin (two studies, 138 participants). Similar to the previous systematic review, comparisons between IP intermittent or continuous antibiotic administration showed no difference in the complete cure and relapse rates (four studies, 338 participants). The results were updated for primary treatment failure (five studies, 522 participants) and the catheter removal rate (1 study, 20 participants); no differences between the two forms of antibiotics were found. A preference for IP antibiotics (vancomycin and tobramycin) over intravenous administration was newly stated based on one study with 75 patients. In addition, based on one study, comparisons of the adverse effects of these antibiotic administration routes were included. No significant differences were observed in the incidence of hypotension (76 participants), cutaneous rash (20 participants), and infusion pain (20 participants). The advantage of simultaneous catheter removal/replacement over urokinase at reducing treatment failure rate was rewritten (one study, 37 participants), but the authors presented new information on comparisons between fibrinolytic agents and non-urokinase or

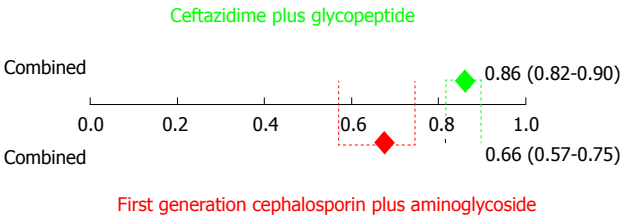

Figure 1 Combined resolution rate and 95%CIs of studies on initial treatment of peritoneal dialysis-related peritonitis with ceftazidime plus a glycopeptide *vs* a first generation cephalosporin plus an aminoglycoside.

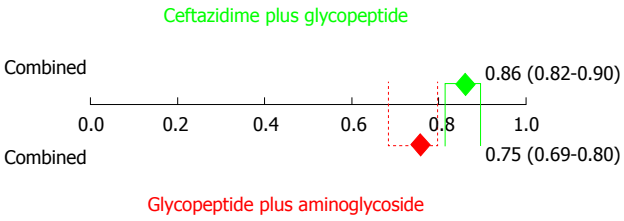

Figure 2 Combined resolution rate and 95%CIs of studies on initial treatment of peritoneal dialysis-related peritonitis with ceftazidime plus a glycopeptide *vs* a glycopeptide plus an aminoglycoside.

placebo. No significant differences were found in the following outcomes: complete cure rate (one study, 88 participants), primary treatment failure (two studies, 99 participants), relapse in persistent peritonitis (2 studies, 101 patients), relapse when fibrinolytic therapy was initiated at the time peritonitis was diagnosed (one study, 80 participants), catheter removal (2 studies, 116 participants), and all-cause mortality (1 study, 88 participants). Finally, the study found that there is no advantage to a 24-h period of peritoneal lavage compared to non-lavage (one study, 36 participants).

PROPORTIONAL META-ANALYSIS

One limitation of systematic review studies is the exclusion of a large number of publications with a large number of patients and episodes of peritonitis. Most of these excluded studies were case series. In turn, their authors have noted the inclusion of many trials with small patient numbers as a limitation[17,18]. In an attempt to overcome these limitations, our center is employing an alternative methodology: the proportional meta-analysis to examine possible differences among therapeutic protocols. This method has been used in other clinical settings[19,20], and it is possible to perform a meta-analysis of results from case series. Accordingly, a review of case series and RCTs concerning the treatment of PD-related peritonitis has been developed, focusing on comparing peritonitis resolution with antibiotics or antibiotic combinations more frequently recommended by the ISPD guidelines for empirical treatment of peritonitis and peritonitis due to gram positive or gram negative bacteria[21].

Studies were obtained between 1966 and January 2013, using the following sources: United States National Library of Medicine, Excerpta Medica database, and Literatura Latino-Americana e do Caribe em Ciências da Saúde. Peritonitis was defined according to the authors in accordance with the contemporary ISPD guidelines[7-12]. The criterion for peritonitis resolution was based on definitions used by authors and can vary greatly; the outcome resolution rate was treated as a dichotomous variable (peritonitis resolution *vs* non-resolution).

For first generation cephalosporins, we included the following: cefazolin, cephalotin, and cephaloridine. The only third generation cephalosporin we analyzed was ceftazidime. For aminoglycosides, we included gentamicin, amikacin, netilmicin and tobramycin. Vancomycin and teicoplanin were considered in the analysis as glycopeptides. Finally, ciprofloxacin, levofloxacin and ofloxacin were the fluoroquinolones included.

After screening by title and abstract, we obtained full paper copies of 140 eligible studies reporting antibiotic therapy for PD-related peritonitis. However, after applying the inclusion and exclusion criteria, only 43 studies (26 case series and 17 RCT) were acceptable for a proportional meta-analysis.

Initial treatment with ceftazidime plus a glycopeptide was used in five[15,22-25] studies with a total of 443 episodes; the pooled resolution rate was 86% (95%CI: 0.82-0.89). This resolution rate was significantly higher than initial treatment with a first generation cephalosporin plus aminoglycosides (pooled proportion of 66%, 95%CI: 0.57-0.75) from 14 studies[25-38] with a total of 1438 total episodes (Figure 1). Initial treatment with ceftazidime plus a glycopeptide also showed a higher resolution rate than a glycopeptide plus aminoglycosides (pooled proportion of 75%, 95%CI: 0.69-0.80), which was used in 16 studies[29-31,38-50] with a total of 574 episodes (Figure 2).

The following comparisons showed no statistically significant differences because their CIs overlapped: a first generation cephalosporin plus aminoglycosides [resolution rate (RR) = 66%, 95%CI: 0.57-0.75] *vs* glycopeptides plus aminoglycosides (RR = 75%, 95%CI: 0.69-0.80); a first generation cephalosporin plus aminoglycosides (RR = 66%, 95%CI: 0.57-0.75) *vs* a first generation cephalosporin plus ceftazidime (RR = 59%, 95%CI: 0.32-0.83); glycopeptides plus aminoglycosides (RR = 75%, 95%CI: 0.69-0.80) *vs* first generation cephalosporin plus ceftazidime (RR = 59%, 95%CI: 0.32-0.83), and a first generation cephalosporin plus ceftazidime (RR = 59%, 95%CI: 0.32-0.83) *vs* ceftazidime plus a glycopeptide (RR = 86%, 95%CI: 0.82-0.89).

For treatment of episodes due to gram-positive rods, the pooled resolution rate from 13[23,39,40,48,49,51-58] studies with a total of 917 episodes was 78% (95%CI: 0.66-0.88) for a glycopeptide, while the rates from five studies[26,37,53,58,59] with a total of 532 episodes for a first generation cephalosporin were 73% (95%CI: 0.55-0.88). There were no significant differences

Table 1 Recommendations for antibiotics choice in peritoneal dialysis-related peritonitis

	Monitoring the etiologies and antimicrobial resistance profile	
	Yes	**No**
Initial (empirical) protocol	Start intraperitoneal antibiotics to cover gram-positive and gram roads, according to local microbiologic profile	Start a glycopeptide (gram-positive coverage) plus ceftazidime (gram-negative coverage), both by intraperitoneal route[1]
After results of culture and *in vitro* susceptibility tests	Culture positive: adjust the treatment according to bacterial susceptibility. If *Pseudomonas spp* on culture, add a second anti-*pseudomonas* drug acting in different ways that organism is sensitive to[2]	Culture positive: adjust the treatment according to bacterial susceptibility. If *Pseudomonas spp* on culture, add a second anti-*pseudomonas* drug acting in different ways that organism is sensitive to[2]
Therapy duration	Culture negative: continue initial antibiotics *Pseudomonas spp*, *Enterococcus*/*Streptococcus spp* = 21 d Non-*pseudomonas* single gram-negative = 14-21 d Culture negative, coagulase negative staphylococcus, other gram-positive roads = 14 d	Culture negative: Continue initial antibiotics

[1]Evidence-based medicine; [2]*E.g.*, quinolone, ceftazidime, cefepime, amiglycoside, piperacillin.

between the schemes.

Comparisons of episodes due to gram-negative rods showed that the pooled proportion resolution rate from nine studies[39,40,49,57,60-63] with a total of 138 episodes was 68% (95%CI: 0.50-0.85) for a quinolone. For ceftazidime, the resolution rate was 61% (95%CI: 0.53-0.70) from three studies[33,63,64] with a total of 117 episodes, and for aminoglycosides the resolution rate was 65% (95%CI: 0.51-0.77) from nine studies[23,26,31,39,40,49,55,60,61] with a total of 211 episodes. There were no significant differences among these antibiotics.

LIMITATIONS

The limitations of narrative reviews are those inherent to this type of publication, which include the use of different types of studies, such as RCTs, case series, and others without a statistical tool for comparisons among the treatments. Moreover, they refer to data published many years ago and may be influenced by an era effect.

Regarding the systematic reviews, their authors emphasize inadequate randomization and concealment methods. In addition, the definitions of peritonitis, successful treatment, and relapse varied among trials[17]. Finally, many trials had small patient numbers, which reduces their statistical power.

The most important limitation of our proportional meta-analysis is the low evidence level of case series included with the RCTs. In addition, there is significant heterogeneity among the studies, which differed considerably in their patient selection, baseline renal disease, number of subjects, antibiotic administration routes, and definition of peritonitis and resolution.

CONCLUSION

According to the results of the systematic reviews, there is no superior antimicrobial agent to treat PD-related peritonitis, although glycopeptide-based regimens achieved a significantly higher complete cure rate. Similar treatment failure rates were found with vancomycin and teicoplanin, while the primary treatment failure rate was lower with teicoplanin. Intermittent or continuous IP antibiotic administration had similar complete cure, primary treatment failure, relapse, and catheter removal rates. The advantages of IP antibiotics over intravenous therapy were reported. In cases of persistent or relapsing peritonitis, catheter removal is associated with better outcomes than with IP urokinase. Finally, no advantages were found to be associated with adjunctive therapies, such as fibrinolytic drugs and peritoneal lavage.

A narrative review of antimicrobial treatment for patients with PD-related peritonitis published in 1991[13] concluded that the optimal empirical treatment was weekly vancomycin and ceftazidime.

Our proportional meta-analysis[21] was able to identify that the combination of a glycopeptide plus ceftazidime in the initial treatment of PD-related peritonitis was superior to a glycopeptide plus an aminoglycoside or the combination of a first generation cephalosporin plus an aminoglycoside. This result strongly suggests that the differences found may be related to better coverage of gram-negative bacilli with third generation cephalosporins than with aminoglycosides. Bacterial resistance of gram-negative bacilli, particularly *Pseudomonas* species, to commonly prescribed antimicrobials has been reported in recent years[6]; this finding may explain the superiority of the protocols employing ceftazidime. This review showed that a treatment regimen with a glycopeptide plus ceftazidime could be a promising initial therapy in patients with PD-related peritonitis. However, this result should be carefully analyzed, because this treatment was only used in four cases series[15,22-24] and one RCT[25] for a total of 443 peritonitis episodes. Moreover, an emphasis should be placed on the necessity of monitoring the local microbiologic profile in each dialysis center to determine the initial therapeutic protocol. Recommendations for antibiotics choice in peritoneal dialysis-related peritonitis are expressed in the Table 1.

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African origins and chronic kidney disease susceptibility in the human immunodeficiency virus era

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Abstract

Chronic kidney disease (CKD) is a major public health problem worldwide with the estimated incidence growing by approximately 6% annually. There are striking ethnic differences in the prevalence of CKD such that, in the United States, African Americans have the highest prevalence of CKD, four times the incidence of end stage renal disease when compared to Americans of European ancestry suggestive of genetic predisposition. Diabetes mellitus, hypertension and human immunodeficiency virus (HIV) infection are the major causes of CKD. HIV-associated nephropathy (HIVAN) is an irreversible form of CKD with considerable morbidity and mortality and is present predominantly in people of African ancestry. The APOL1 G1 and G2 alleles were more strongly associated with the risk for CKD than the previously examined MYH9 E1 risk haplotype in individuals of African ancestry. A strong association was reported in HIVAN, suggesting that 50% of African Americans with two APOL1 risk alleles, if untreated, would develop HIVAN. However these two variants are not enough to cause disease. The prevailing belief is that modifying factors or second hits (including genetic hits) underlie the pathogenesis of kidney disease. This work reviews the history of genetic susceptibility of CKD and outlines current theories regarding the role for APOL1 in CKD in the HIV era.

Key words: Chronic kidney disease; Genetics; African ancestry; Human immunodeficiency virus; APOL1; MYH9; Human immunodeficiency virus-associated nephropathy

Core tip: There are striking ethnic differences in the prevalence of chronic kidney disease, including human immunodeficiency virus (HIV)-associated nephropathy (HIVAN), in people of African ancestry suggestive of genetic predisposition. The APOL1 G1 and G2 alleles

are more strongly associated with the risk for HIVAN than the previously reported MYH9 E1 risk haplotype in individuals of African ancestry. The high prevalence of HIVAN among individuals of African ancestry could be a result of high frequencies of APOL1 risk variants as well as the prevalence of HIV-1 subtypes and modifying factors or second hits underlying the pathogenesis of kidney disease.

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INTRODUCTION

Chronic kidney disease (CKD) is a major public health problem worldwide[1]. Mortality due to CKD nearly doubled worldwide between 1990 and 2010, and is now positioned at 18th as a cause of death in the Global Burden of Disease Study[2] and at 5th position in South Africa[3]. An estimated 3.2 million people were on renal replacement therapy by the end of 2013, approximately 2522000 people undergoing dialysis treatment (haemodialysis or peritoneal dialysis) and 678000 people living with renal transplants[4] and it is also estimated that CKD incidence grows by approximately 6% annually[4]. There are striking ethnic differences in the prevalence of CKD such that, in the United States, African Americans have the highest prevalence of CKD[5]. Diabetes and hypertension, which have been considered the two leading causes of CKD, together with differences in clinical, social-demographic or lifestyle factors, are insufficient to account satisfactorily for the excess risk of end stage renal disease (ESRD) in African Americans[6,7]. Africa, the second largest and the world's second most populous continent, is approximately 30.2 million square km^2 and composed of 54 countries[8] and more than 1.1 billion people as of 2013, accounting for 15% of the world's population[9]. It has been postulated that, by 2030, approximately 70% of the patients with ESRD will be living in low income countries such as those in sub-Saharan Africa where majority of people live on less than one dollar-a-day[10,11]. The increased burden of CKD in Africa could be as a result of various communicable diseases such as leishmaniasis, schistosomiasis, infectious glomerulonephritis and importantly, human immunodeficiency virus (HIV) infection superimposed on non-communicable diseases such as hypertension and diabetes mellitus. These factors have resulted in the increase in CKD; several studies have shown that there is a four-fold increase in CKD in HIV uninfected individuals, compared to 18-50 fold increase in CKD in HIV positive individuals of African descent[12,13].

HIV AND CHRONIC KIDNEY DISEASE

Acquired immune deficiency syndrome (AIDS)-associated nephropathy was originally reported in AIDS patients in the United States in 1984. Subsequently, asymptomatic HIV-infected individuals showed similar clinical and histological features, and the name was later changed to HIV-associated nephropathy (HIVAN)[14]. HIVAN is an irreversible form of CKD which is a pathologically distinct complication of HIV infection with considerable morbidity and mortality[15,16]. The odds of developing HIVAN have increased in recent years to fifty according to the United States Renal Data System[17]. There is a huge regional variation in the prevalence of HIV infection; globally, an estimated 35.3 million people were living with HIV as at 2012; in North Africa, approximately 260000 people are living with HIV; while in sub-Saharan Africa, which comprises two thirds of all people living with HIV, an estimated 25 million people are living with HIV[18]. CKD occurs in approximately 6.0%-48.5% of HIV positive patients in Africa[19]. About 24%-83% of these cases are classic HIVAN in South Africa[20-22]. HIVAN is a clinicopathological condition characterized by the presence of focal glomerulosclerosis with collapsing glomerulopathy and glomerular epithelial cell proliferation, together with microcystic tubular dilatation and interstitial inflammation[23]. Risk factors for HIVAN are older age, lower CD4 counts, high viral load, co-morbidity (such as diabetes mellitus, hypertension and hepatitis C co-infection)[24]. HIVAN is present predominantly in people of African ancestry, indicating a possible genetic predisposition[25,26]. Renal histology in HIV infected patients in South Africa is shown in Table 1. A 30-year review of 1848 renal biopsies by Vermeulen at Chris Hani Baragwanath Hospital in Johannesburg, South Africa found that focal segmental glomerulosclerosis (FSGS) comprised 29.6% of primary glomerulonephritis (GN), 24.4% of membranous GN, 23.8% of membranoproliferative GN, 10.3% of minimal change disease, 4.1% of mesangial proliferative GN and 2.7% of IgA nephritis; 19.7% of the biopsies were in HIV positive individuals (Vermeulen A, MMed, University of the Witwatersrand, 2014).

The mechanism by which HIV induces glomerular injury leading to the pathologic syndrome of HIVAN is not well understood, hence a number of theories have been postulated as to how HIV causes renal injury. First, a direct viral infection of podocytes, renal parenchymal cells, especially the visceral epithelial cells of the glomerulus, and the tubular epithelial cells and as a result, this elicits cytopathic effects including proliferation and apoptosis[27]. Secondly, HIV infects the lymphocytes and macrophages that enter the kidney, resulting in the release of inflammatory lymphokines or cytokines which promote injury and fibrosis[27]. In addition, there are studies that have demonstrated that *CCR5* and *CXCR4,* the two main HIV co-receptors, that mediate entry of HIV strains into susceptible

Table 1 Spectrum of renal histology in human immunodeficiency virus in South Africa

Histology	Durban[21]	JHB[20]	Cape Town[22]	JHB[1]
Biopsy numbers	**30**	**99**	**192**	**364**
Classic HIVAN (%)	83	27	24.4	32.7
FSGS		3	32.8	11.3
HIV Immune Complex Disease (%) (mostly with hepatitis B or C co-infection)		21	30.2	11.8
Mesangial proliferative		6		
Membranoproliferative (type I and III) (%)	7			2.7
Lupus-like (%)				4.4
IgA				
Membranous (%)	13.3	13	5.2	7.7
Exudative-proliferative				
HIV TTP/HUS (thrombotic microangiopathy)				
Various glomerulonephropathies (%) (heterogenous group with different aetiologies)	7	41	24	29.4
Minimal change (%)		2		3.3
Immunotactoid				
Amyloidosis				

[1]Adapted from Vermeulen Alda, MMed Research report, University of the Witwatersrand, 2014[83]. JHB: Johannesburg; HIVAN: Human immunodeficiency virus-associated nephropathy; TTP: Thrombotic thrombocytopaenic purpura; HUS: Haemolytic syndrome.

cells, are not expressed by intrinsic renal cells, but are expressed in circulating and infiltrating leukocytes at sites of tubulo-interstitial inflammation[28].

GENETIC PREDISPOSITION TO CHRONIC KIDNEY DISEASE IN PATIENTS OF AFRICAN ANCESTRY

Genetic variation plays an important role in susceptibility to common forms of disease such as diabetes, hypertension and kidney disease, with marked differences in the prevalence and sometimes the presentation, according to ethnicity and ancestry. African Americans have four times the incidence of ESRD when compared to Americans of European ancestry, supporting a causal role for genetics in the aetiology of kidney disease[12,13,29]. These observations led to the use of ancestry informative population variation data to help explain this disparity. In 2008, two groups published papers back to back in Nature Genetics, heralding the discovery of genetic association of markers in the non-muscle myosin heavy chain 9 (*MYH9*) gene on chromosome 22 with non-diabetic ESRD[30] and FSGS[31] in African Americans (Figure 1 and Table 2). Both groups used genome wide admixture mapping approaches in their analysis, showing that increased African ancestry was correlated with increased susceptibility.

The transatlantic slave trade in the 16th to 19th centuries brought in an estimated 12 million individuals from Africa (mainly West Africa) to enslavement in America[32,33] and this was the driver for the introduction of African genetic variation to America. As a consequence of this population relocation, admixture occurred with Native Americans (Amerindians) and Europeans leading to mixed genomic profiles among the group now referred to as African Americans. African Americans have, on average, about 80% African ancestry, although there are regional differences across the country[34]. Differences in allele frequencies of common and rare variants have occurred as a result of random genetic drift, selection and other forces over thousands of years of separation of the ancestral populations, with Europeans having separated roughly 40000 years ago from African populations[35]. Computational approaches take advantage of ancestry informative markers (AIMs), which are single nucleotide polymorphisms (SNPs) that show marked allele frequency differences among the ancestral populations to infer the global ancestry of individuals. Studies utilizing AIMs have shown that American populations with African, Hispanic and Caribbean origins are admixed with varying substantial components of African continental ancestry[36]. This effect of admixture helped in identifying genetic regions that affect one ancestral population and not others, which drive phenotypic associations[37] and can be measured using mapping by admixture linkage disequilibrium (MALD), which quantifies the degree of ancestry of each locus[37-39]. MALD studies were used to identify genomic regions where admixed African American patients with CKD had an excess of African genomic markers compared to unaffected individual controls[30,31]. These studies identified CKD susceptibility loci in African Americans localised to a specific genomic region on chromosome 22q12 that contains more than 21 genes, and proceeded to pinpoint the association with non-diabetic and hypertensive CKD, to markers in non-muscle myosin heavy chain 9 (*MYH9*) gene.

The *MYH9* gene was an excellent biologically plausible candidate as it has a direct link to the structure of podocytes since it codes for a 1960 amino acid protein (Myosin IIA) expressed in the podocytes and widely-distributed cellular motor protein that is essential for cytoskeleton rearrangement, cell motility, division, and

Table 2 Summary of the studies of *MYH9* and *APOL1* variants

Year	Population (ancestry)	Disease	Variant	Freq.	OR (95%CI)	Ref.
2008	African Americans	Hypertensive ESRD	*MYH9* E1	0.67	1.9 (1.25-2.87)	Kopp *et al*[31]
		HIVAN	*MYH9* E1	0.67	5.3 (2.40-12.90)	
		FSGS	*MYH9* E1	0.67	4.5 (2.92, 7.19)	
	European Americans	T2DM ESRD	*MYH9* E1	0.04	NS	
		FSGS	*MYH9* E1	0.04	9.7 (1.07, 463)	
2008	African Americans	Hypertensive ESRD	*MYH9* E1	0.3	2.1 (1.56, 2.74)	Kao *et al*[30]
		Non-diabetic ESRD	*MYH9* E1	0.3	2.2 (1.73, 2.73)	
		FSGS	*MYH9* E1	0.3	3.7 (2.11, 6.34)	
2009	African Americans	Hypertensive ESRD	*MYH9* E1	0.75	2.4 (NS)	Freedman *et al*[47]
		Non-diabetic ESRD	*MYH9* E1	0.76	2.5 (NS)	
2009	African Americans	T2DM ESRD	*MYH9* E1	0.67	1.4 (NS)	Freedman *et al*[44]
2010	African Americans	Non-diabetic ESRD	*MYH9* E1	NS	2.0 (1.37, 2.92)	Behar *et al*[46]
	Hispanic Americans	Non-diabetic ESRD	*MYH9* E1	NS	3.7 (1.67, 8.20)	
2010	American Indians	Kidney dysfunction	*MYH9* SNPs	0.43	1.04 (0.79, 1.36)	Franceschini *et al*[43] Strong Heart Family Study
2010	African Americans	Non-diabetic ESRD	*APOL1* G1	0.46	4.86 (2.35, 10.06)	Tzur *et al*[29]
	Hispanic Americans	Non-diabetic ESRD	*APOL1* G1	0	15.48 (4.00, 60.00)	
2010	African Americans	Hypertensive ESRD	*APOL1* G1/G2	0.41/0.21	7.3 (5.60, 9.50)	Genovese *et al*[13]
		FSGS	*APOL1* G1/G2	0.47/0.25	10.5 (6.0, 18.4)	
2011	African Americans	HIVAN	*APOL1* G1/G2	0.54/0.28	29.2 (13.10, 68.50)	Kopp *et al*[12]
		FSGS	*APOL1* G1/G2	0.55/0.25	16.9 (11.00, 26.50)	
2014	South African blacks	HIVAN	*MYH9* E1	0.83	2.10 (0.07-60.99)	Kasembeli *et al* (Unpublished observations)
			APOL1 G1/G2	0.56/0.34	89.10 (17.68, 911.72)	

NS: Not stated; SNPs: Single nucleotide polymorphisms; OR: Odds ratio; Freq: Frequencies; HIVAN: Human immunodeficiency virus-associated nephropathy; FSGS: Focal segmental glomerulosclerosis; T2DM: Type 2 diabetes mellitus; ESRD: End stage renal disease; APOL1: Apolipoprotein L1; MYH9: Non-muscle myosin heavy chain 9.

cell-cell adhesion[40]. As a result of these observations, researchers concluded that *MYH9* variants that are associated with susceptibility to CKD likely play a causal role in disease pathology[41]. The high frequency of the *MYH9* associated haplotypes in African populations led to speculations of selection in Africa (Figure 2)[42]. The ethnic specificity of the association was explored with different phenotypes and different populations (Table 2) and subsequent studies provided evidence for a contribution of *MYH9* variants in early stages of CKD as well as diabetic and hypertensive-related CKD in both African Americans and Europeans, but not in Native Americans[43-45]. However, biopsy-proven forms of CKD were lacking in some of these studies and therefore, as in the case of diabetic and hypertensive nephropathies, researchers suggested that the association with *MYH9* could also reflect the presence of non-diabetic and non-hypertensive CKD. This hypothesis was supported by association with *MYH9* SNPs that were strongly associated with these types of CKD[46].

The *MYH9* SNPs with the strongest associations were categorised into three haplotype groups termed as E, S and F[42,46] and E1 was defined as the risk haplotype (rs4821480, rs2032487, rs4821481 and rs3752462), being highly associated with CKD in individuals of African descent. E1 was then further found to be highly distributed in Africa as compared to other regions of the world (Figure 2)[42]. The *MYH9* E1 haplotype explained nearly the entire excess burden of major forms of CKD in African Americans with attributable risks of 100% and 70% for HIVAN and FSGS, respectively, and a significant percentage for hypertensive nephrosclerosis risk[31,40,47]. However, despite major scientific efforts, including *MYH9* re-sequencing experiments and detailed-intense genotyping, no mutations with a clear predicted functional effect could be identified that would impact kidney function and the field began to shift toward exploring neighbouring genes on chromosome 22.

Two research groups re-analyzed the chromosome 22q12 genomic region using data from International HapMap and 1000 Genomes Projects[29,48]. The data from these studies played a vital role in the discovery of candidate SNPs in the neighbouring apolipoprotein L1 (*APOL1*) gene, approximately 20 kb downstream from the 3' end of *MYH9,* that were statistically powered to explain an increased risk of CKD in individuals of African ancestry[13,29]. The studies yielded 7479 SNPs, four of which were non-synonymous mutations in the coding region of the genes in high linkage disequilibrium with the *MYH9* E1 risk haplotype. Two of these (rs73885319 and rs60910145) were missense mutations in the last exon (exon 7) of the *APOL1* gene, which result in amino acid substitutions: Ser342Gly and Ile384Met. These two missense mutations are referred to as the G1 alleles since they were in almost complete linkage disequilibrium (r^2 = 1.0) with each other, and are both highly associated with CKD susceptibility. Another SNP, rs71785313, was also found in exon 7 of *APOL1* and represents a six base pair deletion resulting in loss of two amino acids (Asn388-Tyr389del), and is referred to as the G2 allele. These three codon-changing variants in the *APOL1* gene, encoding apolipoprotein L1, were found to be in strong association with HIVAN odds

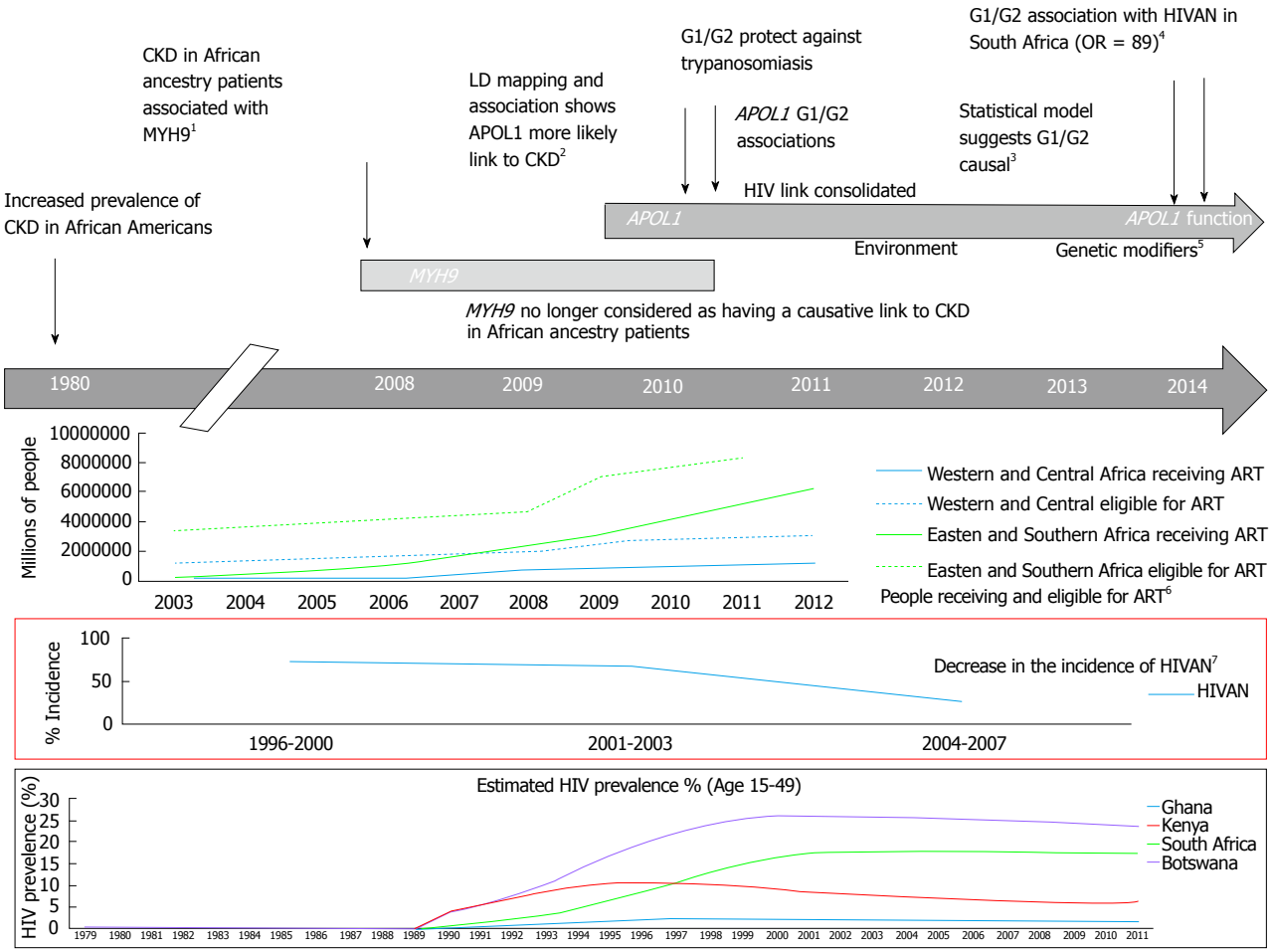

Figure 1 Historical timeline reflecting the discovery of genetic association to chronic kidney disease in populations with African ancestry. [1]Adapted from Kopp *et al*[31] and Kao *et al*[30]; [2]Adapted from Freedman *et al*[49], Genovese *et al*[13], Tzur *et al*[29]; [3]Adapted from Genovese *et al*[84]; [4]Adapted from Kasembeli *et al* (2014 unpublished observations); [5]Adapted from Freedman *et al*[64]; [6]Adapted from UNAIDS report on global AIDS epidemic[18]; [7]Adapted from USRDS 2012 Annual Data Report. APOL1: Apolipoprotein L1; MYH9: Non-muscle myosin heavy chain 9; ART: Antiretroviral therapy; CKD: Chronic kidney disease.

ratio (OR = 29), FSGS (OR = 17) and ESRD (OR = 7) in African Americans, for homozygotes or compound heterozygotes carrying two risk alleles[12,13,29]. They were also absent in individuals of European ancestry but common in African populations. The G1 and G2 alleles were strongly associated with the risk for CKD than the previously examined *MYH9* E1 risk haplotype in a sample of individuals of African ancestry. They are in perfect negative linkage disequilibrium, never occurring on the same parental chromosome, suggesting that these variants arose independently and due to their proximity and high linkage disequilibrium, they have remained mutually exclusive in almost all haplotypes observed[12,13]. The historical timeline reflecting the discovery of genetic association to CKD in populations with African ancestry is shown in Figure 1. The link with increased susceptibility to kidney dysfunction in the presence of HIV infection[15,16] is now well established and the association of *APOL1* risk alleles with HIVAN is of particular concern in sub-Saharan Africa, where the risk allele frequency is high (Figure 2). *APOL1* association with CKD and the postulated mechanism of action and the functional role of *APOL1* in kidney disease are explored further in the next sections.

APOL1 ASSOCIATION WITH CKD IN INDIVIDUALS OF AFRICAN ANCESTRY

The coding variants (G1 and G2) are suggested to be causally related to CKD and provide an explanation for selection of *APOL1*-associated CKD risk polymorphisms as a protective measure against Trypanosomiasis, an infectious disease that was common in Africa. A study by Genovese *et al*[13], (2010), comparing 205 African Americans with biopsy-proven FSGS with 180 African Americans without kidney disease as controls was performed, using SNPs from the 1000 Genomes Project belonging to Yoruba as proxies to the African American population. These SNPs revealed evidence of a strong association within a 10 kb region in the exon 7 of the *APOL1* gene. The strong signal was found to be at non-synonymous coding variants, rs73885319 (S342G) and rs60910145 (I384M) which were in perfect linkage disequilibrium (r^2 = 1.0). The frequency of these variants was 52% in patients and 18% in

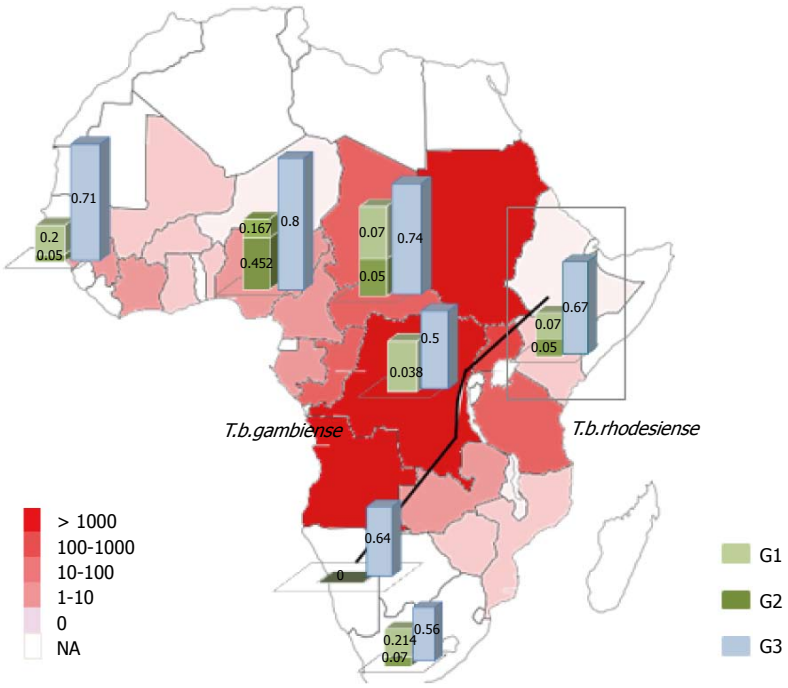

Figure 2 Distribution of Human African Trypanosomiasis (T.b *gambiense* and T.b *rhodesiense*), MYH9 E1 and APOL1 G1 and G2 risk alleles in Africa[12,42]. The frequency of distribution of *APOL1* risk variants in Africa are represented by bar charts and overlap the areas distribution of Human African Trypanosomiasis. The numbers reflect the reported cases of Trypanosomiasis from the WHO, 2010. T.b: *Trypanosoma brucei*.

controls. They further controlled for the effects of these two variants and found a second strong *APOL1* signal, 12 base pairs from I384M. This signal is a 6-base pair deletion represented by rs71785313 which removes two amino acid residues (Asparagine-N and Tyrosine-Y). The frequency of this variant was 23% in patients and 15% in controls. The odds ratio (OR) of association for carrying at least one risk (G1-G2) allele was 10.5 (95%CI: 6.0-18.4). Controlling for both G1 and G2 did not result in significant association with *MYH9.* However, controlling for *MYH9* variants maintained significant *APOL1* signal at G1 and G2. Kopp *et al*[12], (2011), in a larger FSGS and HIVAN cohort confirmed the association but this time, a greater association was observed in HIVAN (OR = 29.2, 95%CI: 13.1-68.5, $P = 6 \times 10^{-22}$) compared to FSGS (OR = 16.9, 95%CI: 11.0 to 26.5, $P = 1.3 \times 10^{-48}$). The authors reported that 50% of African Americans with two *APOL1* risk alleles would develop HIVAN if not on antiretroviral therapy. A study in an indigenous South African black cohort showed an independent high association with HIVAN susceptibility for these G1 and G2 variants of 89-fold, 95%CI: 17.68-911.72, $P = 1.2 \times 10^{-14}$ (Kasembeli *et al*, unpublished observations). In all these studies, there was strong evidence for a contribution of *APOL1* variants to CKD (Figure 1 and Table 2).

The observed mode of inheritance of the *APOL1* risk variants was fully recessive in both FSGS and HIVAN cohorts. However, there have been instances where mild dominant effects (OR of 1.26 for one risk allele and OR of 7.3 for 2 risk alleles) have been observed in larger cohorts of hypertensive-associated CKD and geographically matched control subjects[13]. We therefore cannot fully exclude this mild dominant inheritance because this could be explained by yet "undiscovered variants" or by sporadic mutations that might occur in patients with recessive model. Furthermore, there could be a possibility of additional rare variants in *APOL1, MYH9* or other neighbouring genes that could be involved in CKD susceptibility since extended linkage disequilibrium exists in this region as a result of selective pressure[49].

POSITIVE SELECTION FOR *APOL1*-ASSOCIATED CKD RISK VARIANTS AS A RESULT OF TRYPANOSOMIASIS EPIDEMIC IN AFRICA

It has been shown that harbouring of *APOL1* risk variants protects against Trypanosomiasis disease [Human African Trypanosomiasis (H.A.T)], otherwise known as sleeping sickness, that was epidemic in Africa many years ago and still affects millions of Africans today. This effect explains the high frequencies of these variants in the general African American and indigenous African population (Figure 2)[12,42]. The *APOL1* G1 and G2 alleles show distinct distributions among various African and African-derived populations and evidence shows these mutations to be maintained in these populations. In Yoruba from Nigeria in West Africa, the frequency is greater than 45% for G1 (Figure 2) while in African Americans the G1 frequency is approximately 20%. The battle between host and pathogen (*Trypanosoma* species), resulted in development of *APOL1* mutations (G1 and G2) that provided positive selective advantage to carriers at the expense of increased risk for CKD (Figure 3).

There are three main *Trypanosoma* species; *Trypanosoma brucei brucei, Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*.

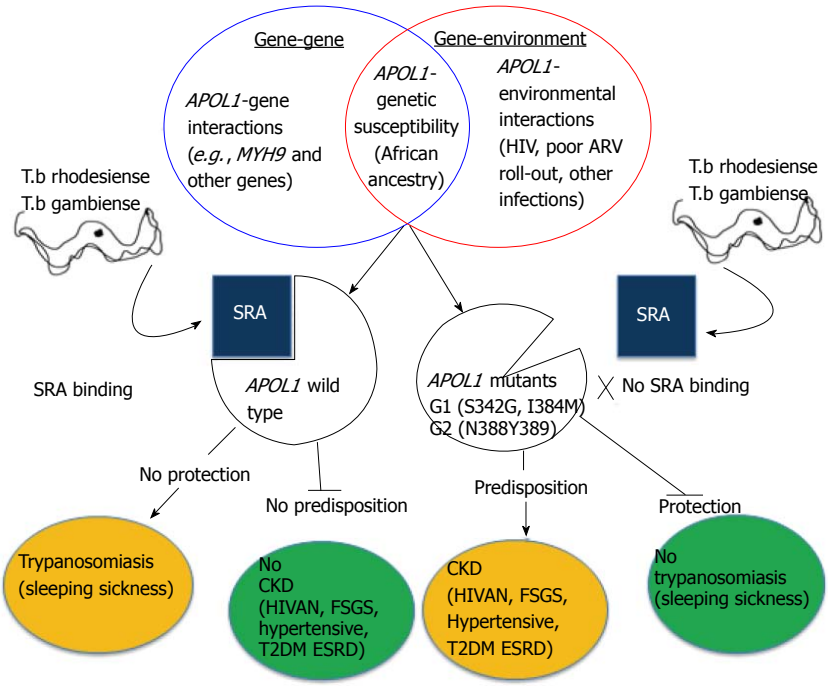

Figure 3 Gene-Gene, Gene-Environment steering contribution to APOL1 associated CKD and the positive selection of APOL1 associated CKD variants as a result of Trypanosomiasis. SRA: Serum resistant associated protein; HIV: Human immunodeficiency virus; T.b: Trypanosoma brucei; APOL1: Apolipoprotein L1; MYH9: Non-muscle myosin heavy chain 9; HIVAN: Human immunodeficiency virus-associated nephropathy; FSGS: Focal segmental glomerulosclerosis; T2DM: Type 2 diabetes mellitus; ESRD: End stage renal disease; CKD: Chronic kidney disease.

Trypanosoma brucei brucei is unable to infect humans because of the complex, trypanolytic factor (TLF) comprising of apolipoprotein L1, high density lipoprotein (HDL) particles, haptoglobin-related protein and apoliprotein A1 that is present in the human serum. This confers innate protection against *Trypanosoma brucei brucei.* However, both *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* have evolved a mechanism to evade lysis by the TLF leading to infection, hence sleeping sickness (Figure 3)[50,51]. Apolipoprotein L1, a protein product of *APOL1* gene is usually part of TLF circulating in the blood. The *APOL1* gene is a member of the *APOL* gene family which is composed of six genes in humans (*APOL1, APOL2, APOL3, APOL4, APOL5* and *APOL6*), grouped within 619kb on chromosome 22[52]. This protein has five functional and structural domains: a secretory domain, pore forming domain, B-cell lymphoma 2 homology domain 3, membrane addressing domain, leucine zipper domain and serum resistant-associated interacting domain (SRA), listed from the N-terminal to C-terminal respectively.

The trypanolytic function of apolipoprotein L1 is the most widely studied function of apolipoprotein L1[13,53]. The secretory domain allows it to be expressed as a circulating protein, which makes it the only circulating *APOL* protein[53-56]. *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* evade the TLF lysis by expressing SRA protein that binds to the C-terminus domain of the apolipoprotein L1, in the process neutralizing its lytic activity[57,58]. However, *APOL1* variants G1 and G2, powerfully associated with CKD arose to modify the C-terminus SRA binding site of the *APOL1* gene resulting in a mutated apolipoprotein L1 that evades neutralization by the *Trypanosoma* SRA protein[58]. By so doing, apolipoprotein L1 exercised its trypanolytic activity, conferring an adaptive advantage in the endemic regions of Africa (Figure 3). This explains the distribution of G1 and G2 risk variants in Africa.

Genovese *et al*[13] reported that both G1 and G2 variants restored the lytic activity of human serum and this provides the selective advantage to carriers of two *APOL1* risk variants against sleeping sickness. These findings corroborated the evidence of the recent evolution of *APOL1* which occurred in the last 10000 years and also suggesting that these variants were selected for within Africa because they conferred protection against lethal trypanosomiasis while at the same time increasing susceptibility to CKD (Figure 3)[49]. A more recent study in a South African black population (Kasembeli *et al*, unpublished data, 2014) has found the odds to have almost doubled. The prevalence of HIVAN in Africa is variable, 24%-83% in South Africa, while in the United States, it is highest in the African American population (15.5%). This is eight-fold greater than that of HIV-infected European Americans[59]. This high prevalence of HIVAN among individuals of African ancestry could be, not only as a result of high frequencies of *APOL1* risk variants, but also the prevalence of HIV-1 subtypes circulating in Africa. For instance, HIV-1 subtype C is highly virulent, accounting for approximately 50% of all HIV infections worldwide and 98% of HIV infections in South, West and East Africa, with corresponding higher viral loads[60,61]. Another more important reason could be because sub-Saharan African countries are resource limited, and therefore roll-out of antiretroviral therapy (ART) may have been delayed, giving more room for the development of HIVAN among individuals carrying two *APOL1* risk variants. An effective roll-out of ART has been shown to reduce the occurrence of HIVAN[22,62,63]; Figure 1. Therefore, there is need for HIV screening,

surveillance, and strict implementation of World Health Organization (WHO) recommendations for ART initiation to reduce the burden of HIVAN and other forms of HIV-related CKD in Africa. At present, WHO ART guidelines 2013 for the treatment of HIV infection in Africa suggest that ART be instituted for individuals with WHO clinical stage 3 and 4 disease and in all HIV positive individuals with CD4 counts < 500 cells/μL.

GENE-ENVIRONMENTAL MODIFIERS OF *APOL1* SUSCEPTIBILITY

APOL1-environmental interactions play a vital role in CKD susceptibility in individuals of African ancestry (Figure 3). These could be social demographic status, lifestyle or presence of other communicable diseases and most importantly, HIV infection coupled with poor ART roll-out. Environmental exposure to HIV was initially thought to trigger HIVAN. However, observations in African American family studies showed that relatives of HIVAN patients, in the absence of HIV infection, suffered ESRD due to other aetiologies[64]. This illustrates that there could be other environmental factors that drive the process. As described, patients with HIVAN harbouring two *APOL1* risk alleles that are untreated or undertreated for HIV infection will suffer rapid progression to ESRD[12]. In striking contrast, HIV patients without the *APOL1* risk genotype are protected from HIVAN even when HIV infection is not properly controlled. This effect is well illustrated in the Ethiopian population who appear to be protected from HIVAN since their genomes lack the *APOL1* risk variants. A study by Behar *et al*[65], in HIV infected individuals of Ethiopian origin reported complete absence of HIVAN. This led to the emphasis of skewed ethnic distribution, inter-individual variability and/or familial aggregation of HIVAN suggesting that host genetic susceptibility plays a major contributing factor. This study genotyped 676 African individuals from 12 populations, including 304 Ethiopians, for mutations in the *MYH9* and *APOL1* risk clusters. The frequency of the G1 and G2 *APOL1* risk variants was zero[65]. However, there was an increasing trend in frequency of the risk variants proceeding towards the west and south in Africa (Figure 2). This led researchers to conclude that the risk of developing HIVAN is not a African-wide problem but rather restricted to Western, Central and Southern Africa, and absent in regions of the North and North-East parts of Africa including Ethiopia. Since sleeping sickness was not an epidemic in Southern Africa, a possible explanation for the increase in prevalence of *APOL1* risk variants could be the result of migration of the bantu-speaking populations from West Africa and East Africa[66,67].

In the United States there has been a steady decline in the incidence of HIVAN with the introduction of HAART, in spite of stable frequencies of the risk variants[68]. Risk factors for progression to ESRD in HIVAN are severity of renal dysfunction, percentage of sclerotic glomeruli[25,69], lack of viral suppression[26,70], 2 *APOL1* risk alleles[63,71], while use of renin angiotensin system blockers were reported to be protective[25]. HIV-infected individuals with non-HIVAN pathology and two *APOL1* risk alleles had an almost 3-fold risk of ESRD, in spite of effective ART-suppression of viral load and use of renin-angiotensin aldosterone blockers; baseline kidney function was the strongest predictor of progression to ESRD in this study[71]. Investigators reviewing the African American study on Kidney Disease and Hypertension (AASK) and The Chronic Renal Insufficiency Cohort (CRIC) found that *APOL1* risk variants in black patients were associated with higher rates of ESRD and progression of CKD[72].

Thus HIV is considered a risk factor for HIVAN when presented with the appropriate genetic susceptibility. Either genetic risk or viral infections alone do not cause the kidney disease. Instead it is the geneenvironment interaction that is fundamental for the pathogenesis. The rapidly changing natural history of *APOL1*-associated HIVAN provides further support that HIV is an environmental risk factor[68]. Additional viral environmental modifiers have been proposed. The John Cunningham (JC) polyoma virus has been shown to maintain a reservoir in the uroepithelium of the kidney after infection and has been proposed to interact with the genetic risk posed by *APOL1* variants[73]. Divers *et al*[73] studied the relationship between the JC virus and genetic risk for kidney disease hypothesising that the presence of the *APOL1* risk variants may predispose individuals to JC infection and that this second hit may act as an additional environmental factor increasing kidney disease risk. However, paradoxically, the reverse scenario was observed where the presence of the high risk *APOL1* variants in the presence of JC virus resulted in less kidney disease. The JC virus in the kidney was postulated to either protect against other nephropathic viruses or alter cellular function as protection against other sources of glomerular injury.

GENE-GENE INTERACTIONS AS MODIFIERS OF *APOL1*-ASSOCIATED NEPHROPATHY

Whilst nephrology research in African ancestry populations has been hampered by the lack of large genome wide- association studies, a number of gene-gene interaction studies have been conducted on pooled GWAS data in non-diabetic ESRD in African Americans and non-nephropathy controls. Results of a gene-gene interaction analysis identified several SNPs that interacted with *APOL1* risk variants (Figure 3)[74]. *MYH9* has been shown to be one of the genes linked to *APOL1* to cause CKD susceptibility. Other studies have also shown a possibility of other genes interaction with *APOL1* gene. In a replication study, eleven SNPs were validated and three genes, podocin (NPHS2; rs16854341); serologically defined colon cancer

antigen 8 (SDCCAG8; rs2802723) and SNP "near bone morphogenetic protein 4" (BMP4; rs8014363) were significant. These interactions were quantified and all show effects on *APOL1* association[75,76]. These three genes show expression in podocytes and are linked to renal disease characterised by FSGS. It has thus been postulated that they play a role in inducing podocyturia and glomerular damage.

APOL1-ASSOCIATED CKD: THE FUTURE

Since there is evidence of *APOL1* association with non-diabetic forms of CKD and the role of selection in the increase in frequencies of the risk variants, it is necessary to move beyond the statistical tests of association to molecular cellular characterization to evaluate the effects of these risk variants in CKD. It has been postulated that cellular and physiologic activities of apolipoprotein L1 include involvement in autophagic and apoptosis pathways[52,77-79]. There is a general agreement that *APOL1* expression occurs in podocytes[80]. But whether there is apolipoprotein L1 expression in the tubular cells, glomerular endothelial cells, and the tunica intima and media of the renal blood vessels is uncertain. Currently, there is no definitive mechanism by which *APOL1* variants cause kidney injury but several possibilities have been proposed. Firstly, apolipoprotein L1 isoform expressed in the kidney cells may be retained in the cells and cause cell destruction *via* the apoptotic pathway since they share structural and functional similarities with proteins from the BcL2 family[77,78]. Secondly, *APOL1* as part of TLF, is directed to lysosomes to induce programmed cell death *via* the autophagic response[13,56,81]. Thirdly, circulating apolipoprotein L1 may also be important in the pathogenesis of CKD since the presence of G1 and G2 could lead to dysfunctional HDL particles leading to inflammation of vascular endothelial cells, with arteriolar nephrosclerosis[49]. There is a proven race specific relationship between *APOL1* genotype and HDL cholesterol concentration and kidney function[82]. In Han Chinese and European American populations, there was a higher HDL level in association with higher eGFR. The inverse association was observed in West Africans and African Americans. However, a significant effect was observed only in African Americans with *APOL1* risk variants (but not in West Africans). These observations have led to the view that the mechanism underlying *APOL1* nephropathy most likely involves HDL cholesterol. In addition, circulating *APOL1*, may be available for uptake by podocytes after passage across the glomerular filtration barrier and exercising their effect on the podocytes. Future studies should define the role of *APOL1* in the pathogenesis of kidney disease.

LESSONS LEARNED FROM CKD POPULATION GENETICS

The remarkable advances in molecular genetics have enabled researchers to unravel the underlying genetic susceptibility to kidney disease in African Ancestry populations. Identification of region of 22q12.3 using MALD studies and identification of *APOL1* risk variants have raised the possibility of a personalised approach to treat several forms of kidney disease that are prevalent in African populations. As regards HIVAN, the priority for the African continent should be targeting of the modifying trigger of the disease, HIV infection, through effective treatment and prevention campaigns. Greater advances in understanding the mechanisms underlying *APOL1* pathogenesis, the identification of modifiable environmental factors and interacting genes offer the promise of novel preventive, prognostic and therapeutic measures to treat *APOL1* associated forms of kidney disease in the genetically susceptible and therefore vulnerable African descent individual.

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Species differences in regulation of renal proximal tubule transport by certain molecules

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Abstract

Renal proximal tubules (PTs) play important roles in the regulation of acid/base, plasma volume and blood pressure. Recent studies suggest that there are substantial species differences in the regulation of PT transport. For example, thiazolidinediones (TZDs) are widely used for the treatment of type 2 diabetes mellitus, but the use of TZDs is associated with fluid overload. In addition to the transcriptional enhancement of sodium transport in distal nephrons, TZDs rapidly stimulate PT sodium transport *via* a non-genomic mechanism depending on peroxisome proliferator activated receptor γ/Src/epidermal growth factor receptor (EGFR)/MEK/ERK. In mouse PTs, however, TZDs fail to stimulate PT transport probably due to constitutive activation of Src/EGFR/ERK pathway. This unique activation of Src/ERK may also affect the effect of high concentrations of insulin on mouse PT transport. On the other hand, the effect of angiotensin Ⅱ (Ang Ⅱ) on PT transport is known to be biphasic in rabbits, rats, and mice. However, Ang Ⅱ induces a concentration-dependent, monophasic transport stimulation in human PTs. The contrasting responses to nitric oxide/guanosine 3',5'-cyclic monophosphate pathway may largely explain these different effects of Ang Ⅱ on PT transport. In this review, we focus on the recent findings on the species differences in the regulation of PT transport, which may help understand the species-specific mechanisms underlying edema formation and/or hypertension occurrence.

Key words: Renal proximal tubule; Thiazolidinediones; Peroxisome proliferator activated receptor γ; Insulin; Angiotensin Ⅱ; Nitric oxide

Core tip: Renal proximal tubule (PT) transport is essential for the regulation of plasma volume and blood pressure. Several species differences are found as to the stimulatory effects of thiazolidinediones, insulin, and angiotensin Ⅱ on PT sodium transport. This review focuses on this topic, which may be relevant to species-specific mechanisms underlying edema formation and/or hypertension occurrence.

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INTRODUCTION

The kidney plays an essential role in the homeostatic regulation of electrolytes, acid-base and plasma volume in the body. Some species differences are known to exist in the distribution patterns of solute transporters as well as the hormonal actions in distal nephron[1,2]. In proximal tubules (PTs), on the other hand, there are no major species differences in the distribution patterns and functions of main sodium transporters such as the apical Na^+/H^+ exchanger type 3 NHE3 and the basolateral electrogenic Na^+-HCO_3^- cotransporter NBCe1[3,4]. While the transport stoichiometry of NBCe1 was reported to be $1Na^+$ to $3HCO_3^-$ in rat PTs *in vivo*[5], it was found to be $1Na^+$ to $2HCO_3^-$ in rabbit PTs *in vitro*[6]. However, this difference was turned out to be due to the differences in experimental conditions[7,8].

Nevertheless, recent studies identified substantial species differences in the regulation of PT transport, which might be important for uncovering the species-specific causes for edema and/or hypertension. Species differences in renal physiologic and/or metabolic responses may be also potentially important for understanding mechanisms for the different effects of several agents against diabetic nephropathy between animal models and human patients. For example, inhibition of advanced glycosylation end products by aminoguanidine or pyridoxamine was reported to be protective in rodent models of diabetic nephropathy[9-12]. However, these agents were not found effective in initial human trials in diabetic patients[13,14]. Effectiveness of a selective PKC-inhibitor Ruboxistaurin found in rodent models of diabetic nephropathy[15,16] was also not confirmed in human type 2 diabetic patients[17]. Indeed, rodent models of diabetic nephropathy exhibit proteinuria and pathological glomerular changes, but do not exhibit progressive renal failure[18]. Dogs, pigs and other non-human primates have been used for toxicology testing. However, ideal animal models have not been established for revealing drug side effects[19]. We focus on species differences in the regulation of PT transport in this review.

EFFECTS OF THIAZOLIDINEDIONES

Thiazolidinediones (TZDs) activate a nuclear receptor peroxisome proliferator activated receptor γ (PPARγ), thereby improving insulin resistance through the transcriptional modulation of the relevant genes[20]. TZDs have been widely used for the treatment of type 2 diabetes. However, fluid retention is a serious clinical problem, which may exert an adverse effect on the cardiovascular system[21].

Initially, PPARγ-mediated transcriptional enhancement of the epithelial Na channel ENaCγ subunit in collecting ducts was proposed to be a main mechanism for TZDs-induced volume expansion[22,23]. Subsequent analysis in renal principal cell culture models, however, did not support the central role of ENaC in TZDs-induced volume expansion[24]. Moreover, TZDs-induced volume expansion was preserved in collecting ducts specific ENaC deficient mice[25]. These results indicate that mechanism(s) other than the activation of ENaC in collecting ducts may be also involved in TZDs-induced volume expansion. Interestingly, Muto and colleagues reported the rapid stimulation of NBCe1 activity by troglitazone in isolated rabbit PTs[26]. Because TDZs were reported to stimulate PT transport also in humans[27], we performed the detailed analysis on the mechanism of TZDs-induced stimulation of PT sodium transport.

We found that TZDs rapidly induced transport stimulation within minutes in isolated PTs from rabbits, rats, and humans. Our subsequent analysis revealed that TZDs-induced PT transport stimulation was dependent on the non-genomic signaling cascade consisting of PPARγ/Src/epidermal growth factor receptor (EGFR)/MEK/ERK[28]. Notably, however, TZDs failed to induce transport stimulation in isolated mouse PTs, despite the definite expression of PPARγ in these tubules. We speculated that some factor(s) specific for mouse PTs might interfere with TZDs-induced rapid signaling. Consistent with this view, Kiley *et al*[29] reported the constitutive activation of Src/EGFR pathway that was found only in mouse PTs. This unique constitutive activation of Src/EGFR, which could potentially explain the different effects of exogenous EGF on unilateral ureteral obstruction in rats and mice[30], was confined to mouse PTs and not found in other mouse tissues[29]. Because TZDs rapidly activated the Src/ERK pathway in kidney cortex of rabbits and rats but not mice, we concluded that the constitutive activation of Src/EGFR prevented TZDs-induced transport stimulation in mouse PTs[28]. Consistent with this conclusion, the constitutive activation of Src was also reported to interfere with the non-genomic signaling of another nuclear receptor for estrogen[31].

Most likely, TZDs-induced volume expansion is multifactorial, depending on both genomic and non-genomic actions of PPARγ on tubular sodium transport. One of potential targets of non-genomic actions of PPARγ may be distal nephron, where WNK kinases regulate the balance between renal NaCl absorption and K^+ secretion[32,33]. WNK4 is also known to affect Cl^- transport in extrarenal epithelia[34]. It remains to be determined whether PPARγ regulates the WNK kinase system.

EFFECTS OF INSULIN

Insulin is thought to enhance renal sodium retention by activating sodium transport in several nephron segments[35]. In PTs, insulin stimulates the major sodium transporters NHE3, NBCe1, and the Na^+/K^+-ATPase[36-38]. In isolated rabbit PTs, Baum found that insulin, at the concentrations between 10^{-10} mol/L and 10^{-8} mol/L, dose-dependently stimulates volume and bicarbonate absorption[39]. Recently, we also found the similar dose-dependent stimulation of NBCe1 by up to 10^{-8} mol/L insulin in isolated PTs from rats

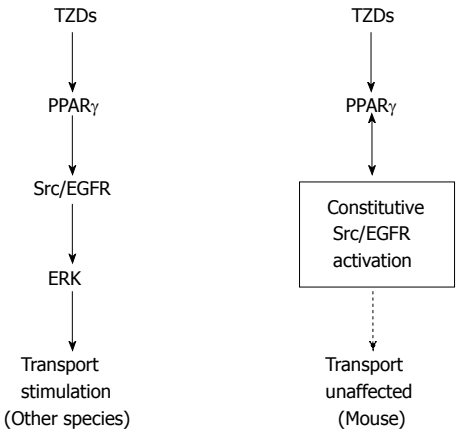

Figure 1 Species differences in thiazolidinediones effects on proximal tubule transport. Non-genomic activation of ERK by TZDs *via* PPARγ/Src/EGFR pathway is suppressed in mouse proximal tubule probably due to constitutive activation of Src/EGFR. TZDs: Thiazolidinediones; PPAR: Peroxisome proliferator activated receptor; Src: Sarcoma; EGFR: Epidermal growth factor receptor; ERK: Extracellular signal-regulated kinase.

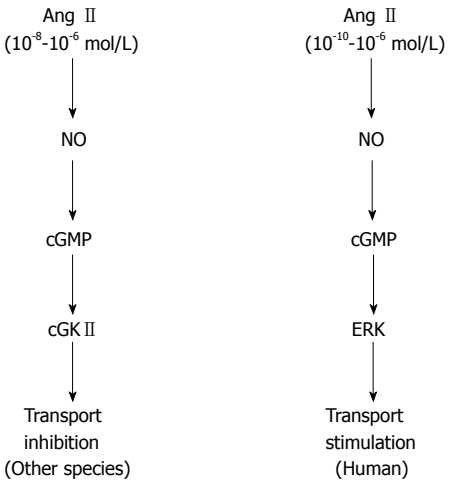

Figure 2 Species differences in angiotensin Ⅱ effects on proximal tubule transport. In human, NO/cGMP pathway mediates the concentration-dependent stimulatory effect of Ang Ⅱ. In other species, NO/cGMP pathway mediates the inhibitory effect of high concentrations of Ang Ⅱ. Ang Ⅱ: Angiotensin Ⅱ; NO: Nitrox oxide; cGMP: Guanosine 3',5'-cyclic monophosphate; cGKⅡ: cGMP-dependent kinase Ⅱ; ERK: Extracellular signal-regulated kinase.

and humans[40]. This stimulatory effect of insulin on PT sodium transport, which is completely preserved in insulin resistant rats and humans, may play an important role in the pathogenesis of hypertension associated with insulin resistance[40].

Insulin actions are initiated by the activation of tyrosine kinase in the cell membrane receptor, which induces a series of phosphorylation in multiple insulin receptor substrates (IRSs). The two major IRS proteins, IRS1 and IRS2 often mediate distinct insulin actions, and defects at the levels of IRS1 or IRS2 may be responsible for the occurrence of selective insulin resistance in several tissues[41]. We have shown that IRS2 but not IRS1 mediates the stimulatory effect of insulin on PT transport in both mice and rats. Moreover, we have confirmed that insulin activates Akt in kidney cortex of both mice and rats[40,42]. We found that up to 10^{-9} mol/L insulin stimulated mouse PT sodium transport. Unlike in rats and humans, however, the higher concentrations of insulin (*i.e.,* more than 10^{-8} mol/L) failed to stimulate PT transport in mice[42]. In isolated mouse collecting ducts, by contrast, insulin at the high concentrations up to 10^{-7} mol/L was reported to activate ENaC[43]. Importantly, insulin is known to activate both Akt and ERK pathways, and these two pathways are interconnected with each other[44,45]. It is therefore possible that the constitutive activation of Src/ERK may somehow interfere with the effects of high concentrations of insulin in mouse PTs.

EFFECTS OF ANGIOTENSIN Ⅱ

The stimulation of PT sodium transport by Angiotensin Ⅱ (Ang Ⅱ) may be essential for Ang Ⅱ-induced hypertension[46,47]. Actually, however, Ang Ⅱ is known to regulate PT transport in a biphasic way: transport is stimulated by low (picomolar to nanomolar) concentrations of Ang Ⅱ, but inhibited by high (nanomolar to micromolar) concentrations of Ang Ⅱ. This biphasic regulation of PT transport by Ang Ⅱ has been confirmed in rats, mice, and rabbits[48-51]. Regarding the receptor subtype(s) responsible for the biphasic effects of Ang Ⅱ, controversial results had been reported[52-54]. However, the analyses on isolated PTs from type 1A Ang Ⅱ receptor (AT_{1A}) deficient mice have clearly shown that AT_{1A} mediates both the stimulatory and inhibitory effects of Ang Ⅱ[49,51].

The stimulatory effect of Ang Ⅱ is dependent on the activation of protein kinase C and/or the decrease in the intracellular cAMP concentration, which may result in the activation of ERK[55,56]. On the other hand, the inhibitory effect of Ang Ⅱ is dependent on the activation of phospholipase A_2/arachidonic acid/5,6-epoxyeicosatrienoic acid (EET) pathway and/or nitric oxide (NO)/guanosine 3',5'-cyclic monophosphate (cGMP) pathway[53,55,57]. Because little had been known about the direct effects of Ang Ⅱ on human PT transport, we tried to clarify this issue using isolated, intact human PTs obtained from nephrectomy surgery.

Surprisingly, we found that the inhibitory effect of Ang Ⅱ is lost in human PTs. Actually, up to 10^{-5} mol/L Ang Ⅱ dose-dependently stimulated human PT transport. In view of high intrarenal concentrations of Ang Ⅱ, these data suggest that Ang Ⅱ may play an even more important role in the regulation of plasma volume and blood pressure in humans than in other species[58].

The detailed analysis revealed that the contrasting responses to NO/cGMP could explain the different modes of PT transport regulation in humans and other species. While the NO/cGMP/ERK pathway mediates the dose-dependent stimulatory effect of Ang Ⅱ in

humans, the NO/cGMP/cGMP-dependent kinase Ⅱ (cGKⅡ) pathway mediates the inhibitory effect of high concentrations of Ang Ⅱ in mice[58]. In cGKⅡ-deficient mice, the inhibitory effect of Ang Ⅱ was lost, but the NO/cGMP pathway failed to stimulate PT transport. These results indicate that the loss of cGKⅡ alone in mice cannot reproduce the NO/cGMP/ERK-dependent stimulatory effect of Ang Ⅱ in humans.

Currently, the reason why the NO/cGMP pathway exerts different effects on PT transport in humans and other species remains unknown. Nevertheless, several previous reports supported the existence of such species differences. For example, renal NO production was enhanced by salt loading in rodents, which might facilitate sodium diuresis and prevent blood pressure elevation[59,60]. Thus, renal NO is thought to induce a natriuretic response to salt loading in rodents[61]. However, renal NO production was not significantly enhanced by salt loading in humans[62,63], and an adaptive role of renal NO to salt loading is less clear in humans[64,65]. In any case, the stimulatory effect of NO/cGMP pathway on PT transport may represent a human-specific target for hypertension.

The NO/cGMP pathway is thought to be inhibitory on sodium transport in thick ascending limb and collecting ducts[61]. Therefore, it will be interesting to examine whether the similar species differences may also exist in the effects of NO/cGMP pathway on these nephron segments. Such knowledge may help understand the mechanism of fluid overload by selective endothelin receptor antagonism[66].

CONCLUSION

In summary, non-genomic stimulation of PT transport by TZDs is uniquely absent in mice probably because of the constitutive activation of Src/EGFR as shown in Figure 1. As shown in Figure 2, on the other hand, the inhibitory effect of Ang Ⅱ is lost in human PTs, where the NO/cGMP pathway is stimulatory unlike in other species. These species differences may be at least partially responsible for the species-specific mechanisms underlying edema formation and/or hypertension occurrence.

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ORIGINAL ARTICLE

Case Control Study

Histopathology of renal asphyxia in newborn piglets: Individual susceptibility to tubular changes

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Informed consent: The research complies with the guidelines for human studies and animal welfare regulations. The authors declare that subjects have given their informed consent.
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Abstract

AIM: To analyze the effects on the kidney of hypoxia-reoxygenation in an experimental model of normocapnic asphyxia.

METHODS: To this end, 40 newborn Landrace/Large-White piglets aged 1-4 d were studied in this work. Hypoxia was induced by decreasing the inspired fiO2 to 0.06-0.08. Animals were resuscitated with different fiO_2 and subdivided into 4 groups: group 1, 2, 3 and 4 received 18%, 21%, 40% and 100% O_2 respectively. Macroscopic examination was carried out to evidence possible pathological features. Tissue sample were obtained from both kidneys. Four or five micron paraffin sections were stained with H-E and PAS stain and examined under an optical microscope.

RESULTS: Pathological changes, mainly affecting tubular cells, were observed in the vast majority of kidneys of asphyxiated piglets. The most frequent tubular changes were: tubular casts (95%), tubular dilatation (87.5%), tubular vacuolization (70%), tubular eosinophilia (52.5%), sloughing (50%), fragmentation of the brush border (50%), oedema (32.5%), apoptosis (15%) and glomerular changes (meningeal cell proliferation, capsular adhesion between the flocculus and Bowman's capsule, glomerulosclerosis and fibrous or cellular crescents associated with collapse of the glomerular tuft). Statistical analysis was carried out on changes observed when the animals were allocated in the 4 groups (χ^2-test 0.05). The statistical analysis showed no evidence of differences regarding kidney lesions among the animals groups.

CONCLUSION: Our data show that renal pathology in newborn piglets is characterized by interindividual variability to hypoxia and is not associated with oxygen

concentration.

Key words: Asphyxia; Kidney; Tubular eosinophilia; Tubular dilatation; Vacuolization; Sloughing; Apoptosis; Brush border fragmentation

Core tip: This work studied pathological renal changes following hypoxia-reoxygenation using an established experimental model of normocapnic asphyxia. Tubular dilatation, vacuolization, tubular eosinophilia, sloughing, fragmentation of the brush border and apoptosis were the most frequent changes detected in proximal tubules. Tubular dilatation, vacuolization and sloughing were the earliest lesions, interstitial oedema and apoptosis the late ones. In newborn piglets undergoing asphyxia, renal pathology was not associated with oxygen concentration used during resuscitation.

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INTRODUCTION

Perinatal asphyxia is generally considered an important aetiological factor at the basis of neonatal mortality and morbidity in survivors, with involvement of all organs, including the kidney[1-3]. The kidney is particularly susceptible to hypoxia[4], since the renal tubular cells are characterized by a high metabolism rate, due to high demand and consumption of oxygen[5-7].

Neonates on the first or second day post-asphyxia, often present with symptoms of acute renal impairment or failure, including oliguria, anuria, oedema, and hyperkalemia[8]. Acute renal injury is a common condition encountered in neonates admitted to Neonatal Intensive Care Units[9,10], occurring in as many as 8% of them, and carrying a mortality rate of around 40%[11,12]. Acute kidney injury is generally considered to be related to severe pathological changes in proximal tubular cells, which are considered the typical diagnostic signs of the disease[13,14].

The most severe renal damage has been described to occur 2 h post-asphyxia. It is mainly attributed to the reperfusion injury of the tubular epithelial cells by free oxidant radicals accumulation and the influx of Ca^{2+}[15]. This overload may activate the cysteine protease calpain in tubular cells, leading to hydrolysis of integrins and cytoskeleton components such as F-actin, talin, alpha-actin, and filamin, resulting in tubular cell membrane damage[16].

Acute renal failure has been reported to cause structural changes in tubular renal cells, including loss of brush border, vacuolization of tubular cells, apoptosis and cast formation of necrotic epithelial cells[6]. In experimental animal models of hypoxia, only minor histological changes, including mild brush border loss and vacuolization of tubular cells, are present in the kidney at 22 h post-peritonitis-induced septic shock in pigs[17]. Piglets subjected to mild hypothermic cardiopulmonary bypass exhibit tubular dilatation, vacuoles, leukocyte infiltration, epithelial destruction and interstitial oedema[18]. In cultured glomerular endothelial cells, hypoxia has been shown to induce apoptosis[19].

Resuscitation strategies modify the relationship between the inhibitors and the factors that promote angiogenesis. In particular, reoxygenation using 100% oxygen of neonatal piglets has been shown to decrease serum levels of angiostatin[20].

On clinical grounds, several questions remain unanswered at the moment: how does asphyxia cause acute renal tubular injury in neonates and what histological change corresponds to tubular dysfunction? What is the role of oxygen concentration used during resuscitation in determining kidney injury?

In the present study we investigated the renal injury caused at the histological level by hypoxia-reoxygenation in an experimental neonatal swine model previously described by our group[21].

MATERIALS AND METHODS

Forty male Landrace/Large White newborn piglets, weighing 2.3-3.8 kg and aged 1-4 d were studied in this work. All experimental piglets came from the same breeding unit. Experimental procedures were previously approved by the General Directorate of Veterinary Services (Permit no. 404/21-04-09)[21].

Briefly, following sedation with 10 mg/kg ketamine and 0.5 mg/kg midazolam, anesthesia was induced with 1 mg/kg propofol and 10 mg/kg fentanyl, administered through a peripheral vein. Hypoxia was induced by decreasing the inspired fiO_2 to 0.06-0.08; resuscitation efforts were carried out according to the Newborn Life Support (NLS) algorithm[1]. Animals were resuscitated with different fiO_2 and subdivided into 4 groups: groups 1, 2, 3 and 4 received 18%-21%-40% and 100% O_2 respectively (Table 1). Surviving animals were euthanized by intravenous infusion of 30 mg/kg sodium thiopental (Pentothal, Hospira Enterprises BV, The Netherlands). A macroscopic examination was carried out to evidence possible pathological features. Tissue samples were obtained from both kidneys, fixed in 10% formalin, routinely processed, paraffin embedded. Four or five micron paraffin sections were stained with H-E and PAS stain and examined under an optical microscope. Depending on recovery time, the experimental animals were subdivided into 5 groups (Table 2): group A: fast recovery (< 15 min); group

Table 1 Animal groups according to oxygen concentration used for resuscitation

	F. O_2	Deaths	Survivors	Mean time of resuscitation (min)
Group 1	18%	3	7	5-26
Group 2	21%	1	9	7-75
Group 3	40%	2	8	12-142
Group 4	100%	3	7	30-120

B: medium (15-45 min); group C: slow (45-90 min); group D: very slow recovery (> 90 min), and group E: dead animals.

Kidney samples from 4 male Landrace/large White newborn piglets not submitted to hypoxia served as subjects for the control group. Statistical analyses were based on the use of the χ^2 test.

RESULTS

Kidneys from the control group preserved their architecture. In the subcapsular regions, active glomerulogenesis was detected, represented by the tubule-glomerular nodules recently reported by our group as the typical developing unit in piglets[21]. Moreover, in the deep cortex, some senescent glomeruli with different degrees of sclerosis were detected and were occasionally associated with cellular or fibrotic crescents. Few scattered PAS-positive tubular casts were also observed in all control kidneys.

Pathological changes, mainly affecting tubular cells, were observed in the vast majority of kidneys from asphyxiated piglets. The most frequent tubular changes are illustrated in Figure 1.

Tubular dilatation, mainly affecting distal tubules, was observed in 35/40 cases (87.5%); in 7 of the 35 positive cases, tubular dilatation was marked and diffuse. Dilatation was found to occur even in the subcapsular regions, in developing nephrons and, in particular, in renal vesicles and S-shaped bodies.

Tubular vacuolization was detected in 28/40 (70%) cases with an incidence similar to that of tubular dilatation. In the majority of cases, tubular vacuolization was focal, whereas in 7 cases it was diffuse. In kidneys with focal tubular vacuolization the lesions were mainly observed in the deep cortex at the cortico-medullary boundary. Only in rare cases did vacuolization affect tubular structures in the subcapsular regions. Vacuolization was mainly observed in proximal tubules. In the majority of affected kidneys, the size of vacuoles varied widely among cases: multiple microvacuoli were observed but, in few cases, larger vacuoles were found, sometimes occupying entirely the cytoplasm of affected tubular cells (Figure 2).

Apoptosis: this was diagnosed on the basis of morphological changes. It was detected only in 6/40 (15%) cases, was mainly focal and occurred in proximal tubular cells. Cells undergoing apoptosis showed cell fragmentation with the formation of multiple eosinophilic globules, some of which contained nuclear remnants (Figure 3). Apoptotic bodies often appeared strongly PAS-positive.

Tubular eosinophilia was observed in 21/40 (52.5%) cases. Eosinophilic changes were mainly focal, localized in tubular structures of single nephrons with their cytoplasm intensely stained by eosin. Eosinophilia was detected in the superficial and deep cortical regions: subcapsular areas with active nephrogenesis were frequently affected. At high power, tubules affected by eosinophilia often showed associated degenerative changes, including vacuolization.

Tubular casts were observed in 38/40 cases (95%), as well as in the kidneys from the control group. Casts were mainly hyaline, detected in areas affected by interstitial oedema and tubular cell vacuolization. Occasionally, granular casts were found in areas with tubular cells apoptosis.

Oedema was observed in the interstitial space of the cortical region in 13/40 cases (32.5%): in 10 cases it was focal and mainly localized in the subcortical regions; in 3 cases it was diffuse in the whole cortex, including the deep areas.

Sloughing. Detachment of tubular cells from the basal membrane was observed in 20/40 cases (50%) mainly in proximal tubuli. Inside the affected tubules, sloughing was focal, with scattered cells detaching from the basal lamina of tubuli. Sloughing was unevenly distributed throughout the entire cortex, with no preference for superficial or deep cortical zones.

Fragmentation of the brush border. In 20 out of 40 cases (50%), proximal tubules at high power revealed a previously underestimated lesion: the brush border had lost its integrity and appeared fragmented. Brush border fragmentation was often associated with sloughing and with other tubular changes, including dilatation and cytoplasmic vacuolization.

Glomerular changes were found in all samples examined, most frequently in the deep cortex and at the cortico-medullary junction. Mesangial cell proliferation, capsular adhesion between the flocculus and Bowman' s capsule, glomerulosclerosis and fibrous or cellular crescents associated with collapse of the glomerular tuft (Figure 4) were detected. In some cases, these glomerular lesions were also detected in the subcapsular regions, affecting glomeruli during the initial phases of their development. Focal glomerulosclerosis and hyalinization were observed even in controls, but always restricted to the deep cortex.

As for the glomerular changes, these lesions will not be discussed, given their presence in all the control kidneys examined. They might represent physiological senescence of "not well developed" glomeruli.

Statistical analysis was carried out on changes observed when the animals were allocated in the 4 groups depending on the concentration of oxygen used

Table 2 Animal groups according to time of resuscitation

	Resuscitation				
	Group A Fast < 15′ (%)	Group B Middle 16-45′ (%)	Group C Slow 45-90′ (%)	Group D Very slow > 90′ (%)	Group E Death (%)
Oedema	12.5	50	28.50	0	44.40
Eosinophilia	50	50	42.80	100	44.40
Tubular dilatation	87.50	83.30	85.70	75	100
Casts	100	100	85.70	100	88.80
Tubular vacuoles	75	83.30	42.80	75	66.60
Sloughing	75	41.60	42.80	25	55.50
Brush border fragmentation	75	41.60	42.80	25	55.50
Apoptosis	25	8.30	0	25	22.20

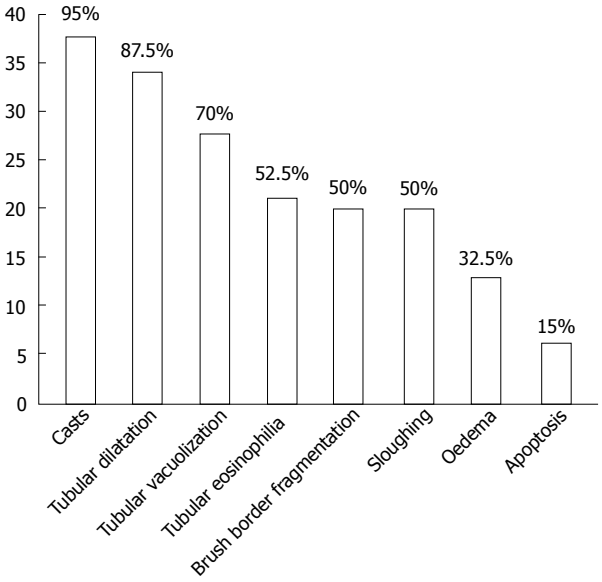

Figure 1 Percentage of the elementary lesions in the kidney.

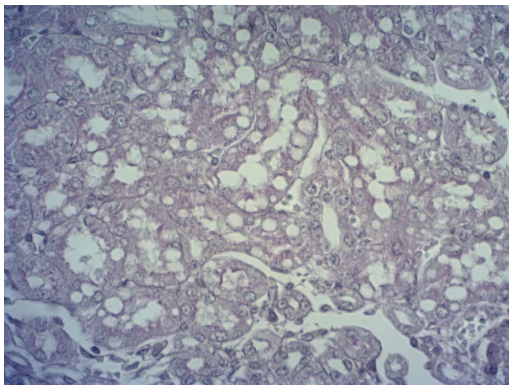

Figure 2 Vacuolation of the proximal tubules.

during resuscitation and when they were allocated in 5 groups on the basis of the time of resuscitation. Statistical analysis showed no evidence of differences regarding kidney lesions among animals groups.

DISCUSSION

Despite improvement in knowledge over the last

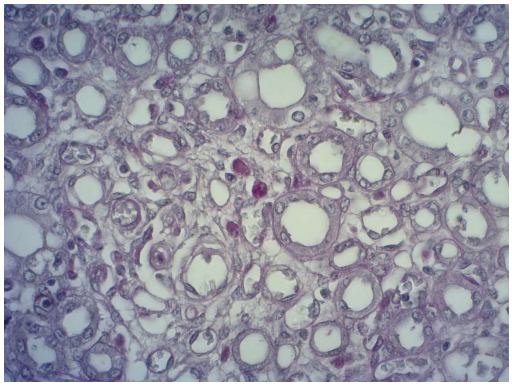

Figure 3 Apoptosis.

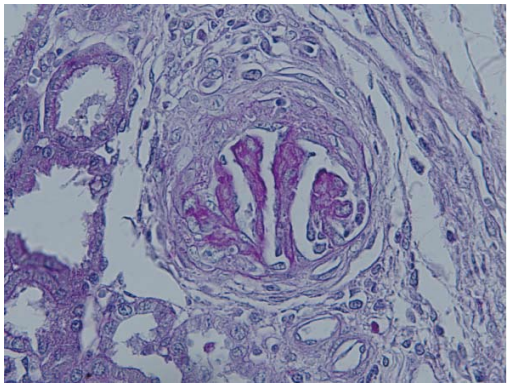

Figure 4 Fibrous and cellular crescents associated with collapse of the glomerular tuft.

decade[5] regarding the molecular mechanisms by which perinatal asphyxia causes tissue damage, the insult leading to acute kidney injury is partly unknown and still under discussion. Some authors claim that the predominant role in tubular cell death is played by hypoxia *per se*, while others attribute this to dysoxia 4 or reoxygenation[20].

This study presents data on histological changes occurring in kidneys of newborn piglets exposed to acute hypoxia-reoxygenation. The degenerative and necrotic changes were predominantly localized in the proximal tubules, followed by distal tubules, in absence of significant pathological changes in glomerular cells

and vascular structures. These data confirm that kidney proximal tubular cells are the main target of hypoxia, due to their high physiological metabolic rate[4] and high rate of aerobic glycolysis[5]. Distal tubular cells, despite their lower metabolism rate, were however not devoid of pathological changes in this study, with tubular dilatation being the main one. Tubular vacuolization was detected both in proximal and distal tubules.

As for the role of oxygen concentration used for resuscitation in acute kidney injury, the present data suggest that it did not play a significant role in acute kidney injury following hypoxia-reoxygenation in our experimental model. No significant difference in the incidence of renal pathological changes among animals of groups 1-4 were detected (Table 1). However, we cannot exclude long-term effects of different oxygen concentrations in the kidney.

Interesting data on the changes of renal cells were obtained when piglets were subdivided into groups A-E depending on the time required for resuscitation (Table 2). Interstitial oedema appeared to be a "late" lesion, detected in only 12.5% of animals in group A. On the contrary, eosinophilia of tubular cells should be considered an "early" lesion as it was found in 50% of piglets in group A, and a similar percentage was found in deceased animals. Tubular dilatation appeared frequently in group A (87.5%) thus suggesting that it is a "very early" response of kidney structures to hypoxia. The observation of dilated renal tubules in 100% of deceased piglets reinforces the hypothesis of a major role of this early pathological lesion in the pathogenesis of acute kidney injury following hypoxia. Tubular casts were observed in the majority of animals, ranging from 85.7% up to 100% of cases (Figure 1). This association with their detection in control kidneys suggests a minor, if any, role of casts in acute kidney injury.

As for the vacuolization occurring in the cytoplasm of proximal and distal tubular cells, it appears to be an "early" lesion, observed in 75% of piglets in group A. Contrary to other lesions, the incidence of this pathological change had not significantly increased in animals with a longer time of resuscitation and was found in 66.6% of deceased animals, thus suggesting that some of them probably did not survive long enough after asphyxia for cytoplasmic vacuoles to appear. Sloughing and brush border changes appeared to be "early" lesions observed in 75% of animals in group A and decreased in piglets with late and very late (> 90 min) resuscitation, finally to be observed in about 50% of deceased animals (Table 2).

The rare presence of apoptotic globules with nuclear condensation and fragmentation in animals of group A may be attributed to the short time to complete the whole process of apoptotic cell death. The same explanation can be applied to deceased animals: as apoptosis is an active process, we can speculate that the majority of deceased piglets did not survive long enough to allow apoptosis to occur. On the contrary, the incidence of apoptotic globules in kidneys of animals characterized by a very late resuscitation time (> 90 min) suggests an important role of apoptosis in asphyxia-related acute kidney injury. We may also speculate that sloughing should be considered the morphological sign of tubular cell apoptosis: the detachment of tubular cells from each other and from the basal lamina and their removal by the urinary flow may be a limit for the development of the complete sequence of the apoptotic process, thus limiting their finding to few kidney samples.

Our data clearly show that the Landrace/Large White piglet model provides useful data for the study of hypoxia-induced kidney injury. Tubular dilatation, tubular cell vacuolization, sloughing and brush border changes are the main, earliest and most severe lesions in the post-asphyxia kidney. A strong interindividual variability in the severity of renal changes to asphyxia was observed. The extent of kidney lesions was not associated with the concentration of oxygen used during resuscitation, thus suggesting a previously unreported individual susceptibility to hypoxia. Further studies on pathological kidney changes from human and non-human models are needed to verify their role in the short- and long-term kidney damage following hypoxia-reoxygenation.

COMMENTS

Background

The study focuses on the histological renal change following asphyxia in a experimental model of neonatal asphyxia. On the basis of recent data suggesting a role of reoxygenation in the development of renal lesions, the authors evaluated the presence and degree of renal lesions in piglets submitted to different oxygen percentages following asphyxia.

Research frontiers

The study is mainly related to the research field of tissue damage following asphyxia and reoxygenation in the perinatal period.

Innovations and breakthroughs

The most important data of the study regard the usefulness of the Landrace/Large White piglet model for the study of hypoxia-induced kidney injury. The strong interindividual variability in the severity of renal changes to asphyxia described in the study represents a new finding that may lead the neonatologist towards an individualized sartorial approach in each newborn affected by perinatal asphyxia.

Applications

In the study, tubular dilatation, tubular cell vacuolization, sloughing and brush border changes are the main, earliest and most severe lesions observed in the post-asphyxia kidney. These data may help pathologists involved in the study of asphyxia-induced renal pathology.

Peer-review

The study is interesting and well-conducted.

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CASE REPORT

Prolonged hypernatremia triggered by hyperglycemic hyperosmolar state with coma: A case report

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Ethics approval: NA.
Informed consent: Approval for this case report was obtained from the human research committee of the Raymond G Murphy VA Medical Center.
Conflict-of-interest: The authors have no conflicts of interest to declare.

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Abstract

A man with past lithium use for more than 15 years, but off lithium for two years and not carrying the diagnosis of diabetes mellitus or nephrogenic diabetes insipidus (NDI), presented with coma and hyperglycemic hyperosmolar state (HHS). Following correction of HHS, he developed persistent hypernatremia accompanied by large volumes of urine with low osmolality and no response to desmopressin injections. Urine osmolality remained < 300 mOsm/kg after injection of vasopressin. Improvement in serum sodium concentration followed the intake of large volumes of water plus administration of amiloride and hydrochlorothiazide. Severe hyperglycemia may trigger symptomatic lithium-induced NDI years after cessation of lithium therapy. Patients with new-onset diabetes mellitus who had been on prolonged lithium therapy in the past require monitoring of their serum sodium concentration after hyperglycemic episodes regardless of whether they do or do not carry the diagnosis of NDI.

Key words: Hypertonicity; Lithium; Hypernatremia; Hyperglycemia; Nephrogenic diabetes insipidus

Core tip: Hyperglycemic coma with large losses of body water may aggravate lithium-induced nephrogenic diabetes insipidus (NDI) which had been asymptomatic and undiagnosed for years after cessation of lithium therapy. The development of conditions leading to loss of water and consciousness in patients who were on long term lithium therapy should trigger surveillance for NDI even when they were asymptomatic in the past.

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INTRODUCTION

Hypertonicity resulting from excessive losses of body water through the kidneys, the respiratory tract, the skin, the gastrointestinal tract and/or gain in body solute, causes neurological manifestations that may become life threatening[1,2]. Hypernatremia[3] and hyperglycemia[4] are the two common causes of hypertonicity. Severe hyperglycemia developing on the ground of another condition potentially causing hypernatremia may lead to extreme hypertonicity. We present a patient who developed coma from hyperglycemic hyperosmolar state (HHS) followed by prolonged hypernatremia. Nephrogenic diabetes insipidus (NDI) secondary to chronic lithium intake was diagnosed during the period of hypernatremia. NDI had apparently persisted despite discontinuation of lithium two years prior to the HHS, but had not been diagnosed because of absence of hypernatremia and lack of symptoms of hypertonicity.

CASE REPORT

Calculated values, summary statistics

Calculated values: Serum tonicity (effective osmolarity), mOsm/L[5] = 2 × serum sodium concentration ([Na]) + serum glucose concentration ([Glu])/18. Corrected serum sodium concentration[6]: [Na] at hyperglycemia corrected to a [Glu] value of 100 mg/dL by the use of Katz's[7] correction factor, which computes that a 100 mg/dL rise in [Glu] causes a 1.6 mmol/L depression in [Na]:

$[Na]_{Corrected}$, mmol/L = [Na] + 0.016 × ([Glu] - 100)

Calculated serum osmolarity, mOsm/L[5] = 2 × [Na] + [Glu]/18 + blood urea nitrogen ([BUN])/2.8

Summary statistics: Parametric variables are presented as mean ± SD.

Patient report

A 58-year-old man with bipolar disorder was admitted with HHS and coma. He had been treated in the past with lithium carbonate for more than 15 years. During that period, serum lithium level was 0.72 ± 0.27 mmol/L (36 determinations) with two values, 1.3 and 1.4 mmol/L above the therapeutic range (0.5-1.2 mmol/L); in 22 determinations, average [Na] and [Glu] values were within the normal range (Table 1), with one [Na] value, at 146 mmol/L, above the upper normal limit of 145 mmol/L and one [Glu] value was in the hyperglycemic range (171 mg/dL); and in 15 determinations urine specific gravity was 1.008 ± 0.004. The urine specific gravity of all five urinalyses obtained in the last five years of this period was ⩽ 1.005.

Two years prior to the admission he moved to another town and discontinued the intake of lithium. Two months prior to admission with HHS, he resumed his visits to the outpatient clinics of this hospital after a large left lung mass was diagnosed. Positron emission tomography (PET) study showed a left lung mass, 10.9 cm in diameter invading the left main bronchus and the wall of the left pulmonary artery and involvement of several lymph nodes. Lung biopsy revealed squamous cell carcinoma.

He refused treatment for his tumor and opted for palliative management. He did not carry the diagnosis of diabetes mellitus or diabetes insipidus up to that time. During subsequent outpatient visits, progressive hyperglycemia was noted in. Three successive blood samples (Table 1). He refused admission when [Glu] was 809 mg/dL, but was admitted in deep coma three days later. On admission, blood pressure was 147/87 mmHg and heart rate 87 beats per minute. His mucosae were dry. Initial serum chemistries revealed hyperglycemia and profound hypertonicity (Table 1). In addition, BUN was 67 mg/dL, and serum potassium 3.7 mmol/L, total carbon dioxide 16 mmol/L, creatinine (previously in the normal range) 2.49 mg/dL, phosphorus 6.2 mg/dL, magnesium 4.2 mg/dL, lactate 3.4 mmol/L and calculated serum osmolarity 428.6 mOsm/L. The urine had a specific gravity of 1.016 and contained > 500 mg/dL of glucose, but no acetone. Arterial blood pH was 7.01, P_aO_2 102 mmHg (on nasal oxygen supplementation), P_aCO_2 71 mmHg and calculated bicarbonate 13.2 mEq/L. Chest X-ray showed a large mass in the left lung displacing the trachea to the right and several enlarged noncalcified lymph nodes in both lung fields. These findings were unchanged from those in recent earlier chest X-rays.

He received endotracheal intubation with mechanical ventilation, continuous infusion of insulin and large volumes of hypotonic saline containing potassium chloride. Large urine output was noted from the onset of treatment. Progressive decline in [Glu] was documented (Table 1). In a blood sample obtained four hours after onset of treatment, BUN was 66 mg/dL, serum creatinine 2.33 mg/dL, and calculated osmolarity 418.2 mOsm/L, while a simultaneously measured serum osmolality was 424 mOsm/kg. Following these measurements he received larger volumes of water in his infusions and through a gastric tube.

Hyperglycemia, hypokalemia, and hyperphosphatemia were corrected by 48 h after initiation of treatment. At that time, BUN was 48 mg/dL and calculated serum osmolarity 451.5 mOsm/L. Serum creatinine and magnesium declined progressively and reached normal levels by 72 h after the start of treatment. He was extubated on the fourth hospital day. However,

Table 1 Serum chemistries and calculated values related to tonicity

Time	[Glu]	[Na]	Tonicity	[Na]Corrected
	mg/dL	mmol/L	mOsm/L	mmol/L
-15 to -2 yr[1a]	99.6 ± 22.0	141.1 ± 2.7	287.6 ± 5.6	141.0 ± 2.7
-21 d[1]	235	139	291.1	141.6
-14 d[1]	304	141	298.9	144.3
-3 d[1]	809	135	314.9	146.3
Admission	1236	168	404.7	186.2
+2 h[2]	1141	169	401.4	185.7
+4 h[2]	982	170	394.6	184.1
+10 h[2]	771	165	372.8	175.7
+14 h[2]	650	163	362.1	171.8
+18 h[2]	611	160	353.9	168.2
+22 h[2]	548	165	360.4	172.2
+24-48 h[2]	233.5 ± 151.7	164.0 ± 1.9	341.0 ± 9.7	166.0 ± 3.3
+48-72 h[2]	176.6 ± 72.3	168.0 ± 1.9	345.8 ± 7.6	169.2 ± 3.0
+72-96 h[2]	151.8 ± 23.0	165.3 ± 2.6	338.9 ± 4.7	166.1 ± 2.1
+96-120 h[2]	185.0 ± 99.0	176.5 ± 2.1	363.3 ± 1.3	177.9 ± 0.5
+120-144 h[2]	131	172	351.3	172.5
+144-168 h[2]	135	157	321.5	157.6
+168-192 h[2]	186.5 ± 139.3	158.0 ± 9.9	326.4 ± 27.5	159.4 ± 12.2
+9-14 d[2]	175.5 ± 53.7	146.8 ± 1.8	303.5 ± 4.4	147.9 ± 1.8
+14-21 d[2]	241.3 ± 55.6	143.0 ± 3.9	299.4 ± 7.0	145.2 ± 3.7
+3 mo[2]	240	155	323.3	157.2

[1]Time before admission; [2]Time after onset of treatment; [a]Period of lithium intake; [Glu]: Serum glucose concentration; [Na]: Serum sodium concentration; [Na]Corrected: Serum sodium concentration corrected to a serum glucose level of 100 mg/dL; Values reported as mean ± SD represent 2-22 measurements.

production of copious volumes of dilute urine, confusion and severe hypernatremia persisted despite the combined administration of up to 400 mL per hour of 5% dextrose intravenously and free water by nasogastric tube. Over the four days following normalization of glycemia, he received progressively larger injections of desmopressin (from 1 to 4 mcg), but post-injection urine osmolality values ranged between 139 and 180 mOsm/kg, while [Na] ranged between 164 and 171 mmol/L, [Glu] between 84 and 281 mg/dL and [Na]Corrected between 164.7 and 173.7 mmol/L.

On day seven of admission, simultaneous serum and urine measurements revealed the following values: [Na] 161 mmol/L, serum osmolality 330 mOsm/kg, serum vasopressin 4.6 pg/mL, serum lithium undetectable and urine osmolality 279 mOsm/kg. Immediately following these measurements, he received an injection of 5 units of vasopressin. One hour post-injection, urine osmolality was 290 mOsm/kg. Over the next 20 d, his mental status improved slowly, [Glu] ranged between 69 and 304 mg/dL, while [Na] and [Na]Corrected remained elevated (Table 1).

Hypernatremia improved slowly after increase in water intake and administration of amiloride and hydrochlorothiazide. His last two [Na] values were in the normal range. He left the hospital against medical advice after he was declared competent to make treatment decisions by a Psychiatrist. He was advised to continue the medications for hypernatremia and to have a liberal water intake. Two months later he returned with progressive dyspnea. Computed chest tomography revealed increases in the size of lymph nodes and a large clot in the right pulmonary artery. [Na] was elevated (Table 1). He expired in respiratory failure within 48 h of his last admission. Table 1 shows tonicity values throughout his follow-up.

DISCUSSION

This report of a patient developing protracted hypernatremia following treatment of severe HHS illustrates the following clinical points: (1) the level of hypertonicity can become extreme in patients with HHS that remains untreated for several days; (2) Lithium-induced NDI that remained asymptomatic and undiagnosed for years after cessation of lithium therapy can cause severe hypernatremia in patients who encounter difficulties in consuming adequate volumes of water.

Tonicity of the serum is its property to cause osmotic transfers of water into or out of cells suspended in it. [Na] is, in general, an accurate indicator of serum tonicity[8]. Gain in extracellular solutes other than sodium salts, such as glucose, is the main exception to this rule. Hypertonicity in hyperglycemia should be evaluated in two steps: (1) At presentation, the degree of hypertonicity, which results from extracellular accumulation of solute (glucose)[9] and loss of water through osmotic diuresis[10,11], determines the severity of the presenting clinical manifestations is calculated by the tonicity formula[1,5]; (2) The prescription of the tonicity (*i.e.*, sodium plus potassium concentration) of the replacement solutions should be based on [Na]Corrected[12], reflecting the fact that correction of hyperglycemia without any further changes in the external balances of water and monovalent cations leads to rise in [Na], but decrease in serum effective osmolality[12]. Monitoring of the clinical status and serum chemistries is imperative during treatment of severe HHS[12].

Both serum tonicity and [Na]Corrected were at admission extremely high, indicating profound water deficit, in the patient of this report, who despite infusions of large volumes of hypotonic fluids exhibited subsequently protracted hypernatremia and was eventually diagnosed with NDI by formal testing[13]. Persistently low urine specific gravity values during and after lithium therapy identified lithium as the probable cause of NDI.

Lithium use is associated with a variety of renal functional and structural abnormalities[14,15]. NDI is the most prevalent lithium-induced disorder. Lithium enters the principal cells of the collecting ducts through luminal (apical) epithelial sodium channels (ENaC) and inhibits the signaling pathways that involve glycogen synthase 3-beta causing disruption of the aquaporin-2 structure and function and NDI[16,17].

Amiloride is effective in the prevention and treatment of lithium-induced NDI in part because it is an inhibitor of ENaC, while hydrochlorothiazide affects several transport

proteins[18].

Lithium-induced NDI may persist for years after cessation of lithium therapy[19]. Most available reports have found an association between the duration of lithium use and reduced renal concentrating ability supporting a progressive deficit[20]. Movig *et al*[21] reported that 37% of 75 patients receiving lithium developed polyuria (> 3 L/24 h). Polyuria was strongly associated with simultaneous use of serotonergic antidepressants and duration of lithium therapy. Although lithium-induced NDI is often reversible with median duration of therapy (< 6 years), the renal concentrating defect may be permanent after prolonged (> 15 years) therapy with lithium[22]. In large studies with long term follow-up, approximately 15% of patients using lithium demonstrate an irreversible impairment of renal concentration[22]. Several cases of NDI persistence after discontinuation of lithium therapy have been reported[23-29]. Special care is required for patients with this syndrome when they develop medical conditions preventing spontaneous fluid consumption[28].

Another characteristic of lithium-induced NDI is that it may go undiagnosed for years. Patients are able to compensate for this form of NDI, in which the defect in urinary concentration is usually partial, by consuming large fluid volumes. For example the urine volume that is needed for excretion of a solute load of 900 mOsm at a urine osmolality of 300 mOsm/kg is 3 L and can easily be achieved without the development of hypernatremia by patients with normal thirst mechanism.

Lithium-induced NDI can cause severe hypernatremia[30,31] especially after the development of stressful conditions leading to inability of the patients to drink adequate amounts of fluid. We found three reports of four patients on lithium who developed severe hypernatremia secondary to previously undiagnosed lithium-induced NDI in the immediate post- operative period[32-34]. In contrast to these subjects, our patient had stopped lithium intake two years before his admission.

Finally, our patient illustrates the association of manifestations of diabetes mellitus and lithium-induced NDI. Two patients presenting with clinical manifestations of lithium-induced NDI and diabetic ketoacidosis[35] or severe hyperglycemia[36] have been reported. Potential mechanisms of induction of glucose intolerance by lithium were discussed[36]. In addition to the possibility that lithium triggered the development of diabetes mellitus, it is probable that lithium-induced NDI aggravated the water loss secondary to osmotic diuresis in our patient. In osmotic diuresis osmolality values are higher in urine than plasma in all patients except those with diabetes insipidus who exhibit osmolality values lower in urine than in plasma. Thus, water losses from osmotic diuresis are comparatively larger and the hypertonic state that ensues is comparatively more severe in the patients with diabetes insipidus.

Lithium-induced NDI that remained asymptomatic and undiagnosed for years after cessation of lithium therapy may cause severe clinical manifestations of hypertonicity during clinical episodes affecting the patients' access to fluid intake. If these episodes consist of hyperglycemic emergencies, water loss trough combination of hyperglycemic osmotic diuresis and NDI may be massive leading to severe hypertonicity. Patients with severe hyperglycemia who had been on long-term lithium therapy require prolonged attention to their fluid balance after correction of the hyperglycemic episode.

COMMENTS

Case characteristics

Development of hyperglycemic hyperosmolar state (HHS) with profound coma followed by protracted hypernatremia in a patient who had stopped lithium therapy two years in the past.

Clinical diagnosis

Lithium-induced nephrogenic diabetes insipidus (NDI) diagnosed after correction of the HHS by lack of response of the urinary concentration to a formal vasopressin infusion test.

Differential diagnosis

Other causes of hypernatremia including central diabetes insipidus, persistent osmotic diuresis, and inadequate water intake were excluded by appropriate testing.

Laboratory diagnosis

Extreme hyperglycemia and serum effective osmolarity at presentation was followed by protracted hypernatremia which was shown to be the result of NDI by lack of response of urine osmolality to vasopressin infusion.

Imaging diagnosis

Inoperable lung malignant tumor found in chest X-rays, and computed tomography and positron emission tomography scans.

Pathological diagnosis

Squamous cell carcinoma of the lung found on a biopsy of the tumor.

Treatment

Insulin infusion, large volumes of hypotonic fluids given parenterally, by nasogastric tube, and later by mouth, amiloride and hydrochlorothiazide for the HHS and later the NDI, refusal of the patient to receive treatment for his lung tumor.

Related reports

Reports in the literature suggest that lithium-induced NDI may be permanent after cessation of lithium treatment when the duration of lithium therapy exceeded 15 years, while other reports suggest that lithium-induced NDI may cause severe hyponatremia following episodes of severe hyperglycemia.

Experience and lessons

Patients who had been in the past on long-term lithium therapy are at risk of developing severe hypernatremia during episodes that limit their ability to drink water and should have their serum sodium concentration closely monitored during these episodes even if they had not been diagnosed with nephrogenic diabetes insipidus in the past.

Peer-review

This reviewer thinks that it is worth sharing this case with "prolonged hypernatremia triggered by hyperglycemic hyperosmolar state after discontinuation of lithium therapy" by physicians.

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EDITORIAL

Asymptomatic hyperuricemia following renal transplantation

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Abstract

Evidence is accumulating indicating a role for uric acid in the genesis and progression of kidney disease, and a few studies are beginning to show a possible beneficial effect of urate-lowering therapy. Whether this holds true for renal allograft recipients is not clear. In this short review evidence from epidemiological as well as intervention studies is summarized and discussed, with some practical considerations presented at the end.

Key words: Uric acid; Renal transplant; Urate lowering therapy; Allopurinol; Febuxostat

Core tip: Hyperuricemia is a common finding following renal transplantation; its clinical, as well as prognostic, significance, however, is not known. We have summarized available evidence from human epidemiological and intervention studies and concluded that, in the absence of gout, evidence in support of treatment for this condition in renal graft recipients is insufficient at present, although, when required, treatment with low-dose allopurinol or febuxostat appears to be safe.

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INTRODUCTION

A body of evidence, accumulated mainly in the last 15 years, based on animal and human experimental studies, as well as prospective observational and a few intervention studies, reviewed elsewhere[1-5], has lent support to the hypothesis that hyperuricemia may be linked to incident renal disease and the progression of chronic kidney disease (CKD). Fewer data are available regarding the effect of uric acid (UA) and hyperuricemia on graft function and survival following renal transplantation. Aim of this short review, which does not mean to be either exhaustive or comprehensive, is to summarize available evidence gathered from observational and intervention studies on the latter topic in adult patients; we will not cover incidence and treatment of gout after renal transplantation, referring the reader interested in a more detailed discussion to the excellent review by

Stamp *et al*[6].

Literature search was performed on the PubMed, EMBASE and Science Direct databases using the following search terms: Uric, Uric Acid, Urate, Hyperuricemia and Renal, Kidney Transplant, Transplantation, Graft, Allograft.

EPIDEMIOLOGY OF HYPERURICEMIA FOLLOWING RENAL TRANSPLANTATION

Hyperuricemia (serum UA greater or equal to 6.0 mg/dL in women and 7.0 mg/dL in men) is fairly common following renal transplantation. The prevalence of hyperuricemia in recipients of a renal allograft has been shown to range from 19% to 55% in patients whose immunosuppressive regimen did not include cyclosporin A (CsA) and from 30% to 84% in patients treated with CsA[7]. In the same series, incident gout was not observed in non-CsA treated patients, whereas it ranged from 2.0% to 28% following CsA therapy. More recently, Kalantar *et al*[8] have measured serum UA in 12767 samples from 2961 renal graft recipients; they detected hyperuricemia in 1553 patients (52.3%, 61% men, 39% women). In another study[9] of 302 patients with a well functioning kidney graft, at a median 7.6 years after transplantation, hyperuricemia was present in 42.1% of patients. Kim *et al*[10] investigated the prevalence of hyperuricemia in 356 transplanted patients with stable renal graft function (estimated GFR > 60 mL/min per 1.73 m^2). In this subgroup of patients they found raised UA levels in 55 (15.45%). Numakura *et al*[11] found hyperuricemia to be present in 38% of patients one year after transplantation; in their cohort male gender and dialytic vintage before transplantation were predictors of post-transplant hyperuricemia. According to the various studies, risk factors for hyperuricemia following renal transplantation include decreased glomerular filtration rate (GFR), diuretic use, pre-existent history of hyperuricemia, treatment with calcineurin inhibitors, in particular CsA, use of diuretics, male gender, diabetes mellitus, hypercalcemia, and higher body weight[7-11]; tacrolimus has been associated with lower odds of developing hyperuricemia, compared to CsA[9].

OBSERVATIONAL STUDIES EXPLORING THE ASSOCIATION BETWEEN HYPERURICEMIA AND GRAFT FUNCTION/SURVIVAL

Table 1 summarizes the most relevant studies exploring this relationship[10,12-32]. Although most studies tend to favour an influence of UA on graft function and survival, there are notable exceptions: for instance, Meier-Kriesche *et al*[20], reviewing their data from the SYMPHONY Study, in which a cohort of 1645 was followed-up for 3 years, found that the association of baseline UA with follow-up eGFR, disappeared when adjusting for baseline eGFR. Conversely, in the study by Haririan *et al*[21], after a mean 68 mo follow-up, hyperuricemia was associated with a 1.26 (95%CI: 1.03-1.53) hazard ratio (HR) of graft loss. Kim *et al*[24] recently reported their review of patients transplanted between 1990 and 2009, and they observed that hyperuricemia conferred an 1.45 ($P < 0.001$) HR of graft loss; however the same group, in a study enrolling transplanted patients with preserved renal function[10] found hyperuricemia to be associated with decreased renal function, but not with graft survival. Choi *et al*[27] have investigated the effect of hyperuricemia on graft survival in recipients of living-donor kidney transplants, and found a nearly double incidence of graft loss in hyperuricemic patients (22.2% *vs* 11.4%). Hart *et al*[29], in a post-hoc analysis of patients participating in the ABCAN Trial, undergoing protocol biopsies of the graft, found an association between serum UA levels and the degree of interstitial fibrosis and tubular atrophy. In patients undergoing biopsy for acute allograft dysfunction, Weng *et al*[30] found hyperuricemia to be associated with a greater cumulative incidence at one year of the combined end-point of doubling serum creatinine or graft loss (29.8% *vs* 14.9%, $P = 0.02$) compared to normouricemia. As far as cardiovascular outcomes are concerned, Dahle *et al*[28] after a 7.4 year follow-up of 2200 patients found a J-shaped association between serum UA levels and cardiovascular as well as all-cause mortality, with a significant increased HR in the 5th UA centile, and a similar tendency (though not reaching statistical significance) for the lowest UA quintile. Other studies have yielded conflicting results, although those with a longer follow-up, and those assessing graft survival (rather than eGFR) as an end-point, tend to favour an adverse effect of hyperuricemia. The reason for the discrepancies among studies are not completely clear, however differences in the definition of hyperuricemia, duration of follow-up, end-points evaluated, populations studied and in adjustment for confounders and comorbidities may have played a role. Recently Huang *et al*[33] have published a meta-analysis, including 12 studies judged to be of medium-high quality according to the Newcastle-Ottawa quality assessment scale; the results of the meta-analysis showed that hyperuricemia was a risk factor for chronic allograft nephropathy [unadjusted Odds ratio (OR) = 2.85, 95%CI: 1.84-4.38, adjusted HR = 1.65, 95%CI: 1.02-2.65] and graft loss (Unadjusted OR = 2.29, 95%CI: 1.55-3.39; adjusted HR = 2.01, 95%CI: 1.39-2.94). The authors of this meta-analysis concluded that hyperuricemia may be an independent risk factor of allograft dysfunction and may increase slightly the risk of poor outcomes.

At the moment, the evidence supporting a causative or prognostic role for serum UA in renal transplant recipients is not conclusive.

Table 1 Studies investigating the association between serum uric acid and renal function/graft survival in patients with kidney transplantation

Author	Numerosity	Average follow-up	Major findings	Ref.
Gerhardt *et al* (1999)	375	5 yr	Hyperuricemia (> 8.0 mg/dL in men and > 6.2 mg/dL in women), associated with reduced graft survival	[12]
Armstrong *et al* (2005)	90	2.2 yr	UA independent predictor of follow-up eGFR, but not of eGFR change over time	[13]
Akgul *et al* (2007)	133	3 yr	No association found between serum UA and the development of chronic allograft nephropathy	[14]
Saglam *et al* (2008)	34	Not reported	Serum UA associated to development of cyclosporine A nephropathy (biopsy proven)	[15]
Akalin *et al* (2008)	307	4.3 yr	Hyperuricemia 6 mo after transplantation significantly associated with new cardiovascular events and graft dysfunction	[16]
Bandukwala *et al* (2009)	405	2 yr	Hyperuricemia associated with cardiovascular events, and, inversely with eGFR	[17]
Meyer-Kriesche *et al* (2009)	1645	3 yr	UA levels one month after transplantation not associated with follow-up eGFR, after adjustment for baseline renal function	[20]
Karbowska *et al* (2009)	78	Not reported	Hyperuricemia associated with markers of endothelial dysfunction and inflammation	[19]
Min *et al* (2009)	368	58 ± 23 mo	Early-onset moderate-to-severe hyperuricaemia (serum UA ⩾ 8.0 mg/dL) was found to be a significant risk factor for chronic allograft nephropathy (P = 0.035) and a poorer graft survival (P = 0.026) by multivariate analysis, whereas mild hyperuricaemia was not	[18]
Haririan *et al* (2010)	212	68 ± 27 mo	Serum UA during the first 6 mo postransplant, is an independent predictor of graft survival	[21]
Kim *et al* (2010)	356	102.6 ± 27.2 mo	Patients with eGFR> 60 mL/min per 1.73 m^2. Hyperuricemia associated with decreased eGFR	[10]
Boratyńska *et al* (2010)	100	34 ± 12 mo	Serum UA not associated to graft survival during 30 mo of follow-up	[22]
Chung *et al* (2011)	351	10 yr	Hyperuricemia increased risk of cardiovascular complication; graft survival at 5 and 10 yr lower in hyperuricemic *vs* normouricemic patients (89% *vs* 96% and 81% *vs* 93% respectively, P = 0.02)	[23]
Kim *et al* (2011)	556	Not reported	Serum UA levels affect graft function, even after adjustment for baseline eGFR	[24]
Wang *et al* (2011)	524	10 yr	Retrospective study: UA significantly lower in patients with longer graft survival	[25]
Park *et al* (2013)	428	120 ± 58 mo	Serum UA associated with allograft loss, but rate of eGFR decline more potent predictor	[26]
Choi *et al* (2013)	378	10 yr	Graft survival (living donor renal transplantation) 88.6% in normouricemic *vs* 78.8% in hyperuricemic patients	[27]
Dahle *et al* (2014)	2200	7.4 yr	Highest serum UA quintile independently associated with increased HR (2.87, 95%CI: 1.55-5.32) of cardiovascular and all-cause (1.55, 95%CI: 1.09-2.25) mortality	[28]
Hart *et al* (2014)	149	5 yr	Post-hoc study of the ABCAN trial. Serum UA independently associated with increased odds of composite outcome of doubling of interstitium or ESRD from Interstitial Fibrosis/Tubular Atrophy, after adjusting for eGFR	[29]
Weng *et al* (2014)	880	43.3 ± 26.3 mo	Hyperuricemia associated with poorer graft survival (60.5% *vs* 75.8%, P = 0.007), no difference in all-cause mortality	[30]
Boratyńska *et al* (2014)	637	10 yr	Retrospective study. Hyperuricemia associated with chronic allograft dysfunction	[31]
Weng *et al* (2014)	124	14.3 mo	Patients undergoing biopsies for acute allograft dysfunction. Hyperuricemia associated with a greater cumulative incidence at one year of doubling serum creatinine or graft loss (29.8% *vs* 14.9%, P = 0.02) compared to normouricemia	[32]

UA: Uric acid; eGFR: Estimated glomerular filtration rate.

INTERVENTION STUDIES

Currently, no randomized, double-blind, controlled clinical trials of urate-lowering treatment on graft function and survival in renal allograft recipients are available. Table 2 shows the few published studies[11,34-37], all suffering from drawbacks such as low numerosity, lack of a placebo arm, single center, inconsistent reporting of adverse events and/or absence of blinding. In a study published in 2003, Perez-Ruiz *et al*[34] Studied 279 renal allograft recipient with hyperuricemia, 89 treated with allopurinol(mean dose 185 mg/d), and 190 with the uricosuric agent benziodarone(mean dose 73 mg/d); the immune-suppressive regimen included azathioprine in 49.1% of patients. Both drugs were effective in reducing serum UA, with similar withdrawal rate (11% for allopurinol and 8% for benziodarone). Major adverse events were rare, 3 in the allopurinol group (one case of pancytopenia, one hepatitis and one unexplained fever, all on high-dose treatment, 600 mg/d) and 2 in the benziodarone group (hypothyroidism). It must be remembered, however, that benziodarone was withdrawn from the market in many countries due to liver toxicity. More recently Numakura *et al*[11] studied 46 patients with post-transplant hyperuricemia treated with allopurinol (100-200 mg/d) compared

Table 2 Studies of uric-acid-lowering therapy in renal allograft recipients

Ref.	Study population	Average follow-up	Intervention/outcome(s)	Main study findings
Perez-Ruiz *et al*[34]	279 renal allograft recipients with hyperuricemia	38.6 ± 18.4 mo	Allopurinol, benziodarone/ serum UA levels	Both drugs effective in lowering serum UA; benziodarone safer in patients on azathioprine
Numakura *et al*[11]	121 renal allograft recipients with and without hyperuricemia	Up to 10 yr, mean not reported	Allopurinol/eGFR, graft survival	Hyperuricemia associated with reduced eGFR, but graft survival similar in normo and hyperuricemic patients
Osadchuck *et al*[35]	108 renal allograft recipients (54 patients treated *vs* 54 controls)	2 yr	Allopurinol/Serum UA levels, eGFR, graft survival	Reduced serum UA, preservation of eGFR in allopurinol treated patients; no differences in graft survival and blood pressure
Sofue *et al*[36]	93 renal allograft recipients (42 normouricemic, 51 hyperuricemic, 26 treated, 25 not treated)	1 yr	Febuxostat/serum UA levels, eGFR	Serum UA lower and eGFR stable in patients treated with febuxostat
Tojimbara *et al*[38]	23 renal allograft recipients with hyperuricemia	12 ± 2 mo	Febuxostat/serum UA, eGFR	Serum UA lower after treatment with febuxostat; eGFR stable

UA: Uric acid; eGFR: Estimated glomerular filtration rate.

to 75 normouricemic patients, followed up to 10 years. The former group had a lower eGFR, with a tendency for graft survival at 5 and 10 years to be reduced, with borderline statistical significancy. Rates of withdrawal from treatment or incidence of adverse events were not reported in this study. Osadchuk *et al*[35] in a retrospective case-control study, evaluated 54 hyperuricemic patients taking allopurinol because of gout, compared to 54 untreated controls matched for eGFR and time from transplant; mean baseline serum UA was 8.0 mg/dL in the allopurinol group and 6.8 mg/dL in the controls. At the end of the observation period (2 years) serum UA was reduced, and eGFR greater in the treatment group compared to controls, whereas no difference in graft survival was recorded. The dose of allopurinol used is not stated, and neither rate of withdrawal from treatment nor the incidence of adverse effects is reported. Sofue *et al*[36] studied 93 renal allograft recipients with stable renal function, 51 of them being hyperuricemic, 42 normouricemic. They treated 26 hyperuricemic patients with low-dose (10-20 mg/d) febuxostat, a novel xanthine-oxidase inhibitor associated with fewer adverse events than allopurinol[37]. After one year of treatment the majority of treated patients had achieved target serum UA levels and eGFR was stable. No serious adverse events were recorded and liver function tests were not altered by febuxostat. Finally, Tojimbara *et al*[37] assessed 22 hyperuricemic renal allograft recipients treated with low-dose febuxostat (10-20 mg/d). Despite the low dose administered, 73% of the patients achieved target serum UA levels (< 6.0 mg/dL). No serious adverse events were recorded, and only one patient withdrew from the study because of numbness in the arms. Immuno-suppressive drug levels were not affected by the co-administration of febuxostat.

CONCLUSION

Available evidence does not support widespread use of urate lowering therapies in asymptomatic hyperuricemic recipients of a renal allograft. At present, treatment should be limited to patients with gout, although patients with severe hyperuricemia (> 8.0 mg/dL) might benefit from serum UA lowering therapy; it is not known what serum UA target should be achieved, however, a recently published[39] long-term follow-up of a randomized, controlled clinical trial of allopurinol treatment in patients with CKD, has shown that nefro-protection can be attained by lowering serum UA just below its crystallization threshold (6.8 mg/dL). The therapeutic armamentarium is currently limited to xanthine-oxidase inhibitors, as uricosuric agents, with the possible exception of losartan, are mostly not indicated, or ineffective, in patients with CKD and/ or kidney transplant, uricase and its analogues are expensive, must be administered parenterally, and have important side effects; the discovery and isolation of urate transporters in the renal tubules, has led the way to the development of new hypouricemic drugs, currently under evaluation[40] but not immediately available for clinical use. The good news is that the data at hand seem to show that both allopurinol and febuxostat can be administered safely, at low doses, in renal transplant recipients, with the exception of those treated with azathioprine, the side-effects of which could be potentiated by xanthine-oxidase inhibition. In conclusion, randomized controlled trials of urate-lowering therapy are badly needed in this population of patients, to establish whether preservation of renal function and prolongation of graft survival can be achieved.

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REVIEW

Pharmacokinetic and pharmacodynamic considerations of antimicrobial drug therapy in cancer patients with kidney dysfunction

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Abstract

Patients with cancer have a high inherent risk of infectious complications. In addition, the incidence of acute and chronic kidney dysfunction rises in this population. Anti-infective drugs often require dosing modifications based on an estimate of kidney function, usually the glomerular filtration rate (GFR). However, there is still no preferential GFR formula to be used, and in acute kidney injury there is always a considerable time delay between true kidney function and estimated GFR. In most cases, the anti-infective therapy should start with an immediate and high loading dose. Pharmacokinetic as well as pharmacodynamic principles must be applied for further dose adjustment. Anti-infective drugs with time-dependent action should be given with the target of high trough concentrations (*e.g.*, beta lactam antibiotics, penems, vancomycin, antiviral drugs). Anti-infective drugs with concentration-dependent action should be given with the target of high peak concentrations (*e.g.*, aminoglycosides, daptomycin, colistin, quinolones). Our group created a pharmacokinetic database, called NEPharm, hat serves as a reference to obtain reliable dosing regimens of anti-infective drugs in kidney dysfunction as well as renal replacement therapy. To avoid the risk of either too low or too infrequent peak concentrations, we prefer the eliminated fraction rule for dose adjustment calculations.

Key words: Anti-infective drugsl; Cancer; Kidney function; Pharmacodynamics; Pharmacokinetics; Dose adjustment; NEPharm

Core tip: Cancer patients are at an increased risk for both infection and kidney dysfunction. Infections need immediate treatment; during the further course, kidney function must be taken into account. Almost any drug can be adjusted to any kidney function in every patient. Observation of the pharmacokinetic principles allows avoiding adverse events.

Observation of the pharmacodynamic principles is needed to obtain anti-infective success. The target concentration for anti-infective drugs with a concentration-dependent effect is the high peak level. The target concentration for anti-infective drugs with a time-dependent effect is the high trough level. When in doubt, the peak should be the target.

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INTRODUCTION

The number of patients requiring anticancer therapy is rising due to the increase in life expectancy. Presently, there is almost no malignancy without an option for either curative or palliative, adjuvant or neo-adjuvant chemotherapy. Anticancer drugs bear not only the risk of infection and "febrile neutropenia"[1] but also the risk of nephrotoxicity[2].

Acute kidney injury (AKI) of any cause is a known risk factor for and a consequence of infectious complications. AKI can also be potentiated by the nephrotoxicity of the chemotherapeutics. In cancer patients, the incidence of AKI is estimated at 15%-45% per year[3]. The prevalence of chronic kidney disease (CKD) is reported at 15%-50% in cancer patients[4,5]. This high prevalence can be due to demographic trends but in contrast to previous speculations, CKD is not a risk factor for non-renal malignancies[3].

This review addresses the pharmacokinetics and pharmacodynamics (PK-PD) of anti-infective therapies in cancer patients with impaired kidney function.

Case report

The therapeutic dilemma might be illustrated by the case of a 73-year-old female with fever and leukopenia. The diagnosis of multiple myeloma had been made 18 mo before admission. As a third-line chemotherapy, she had received 4 cycles of bendamustine and prednisolone. Now she was referred from another hospital because of acute on chronic kidney failure requiring hemodialysis (HD). After persistent fever while on piperacillin-combactam and radiological evidence of pneumonia, she received 1000 mg meropenem every 12 h as rescue therapy. Since the half-life was assumed to increase from 1.0 to 9.7 h, the administration interval was prolonged from 8 to 12 h (Table 1). The renal failure dose of 500 mg twice daily as recommended by the manufacturer was considered to be under-dosed - in agreement with recent publications[6]. She remained dialysis-dependent but could be discharged home 3 wk later.

KIDNEY FUNCTION AND DRUG DOSE ADJUSTMENT

Anti-infective treatment is given with a therapeutic or a prophylactic indication. The preemptive treatment is distinguished from the induction therapy and the empirical differs from the sequential mode of therapy. For any mode of treatment, adjustment of anti-infective drug dose to the kidney function is recommended based on estimates of glomerular filtration rate (GFR) as well as pharmacokinetic and pharmacodynamic principles.

Kidney function

The kidney function can be measured by the GFR as this quantitates the primary and principal function of the nephron. It is an anachronism to use the endogenous creatinine clearance since urine collection errors are frequent[7]. This makes such estimates unreliable, resulting in under-dosing of anti-infective and anticancer drugs. For classifying the kidney dysfunction into one of the 5 stages of CKD, the standardized chronic kidney disease epidemiology collaboration (CKD-EPI) formula is currently preferred[8]. For drug dose adjustment, the GFR estimate easiest to access is the most appropriate[9]. Both, the modification of diet in renal disease (MDRD) or CKD-EPI equations estimate the GFR (eGFR) for a standard 1.73 m^2 body surface area (BSA). To estimate the BSA, we use the Mosteller formula[10].

$$GFR = eGFR \cdot \frac{BSA}{1.73}$$

$$BSA\ (m^2) = \frac{\sqrt{Height\ (cm) \cdot Weight\ (kg)}}{60}$$

The eGFR value is automatically calculated in most laboratories with the standardized MDRD and the CKD-EPI equations. Weight or body surface area are important determinants of the distribution volume and thus of the dose. Since oncologists are familiar with the use of BSA, the MDRD and CKD-EPI GFR might have advantages for dose adjustment calculations.

In the Cockcroft and Gault (C and G) formula the body weight is considered; it originally estimated the creatinine clearance. Like the other creatinine-based formulas, the C and G equation can also be used as an estimate of the GFR for drug dose adjustments[11]. Luzius Dettli proposed a coefficient-free version of the C and G equation[12] that was validated recently with the new calibrated serum creatinine measurements[13].

$$GFR = \frac{150 - Age\ (yr)}{Crea\ (mcmol/L)} \cdot Weight\ (kg)$$

Since the GFR is the independent and the serum creatinine is the dependent variable, there can be a time lag of 1 to 2 d behind the actual true kidney function and all creatinine-based GFR estimates in acute kidney injury (AKI). An interesting extension, therefore, is the so-called kinetic GFR for increasing and decreasing kidney function in patients with AKI[14]. The published

equation can be derived from the C and G equation and rearranged for readily available measurements of the initial serum creatinine ($Crea_0$) and differences (deltaX) between subsequent creatinine values ($Crea_{1,2\ldots}$).

$$kinetGFR = \frac{[150 - Age\ (yr)] \cdot Weight\ (kg)}{Crea_0\ (mcmol/L)} \cdot \left[1 - \frac{Crea_2 - Crea_1}{t_2 - t_1} \cdot \frac{24\ (h)}{182(mcmol/L)}\right]$$

This approach holds for changing creatinine and is based on creatinine production. It relates the increase in serum creatinine within a specified time interval to the maximum increase in creatinine within one day. Since creatinine production and renal excretion is constant at about 1000 mg/d and the creatinine distribution volume is 42 L, the maximum 24 h increase in serum creatinine is 182 μmol/L if GFR is zero (the original publication says 133 μmol/L). If AKI is progressing and the creatinine is increasing, the above 1 - deltaX term is < 1.0 whereas the 1 - deltaX term is > 1.0 for decreasing creatinine values and restitution of AKI. The kinetic GFR estimate makes the general GFR-based dose adjustment rules (see below) also applicable to AKI and the intensive care condition with renal replacement therapy[14].

Pharmacokinetics

The main pharmacokinetic parameters are clearance, volume and half-life. Malcolm Rowland claimed the primacy for the clearance term since elimination is driven by clearance not half-life[15]. Where clearance reflects a mechanistic model, however, the half-life reflects a mathematical approach. Friedrich Hartmut Dost argued that the clearance estimate depends on bioavailability and body weight - as does the volume as well - whereas half-life does not[16].

There is a close relationship between the three parameters of clearance (Cl), volume (Vd) and half-life ($T_{1/2}$) where the half-life is inversely proportional to the elimination rate constant (Ke).

$$Cl = Ke \cdot Vd$$

$$T_{1/2} = \frac{In\ (2)}{Ke} = \frac{0.693}{Ke} = 0.693 \cdot \frac{Vd}{Cl}$$

As discussed for antiviral drugs, the half-life is the pharmacokinetic parameter that most impacts drug action[17]. Since the half-life indicates how long an administration interval should be selected, and since the duration of drug action is correlated to the half-life, we consider the elimination half-life to be the most useful pharmacokinetic parameter for drug dosing[18]. In some cases the special half-life that represents the largest part of the area under the curve should be considered - Luzius Dettli coined it the "dominant half-life". Generally, the effect-indicative half-life at target concentrations should be used for dose calculations[18].

An increase and prolongation of the half-life was first reported by Kunin *et al*[19] for special drugs in patients with impaired kidney function. If the half-life is prolonged, drug accumulation kinetics will produce higher peak and higher trough concentrations with an increased risk for drug toxicity. According to the accumulation kinetics, the steady-state peak (C_{peak}) and the trough concentrations (C_{trough}) depend on the initial concentration after the first dose (C_0), on half-life ($T_{1/2}$) and administration interval (Tau).

$$C_{peak} = \frac{C_0}{1 - \exp(-\frac{0.693}{T_{1/2}} \cdot Tau)}$$

$$C_{trough} = \frac{C_0}{\exp(\frac{0.693}{T_{1/2}} \cdot Tau) - 1}$$

The relation between kidney function and half-life is as complex and hyperbolic as that between GFR and serum creatinine. It was a great advantage for drug dose adjustment that Luzius Dettli demonstrated the linear relationship between drug elimination and kidney function. This dependence was originally described as a linear function between the elimination rate constant and the creatinine clearance[20]. The modern approach describes this dependence as a linear function between drug clearance (Cl) and GFR.

$$Cl = a + b \cdot GFR = Cl_{nonren} + b \cdot GFR = Cl_{fail} + \frac{Cl_{norm} - Cl_{fail}}{GFR_{norm}} \cdot GFR$$

Based on this fundamental equation, the dose can be adjusted to the individual GFR in proportion to the decrease in drug clearance (Figure 1). The dose can also be adjusted in inverse proportion to the increase in half-life since in many published investigations, the inverse half-life, namely the elimination rate constant (Ke) has been related to the GFR. Based on the ideas of Luzius Dettli and for practical purposes, the fraction eliminated by the renal route (fren) has been proposed as the leading parameter for drug dose adjustment[21].

$$fren = \frac{A_{urine}}{D} = \frac{Cl_{ren}}{Cl_{tot}} = 1 - \frac{Cl_{fail}}{Cl_{norm}} = 1 - \frac{T_{1/2norm}}{T_{1/2fail}}$$

$$Cl = Cl_{norm} \cdot [1 - fren\ (1 - \frac{GFR}{GFR_{norm}})]$$

$$D = D_{norm} \cdot \frac{T_{1/2norm}}{T_{1/2}} = D_{norm} \cdot [1 - fren \cdot (1 - \frac{GFR}{GFR_{norm}})]$$

Since pharmacokinetics of anticancer drugs is rarely investigated in patients with CKD or AKI, it is an advantage that this fraction can be derived in volunteers with normal kidney function. However, kidney dysfunction also influences non-renal clearance, bioavailability and drug metabolism by the liver and intestines[22]. Therefore, the pharmacokinetics as determined in real patients with failing kidney function (CKD or AKI) should be the preferred source for drug dose adjustment calculations (*e.g.,* half-life estimates).

$$T_{1/2} = \frac{T_{1/2norm}}{1 - fren\ (1 - \frac{GFR}{GFR_{norm}})} = \frac{T_{1/2norm}}{1 - (1 - \frac{T_{1/2norm}}{T_{1/2fail}}) \cdot (1 - \frac{GFR}{GFR_{norm}})}$$

Table 1 Proposals for adjustment of an anti-infective drug dose to the estimated kidney function or to intermittent hemodialysis and continuous hemofiltration

Drug	Half life (h)		Loading dose	Normal kidney function (GFR = 100 mL/min)		Kidney impairment (GFR ≈ 30 mL/min)		Failure (GFR ⩽ 5 mL/min) and hemodialysis			Hemofiltration (2 L/h) and continuous dialysis	
								Off dialysis day Dfail		$D_{HD} = D_{fail} + D_{sup}$		
	Normal	Failure	D_{start} (mg)	Maintenancedose (mg)	Dose interval (h)	Maintenancedose (mg)	Dose interval (h)	Maintenancedose (mg)	Dose interval (h)	Post dialysis D_{HD} (mg)	Maintenancedose (mg)	Dose interval (h)
Abacavir (po)	1.5	2.1	600	600	12	600	12	600	12			
Aciclovir	2.5	25	750	750	8	500	12	500	24	750	750	24
Adefovir (po)	1.6	160	10	10	24	10	48	10	168	10		
Albendazole (po)	8	8	400	400	12	400	12	400	12			
Amantadine (iv)	13	600	200	200	8	200	72	200	168	200	200	72
(po)	20	610	100	100	12	100	72	100	168	100		
Amikacin	2	40	Norm/Failure 1500/750	1500	24	500	24	250	24	750	750	24
Amoxicillin (po)	1.2	12	1000	1000	8	1000	12	500	12	1000		
Amoxicillin + Clavulanic acid	1.2 + 1.2	12 + 4.3	500 + 125	500 + 125	8	500 + 125	12	500 + 125	12	500 + 125	500 + 125	12
			875 + 125	875 + 125	8	875 + 125	12	500 + 125	12			
Amphotericin B	24 (360)	35 (360)	70	70	24	70	24	50	24	50	50	24
Amphotericin B liposomal	24/92	24/160	200	200	24	200	48	200	24	200	200	24
Ampicillin	1	13	2000	2000	8	2000	12	1000	12	2000	2000	12
+ Sulbactam	+ 1	+ 6.6	+ 1000	+ 1000	8	+ 1000	12	+ 500	12	+ 1000	+ 1000	12
Amprenavir	8	8	1200	1200	12	1200	12	1200	12			
Anidulafungin	26	26	200	100	24	100	24	100	24	100	100	24
Artesunate	0.5			180								
Atazanavir (po)	9			300	24							
Atovaquone (po)	63	63	750	750	12							
Atovaquone +	63	63	250 + 100	250 + 100	24	250 + 100	24	250 + 100	24			
Proguanil (po)	14	23										
Azidothymidine	1	1.9 (52)	200	200	8	100	8	100	8	200	200	24
Azithromycin	39	40	1000	500	24	500	24	500	24	500	-	-
Brivudin (po)	14 (144)		125	125	24 for 7 d							
Caspofungin	10	10	70	50	24	50	24	50	24	50	50	24
Cefaclor (po)	0.7	3	1000	1000	8	1000	12	1000	12	1000		
Cefazolin	2.2	34	2000	2000	8	2000	12	500	12	1500	1000	12
Cefotaxime	1.2	7 (10)	2000	2000	8	2000	12	1000	12	2000	2000	12
Cefotiam	1	8	2000	2000	8	2000	12	1000	12	2000	2000	12
Ceftaroline fosamil	2.7	6	600	600	12	600	12	600	12	600	600	12
Ceftobiprol-medocaril	3.3	11	1000	1000	8	1000	12	500	12	1000	1000	12
Ceftazidime	2.1	25	2000	2000	8	2000	12	1000	24	2000	1000	12
Ceftriaxone	8	15	2000	2000	24	2000	24	2000	24	2000	2000	24
Cefuroxime (iv)	1.1	18	1500	1500	8	1500	12	750	24	1500	750	12
(po)			500	500	8	500	12	500	24			
Chinin = Quinine	13	15	600	600	12	600	12	600	12	600	600	12
Chloramphenicol	2.5	7	1000	1000	8	1000	8	1000	12	1000	1000	12
Chloroquine	48/212	300	250 mg/8 h	150	8	75	24					
Cidofovir	3.4	45	375 mg/168 h	375	336 h = 14 d	70	336 = 14 d	35	336 = 14 d	70	140	336 = 14 d
Ciprofloxacin (iv)	4.4	10	400	400	12	400	12	400	24	400	400	12
(po)			500	500								

Clarithromycin	6.8	17	500	500	12	500	12	500	24				
Clindamycin	3	3	900	600	6 - 8	600	6 - 8	600	6 - 8	600	600	6 - 8	
Colistin colistimethate Na	3 (9)	24 (11)	480 - 720 = 7 Mio IE	240 = 3 Mio IE	8	240 = 3 Mio IE	12	240 = 3 Mio IE	24	240 = 3 Mio IE	320 = 4 Mio IE	12	
Colistin (po)	3	16	160 mg = 2 Mio IE	2 Mio IE	12								
Co-trimoxazole	11/10	31/28	160/800	160/800	12	160/800	24	160/800	24	160/800	160/800	12	
Dalbavancin	336		1000	500	168								
Dapsone (po)	24	31	200	200	24	200	24	200	24	200			
Daptomycin	8	33	500	500 6-10 mg/kg	24	500 6-10 mg/kg	48	500 6-10 mg/kg	48	500 6-10 mg/kg	350	24	
Darunavir (po)	8			600	12								
Delavirdine	5.8			400	8								
Didanosine (po)	1.4	4.5	200	200	12	200	12	200	24				
Doripenem	1	8	1000	1000	8	1000	8	1000	12	1000	1000	8	
Doxycycline	15	23	200	100	24	100	24	100	24	100	100	24	
Efavirenz (po)	46.8	47	600	600	24	600	24	600	24				
Emtricitabine (po)	8.7	36	200	200	24	200	24	200	72				
Enfuvirtide	30		90		12								
Entecavir (po)	24 (138)	67 (384)	1.0	1.0	24	0.5	48	0.5	72	0.5			
Ertapenem	4.1	14.4	1000	1000	24	1000	24	1000	24	1000	1000	24	
Erythromycin	2.3	5	1000	1000	8	1000	12	1000	12	1000	1000	8	
Ethambutol	3.1	9.6	1600 20 mg/kg	1600	24	1200	24	1000	48	1600	1600	24	
Famciclovir (po)	2.2	14	250	250	12	250	12	250	24				
Flucloxacillin	0.8	3	2000	2000	8	2000	8	2000	8	2000	2000	8	
Fluconazole	25	110	800 or 400	800	24	400	24	400	48	400	800	24	
Flucytosine	4	150	2500	2500	6	2500	12	2500	48	2500	1250	24	
Fosamprenavir	19			700	12								
Foscarnet	4.5	100	6000	6000 4000	12 8	3000	24	3000	72	4000	3000	24	
Fosfomycin (iv) (po)	1.5	20	5000 3000	5000 single dose	8	5000	24	2500	24	5000	5000	12	
Ganciclovir	4.2	60	500 5 mg/kg KG	500	12	500	24	300	24	400	200	12	
Gentamicin	2	48	Norm/Fail 240/120	240	24	120	24	40 0	24 48	120 320 before HD	120	24	
Hydroxy-chloroquine	400			200	8								
Imipenem/ + Cilastatin	0.9/ 0.9	3.3/ 13.8	1000	1000	8	1000	12	500	12	1000	1000	12	
Indinavir (po)	1.8	2.1	800	800	8	800	8	800	8				
Isoniazid	1/3.3	5/12	300	300	24	300	24	300	24	300	300	24	
Itraconazole (po)	16	25	200	200	24	200	24	200	24	200			
Ketoconazole (po)	3	2	200	200	12	200	12	200	12	200			
Lamivudine (po)	6.2	21	150	150	12	150	24	100	24	150			
Levofloxacin	7.3	35	750	500	12	500	24	250	24	500	500	12	
Linezolid	4.9	6.9	600	600	12	600	12	600	12	600	600	12	
Lopinavir/Ritonavir	7/3.7	7/6.3	400 + 100	400 + 100	12	400 + 100	12	400 + 100	12				
Maraviroc (po)	36	36	300	300	12	300	12	300	12				

Mebendazole (po)	5		2 x 500	1000	8								
Mefloquine (po)	336	340	250	250	168	250	168						
Meropenem	1	9.7	1000	1000	8	1000	12	1000		1000		12	
Metronidazole (iv) (po)	10	11 (34)	500	500	8	500	12	500	500	500		12	
			400	400		400							
Micafungin	13	14	100	100	24	100	24	100					
Miconazole	24	24	1200	1200	24	1200	24	1200					
Moxifloxacin	12	15	400	400	24	400	24	400	400	400		24	
Nelfinavir (po)	4.5	4	750	750	8	750	8						
Nitrofurantoin (po)	1.0	1.2	100		8								
Nevirapine (po)	28	22	200/24	200	12	200	12						
Oritavancin	336		1200										
Oseltamivir (po)	7	(80)	75	30	24	75	72	75					
Paromomycin	2	40	500	500	24	500	48						
Penicillin G = Benzylpenicillin	0.5	10	10 mega	10 mega	8	10 mega	12	5 mega	5 mega	5 mega	5 mega	8	
Penicillin V (po)	0.6	4.1	1 mega	1 mega	8	1 mega	8	1 mega					
Pentamidine (iv) (inhaled)	60	96	300	300	24	300	24	300					
			600		24								
			300		4 wk								
Piperacillin	1.1	4	4000	4000	8	4000	12	4000	12	4000	4000	12	
+ Sulbactam	1	8	500	500	8	500	12	500	12	500	500	12	
Piperacillin	1.1	4	4000	4000	8	4000	12	4000	12	4000	4000	12	
+ Tazobactam	1	8	500	500	8	500	12	500	12	500	500	12	
Posaconazole (po)	24	29	2 x 300	300	24	300	24	300	24				
Primaquine (po)	6.3	6.4	30	30	24	30	24	30	24				
Proguanil (po)	14	23		200	24								
Propicillin (po)	1		700 = 1 mega	700	8								
Prothionamide (po)	1.5		1000	1000	24	500	24	250	24	500	?	?	
Pyrazinamide (po)	9.1	19	2000	2000	24	2000	24	2000	48	2000	-	-	
Pyrimethamine	92	80	75	50	24	50	24	50	24				
Pyrvinium embonate	?	?	50	Single dosing									
Quinine	13	15	600	600	12	600	12	600	12	600	600	12	
Raltegravir (po)	5.5	2.5	400	400	12	400	12	400	12				
Ribavirin aerosol	44	26	6000	6000	12	6000	12	6000	12	6000	6000	12	
Ribavirin (po)	4/250	24/672	600	600	12	400	24	200	24	400	400	72	
(iv)			1000	1000	8	500	12	500	48	500	500	48	
Rifabutin (po)	25	37	600	600	24	600	24	300	24				
Rifabutin +	25	37	600	600	24	600	24	300	24				
Clarithromycin	6.8	17	300	300	24	300	24	300	24				
Rifampicin (iv)	4.5	4.5	600	600	24	600	24	600	24	600	600	24	
(po)			450	450	12	450	12	450	12				
Rifaximin (po)	intestine	unch	400	400	12	400	12	400	12				
Ritonavir (po)	3.7	6.3	600	600	12	600	12	600	12				
Roxithromycin	12	15	300	300	24	300	24	300	24	-	-	-	
Saquinavir (po)	7	13	2 x 500	1000	12	1000	12	600	12				
Stavudine (po)	1.5	6.0	40	40	12	40	12	40	12	40			
Sofosbuvir	1 (18)	(25)	400	400	24	400	24	300	24	400			
Streptomycin	2.6	100	1000	1000	24	500	48	250	72	250	500	24	

Teicoplanin	52	348	3 x (800/24)	1200	24	400	24	400	48	800	400	24
Telavancin	7.3	25	750	750	24	500	24	250	24	500	750	24
Telbivudine (po)	22		600	600	24							
Tenofovir (po)	14	28	245	245	24	245	24	245	48	245		
Terbinafine (po)	16	16	250	250	24	250	24	250	24			
Tetracycline (po)	8.9	83	500	500	8							
Tigecycline	40	47	100	50	12	50	12	50	12	50	50	12
Tipranavir (iv)	2.8	2.8	500	500	12							
+ Ritonavir (po)	3.7	6.3	+ 200	+ 200								
Tobramycin	2	48	Norm/Fail 240/120	240	24	120	24	40	24	120	120	24
Trimethoprim (iv) (po)	11	31	200	150	12	150	24	150	24	-	-	-
			100	100	24	100	24					
Trimethoprim +	11	31	160	160	12	160	24	160	24	160	160	12
Sulfamethoxazole	10	28	+ 800	+ 800	12	+ 800	24	+ 800	24	+ 800	+ 800	12
Trimethoprim +	11	31	400	400	8	320	12	400	24	400	400	12
Sulfamethoxazole (Pneumocystis)	10	28	+ 2000	+ 2000	8	+ 1600	12	+ 2000	24	+ 2000	+ 2000	12
Valacyclovir (po)	2.5	25	1000	1000	8	1000	12	500	24	1000		
Valganciclovir (po)	3.0	68	900	900	12	450	24	450	72	900		
Vancomycin	6	150	1000	1000	12	1000	24	500	72	1000	1000	24
Voriconazole	8	12	2 x 400/24	200	12	200	12	200	12	200	200	12
Zalcitabine (po)	1.8	11	0.75	0.75	8	0.75	12	0.75	24			
Zanamivir	2.5	13.7	10	10	12	10	12	10	12		10	24
Zidovudine	1	1.9 (52)	200	200	8	100	8	100	8	200	200	12

Drugs are listed in alphabetical order and the parameter values for the drug (or active metabolite) are taken from our NEPharm database. If the individual GFR is not exactly 100 mL/min, or 30 mL/min, or 5 mL/min, the dose could be estimated by interpolation between the stated proposals. GFR: Glomerular filtration rate.

Dose adjustment rules

According to the proportional dose adjustment rules as proposed by Luzius Dettli, either the dose (D) should be reduced or the interval (Tau) extended (Figure 2). When the dose is reduced (Dettli 1) the peak levels are lower than in normal conditions but the trough levels are higher. When the administration interval is extended (Dettli 2) the peak and the trough concentrations are kept constant but the dosing frequency will decrease.

$$\frac{D}{Tau} = \left(\frac{D}{Tau}\right)_{norm} \cdot \frac{T_{1/2norm}}{T_{1/2fail}}$$

The dosing alternative proposed by Calvin Kunin states: The loading dose is the normal dose ($D_{start} = D_{norm}$) and the maintenance dose is one half of the loading dose where the administration interval corresponds to one half-life[23]. The Kunin rule leads to normal peak levels but higher troughs, a larger area AUC and more frequent peaks than those obtained with the Dettli rule 2.

$$\frac{D}{Tau} = \frac{(1/2) \cdot D_{start}}{T_{1/2}} = \frac{(1/2) \cdot D_{norm}}{T_{1/2}}$$

The Kunin rule can be illustrated with the example of ampicillin. In kidney failure, the ampicillin dose is decreased from 2000 mg every 8 h to 1000 mg every 12 h, since the half-life increases from 1.0 to 13 h (Table 1). For a GFR of 30 mL/min, the ampicillin half-life can be estimated at 3.8 h, giving reason to extend the administration interval from 8 to 12 h but to not change the 2000 mg dose since the half-life is shorter than the administration interval.

A general dosing rule that combines the Kunin rule with the Dettli rule 2 has been mentioned by Luzius Dettli: the eliminated fraction rule (Dettli 3). With the Dettli

rule 3, the administration interval is selected according to the target trough concentration while the peak is kept constant (Figure 3).

$$D = D_{norm} \cdot \frac{1 - \exp(-0.693 \cdot \frac{Tau}{T_{1/2}})}{1 - \exp(-0.693 \cdot \frac{Tau}{T_{1/2}})_{norm}}$$

$$= D_{start} \cdot [1 - \exp(-0.693 \cdot \frac{Tau}{T_{1/2}})]$$

$$= D_{start} \cdot [1 - (\frac{C_{trough}}{C_{peak}})_{target}]$$

$$Tau = \frac{T_{1/2}}{0.693} \cdot \ln(\frac{C_{peak}}{C_{trough}})_{target}$$

For the condition where peak as well as trough concentrations are constant and maintained as in the normal situation, the Dettli rule 3 corresponds to the Dettli rule 2 with a proportional extension of the administration interval. For the condition where the peak is constant but the trough should be no less than one half of the peak, the Dettli 3 rule corresponds to the Kunin rule.

Which rule should be applied cannot be decided by pharmacokinetic principles alone, but pharmacodynamic principles must be considered too. In addition, whenever possible, therapeutic drug monitoring should be utilized. In times where tandem mass spectrometry LC-MS/MS is possible, nearly every drug could be measured.

Therapeutic drug monitoring

Amikacin, gentamicin, tobramycin, teicoplanin and vancomycin, but recently also colistin, piperacillin, meropenem and linezolid are anti-infective drugs that routinely can be measured. When drug levels are measured for optimizing antimicrobial therapy, two important peculiarities must be observed. If impaired kidney function impacts pharmacokinetics, higher trough concentrations must be accepted to obtain efficient peak concentrations - this can be seen when the Dettli rule 1 or the Kunin rule are applied for dose adjustment (Figures 2 and 3). This was demonstrated by the use of aminoglycosides in HD patients where only troughs of at least 3 ng/mL are associated with peaks above 7 ng/mL and both peaks and troughs were significantly higher in those patients surviving than in those without anti-infective success[24,25].

In line with these statements, the target trough concentration for vancomycin has consistently been increased in the last 25 years. The area under the curve should be > 400 h x mg/L (= 24 h x Css; Css > 17 mg/L) to obtain an antimicrobial response with vancomycin[26]. The new targets are troughs of 15 ng/mL needed to guarantee peaks of 30 to 40 ng/mL[27]. The further increase in vancomycin dose and higher trough concentrations, however, might be associated with an increased risk of nephrotoxicity[28].

Counterintuitively, plasma binding does not have much impact on drug dosing since the absolute free drug concentration value (C_{free}) is unchanged when bound concentrations change[29].

$C_{free} = C - C_{bound} = (C - \Delta C_{bound}) - (C_{bound} - \Delta C_{bound}) = const$

If the binding decreases, only the total (C_{tot}) and the bound (C_{bound}) concentrations and not the free (C_{free}) concentration will decrease. Since the effect is supposed to depend on free concentrations, lower total concentrations do not need a change in dosage. However, plasma binding does have an effect on drug monitoring as far as total concentrations are measured ($C_{tot} = C_{initial} - \Delta C_{bound}$) and lower than normal concentrations must be the target when binding is less. This mainly applies to antibiotics with high plasma binding such as teicoplanin and ceftriaxone. And again, the decision as to which concentration should be the target can be made most rationally by considering pharmacodynamic criteria too.

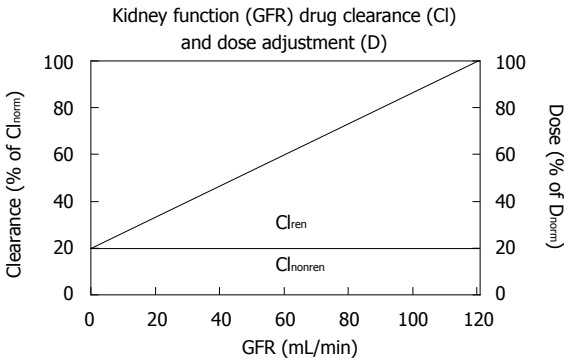

Figure 1 Linear correlation between drug clearance and the glomerular filtration rate as a measure of kidney function[20]. The dose can be adjusted in proportion to the reduced drug clearance, where $Cl = Cl_{ren} + Cl_{nonren}$. GFR: Glomerular filtration rate.

Pharmacodynamics

Pharmacokinetics is a necessary requirement for drug dose adjustment, but only the combined use of pharmacokinetics and pharmacodynamics is the sufficient condition for drug dose adjustment. Although some drug action might follow the dynamics of an irreversible effect, the most general concept of pharmacodynamics is based on the sigmoid Hill equation describing reversible effects. Even after mechanistic analysis of bacterial growth and killing dynamics, the Hill equation applies also to modeling the antimicrobial effect[30,31]. The actual effect (E) is a function of the maximum effect and of the concentration producing the half-maximum effect (CE_{50}). The Hill coefficient (H) gives a measure of the sigmoidicity of the effect concentration correlation.

$$E = \frac{E_{max}}{1 - (\frac{CE_{50}}{C})^{H}}$$

From the above equation, the threshold concentration (CE_{05}) and the ceiling concentration (CE_{95}) can be derived[32]. The threshold concentration produces only 5% of the maximum effect and the ceiling concentration produces 95% of the maximum effect. The higher the Hill coefficient, the higher the threshold concentration is,

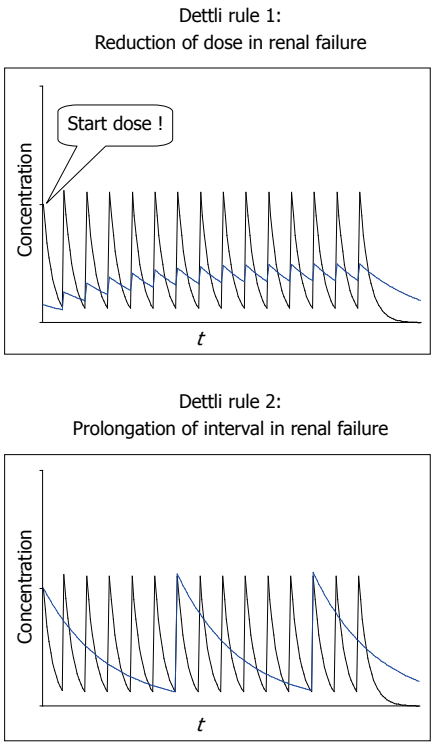

Figure 2 Dettli rules 1 and 2 for drug dose adjustment in kidney dysfunction. Dettli rule 1 leads to higher trough concentrations but lower peaks. To obtain an immediate antimicrobial effect, a loading dose must be given. With Dettli rules 1 and 2, the area under the curve AUC remains constant.

but the lower the ceiling concentration and the narrower the range of lower and upper target concentrations are (Figure 4).

$CE_{05} = CE_{50} \cdot 19^{-1/H}$

$CE_{95} = CE_{50} \cdot 19^{1/H}$

The ceiling concentration can be considered to be the upper limit of the target peak levels ($C_{peak} < CE_{95}$), whereas the threshold concentration marks the lower limit of effective trough levels ($C_{trough} > CE_{05}$). The distance between the ceiling and the threshold concentrations depends on H, not on CE_{50}, and the ceiling-to-threshold time $t_{ceiling\text{-}threshold}$ can be measured by multiples of the respective elimination half-life. For a drug with a short half-life and a high Hill coefficient, the therapeutic range of target concentrations can be very narrow (Figure 4).

$$CE_{05} = CE_{95} \cdot \exp\left(-\frac{\ln(2)}{T_{1/2}} \cdot t\right)$$

$$t_{ceiling\text{-}threshold} = T_{1/2} \cdot \frac{2}{H} \cdot \frac{\ln(19)}{\ln(2)} = T_{1/2} \cdot \frac{8.5}{H}$$

This conclusion might be illustrated with the beta lactam antibiotic ceftazidime where the half-life is 2.1 h and short in patients with normal kidney function (Table 1) but the Hill coefficient is 3.7 and high[33]. These values yield a short peak to trough or ceiling-to-threshold time $t_{ceiling\text{-}threshold}$ = 5 h, indicating that ceftazidime should be given at least every 6 h to maximize efficacy. In contrast, for gentamicin, the half-life is also 2 h (Table 1), but the Hill coefficient is 1.3 and low[33].

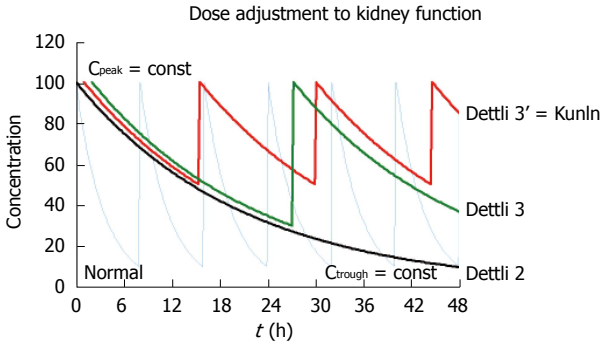

Figure 3 It is most practical to keep the peak concentration constant when the drug dose is adjusted to impaired kidney function[9]. With the eliminated fraction rule (Dettli 3), any dose and any interval can be estimated and selected. The Kunin rule is a special case of the Dettli rule 3 for the condition C_{trough} = 1/2 C_{peak}. With the Kunin rule and the Dettli rule 3, the area AUC is higher than under conditions with normal kidney function.

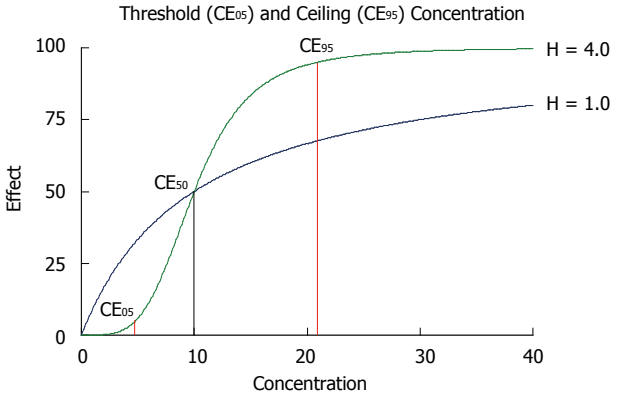

Figure 4 Pharmacodynamics. The threshold concentration CE_{05} produces 5% and the ceiling concentration CE_{95} produces 95% of the maximum effect. With a Hill coefficient of H = 1.0, the concentration is CE_{05} = 0.5 units and the CE_{95} = 190 units whereas for a higher Hill coefficient of H = 4.0, the threshold is high with CE_{05} = 6.0 units but the ceiling is low with CE_{95} = 21 units.

Thus, the estimated peak-to-trough time $t_{ceiling\text{-}threshold}$ is longer than 13 h: Here the administration interval could be extended to 12 h or more (Tau = $t_{ceiling\text{-}threshold}$).

The clinical progress in anti-infective dosing that has had the greatest impact has probably been achieved with the differentiation of drugs with time-dependent from drugs with concentration-dependent action[34,35]. Specific examples are the penicillins, cephalosporins, vancomycin, teicoplanin, the penems and the antiviral drugs with a time-dependent effect whereas gentamicin, amikacin, daptomycin, colistin, ciprofloxacin or levofloxacin possess a concentration-dependent activity.

It has been shown that anti-infective drugs with a time-dependent effect have a significantly higher Hill coefficient than those with concentration-dependent action[33]. This difference translates into practical consequences for the threshold and the ceiling concentration. A high Hill coefficient is associated with a relatively low ceiling concentration but simultaneously with a high threshold concentration (Figure 4). Thus, the time interval should be short between dosing of time-dependent

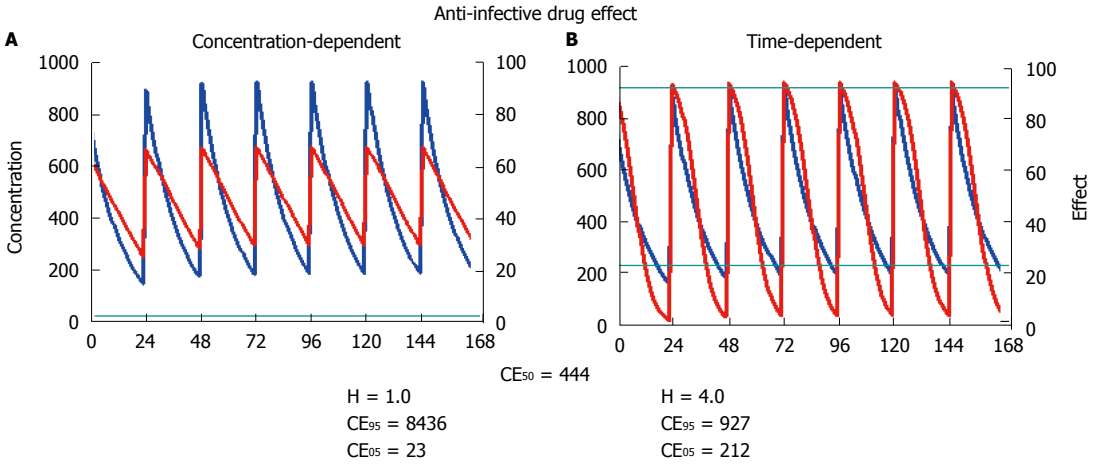

Figure 5 Pharmacodynamics of anti-infective drugs. The pharmacokinetics and the concentration curves are equal in both diagrams. Also the concentration producing the half-maximum effect is the same but the Hill coefficient is different. A: Concentration-dependent effect: With a Hill coefficient of H = 1.0, the calculated peak effect is only 60% and far from the ceiling effect CE_{95}. Thus, the concentration-dependent effect could be strengthened by increasing the dose; B: Time-dependent effect: With a Hill coefficient of H = 4.0, the calculated trough effect falls below the threshold concentration CE_{05} at the second part of the administration interval. Thus, the time-dependent effect could be strengthened by dosing more frequently.

anti-infective drugs and it makes no sense to increase the dose above the ceiling concentration. In contrast, a low Hill coefficient is associated with a high ceiling concentration and a low threshold concentration. Thus, it might increase the effect of concentration-dependent anti-infective drugs to give a single high bolus dose but it is not so critical to extend the administration interval - as proposed for aminoglycosides[36]. On a practical level, it might prove optimal to administer anti-infective drugs with time-dependent action more frequently, or even as a continuous infusion[37,38]. By contrast, anti-infective drugs with concentration-dependent action should be given with a bolus and a high maintenance dose to increase efficacy (Figure 5).

The usual measures of the antimicrobial effect such as the time over minimal inhibitory concentration MIC, or the AUC over MIC, or the peak over MIC can be unified by the following concept: A close correlation of the MIC and the concentration producing the half-maximum effect can be predicted. However, it has been shown[33] that for concentration-dependent antimicrobial action, the minimal inhibitory concentration could fall considerably below the concentration producing the half-maximum effect (MIC << CE_{50}). Consequently, it might be more reasonable to compare the bacteriological MIC with the pharmacodynamic parameter of a threshold concentration. Frequently the concentration target is stated as high as 4 times above the MIC. If this target corresponds to the CE_{50}, this translates into an average sized Hill coefficient of H = 2.1 since the following condition might hold true.

$C_{threshold} = MIC = CE_{05} = CE_{50} \cdot 19^{-1/H}$

In agreement with this equation, the Hill coefficient of meropenem is reported at H = 3.1 for the MIC of 1.0 mg/L and a CE_{50} at 2.6 mg/L[33].

Potency is also a significant measure of microbiology. The potency is inversely proportional to the concentration CE_{50} producing the half maximum effect. Therefore, resistance of the strain is just another word for a change in the CE_{50} and thus for reduced potency of the drug.

potency = $1/CE_{50}$

To overcome resistance, a higher dose might be necessary since a high concentration CE_{50} is required to produce the half-maximum effect. This concept allows a distinction to be made between relative resistance and absolute drug resistance. A pathogen with relative resistance can be made sensitive by increasing the dose[39-41]. Thus, it has been recommended to treat severe infections with resistant strains by increasing the standard meropenem dose to 3 x 2000 mg per day[42,43] or the daptomycin dose to > 8 mg/kg per day[44] with careful monitoring of side effects.

From the concept of potency and the interpretation of the Hill coefficient, it can be considered plausible that the time-dependent action and the concentration-dependent action are only the extreme positions of a continuum. Every drug can be considered both concentration-dependent and time-dependent - more or less, either the one or the other[31]. The antimicrobial drug effect needs the presence of leukocytes, and less bacterial killing is reported in neutropenia[31]. Therefore, these patients need a 1.5 to 2 times higher than usual dose of anti-infective drugs[45]. In addition, the increasing rate of drug resistance in febrile neutropenia also strongly supports the concept of high dosing[31,46].

Dose adjustment

Anticancer drugs and anti-infective drugs should be used differently. The adjustment of anticancer drugs must not only be based on the kidney function but also on the physical condition of a patient. Tumor patients are older and anticancer drugs have a considerable potential for toxicity. Therefore, anticancer chemotherapy must be adjusted to both kidney function and to the general medical condition (in cases with Karnofsky index < 40% or ECOG > 2 performance status). In contrast to

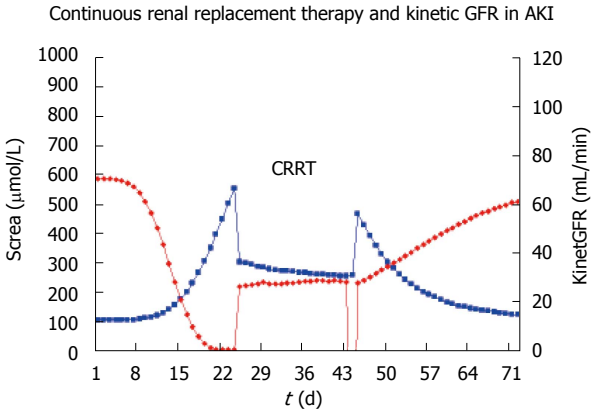

Figure 6 Serum creatinine (Screa) and estimated kinetic glomerular filtration rate in acute kidney injury. The kinetic GFR can also be estimated during continuous renal replacement therapy continuous hemofiltration (CRRT)[14]. GFR: Glomerular filtration rate; AKI: Acute kidney injury.

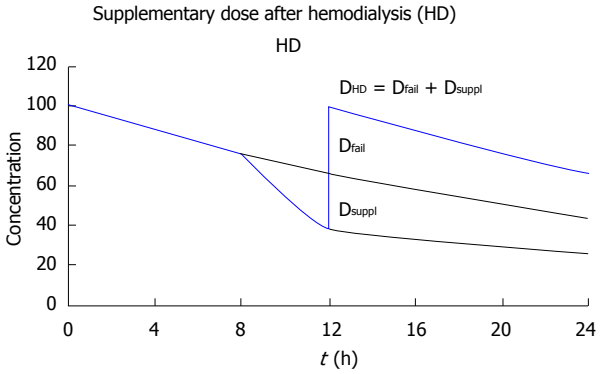

Figure 7 The dose after dialysis (D_{HD}) replaces both the dose adjusted for kidney failure (D_{fail}), and the supplementary dose (D_{suppl}) that compensates for the fraction (FR) removed during hemodialysis (HD).

anticancer drugs, however, the anti-infective therapy should be adjusted to kidney function alone, but a compromised or even poor general condition should not result in a reduced dose or selection of less active anti-infective therapy. An immediate and sufficiently high antimicrobial therapy is needed in the most vulnerable, that is, in elderly and immunocompromised cancer patients. Where the risk is low, oral dosing of anti-infective drugs is sufficient in febrile neutropenia[47]. In most cases, however, intravenous dosing might be preferable with sequential oral dosing only in responders.

Ehrlich[48] stated the principle of anti-infective therapy: "frapper vite et frapper fort" meaning "hit fast, hit hard".

For anti-infective drug therapy, the immediate and high loading dose is very important[49,50]. According to the "Tarragona strategy" the antibiotic regimen should be started fast and with a loading dose, whereas the dose adjustment follows the course and clinical condition[51]. It can be a deleterious mistake to adjust the dose to the impaired kidney function but to give no loading dose (Figure 2). The loading dose is usually the normal standard dose. However, many patients in the intensive care unit are over-hydrated and the distribution volume is much larger than under normal conditions[34]. The loading dose could well be adjusted to such volume changes by applying the BSA.

$$D_{start} = D_{norm} \cdot \frac{Vd}{Vd_{norm}} = D_{norm} \cdot \frac{Weight + V_{water}}{Weight_{norm}}$$

$$= D_{norm} \cdot \frac{BSA}{1.73\ m^2}$$

Thus, the required loading dose can be higher than the normal standard dose. In patients with sepsis, the gentamicin distribution volume was 0.35 L/kg *vs* 0.29 L/kg and significantly larger compared to intensive care patients without sepsis[52]. The need for a higher dose to initiate antimicrobial therapy can be stated as the rule when the immediate and high blood level is the target as with anti-infective therapy. The immediate start of treatment and an initially high concentration are also needed to avoid selection of resistant strains. Therefore, the antimicrobial treatment starts with a normal or even higher loading dose in the intensive care patients. Afterwards, the adjustment with a reduced maintenance dose is usually not needed before day 2 or 3 of anti-infective treatment[53].

A special problem occurs in the case of aminoglycosides: It is now standard practice to administer one single bolus dose per day instead of three divided doses[36]. Such a single high bolus dose will be associated with a 20-fold increase in the AUC if renal failure is present and the half-life increases from 2 to 40 h. For aminoglycosides, we propose administering only 50% of the standard high bolus loading dose to avoid excessive exposure in kidney failure or dialysis patients (Table 1). Following the loading dose, the maintenance dose can be estimated by one of the three Dettli rules, or the Kunin rule.

In addition to the case of over-hydration with an increase in distribution volume, the so-called augmented renal clearance has been brought into debate[54]. Augmented renal clearance is estimated from serum creatinine or endogenous creatinine clearance. If a patient is overhydrated, however, the serum creatinine is diluted, making creatinine clearance and creatinine-based GFR estimates falsely high. Since the clearance can be seen as the arithmetic product of elimination rate constant and distribution volume, the higher creatinine clearance in the patients with the systemic inflammatory response syndrome and sepsis could be explained by two mechanisms, augmented renal elimination and over-hydration. The consequences are different: augmented renal elimination needs a higher maintenance dose but over-hydration requires both an increase in the loading dose and a higher maintenance dose (= weight-based dosing as in pediatrics).

Renal replacement therapy

In the intensive care unit (ICU), three modalities are used as renal replacement therapy: Continuous hemofiltration (CRRT), sustained low efficiency daily dialysis (SLEDD) and intermittent HD. The hemofiltration is applied with variable modifications either of the surface area, of

the filter membrane, with predilution or post-dilution replacement fluid, and variable ultrafiltration rates that are used along with the corresponding flow rate of the substitution volume. Therefore, a global measure of the effect of hemofiltration on drug elimination will be very useful and the total creatinine clearance or the other creatinine-based measures of the GFR have been proposed for this purpose[9,55,56]. The recently introduced kinetic GFR applies also to patients with CRRT[14], and thus has clear advantages in the intensive care unit where the medical conditions can change rapidly (Figure 6).

$totalCL_{crea} = Filtration_{kedney} + Filtration_{CRRT}$

$totalCL_{crea} = eGFR = MDRD_{GFR} = CKD\text{ - }EPI_{GFR} = C\text{ and }G_{GFR}$

$totalCL_{crea} = kinetGFR$

There is a trend to underestimate drug elimination by CRRT and consequently under-dose antimicrobials in the ICU[57]. By using the total creatinine clearance, the creatinine-based GFR estimates or the kinetic GFR, the dose can be adjusted according to the rules of Dettli and Kunin also for patients on CRRT. As a rule and to avoid under-dosage, the normal standard dosage should be given and not be reduced if the total creatinine clearance is above 60 mL/min.

A combination of continuous and intermittent renal replacement is the SLEDD. The frequency of under-dosage is estimated with a median value of 70% whereas the risk of over-dosage was only 5% while on SLEDD[6,58]. If this kind of treatment is applied, the daily dose at least corresponds to the post HD dose (see below) but recommendations vary widely.

$D_{SLEDD} = D_{HD} \approx D_{start}$

More complex is the drug dosing when intermittent HD is performed (Figure 7). Off dialysis, the dose must be adjusted to the failing kidney function. For intermittent HD, we argue that it is better to give the dose not at the beginning but at the end or immediately after HD. With a pre-dialysis dose, no anti-infective effect will be maintained in the interval off dialysis[59].

If the drug is given after dialysis, the post-dialysis dose should replace first the amount eliminated during the interval off dialysis, that is, the dose for failing kidney function (D_{fail}). In addition to that, the effect of HD should be compensated by a supplementary dose (D_{suppl}) replacing the fraction eliminated on dialysis (FR).

$D_{HD} = D_{fail} + D_{suppl}$

$D_{suppl} = FR \cdot (D_{start} - D_{fail})$

$FR = 1 - \exp[(-0.693/T_{1/2on}) \cdot t_{on}]$

Thus, the dose after HD is higher than the adjusted maintenance dose[9]. In many cases the dose after HD is another loading dose (D_{start}). The post-dialysis dose (D_{HD}) can again be illustrated with the example of ampicillin: The fraction eliminated by dialysis is implicitly stated in NEPharm (40%) and the dose after dialysis is 2000 mg corresponding to the size of the normal loading dose (Table 1).

$D_{HD} \approx D_{start}$

In contrast to the usual post-dialysis dosing, it might be a good option to perform HD after drug administration for removal of high-dose anticancer therapy administered before dialysis. In analogy, the dosing immediately before dialysis has been also proposed for aminoglycosides[60]. With a pre-dialysis regimen, however, aminoglycosides must be given at a higher dose (gentamicin up to 400 mg) and HD should be performed on a daily basis in order to not miss the antimicrobial effect in the interval off dialysis.

CONCLUSION

The prevalence of CKD and incidence of AKI are high in patients with malignancies. This generally makes dose adjustment necessary, usually ending in a lower dose than normal. Since 1978, we have documented pharmacokinetic parameters in the NEPharm database from extracted PubMed citations[61-63]. With the parameters recorded in NEPharm and based on the above pharmacokinetic/pharmacodynamic considerations, we have made explicit dose proposals. These recommendations are used in our institution and subjected to continuous updates (Table 1).

Anti-infective therapy should start immediately without any delay and with a high dose. Dose adjustment follows on day 2 or later in the course of treatment[53]. A loading dose that takes into account the real volume especially in volume-expanded patients should be given. When in doubt, we propose that the peak level should be the target and the standard dose should be given with an extended administration interval when kidney function is impaired[9].

The anti-infective therapy should be optimized by therapeutic drug monitoring whenever possible (gentamicin, tobramycin, amikacin, vancomycin, teicoplanin, colistin, piperacillin, meropenem, linezolid). However, the adequate practical consequences should be drawn from the measured concentrations. In patients with impaired kidney function, higher trough concentrations result from the dose adjustment according to Dettli 1, Dettli 3 or Kunin. Only the Dettli rule 2 is associated with the same peak and trough concentrations as under normal conditions. On the other hand, the plasma binding of many drugs can decrease in kidney dysfunction. In this case, lower trough concentrations are acceptable (ceftriaxone, teicoplanin) since the absolute free concentration does not change when the bound fraction decreases but free concentrations produce the effect.

The modern distinction between time-dependent and concentration-dependent effects can be parameterized by the Hill coefficient. A high Hill coefficient (> 2.1) indicates time-dependent drug action, whereas a low Hill coefficient (< 2.1) indicates concentration-dependent action. Based on the Hill equation, the threshold concentration can be distinguished from the ceiling concentration. A high Hill coefficient determines that the ceiling concentration is low but the threshold concentration is relatively high (Figure 4). In contrast, a low Hill coefficient determines that the ceiling concentration is relatively high but the

threshold concentration is low. We suggest that the minimal inhibitory concentration from microbiology be correlated to the threshold concentration. The target concentration should not be less than the threshold concentration for time-dependent effects, but the target concentration could be as high as the ceiling concentration for concentration-dependent effects.

To decide between the pharmacokinetic dosing alternatives (Dettli 1-3), pharmacodynamic considerations can give an answer to whether the dose should be reduced or the interval extended in kidney dysfunction: (1) For time-dependent anti-infective action, more frequent dosing is more effective than maintaining the single high dose[35]: The target trough levels should be kept above the threshold concentration (Figure 5). The beta lactam antibiotics oxacillin or piperacillin are considered to exhibit a time-dependent action. Accordingly, it has been shown that continuous infusion produces a better antimicrobial response than intermittent dosing of the respective daily dose[37,38]; and (2) For concentration-dependent anti-infective action, however, the extension of the interval is less disadvantageous than reducing the single dose (Figure 5). The target peak levels should be close to the ceiling concentration and kept as high as possible[35]. The quinolone ciprofloxacin exhibits concentration-dependent action. Here, the high bolus dosing produced a more rapid bactericidal effect than the more frequent application of a lower dose[33,64]. Also for aminoglycosides, a high peak concentration is superior to more frequent dosing to induce bacterial killing[36,65].

For drugs with a high Hill coefficient, the area under the effect time curve may fall disproportionally less and result insufficient with a lower dose[61]. Therefore, we discourage proportional dose reduction, especially Dettli 1, if the Hill coefficient is unknown. The risk of selecting resistant strains is also less when the initial dose is high[31].

The time above MIC reflects effect duration. A pharmacodynamic measure for the duration of drug effect, the time of effect duration (TED), can be derived from the elimination half-life[18]. The intuitively most evident effect duration time is the effect bisection time (TED_{50}) that is correlated to the elimination half-life ($T_{1/2}$), the peak concentration (C_{peak}) and the Hill coefficient (H) along with the concentration (CE_{50}) producing the half-maximum effect[18].

$$TED_{50} = T_{1/2} \cdot \left(\frac{1.44}{H}\right) \cdot \ln\left[2 + \left(\frac{C_{peak}}{CE_{50}}\right)^{H}\right]$$

The longer the half-life and the higher the peak concentration - but the less the CE_{50} - the longer lasting the effect is. The half-life is 1.0 h (Table 1) and the Hill coefficient is stated at H = 3.1 for meropenem[33]. If the MIC of 6 mg/l[44] is equated to the threshold concentration (CE_{05} = MIC), the CE_{50} can be estimated at 37 mg/L. With a dose of 500 mg every 8 h and a peak concentration of 50 mg/L[44], the effect bisection time will be estimated at TED_{50} = 0.71 h. Doubling the dose, however, will more than double the effect bisection time TED_{50} to 1.5 h, thus extending the drug action while the pharmacokinetic half-life of 1.0 h is the same. However, the standard dose administered more frequently would not increase the effect bisection time.

The dose in patients with continuous renal replacement therapy can be derived from the creatinine-based GFR estimates or in case of changing kidney function, from the "kinetic GFR" (Figure 6). If this GFR estimate is above 60 mL/min, no dose adjustment is required. For intermittent HD a supplementary dose should be given after dialysis (Figure 7). The supplementary dose adds with the dose adjusted to renal failure to the post-HD dose that can be as high as the loading dose. This practice might be prudent also in cases where the drug fraction eliminated during HD is not known.

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REVIEW

Estimating glomerular filtration rate in kidney transplantation: Still searching for the best marker

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Abstract

Kidney transplantation is the treatment of choice for end-stage renal disease. The evaluation of graft function is mandatory in the management of renal transplant recipients. Glomerular filtration rate (GFR), is generally considered the best index of graft function and also a predictor of graft and patient survival. However GFR measurement using inulin clearance, the gold standard for its measurement and exogenous markers such as radiolabeled isotopes (^{51}Cr EDTA, ^{99m}Tc DTPA or ^{125}I Iothalamate) and non-radioactive contrast agents (Iothalamate or Iohexol), is laborious as well as expensive, being rarely used in clinical practice. Therefore, endogenous markers, such as serum creatinine or cystatin C, are used to estimate kidney function, and equations using these markers adjusted to other variables, mainly demographic, are an attempt to improve accuracy in estimation of GFR (eGFR). Nevertheless, there is some concern about the inability of the available eGFR equations to accurately identify changes in GFR, in kidney transplant recipients. This article will review and discuss the performance and limitations of these endogenous markers and their equations as estimators of GFR in the kidney transplant recipients, and their ability in predicting significant clinical outcomes.

Key words: Glomerular filtration rate estimation; Creatinine; Cystatin C; Kidney transplantation; Clinical outcomes

Core tip: An accurate evaluation of allograft function is essential in the management of kidney transplant. Glomerular filtration rate (GFR), is generally considered the best index of graft function. Endogenous markers, such as serum creatinine or cystatin C, are used to estimate kidney function, and equations using these markers adjusted to other variables, are an attempt to improve accuracy in estimation of GFR. This article will review and discuss the performance and limitations of these endogenous markers and their equations as estimators of GFR in the kidney transplant recipients, and their ability in predicting clinical outcomes.

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INTRODUCTION

Kidney transplantation is the treatment of choice for end-stage renal disease. A successful kidney transplant improves the quality of life, reduces the mortality risk for most patients and is less costly when compared with maintenance dialysis[1-3]. The significant progress that has occurred over the last two decades in renal transplantation is mostly driven by improvements in short-term graft survival whereas long-term outcomes remained largely unchanged[4,5]. Nowadays, with the traditional short-term outcomes, namely the 1-year graft and patient survival rates in excess of 90% and 1-year acute rejection rate of less than 15%, the question arises if any further improvements are possible or even necessary[6]. However, these outstanding results have failed to anticipate long-term survival, so it becomes clear that identification of new, short-term end points capable of correlating with long-term graft outcome is necessary[7] ideally translating in longer graft maintenance.

Renal allograft function seems to be a tempting candidate as surrogate marker for research studies on transplantation[8], also for the assessment of new drugs[9,10], although its use as an outcome marker for graft loss is controversial[11,12]. In generally, the glomerular filtration rate (GFR), is considered to be the best index of overall kidney function[13,14], also an indicator of long-term graft survival[15], and an independent risk factor for cardiovascular mortality[16,17], the primary cause of death in kidney transplant recipients[17,18]. Of note, like in non-transplant chronic kidney disease, prevalence of complications related to loss of renal function such as hypertension, anemia and abnormal mineral metabolism increases significantly as the GFR declines[19]. Another important point is that the decline in GFR is also related with increased health care costs, and over the two years, transplantation was both more effective and less costly than dialysis[2,3]. Therefore, an accurate evaluation of renal allograft function is crucial in the clinical management of kidney transplant recipients.

Methods to measure GFR using exogenous markers, such as inulin clearance, the gold standard, and others such as radiolabeled isotopes (^{51}Cr EDTA, ^{99m}Tc DTPA or ^{125}I Iothalamate) and non-radioactive contrast agents (Iothalamate or Iohexol), are laborious as well as expensive, being rarely used in clinical practice. Therefore, endogenous markers, such as serum creatinine (SCr) or cystatin C (CyC), are used to estimate kidney function. Mathematical formulas employing these markers adjusted to other variables (mainly demographic) are an effort to ameliorate GFR estimation (eGFR) accuracy.

However, there is some concern about the accuracy of the available eGFR equations in kidney transplant recipients and guidelines still provide conflicting recommendations about GFR estimation methods in this population[20].

In this article, we aim to review the performance and limitations of these endogenous markers and their equations as estimators of GFR in the kidney transplant recipients, and their ability in predicting significant clinical outcomes.

ENDOGENOUS MARKERS

SCr

SCr concentration is the best known and most commonly used marker for estimation of GFR, since it was first described as a GFR marker in 1937[21], and SCr analysis is inexpensive and generally accessible. Creatinine is a breakdown product of creatinephosphate in muscle tissue, produced at a relatively constant rate, depending on the muscle mass, and filtered in the glomerulus but also actively secreted in the proximal tubule[22]. Tubular secretion contributes normally to 10% of renal Cr removal, but increases when GFR decreases[23], causing SCr to remain in the normal range until GFR drops below 60-70 mL/min. Some Cr is also incorporated from the diet. Ingestion of meat contributes substantially to the urinary Cr excretion, both as a result of expansion of the total creatine pool and as a result of gastrointestinal absorption of Cr[22]. Thus, multiple factors contribute to reduce the accuracy of SCr as an indicator of the GFR, including sex, age, race, muscle mass and dietary protein intake.

Particularly, in renal transplantation there are other determinants that may interfere with Cr metabolism such as corticosteroids, which have a direct catabolic effect[24] and cause a changed muscle mass ratio to total body weight[25]. Catabolic illnesses such as infection and acute rejection, and prolonged dialysis, can also be partly responsible[26].

Cr tubular secretion can be blocked by some drugs such as trimethoprim, commonly used in kidney transplantation[27]. Also, chronic rejection and acute tubular necrosis, can contribute, because tubular secretion of creatinine is reduced.

Because Cr secretion is not predictable, the GFR can decrease to nearly half the normal value before the SCr increases[13], with remarkable consequences in kidney transplant outcome, where subclinical progressive damage, such as calcineurin toxicity and rejection will not be early identified. Several studies in kidney transplantation demonstrated that the SCr and GFR were barely correlated[26,28].

In addition, SCr measurement by the most common method (Jaffé) is subject to interferences by chromogens such as bilirubin, glucose and uric acid, and the enzymatic method is prone to interference by bilirubin and some antibiotics. Considerable variations between SCr assays calibration may also cause inaccuracies in its determination[29]. An attempt to standardize measurement

has been recently introduced by adoption of a common calibration to isotope dilution mass spectrophotometry standard (IDMS) with substantial improvement and traceability of SCr measurements[30].

Nonetheless, SCr is recommended as a screening test for changes in allograft function[31], adjustments of immunosuppressive drugs[32], and it was shown that SCr by itself may be a predictor of long-term graft and patient survival[33].

Creatinine clearance

Creatinine clearance (CCr) as measured from 24-h urine collection is often used in clinical practice to calculate GFR, but it overestimates GFR due to the secretion of Cr by the renal tubules and the inherent limitations of SCr as a kidney marker. However, this calculation does not correct for tubular secretion, and overestimates GFR also in transplant populations[28,34,35], with additional errors in urine collection. Measurement of CCr using this method becomes more reliable after the administration of cimetidine, which inhibits tubular secretion[36], but still does not supply additional knowledge about renal function than other Cr-based methods[13,14].

Serum CyC

CyC is a 122-amino acid, 13-kDa protein that is a member of a family of competitive inhibitors of lysosomal cysteine proteinases. Its functions include involvement in extracellular proteolysis, immune modulation, and antibacterial and antiviral activities.

CyC has certain characteristics that make it an acceptable candidate as a kidney function marker, including a constant production rate, free glomerular filtration, complete reabsorption and catabolism by the proximal tubules with no reabsorption, and no tubular secretion[37].

Several clinical data demonstrated that serum CyC levels correlate better with GFR than does Cr alone, especially at higher levels of GFR, and it was also thought to be less influenced by certain demographic factors such as age, race, gender, or muscle mass compared with SCr[38,39]. However, some emerging new data have shown that serum CyC may be influence by these and other variables.

A recent study concluded that CyC was 9% lower in women and 6% higher in blacks for a given GFR[40]. In a cross-sectional study, Knight *et al*[41], found that older age, male gender, greater weight, greater height, current cigarette smoking, and higher serum C-reactive protein levels were independently associated with higher serum CyC levels after adjusting for CCr.

Moreover, in certain clinical settings, CyC level may be biased as a marker of kidney function, such as in patients with uncontrolled thyroid disease, rapid cell turnover, and those under steroid therapy[42], like kidney transplant recipients. Also, CyC is quite costly and unavailable in many transplant centers.

GFR ESTIMATION FROM SCR BASED EQUATIONS

To overcome some of the limitations of Cr as a marker for GFR, several formulas have been constructed to correct for the influences of weight, age, gender and/or race[26,43-46].

Some of these equations have been evaluated in renal transplant patients, and the most commonly used are the Modification of Diet in Renal Disease (MDRD) study[44], Cockcroft-Gault[43], and Nankivell[26] equations. The KDIGO position statement includes the proposal that Cr-based eGFR equations should be used to evaluate renal function in the everyday management of renal transplant recipients[14].

The Cockcroft-Gault equation was derived in 236 (96% male) hospitalized patients with a wide range of GFR values[43]. The MDRD equation, published in 1999 were derived in 1628 patients with chronic kidney disease (mean GFR, 40 mL/min per 1.73 m^2)[44], and this was simplified in 2000[47] and reexpressed in 2005, after standardization of the SCr assays to the reference method using IDMS[48,49]. The Nankivell equation is the only one that was derived from kidney transplant recipients[26], however some of these transplant patients were in an early post-transplant phase or with acute dysfunction, which has implications in prediction of GFR.

More recently, a new formula was published by the chronic kidney disease epidemiology collaboration (CKD-EPI)[50], to overcome the systematic underestimation of GFR and lack of precision of the MDRD formulas in patients with relatively well-preserved kidney function, but only 4% of the CKD-EPI derivation cohort consisted of organ transplant recipients.

PERFORMANCE OF CREATININE-BASED GFR ESTIMATION EQUATIONS IN KIDNEY TRANSPLANTATION

To certify graft function as a valid surrogate marker, we must know for certain that we use a solid measure of kidney function.

The eGFR equations were an alternative to estimate GFR in clinical context, as they allow us to overpass some of the limitations of the SCr[51].

To determine the performance of a given eGFR equation the K/DOQI guidelines[13] proposed a methodological approach according to simple and reproducible criteria: "BIAS", "PRECISION" and "ACCURACY". The absolute BIAS expresses the systematic deviation from the gold standard measurement of GFR, and was given by the mean difference between estimated GFR and gold standard clearance (true GFR). The relative BIAS, hereafter named percent BIAS, is expressed as the proportion of true GFR represented by the absolute bias, and was calculated as: absolute BIAS/true GFR × 100. PRECISION expresses the

variability or dispersion of predictions around the true GFR and corresponds to the standard deviation of the difference between the true and estimated GFR. The distribution of the differences between estimated and true GFR accounts for the ACCURACY of the GFR estimates (*e.g.*, 30% accuracy is the proportion of predicted GFR within ± 30% of the true GFR).

In several studies in kidney transplantation, the efficiency of MDRD, Cockcroft-Gault and Nankivell equations has been consistently reviewed[52], with a significant heterogeneity between studies, with low precision inducing limited accuracies, and this can be attributed to varied patient characteristics, differences in measure GFR methods and Cr assay calibration and, potentially, some inherent differences in this specific population of transplant recipients[52]. In the majority of these studies, all of these equations persistently testified progressive decrease in GFR overestimation and/or increase in GFR underestimation as graft function ameliorated [28,34,53].

The CKD-EPI equation[50] introduces a correction term to overcome the systematic underestimation of GFR of the MDRD formulas in patients with relatively well-preserved kidney function, as mentioned above. In a cohort of 207 stable Kidney transplant recipients[54] CKD-EPI shows improved estimation ability compared with MDRD equation, but still with suboptimal precision that limit the value of the CKD-EPI for monitoring changes in kidney function over time[54]. Other studies compare the performances of the MDRD and CKD-EPI equations in a large transplant patient's cohort[55,56] and the authors concluded that the latter equation does not offer a better GFR estimation in this population.

More recently, Shaffi *et al*[57], conducted a systematic evaluation of the development methods of all published Cr-based eGFR equations, and assess their performance in a large population (n = 3622) of solid-organ transplant recipients, including 53% kidney transplant recipients. They founded that the CKD-EPI[50] and IDMS-traceable 4-variable MDRD Study equations[48] were more accurate than the alternative equations, including those developed in populations including only transplant recipients, and as accurate as observed in non-transplanted populations. Nevertheless, we can't forget that these equations still misestimate true GFR by > 30% in 1 of 5 patients.

They also concluded that there was no difference between these two equations in the overall study population, but CKD-EPI equation showed better performance at higher GFRs compared with better performance of MDRD Study equation at lower GFRs, which is in agreement with the results of the systematic review performed by Earley *et al*[58]. This study[57] may have implications in clinical practice, support the use of these eGFR equations to routine access renal function in transplant patients as in other populations. Even though it was a good diagnostic test study design with a standardized reference test, the study population included few nonwhites and individuals with solid organ transplants other than liver and kidneys; therefore assessment of the equation performance in these subgroups is limited[57].

However we can't ignore that SCr levels are affected by factors besides GFR, and several studies suggest worse stage-based care in kidney transplant patients compared with native kidney diseases[59,60], so any eGFR equations based on SCr still have limitations.

PERFORMANCE OF CYSTATIN-BASED GFR ESTIMATION EQUATIONS IN KIDNEY TRANSPLANTATION

As with SCr, it is the CyC-based GFR, rather than the CyC itself, that is of greater clinical interest. Over the last decade, several serum CyC-based equations have been developed and proposed to estimate the GFR[61-67].

Only two of these equations (Rule *et al*[64] and Le Bricon *et al*[67]) were exclusively derived from a population of kidney transplant recipients.

Several studies in the renal transplant population, showed discordant results with some indicate advantage of CyC-based equations over Cr-based equations, whereas others showed no superiority of CyC over SCr[20,34,68]. One of the limitations of CyC-based eGFR formulas in this population is that the treatment with corticosteroids increases CyC levels by increasing the production of CyC[69]. Although the KDIGO recommendations on kidney transplantation comment the possible interest of using CyC to GFR estimation, they do not advocated its regular clinical use, due to the paucity of validation studies in this group of patients[20].

A recent systematic review[70], identified 10 studies, evaluating the accuracy of 14 different CyC-based eGFR equations in renal transplant recipients. The authors conclude that the Le Bricon equation[67] was the highest accurate, and the majority of the CyC-based equations exhibited 30% and 50% accuracy improvements compared with the Cr-based MDRD equation. However, as with the Cr equations, there was substantial variability between the studies. Much of this variability is consequence of different study populations, differences in the GFR reference standard measurement, and in variation in the calibrators for the CyC measurement, and this latter contributes to the greatest source of variation. Standardized reference material for CyC has already been developed[71], but none of the studies involved in this analysis[70], adopted this methodology.

In 2008 a new Cr- and CyC-based formula (CKD-EPI CyC equation) was developed[40], which besides serum CyC includes the variables of gender, age and race, and seems more accurate than the formulas based on Cr or CyC alone, but this formula requires further testing in various patients groups.

Recently, the CyC-based estimating equations were re-expressed for use with the standardized CyC reference material (ERM-DA47/IFCC)[72]. These and the equations with CyC in combination with SCr[40], improved in 2012 with lesser bias at GFR > 60 mL/min

(CKD-EPI Cr-CyC 2012)[73], were validate in a European cohort of renal transplants patients[74] but their accuracy needs to be evaluated in more studies with this population.

CREATININE-BASED AND CYC-BASED eGFR EQUATIONS AS PREDICTORS OF CLINICAL OUTCOMES

Although, it has been demonstrated that eGFR is a predictor of patient and transplant survival[75], disappointing results have been reported when several of Cr-based eGFR formulas where assessed against the most important outcome measures such as mortality and graft failure, with limited utility and no benefit over the use of SCr alone[76].

Another relevant problem in clinical practice is whether the eGFR equations were able to precisely predict variations in graft function over time. Several studies reported considerable variability of the Cr-based eGFR equations performance at different times post-transplant[28,77] with less accuracy within the first year of transplantation[78], indicating that those Cr-based equations must be worn with caution for GFR monitoring through time[79].

Nowadays there is an increasing interest in CyC-based equations as an outcome predictor in kidney transplantation. In general population, CyC-based eGFR equations are a stronger predictor of the risk of death and cardiovascular events, when compared with Cr[80,81], as well as the correlation of serum CyC with all-cause and cardiovascular mortality in chronic kidney disease (CKD)[82]. Recently, a meta-analysis of 11 general-population studies and 5 studies of cohorts with CKD[83], shows that the utilization of CyC alone or in combination with Cr reinforce the power of eGFR as a predictor of end-stage renal disease and death .

Whether this outcome prediction is true for transplant recipients needs to be confirmed. Although, some studies showed that CyC and or CyC-based equations predicted both patient mortality and graft outcome better than Cr-based eGFR equations[34,84], others founded that CyC and SCr were equally reliable predictors of graft outcome[85].

Interestingly, very recently, a study examined the extent to which the addition of serum CyC improves GFR estimation and mortality prediction, in comparison to various eGFR equations, in a population of 401 liver transplanted patients. In this work, the authors founded that CyC, by itself or as a part of an eGFR, was a significant predictor of mortality[86].

Another approach is a multimarker management, including combination of different markers of graft function, such as SCr, CyC, and kidney pathologic markers, such as proteinuria and/or albuminuria. Models that include Cr-based or CyC-based eGFR and albuminuria show better prediction to end-stage renal disease in general population[87,88], and CKD patients[89].

A clinical score constructed from a cross-validated French database of 2169 kidney transplant recipients, combining risk factors of graft loss, including SCr and proteinuria, demonstrated to be highly predictive of long-term kidney graft survival[90], and other study demonstrated that the combination of low-grade albuminuria and decreased eGFR was related with graft loss and mortality[91].

In a similar way, a recent small-sample single-center study[92] founded that predictors combining albuminuria and Cr- or CyC-based eGFR, performed better than those markers alone, to predict death censored graft loss, in kidney transplant recipients. Moreover, the best predictor of graft failure in this work was a product of CyC and the logarithm of albuminuria, and CyC-based predictors performed better than Cr-based predictors.

More recently, there has been some enthusiasm in new markers of kidney injury such as neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1), and it potential in prediction of kidney function, not influenced by age, gender, race, body fat and muscle mass. Particularly, in kidney transplant recipients, it was demonstrated that urinary excretion of KIM-1, a proximal tubular protein, independently predicts graft failure[93]. However, more trials are required to validate these results in clinical setting.

PROTEINURIA

Proteinuria, although not a direct GFR marker, is also an important indicator of allograft dysfunction[94,95], associated with a reduced long-term graft survival[94,95] and increased patient mortality[94,96].

One of the limitations of proteinuria as an accurate marker of graft dysfunction is the native kidney excretion, and in that cases, should have a baseline value before transplantation, particularly in the setting of pre-existing glomerular disease. However, pretransplant proteinuria decreases or disappears after successful transplantation and *de novo* or increasing proteinuria is indicative of graft pathology[97].

Proteinuria can signal pathologic changes including recurrent or *de novo* glomerular disease, calcineurin inhibitor toxicity, alloantibody-mediated injury and chronic allograft nephropathy[98]. In this way, graft biopsy helps to determine the etiology of proteinuria[20,99] and to manage some treatable causes of graft injury. KDIGO guidelines[20] proposed monitoring of proteinuria as part of routine transplant follow-up.

CONCLUSION

An accurate evaluation of allograft function is crucial in the management of kidney transplant, and most importantly in predicting clinical outcomes. However any endogenous kidney function marker has limitations, and understandably, eGFR formulas derived from them will present similar barriers. Also, these prediction equations have inherent problems, namely the selected

populations used for their derivation, usually non-transplanted patients.

The Cr-based eGFR equations were the much widely used and recent studies, accessing the performance of MDRD study and CKD-EPI equations in kidney transplantation, support their use to routine access renal function in transplant patients as in other populations. But, we can't forget that Cr-based eGFR equations have never been demonstrated to improve the clinical recognition of changes in transplant function, compared to the use of Cr alone, and many transplant injuries occur without change in SCr level or eGFR.

In the last years, our attention is moving toward another markers and CyC seems to be a promising one. Although, some conflicting results, several studies in kidney transplants confirm the better performance of CyC-based equations over Cr-based equations in estimating GFR. The use of CyC alone or in combination with Cr reinforces the eGFR power as a predictor of end-stage kidney disease and death, in general and CKD population, but we need to confirm this outcome prediction in transplant recipients. However, like Cr, CyC is also influenced by non-GFR determinants, is more expensive than SCr and has suboptimal standardization, therefore its use is not widespread implemented.

Finally, a model combining different markers such as SCr, CyC, proteinuria and/or albuminuria can be useful in clinical practice, providing an improvement in outcome prediction. At moment, and regarding the kidney transplant management, we are still searching for the optimal combination and for the best marker.

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MINIREVIEWS

Modern approaches to incompatible kidney transplantation

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Abstract

The presence of human-leukocyte antigen (HLA)-antibodies and blood group incompatibility remain a large barrier to kidney transplantation leading to increased morbidity and mortality on the transplant waiting list. Over the last decade a number of new approaches were developed to overcome these barriers. Intravenous immunoglobulin (IVIG) remains the backbone of HLA desensitization therapy and has been shown in a prospective, randomized, placebo controlled trial to improve transplantation rates. Excellent outcomes with the addition of rituximab (anti-B cell) to IVIG based desensitization have been achieved. There is limited experience with bortezomib (anti-plasma cell) and eculizumab (complement inhibition) for desensitization. However, these agents may be good adjuncts for patients who are broadly sensitized with strong, complement-fixing HLA antibodies. Excellent short and long-term outcomes have been achieved in ABO incompatible transplantation with the combination of antibody removal, B cell depletion, and pre-transplant immunosuppression. Kidney paired donation has emerged as a reasonable alternative for programs who cannot provide desensitization or in conjunction with desensitization. Future therapies directed toward cytokines that alter B cell proliferation are under investigation.

Key words: Desensitization; Antibodies; Intravenous immunoglobulin; Rituximab; ABO incompatible; Eculizumab; Bortezomib

Core tip: Intravenous immunoglobulin (IVIG) remains the backbone of human-leukocyte antigen (HLA) desensitization therapy and excellent outcomes with the addition of rituximab (anti-B cell) have been achieved. Bortezomib (anti-plasma cell) and eculizumab (complement inhibition) may be good adjuncts for patients who are broadly sensitized with strong, complement-fixing HLA antibodies. Excellent outcomes have been achieved in ABO incompatible transplantation with the combination of antibody removal, B cell depletion, and pre-transplant immunosuppression. Kidney paired donation has emerged as a reasonable alternative for programs who cannot provide desensitization or in conjunction with

desensitization.

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INTRODUCTION

Kidney transplantation is the gold standard for treating end-stage kidney disease and remarkable strides have been made over the last thirty years. However, there are now over 100000 people awaiting kidney transplantation in the United States according to the Organ Procurement and Transplantation Network. A significant proportion of these patients are broadly human-leukocyte antigen (HLA) sensitized and will have to wait longer to find an acceptable match; some may never. There are also those on the wait list with living donors who are blood type incompatible (ABOi), but would otherwise be an acceptable match. The long wait times incurred lead to increased mortality on the kidney transplant list[1]. The ability to provide a blood type or HLA incompatible transplant decreases mortality and gives hope to those languishing on the wait list.

Desensitization therapies started to emerge in the 1980's. Donor specific blood transfusions were performed for HLA desensitization with limited success. There was more success with ABOi transplantation during this time period with techniques employing a combination of plasma exchange (PLEX) and splenectomy. HLA antibody desensitization with intravenous immunoglobulin (IVIG) was first reported in the mid-1990's and ushered in a new era of transplantation. New immunomodulatory therapies have since emerged that successfully allow HLA and blood type incompatible transplant. In this review, we will discuss the current approaches and future directions of desensitization therapies.

IVIG AND RITUXIMAB (ANTI-B CELL)

IVIG is a complex preparation derived from the gamma globulin fraction of pooled human plasma used to treat primary hypogammaglobulinemia, acquired antibody deficiency, and various autoimmune disorders. It modulates the auto- and allo-immune response *via* broad-acting mechanisms. These mechanisms include neutralization of circulating antibodies, inhibition of B and T cell proliferation *via* interactions with Fc receptors, alteration of cytokine production, and down-regulation of complement. It therefore has powerful immunomodulatory effects and is now widely used for desensitization and treatment of antibody-mediated rejection (ABMR).

The efficacy of high-dose IVIG (1-2 g/kg per dose) was initially described separately by Glotz *et al*[2] and Tyan *et al*[3]. IVIG was administered on a monthly basis to those awaiting either a living or deceased donor kidney transplant. An improvement in panel reactive antibodies (PRA) and transplant rates was observed. These early successes lead to the first randomized, multicenter, placebo-controlled trial for desensitization. The National Institute of Health Ig02 trial included a total of 101 highly sensitized patients with a PRA greater than 50%. Subjects were randomized to receive dialysis with IVIG (2 g/kg) monthly for 4 mo or dialysis with equivalent volume of placebo[4] (Figure 1). Patients receiving high-dose IVIG had a statistically significant reduction in PRA and an improved rate of transplantation with a shorter wait time (4.8 years *vs* 10.3 years). There was a higher rate of acute rejection observed in the IVIG group (53%) compared with the placebo group (10%). However, the 2-year graft survival rates were not significantly different. This approach was effective for both living and deceased donor transplants.

Another approach utilizes low-dose IVIG (100 mg/kg) plus PLEX (Figure 1). Montgomery *et al*[5] demonstrated the efficacy of this combined therapy to rescue three living donor kidney transplant recipients who experienced ABMR and to preemptively eliminate donor specific antibody (DSA) in four recipients scheduled for a living donor kidney transplant. Recently, Montgomery *et al*[1] reported a significant survival benefit of desensitization with the low-dose IVIG/PLEX regimen in 211 HLA sensitized patients compared to patients who remained on the waiting list for eight years[1]. This low-dose IVIG/PLEX regimen is primarily limited to living donor kidney transplantation due to rebound of HLA-antibody that is often seen within days following therapy.

Rituximab, a chimeric anti-CD20 (anti-B cell) monoclonal antibody, has emerged as an important drug for modification of B cell and antibody responses. It is approved for treatment of lymphoma and rheumatoid arthritis and has demonstrated a significant benefit in a number of autoimmune disorders[6]. Clinical data suggest that the beneficial effects of rituximab are likely related to modification of dysfunctional cellular immunity rather than simply a reduction in antibody. Rituximab binds to CD20 and marks the cell for destruction by antibody-dependent cell mediated cytotoxicity, complement-dependent cytotoxicity and cell-mediated apoptosis *via* CD20 cross-linking[7,8]. Rituximab depletes $CD20^+$ B-cells in the bone marrow, spleen and lymph nodes. It does not deplete plasma cells as they are CD20 negative. Rituximab may have some effect on plasmablasts that emerge primarily from the spleen. Data suggest that splenectomy is effective in treating ABMR because it removes DSA secreting plasmablasts that are the primary source of DSA production[9].

Over the past several years, rituximab has been studied and incorporated into desensitization protocols based on the synergistic effect with IVIG observed

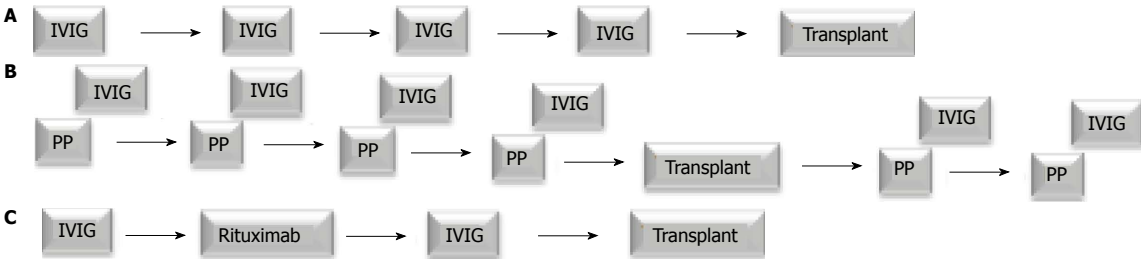

Figure 1 Desensitization protocols. A: The NIH Ig02 trial administered intravenous immunoglobulin (IVIG) in four monthly doses for patients awaiting a living or deceased donor transplant. This was followed by a living or deceased donor transplant once an acceptable crossmatch was achieved; B: Johns Hopkins University used a combination plasmapheresis (PP) with low-dose cytomegalovirus immune globulin following each PP session. The number and frequency of the PP sessions is dependent on the donor specific antibody titer. A living donor transplant occurs when an acceptable crossmatch is achieved. Additional sessions of PP/IVIG are administered after transplant; C: A modified protocol combining IVIG and rituximab was developed at Cedars-Sinai Medical Center. Two doses of IVIG are administered one month apart with one dose of rituximab given in between. A deceased or living donor transplant then takes place when an acceptable crossmatch is obtained.

in patients with autoimmune diseases. Our group evaluated the effect of adding two weekly doses of rituximab to a high-dose IVIG regimen in 20 highly sensitized patients. This protocol reduced PRA from an average 77% to 44%. There was an 80% rate of transplantation with excellent patient and allograft survival. Acute rejection occurred in 50% of patients who received a transplant. Most rejection episodes were diagnosed within the first month after transplantation and were reversible with treatment[10].

We subsequently reported a larger experience evaluating the efficacy of IVIG plus rituximab. Seventy-six highly sensitized patients with a positive cross-match who were treated with a desensitization regimen (IVIG 2 g/kg on day 1 and 30 and rituximab 1 g on day 15), had significant reductions in the T cell flow cytometry crossmatch, and were successfully transplanted. Thirty-one patients received a living donor and 45 patients received a deceased donor kidney transplant. Those awaiting a deceased donor were transplanted, on average, four months following desensitization. This was after waiting an average of 95 mo. There was a 37% rate of ABMR in this cohort, occurring mostly within the first month after transplant. ABMR was treated with pulse steroids, IVIG, and rituximab. PLEX was additionally administered for severe ABMR. The rejection episodes were reversible and did not translate to inferior outcomes. Patient and graft survival were 95% and 84%, respectively, at 24 mo[11].

These studies indicate that IVIG and rituximab offer a significant benefit in reduction of anti-HLA antibodies allowing improved rates of transplantation for highly sensitized patients. However, Marfo *et al*[12] found IVIG and rituximab to lack efficacy in a prospective cohort study that included highly sensitized kidney transplant candidates with a calculated PRA (cPRA) greater than 50%. The cPRA estimates the percentage of deceased donor offers that will be crossmatch incompatible for a candidate taking into account both class Ⅰ and class Ⅱ PRA. After a mean follow-up of 334 d, only two patients received a kidney transplant compared with 14 patients in the non-desensitized group (18% *vs* 52%). Desensitization did not lead to any significant reduction in patients' class Ⅰ and Ⅱ cPRA. There was also no change in the number of unacceptable antigens or their strength as measured by the mean fluorescence intensity (MFI). However, whole blood gene expression analyzed by microarrays demonstrated a down-regulation of immunoglobulin and B cell-associated transcripts after treatment[12].

More recently, our group conducted a double-blind randomized placebo-controlled trial comparing IVIG (2 g/kg, max 140 g administered at weeks 0 and 4) with rituximab (1 g administered at week 2) to IVIG (2 g/kg, max 140 g administered at weeks 0 and 4) with placebo (normal saline administered at week 2). Initially, 13 of 15 randomized patients received deceased donor transplants. The number of serious adverse events reported in the control group prohibited the completion of this trial and the study was un-blinded. There were six patients randomized to IVIG with rituximab and seven to IVIG with placebo. The data showed that all ABMR episodes occurred in the IVIG plus placebo group (N = 3, 43%) *vs* IVIG plus rituximab N = 0, 0%) (P = 0.06). The patients with ABMR episodes were treated with IVIG and rituximab with significantly improved renal function post-transplant at 6 and 12 mo. No transplant glomerulopathy was seen on protocol biopsies for the patients in IVIG plus rituximab group. It appeared that both protocols were effective in achieving an acceptable crossmatch allowing for transplantation. However, the combination of IVIG with rituximab was more effective at preventing DSA rebound, ABMR, and transplant glomerulopathy[13]. Desensitization using the combination of IVIG with rituximab was additionally shown to be cost-effective in a separate study[14].

BORTEZOMIB (ANTI-PLASMA CELL)

Bortezomib, a selective inhibitor of the 26S proteasome, was developed and approved by the United States Food and Drug Administration (FDA) for the treatment of multiple myeloma. Bortezomib inhibits antibody production from plasma cells, mediates apoptosis of this cell type and decreases the number of bone marrow-derived plasma cells. Therefore, it is expected to have

strong suppressive effects on humoral immunity and may represent a promising desensitization strategy.

Bortezomib has been used for the treatment of ABMR[15-18]. Nigos *et al*[19] performed a retrospective chart review of six kidney transplant patients with biopsy-proven ABMR. These six patients were treated with PLEX, IVIG (100 mg/kg after each PLEX and 300-400 mg/kg for 1-2 d after the last PLEX with a cumulative dose of 1 g/kg), steroids, and single-dose rituximab (375 mg/m^2) along with bortezomib (1.3 mg/m^2). Four out of the six patients had biopsy proven resolution of ABMR and stable allograft function over a median follow-up of 14 mo[19]. However, in a case series of four kidney transplant recipients with subacute ABMR and persistent DSA, Sberro-Soussan *et al*[16] found that bortezomib (1.3 mg/m^2) did not significantly decrease DSA MFI over a 5 mo follow-up. In this study, bortezomib was used as the sole therapeutic agent and only one dose was administered.

The potential effect of bortezomib on HLA antibody makes it an intriguing choice for desensitization. However, experiences with bortezomib as an alternative desensitizing agent are currently limited. Idica *et al*[20] reported the effect of bortezomib in thirteen highly sensitized patients. They found elimination of DSA in 10 of the patients and reduced MFI in the remaining three. Trivedi *et al*[17] reported a decrease in anti-HLA antibodies, both DSA and non-DSA, to less than 1000 MFI in nine of eleven patients treated with a combination of bortezomib and PLEX. The two patients without successful desensitization had strong HLA-antibodies with a peak MFI greater than 10000. Four patients had reappearance of anti-HLA antibodies after the initial reduction. However, all patients had stable graft function at a mean follow-up of 4 mo post-transplant[17]. Wahrmann used two cycles of bortezomib for pre-transplant desensitization in two highly sensitized kidney recipients[21]. Dexamethasone was added to the second cycle to enhance treatment efficacy. PRA decreased slightly from 87% to 80% in one patient and 37% to 13% in the second patient. However, both patients showed a greater than 50% reduction in the degree of complement fixing anti-HLA antibodies. Reghavan *et al*[22] reported a kidney transplant recipient with a weak binding DSA who successfully received a deceased-donor kidney transplant after using bortezomib in combination with rituximab. The patients cPRA was reduced from 57% to 31% and the DSA, became undetectable after transplant. The reduction in complement fixing antibodies is significant since they are mostly responsible the acute presentation of c4d positive ABMR and are difficult to modify. However, non-complement binding antibodies acting *via* antibody-dependent cell-mediated cytotoxicity are equally deleterious leading to chronic ABMR and transplant glomerulopathy[23].

In most studies, bortezomib shows promising outcomes for HLA desensitization. There is evidence, albeit limited, suggesting that bortezomib may be effective for altering complement fixing HLA-antibodies. These antibodies are difficult to modify with current therapies and are more deleterious to allografts. Proteasome inhibition alone may not provide durable modulation of HLA antibodies since there is no effect on precursor B cells or cytokines that promote antibody production. The main adverse effect of bortezomib is peripheral neuropathy that may occur in about 30% of treated patients. Severe events noted with bortezomib therapy include thrombocytopenia (28%) and neutropenia (11%). Given the limited experience and lack of long-term follow-up, bortezomib may be best utilized as an adjunct to other established therapies. Well-designed placebo-controlled studies are needed to further elucidate the role of bortezomib for HLA antibody desensitization.

ECULIZUMAB (COMPLEMENT INHIBITION)

Eculizumab is a humanized monoclonal antibody that binds to the complement factor C5 with high affinity, inhibiting its cleavage to C5a and C5b. This ultimately prevents the formation of the membrane attack complex. It is approved for the treatment of paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome (aHUS). It has been used primarily in transplantation to treat refractory ABMR and thrombotic microangiopathy. The binding of DSA to the donor endothelium initiates the classical pathway of the complement cascade. This, in turn, leads to the formation of the membrane attack complex (C5b-C9) and ultimately cell destruction. Eculizumab is administered as an adjunctive agent in desensitization to prevent complement dependent cytotoxicity mediated by antibody. Complement directed therapies do not have any depletive effects on HLA antibodies. Locke *et al*[24] presented a single case in which eculizumab was used combined with PLEX and IVIG to salvage an ongoing severe ABMR. Kidney allograft biopsies after treatment with eculizumab showed a dramatic reduction of the membrane attack complex without a significant change in C4d deposition or DSA. This is expected since C5 is located downstream from C4d in the complement activation cascade. Although the C5 epitope bound by eculizumab is located far from the C5a portion of C5, eculizumab can block C5 cleavage effectively. Eculizumab prevents the entry of the substrate molecule C5 into the C5 convertase, which means that C5 cleavage and the formation of C5a and C5b-9 are inhibited, resulting in blockade of the pro-inflammatory, pro-thrombotic and lytic functions of complement. The inhibition of complement activation at the level of C5 creates a functional C5 deficiency[25]. In the context of multiple different interventions, it was difficult to determine the impact of the anti-C5 antibody on the outcome. Burbach *et al*[26] reported the unsuccessful use of eculizumab in ABMR. However, this rejection was characterized by the absence of both C4d deposition in peritubular capillaries and complement binding DSA.

The role of eculizumab for HLA desensitization is not well defined. There is some evidence that eculizumab is effective in preventing ABMR in highly sensitized recipients. Stegall *et al*[27] reported significantly decreased incidence of early ABMR in 26 highly sensitized recipients with a positive crossmatch against their living donor. The incidence of ABMR was 7.7% (2/26) in the eculizumab group compared to 41.2% (21/51) in the control group. The two cases of ABMR in the eculizumab group occurred on post-transplant day seven and 14 in the setting of increased DSA and a biopsy that showed both C4d deposition and glomerular microthrombi. PLEX was instituted resulting in the resolution of the histologic features of ABMR in one week. The percentage of patients who developed high levels of DSA (MFI > 10000) in the first three months after transplant was similar in both groups. As expected, eculizumab did not have an impact on the presence and strength of DSA after transplant[27]. Long-term follow-up of eculizumab treated patients showed a much higher incidence of transplant glomerulopathy. Thus, C5 inhibition, alone, does not provide long-term protection from other forms of antibody-mediated injury. This raises the question of the need for concomitant B cell and antibody reduction therapies to prevent the development of transplant glomerulopathy.

There are some limitations to the use of eculizumab for desensitization. The duration of therapy after transplant has not been well established. Therefore, treatment may need to be continued indefinitely. Furthermore, it has no depletive effect on DSA and thus cannot alter the underlying immune disorder. Eculizumab only has effect against complement binding HLA antibodies. This can prevent acute ABMR but will likely be ineffective for the prevention of chronic ABMR and transplant glomerulopathy since this is mostly mediated by non-complement dependent pathways (antibody-dependent cell-mediated cytotoxicity). Finally, the cost of eculizumab is prohibitive in many settings and may ultimately limit its utility in kidney transplantation.

ABO INCOMPATIBLE TRANSPLANTATION

The development of protocols allowing for the transplantation of ABOi pairs has expanded the donor pool to recipients with a living donor who would otherwise be awaiting a deceased donor. ABOi transplantation has eased the pressure on the deceased donor waiting list and improved outcomes by transplanting recipients before they begin to experience the burden of chronic renal replacement therapy. Early experience in kidney transplantation showed that transplanting blood type incompatible donor/recipient pairs lead to hyperacute rejection and allograft thrombosis. Anti- A/B isoagglutinins bound to antigens on endothelial cells incite a cascade of events that leads to graft failure, often within minutes of graft reperfusion. Anti-A and B isoagglutinins are distinct from HLA antibodies in that they are natural antibodies that are likely produced in the bowel and peritoneum by precursor B1 cells in response to the presence of normal bacteria. Individuals are sensitized to non-self carbohydrate chains that exist on red blood cells and vascular endothelium. These chains are represented by the designation of blood types A, B, and AB. Those with blood type O do not express these chains and therefore develop antibodies to both A and B isoagglutinins.

There is now a wealth of experience with ABOi transplantation which dates back to the 1980s[28-30]. Early experience with ABOi transplantation occurred primarily in Japan where, at that time, there was limited availability of deceased donors. Through this early experience three essential components of successful ABOi transplantation were elucidated. They include antibody removal, B cell depletion, and pre-transplant immunosuppression. Many center specific protocols have been developed based on these principles[31] (Figure 2). PLEX was initially employed to remove anti-A/B antibodies prior to living donor transplantation, but this alone was not successful. It was the addition of B cell depletion by splenectomy that allowed early success[32]. However, the requirement of splenectomy for successful ABOi transplantation limited its wide spread acceptance given the added surgical risk and resultant life-long risk of infection. The development of rituximab eliminated the need for splenectomy allowing the process to be more palatable to both physicians and patients.

Outcomes in ABOi transplantation have markedly improved over the years. In Japan, higher rates of rejection and graft loss were seen prior to the introduction of tacrolimus and mycophenolate mofetil. Toki *et al*[33] analyzed the impact of ABMR on ABOi transplantation. In this study, 58 consecutive ABOi transplants were divided into two groups: those that developed ABMR within months and those that did not. Graft survival was statistically less at 3, 5, and 8 years after transplant (95% *vs* 49%) in the ABMR group. Multivariable analysis revealed the presence of HLA DSA and an anti A/B titer $\geqslant$ 1:32 to be predictive of ABMR while pre-transplant immunosuppression with mycophenolate mofetil was protective. Successful ABOi programs incorporate these elements into their protocol starting immunosuppression with mycophenolate mofetil with or without tacrolimus and steroids one to four weeks prior to transplant, achieving an anti-A/B titer of < 1:32 at the time of transplant, and screening for anti-HLA antibodies (Figure 2).

Isoagglutinin titers have a large impact on the incidence of rejection and graft outcomes after ABOi transplantation. However, recognition of titer rebound and post-transplant PLEX have improved outcomes dramatically. Won *et al*[34] explored the significance of isoagglutinin titers in a retrospective analysis of 95 patients receiving an ABOi allograft. The desensitization regimen consisted of pre-transplant immunosuppression with tacrolimus, mycophenolate mofetil, and steroids seven to ten days prior to transplant. Rituximab, fixed dose (200 mg or 500 mg), was administered two to 18 d prior to PLEX. The goal titer at the time of transplant was $\leqslant$ 1:4. Basiliximab was administered for induction at the

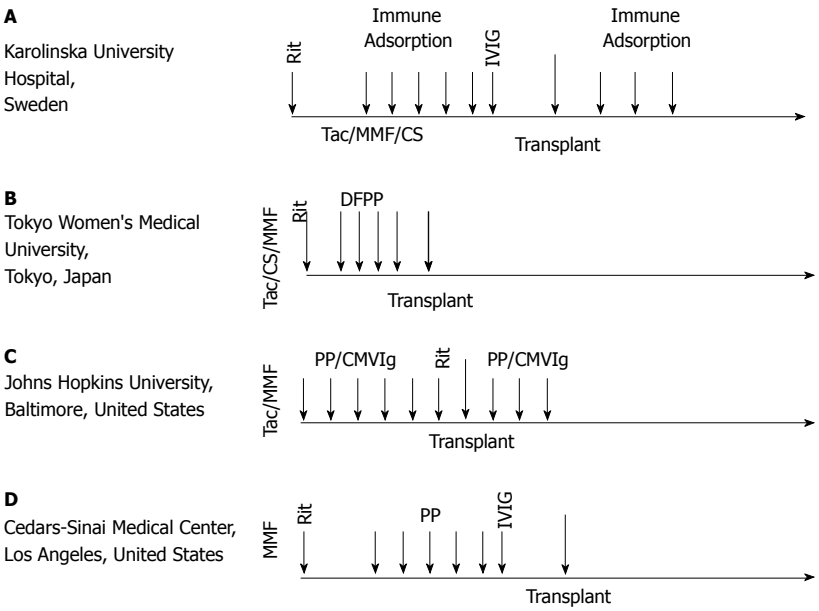

Figure 2 ABOi protocols. Successful ABOi transplantation has been achieved through various protocols around the world. These four protocols all contain the critical components of ABOi desensitization: antibody removal, pre-transplant immunosuppression and B cell depletion. Many protocols also use immune globulin. Rit: Rituximab; Tac: Tacrolimus; MMF: Mycophenolate mofetil; CS: Corticosteroid; DFPP: Double filtration plasmapheresis; PP: Plasmapheresis; CMVIg: Cytomegalovirus immune globulin.

time of transplant. Isoagglutinins rebounded and peaked two weeks after transplant. There were 34 patients (35.8%) that had a rebound in titer to $\geqslant$ 1:16. Titer rebound was associated with an initial pre-transplant titer $\geqslant$ 1:256, rituximab administration $\leqslant$ 7 d prior to initiation of PLEX, and blood type O. Titer rebound was treated with additional post-transplant PLEX. Only one episode of ABMR was reported with at titer of 1:32 at the time of rejection. The addition of immune globulin to the preconditioning regimen may also have the beneficial effect of limiting titer rebound and is used in many protocols around the world (Figure 2).

The use of rituximab facilitated the expansion of ABOi transplantation by obviating the need for splenectomy. Sonnenday *et al*[35] reported in 2004 an early case series of successful ABOi transplantation using a regimen consisting of PLEX, low-dose CMV immune globulin and rituximab. Many other groups have since reported good outcomes with rituximab making it a key ingredient in the current era of ABOi desensitization (Figure 2). More recently, there has been a trend toward minimizing the dose of rituximab in an effort to prevent infections and decrease costs. Some programs have successfully used doses as low as 200 mg[36]. Successful ABOi desensitization has also been described in the absence of both rituximab and splenectomy. Montgomery *et al*[37] reported a series of 24 patients who underwent ABOi transplantation without pre-transplant B cell depletion. Good short-term outcomes were achieved with 100% graft survival. There were three episodes of ABMR. Two were treated with additional PLEX and one with salvage splenectomy. B cell depletion, *via* splenectomy or rituximab, has long-term proven efficacy. A minimalist approach to ABOi transplantation should only be undertaken by experienced transplant centers.

Excellent short and long-term outcomes have been achieved following these principles. Genberg *et al*[38] reported no difference in rejection or allograft survival in ABOi recipients *vs* ABO compatible recipients at three years after transplantation using a protocol consisting of immunoadsorption (antibody removal), pre-transplant immunosuppression with mycophenolate mofetil, and B-cell depletion with rituximab (Figure 2). Analysis of variables associated with rejection revealed elevated anti-A/B titer prior to transplant, absence of pre-transplant immunosuppression, and presence of DSA.

KIDNEY PAIRED DONATION

The recent development of kidney paired donation programs has facilitated the transplant of hundreds of patients in the United States. These programs may be an option for kidney transplant recipients with an HLA or blood type incompatible living donor. Many paradigms exist and range from simple two way exchanges to long, so-called domino chains that have bridging donors (Figure 3). Programs are able to completely avoid unacceptable antigens and blood types removing the need for desensitization in patients with an easy to match phenotype. However, those who are very broadly sensitized with strong HLA antibodies remain a challenge.

A combination of desensitization therapies with kidney paired donation may result in donors with more favorable immunologic profiles. The group at Johns Hopkins recently reported on their experience combining desensitization with kidney paired donation[39]. Mathematical simulations have shown that this approach may improve the rates of transplantation with kidney paired donation

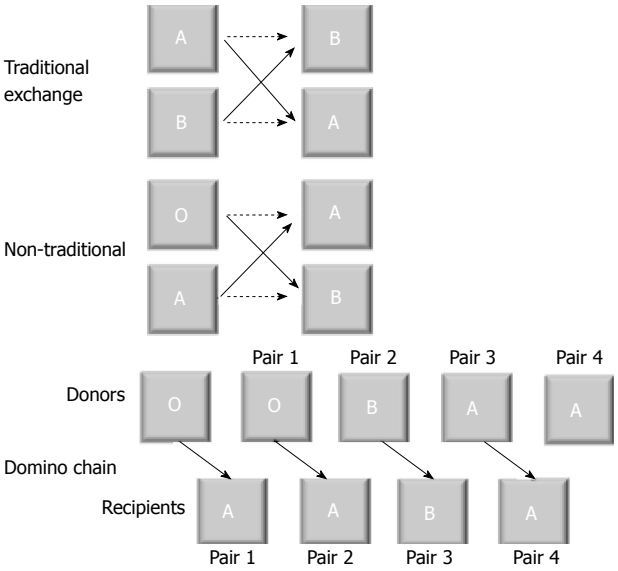

Figure 3 Paired kidney exchange paradigms. Traditional exchange: This exchange swaps two ABO incompatible pairs so that new donor recipient pairs are now ABO compatible. Non-traditional exchange: This exchange uses one human-leukocyte antigen (HLA) incompatible pair and one ABO incompatible pair and trades for two HLA compatible and ABO compatible transplants. Domino chain: This chain starts with a non-directed donor. The recipient of this kidney then has their donor available for another recipient. This occurs progressively until a donor cannot be matched. This donor can act as a bridge to a new chain or can donate to the deceased donor waiting list, thereby ending the chain.

programs. Yabu *et al*[40] recently reported the successful transplantation of five patients with a cPRA of 100% utilizing this approach. Desensitization consisted of IVIG and rituximab with the addition of bortezomib and PLEX in one case. Some centers have also adopted the approach of accepting an ABO incompatible donor in exchange for one that is HLA compatible.

Kidney paired donation provides a good alternative to desensitization in many circumstances; however it is not uniformly effective. A major limitation is that one must have a living donor available to present to the exchange. In addition, favorable donor characteristics including age and blood type must be taken into consideration and effect the likelihood of achieving a match. Patients who are very broadly sensitized with strong binding HLA antibody will be persistently difficult to match without the use of desensitization therapies.

FUTURE APPROACHES

The ongoing development of biologic agents particularly for the treatment of rheumatologic diseases may provide new avenues of exploration for desensitization. All of these agents modulate B cell activity. Epratuzumab targets CD22 on B cells and effectively modulates their activity. It has shown promise in patients with systemic lupus erythematosus (SLE)[41]. Belimumab, an antibody directed against B lymphocyte stimulator (BLyS) was recently approved for SLE and also has B cell modulator effects *via* inhibition of B cell proliferation[42]. Atacicept is currently under study in SLE and acts as a soluble receptor for the B cell proliferation cytokines BLyS and a proliferation-inducing ligand (APRIL) thereby neutralizing their activity. A decrease in total IgG levels has been demonstrated in early phase studies. Tocilizumab is a monoclonal antibody directed against the receptor for interleukin-6, a potent inflammatory cytokine. It is currently approved for rheumatoid arthritis and leads to reductions in IgG and inflammatory responses. It was shown in to modulate the development of DSA in a mouse model of allosensitization[43].

In summary, desensitization therapies with IVIG, rituximab and PLEX have greatly improved the access to and success of incompatible transplantation, both for HLA sensitized and ABOi patients. However, it is important to continue to pursue newer, potentially less toxic approaches that focus on B cells, plasma cells and inhibition of complement-activating antibodies. These basic therapies have also gained acceptance in treatment of ABMR and will likely become more important in transplant medicine as the impact of *de novo* DSA generation post-transplant is better understood.

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MINIREVIEWS

Epigenetics of epithelial Na^+ channel-dependent sodium uptake and blood pressure regulation

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Abstract

The epithelial Na^+ channel (ENaC) consists of α, β, γ subunits. Its expression and function are regulated by aldosterone at multiple levels including transcription. ENaC plays a key role in Na^+ homeostasis and blood pressure. Mutations in ENaC subunit genes result in hypertension or hypotension, depending on the nature of the mutations. Transcription of αENaC is considered as the rate-limiting step in the formation of functional ENaC. As an aldosterone target gene, αENaC is activated upon aldosterone- mineralocorticoid receptor binding to the cis-elements in the αENaC promoter, which is packed into chromatin. However, how aldosterone alters chromatin structure to induce changes in transcription is poorly understood. Studies by others and us suggest that Dot1a-Af9 complex represses αENaC by directly binding and regulating targeted histone H3 K79 hypermethylation at the specific subregions of αENaC promoter. Aldosterone decreases Dot1a-Af9 formation by impairing expression of Dot1a and Af9 and by inducing Sgk1, which, in turn, phosphorylates Af9 at S435 to weaken Dot1a-Af9 interaction. MR attenuates Dot1a-Af9 effect by competing with Dot1a for binding Af9. Af17 relieves repression by interfering with Dot1a-Af9 interaction and promoting Dot1a nuclear export. *Af17*$^{-/-}$ mice exhibit defects in ENaC expression, renal Na^+ retention, and blood pressure control. This review gives a brief summary of these novel findings.

Key words: Gene transcription; Chromatin; Epithelial sodium channel; Histone; Blood pressure

Core tip: The epithelial Na^+ channel (ENaC) is a key player in sodium transport and blood pressure control. This minireview summarizes the epigenetic mechanisms governing the transcription of αENaC. The epigenetic control involves Dot1a-Af9-mediated repression through targeted hypermethylation of histone H3 K79. Aldosterone relieves the repression by decreasing Dot1a and Af9 mRNA levels and by weakening the protein-protein interaction between Dot1a and Af9 interaction *via*

Sgk1-catalyzed Af9 phosphorylation. Aldosterone-independent mechanism involves Af17 as a competitor of Af9 for binding Dot1a and stimulator of Dot1a nuclear export. *Af17*$^{-/-}$ mice exhibit decreased Na^+ reabsorption and lowered blood pressure, indicating the significance of this epigenetic control.

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EPITHELIAL SODIUM CHANNEL IS A KEY PLAYER IN NA^+ METABOLISM

Epithelial sodium channel (ENaC) or the amiloride-sensitive sodium channel contains α, β, and γ subunits[1]. Variations in the production of the subunits, its open probability and/or plasma membrane localization determine the rate of Na^+ entry. ENaC-dependent Na^+ entry occurs in the aldosterone-sensitive distal nephron (ASDN), which consists of the late distal convoluted tubule (DCT2), connecting tubule, and collecting duct[2]. The significance of ENaC in Na^+ metabolism and blood pressure regulation is illustrated by two human genetic diseases, Liddle's syndrome (Liddle syndrome or pseudoaldosteronism) and the autosomal recessive pseudohypoaldosteronism type 1[3].

The manifestations of Liddle's syndrome are similar to those caused by mineralocorticoid excess, including hypertension and, in some patients, hypokalemia and metabolic alkalosis. Moreover, plasma and urinary aldosterone levels are reduced, not increased as in primary aldosteronism. Presentation in most patients takes place at a young age, suggesting the possibility of a genetic disorder rather than an adrenal adenoma. Subsequent studies defined it as an autosomal dominant disorder in which excess loss of potassium and reabsorption of sodium take place in the ASDN. The therapy of the disease consists of a low sodium diet in conjunction with potassium-sparing diuretic medicines such as amiloride. The disorder is extremely rare. Less than 30 families or isolated occurrence have been described in the world as of 2008[4]. Patients with Liddle's syndrome have gain-of-function mutations in either βENaC or γENaC subunit, leading to increased ENaC function that produces inappropriately large Na^+ absorption by ASDN. All of these mutations impact a highly-conserved PPxY domain. Further analyses resulted in identification of the PPxY domain as the ENaC regulatory region. The mutations cause hyperactivity of the channel. Such hyperactivity probably results from changes in protein-protein interactions regulating the channel degradation through Nedd4 ubiquitin ligase[5,6]. Alternatively, the mutations may affect the clathrin-dependent endocytosis[7]. Autosomal recessive pseudohypoaldosteronism type 1 results from reduced ENaC function. The clinical features including aldosterone resistance, sodium wasting, hypovolemia, and hyperkalemia are presented in affected individuals in infancy. These features are similar to those in other forms of hypoaldosteronism in children, with exception of elevated, not reduced plasma aldosterone levels. The disorder results from loss-of-function mutations in any of the three genes encoding ENaC subunits.

Deletion of all three ENaC subunit genes induces perinatal lethality, associated with failure in lung fluid clearance, and/or an acute pseudohypoaldosteronism type 1 featured by metabolic acidosis and severe hyperkalemia[8].

EPIGENETIC CONTROL OF ENAC TRANSCRIPTION BY ALDOSTERONE-SENSITIVE DOT1A-AF9 COMPLEX

Chromatin has been well established to play a critical role in transcription regulation[9]. One of the mechanisms controlling chromatin structure is the post-translational modification of histone N-terminal tails such as acetylation and methylation. According to the histone code hypothesis[9], these histone tails are exposed, unstructured and accessible to various regulatory proteins that recognize a variety of modifications of specific amino acids in the histones or their combinations, giving rise to altered chromatin structures that control particular cellular processes.

Histone methylation can have distinct effects on gene activation, depending on its chromosomal location, the specifically targeted lysines, argines and combinations undergoing posttranslational modifications, and the enzyme (or protein complex) involved in the particular modifications[10]. Members of histone methyltransferase Dot1 family methylate histone H3 K79, which resides in the globular domain. They can modify H3 K79 with one, two, and three methyl groups, leading to mono-, di-, and tri-methylated K79[11]. These methylation events are referred as H3 m1, m2, and m3K79. Such complicity of the modifications may contribute to the functional diversity. In fact, Dot1 proteins have various functions, ranging from telomeric and HM silencing, cell cycle regulation, cell proliferation, meiotic checkpoint, DNA replication, apoptosis, leukemogenesis, to blood pressure control (reviewed in[12]).

Our previous work led to cloning of mouse Dot1-like (*Dot1l*) gene, which is featured by at least five isoforms (Dot1a-e). These isoforms are generated by alternative splicing. Among them, Dot1a is the most characterized variant[13]. The first clue of functional significance of Dot1a in renal physiology came from the observation that aldosterone downregulates Dot1a mRNA level in IMCD3 cells derived from mouse inner medullary collecting duct. Aldosterone regulates Dot1a

mRNA abundance in a time- and dose-dependent manner, resulting in a decrease in overall H3 K79 methylation[14]. Subsequent studies revealed that Dot1a represses α*ENaC* transcription. Chromatin immunoprecipitation (ChIP) coupled by real-time qPCR unearthed the repression associated with targeted H3 K79 hypermethylation at the specific subregions of α*ENaC* promoter. Dot1a is recruited to these subregions, most likely through Dot1a-binding partner ALL1-fused gene from chromosome 9 (*Af9*), a putative transcription factor. There are multiple independent lines of evidence in favor of this hypothesis. First, Dot1a interacts with Af9 in a variety of assays including yeast two-hybrid assays, mammalian two-hybrid assays, GST pulldown, co-immunoprecipitation, colocalization, and re-ChIP. Secondly, aldosterone reduces the levels of Af9 mRNA and protein; thirdly, Af9 overexpression induces hypermethylation of histone H3 K79 at particular subregions of the α*ENaC* promoter and decreases expression of the endogenous α*ENaC* mRNA and α*ENaC* promoter-luciferase reporters. In contrast, depletion of Af9 by specific RNAi causes the opposite results. Fourthly, ChIP assays unearth the association of Dot1a-Af9 protein complex in the corresponding subregions of α*ENaC* promoter[15,16], Finally, we identified the first Af9 cis-element (+78/+92) in the primary site for Dot1a-Af9 interaction and demonstrated Af9 binding to this element in electrophoretic mobility shift assay[17].

Dot1a-Af9-mediated repression can be relieved in an aldosterone-dependent and -independent manner through multiple mechanisms. Aldosterone impairs the formation of Dot1a-Af9 protein complex associated with the α*ENaC* promoter by (1) decreasing abundance of Dot1a and Af9; (2) attenuating the interaction between Dot1a and Af9 *via* Sgk1-catalyzed phosphorylation of Af9 at Ser 435; and (3) counterbalancing the repression through binding to mineralocorticoid receptor (MR) and facilitating its localization in the cell nucleus, where MR and Dot1a compete for binding Af9. Aldosterone-independent de-repression is achieved through the action of ALL1 fused gene from chromosome 17 (*Af17*). We first demonstrated that Af17 upregulates ENaC transcription and benzamil-sensitive Na^+ currents in 293T cells[18]. We showed that the same domain of Dot1a serves as the target for competitive binding by Af17 and Af9. Such competitive binding was mutually verified in a variety of assays. Functionally, Af17 and Af9 had antagonistic effects on expression and activity of ENaC. Af17 promoted decreased Dot1a nuclear expression, at least in part by facilitating its nuclear export, leading to a relief in repression of ENaC mediated by Dot1a-Af9 protein complex[18]. More importantly, whole-cell patch clamping analyses revealed that the alternation in ENaC transcription was translated to the corresponding changes in benzamil-sensitive Na^+ uptake[18]. In more physiologically relevant systems such as M1 and IMCD3 cells, we used equivalent short-circuit current and single-cell fluorescence imaging to examine ENaC activity. We confirmed similar mechanisms by which Dot1a and Af17 regulate ENaC expression and activity[19,20].

AF17$^{-/-}$ MICE HAVE INCREASED NA$^+$ EXCRETION AND DECREASED BLOOD PRESSURE

To demonstrate the functional significance of the epigenetic mechanisms involving Dot1a-Af9-Af17 in regulating Na^+ metabolism and blood pressure control, we created the first *Af17*$^{-/-}$ mice, characterized *Af17* expression pattern during development, and found that *Af17* is not required for hematopoiesis and embryogenesis. Deletion of Af17 has little effect on long-term survival[21], despite increased H3 m2K79 and reduced ENaC function[22]. The impaired ENaC function is a result of downregulated ENaC mRNA and protein levels, lowered channel open probability, decreased active channel numbers, and attenuated effective activity[22]. The abnormalities in sodium handling and blood pressure (BP) were completely corrected when *Af17*$^{-/-}$ mice were treated with a low Na^+ diet, a high K^+ diet, or aldosterone infusion, all of which bolster plasma aldosterone to high levels. These studies establish *Af17* as a potential player for tight regulation of sodium and BP and a potential target for developing new therapeutic strategies in fighting abnormal BP[22].

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How tubular epithelial cells dictate the rate of renal fibrogenesis?

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Abstract

The main threat to a kidney injury, whatever its cause and regardless of whether it is acute or chronic, is the initiation of a process of renal fibrogenesis, since fibrosis can auto-perpetuate and is of high prognostic significance in individual patients. In the clinic, a decrease in glomerular filtration rate correlates better with tubulointerstitial damage than with glomerular injury. Accumulation of the extracellular matrix should not be isolated from other significant cellular changes occurring in the kidney, such as infiltration by inflammatory cells, proliferation of myofibroblasts, obliteration of peritubular capillaries and atrophy of tubules. The aim of this review is to focus on tubular epithelial cells (TEC), which, necessarily involved in the repair process, eventually contribute to accelerating fibrogenesis. In the context of injury, TEC rapidly exhibit phenotypic and functional changes that recall their mesenchymal origin, and produce several growth factors known to activate myofibroblasts. Because they are high-demanding energy cells, TEC will subsequently suffer from the local hypoxia that progressively arises in a microenvironment where the matrix increases and capillaries become rarified. The combination of hypoxia and metabolic acidosis may induce a vicious cycle of sustained inflammation, at the center of which TEC dictate the rate of renal fibrogenesis.

Key words: Epithelium; Fibroblasts; Acute kidney injury; Chronic kidney diseases; Fibrosis

Core tip: In this review, we explain why and how tubular epithelial cells should be regarded not only as victims in the context of chronic kidney disease, but also as actors playing an ambiguous role. In particular, we report on studies which demonstrated that they can actively contribute to fibrogenesis itself, either directly, because their function has been reprogrammed in a way reminiscent of their mesenchymal origin, or from a distance, by influencing endothelial and myofibroblast functions. Last, they are seen as potential targets for new drugs aiming at controlling fibrosis.

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INTRODUCTION

In the clinic, decrease in glomerular filtration rate correlates better with tubulointerstitial damage than with glomerular injury[1]. Myofibroblasts are the main source of extracellular matrix in fibrotic organs, but the view that they merely result from the proliferation of resident interstitial fibroblasts at the onset of an injury is considered simplistic. Bone marrow derived stem cells, vascular smooth muscle cells, epithelial cells, endothelial cells and, more recently, pericytes, have all been suggested as significant sources of myofibroblasts[2-4]. If anything, this three-ring circus reflects a real shift in the paradigm of the cell differentiation and fate process. In contrast to the established idea that cells are terminally differentiated, a more dynamic and plastic vision of how cells behave and react to environmental constraints has emerged[5]. With respect to epithelial cells, a switch to a mesenchymal phenotype (stricto sensu, essentially a cell program that produces the extracellular matrix) makes sense since they are mesenchymal in origin: during embryogenesis the entire nephron, apart from the collecting duct, is derived from the mesenchymal to epithelial transition of the metanephric blastema[6]. The concept of the reverse phenomenon, epithelial to mesenchymal transition (EMT), is well known to embryologists since primary epiblasts acquire mesenchymal proteins in order to disperse, and to oncologists because, at the invasive front of carcinomas, transformed epithelial cells may also acquire migratory properties and metastasize. In 1995, Strutz *et al*[7] extended the concept of EMT to the field of fibrogenesis and its occurrence in adult solid organs, and discussed the possibility that tubular epithelial cells (TEC) might also acquire migratory properties and eventually create *de novo* myofibroblasts[7]. It was proposed that TEC, properly stimulated, would convert and progress from the tubular structure to the interstitium. This major new idea was corroborated by one experimental study[3], but contradicted by other studies[2,8,9]. Overall, the concept of EMT has focused on the TEC phenotype as a potential contributor to fibrogenesis. Rather than suggesting epithelial cells are the main source of myofibroblasts, we use the term "epithelial phenotypic changes" (EPC) to refer to *in situ* EMT[10,11]. Analyzing sequential surveillance biopsies performed in kidney recipients, we and others have demonstrated that EPC are detectable in TEC[12] and are associated with accelerated fibrogenesis and poor graft outcome[10], results confirmed elsewhere. How the external microenvironment influences the phenotype of TEC is an area of intense research, although it is safe to say that the members of the Smad family play a major role. The balance between pro-fibrotic Smads (Smad 2/3) and anti-fibrotic Smads (Smad 1 and Smad 7) is controlled both inside the cells, for example by micro RNAs, and outside, where growth factors such as transforming growth factor β (TGFβ), bone morphogenetic protein 7 (BMP7), hepatocyte growth factor (HGF), their trap proteins [connective tissue growth factor (CTGF), kielin/chordin-like protein (KCP)[13]], and their cognate membrane receptors, all regulate the transient phenotype of "bistable" TEC. Excising *ALK3*, the gene encoding the receptor for BMP7, specifically in TEC, is sufficient to induce a worsening of renal fibrosis in mice subjected to different models of renal injury[14,15]. This demonstrates that TEC exert some control on the process of fibrogenesis. The aim of this review is to provide an update on why EMT is detrimental and contributes *in situ* to renal fibrogenesis. Schematically, EMT reprograms TEC in a way that allows them to produce aberrant amounts of extracellular matrix, activate myofibroblasts from a distance, and eventually impair tissue oxygenation by decreasing the secretion of vascular endothelial growth factor (VEGF) by the epithelium. Table 1 indicates the main molecules produced by TEC and involved in renal fibrogenesis.

TUBULAR EPITHELIAL CELLS AS ABERRANT PRODUCERS OF EXTRACELLULAR MATRIX

The continuous decline in renal function is closely associated with the progressive accumulation of ECM proteins such as collagens and fibronectin. Excessive matrix is scattered between tubular structures, and also around tubules in what pathologists term "tubular atrophy". Beneath the circular ECM that surrounds it, the epithelium often appears flattened, yet Nadasdy *et al*[16] have observed a high cell proliferation rate in those atrophic tubules, *i.e.,* higher than in normal tubules or damaged but non-atrophic tubules[17,18], which suggests that cells are actively engaged in damage repair. In non-atrophic tubules located in non-fibrotic areas, EPC may be detected by immunohistochemistry, using antibodies targeting cytoskeletal proteins typical of myofibroblasts rather than epithelial cells. For example, vimentin, alpha-smooth muscle actin, and even fibroblast-specific protein 1, may be aberrantly expressed in cortical tubules, at the expense of epithelial proteins such as cytokeratins, cadherins, or ZO-1, which are lost. Importantly, this cytoskeletal switch occurs at the same time as increased production of two proteins that help to assemble ECM components: (1) heat shock protein 47 (HSP47), a collagen-specific molecular chaperone which helps to synthesize, process and secrete procollagen from the endoplasmic reticulum, and then acts in the folding and assembly of procollagen

Table 1 Major molecules produced by tubular epithelial cells and involved in renal fibrogenesis

	Role in renal fibrosis	Ref.
Transforming growth factor beta pathway		
TGFβ	Pro-fibrotic agent *via* EMT, activation of myofibroblasts.	[8,15,25-27,30]
CTGF	Trap ligand for TGFβ (promotes its action)	[21,28-31]
BMP7	Anti-Fibrotic agent. Counteracts TGFβ	[14,15]
KCP	Trap ligand for BMP7 (promotes its action)	[13]
Hypoxia pathway		
HIF	Promotes fibrosis through the induction of TGFβ, CTGF, PDGF, and PAI-1. Promotes endothelial survival through the induction of VEGF.	[34-36,41-42]
VEGF	Promotes endothelial fenestration, and survival.	[38-40,42,43]
PAI-1	Pro-fibrotic agent. Inhibitis plasmin formation.	[32,33]
Ph		
Acidotic pH	Induces EMT, enhances angiotensin 2 and endothelin secretion.	[44,50,52-53]

TGFβ: Transforming growth factor β; CTGF: Connective tissue growth factor; BMP7: Bone morphogenetic protein 7; KCP: Kielin/chordin-like protein; HIF: Hypoxia inducible factor; VEGF: Vascular endothelial growth factor; PAI-1: Type 1 plasminogen activator inhibitor.

molecules[19]; and (2) prolyl 4-hydroxylase (P4H), which stabilizes collagen triple helix molecules[20]. We have reported on the *de novo* expression of HSP47 in proximal TEC from human renal allografts, which strongly suggests collagen synthesis[21]. Alpha and beta chains of P4H were similarly found in the tubular cells of most biopsy samples (but not in normal kidneys)[17]. ECM proteins, in particular collagens and laminins, were indeed shown to be synthesized by TEC: Rastaldi *et al*[17], using *in situ* hybridization, were the first to demonstrate that, in a number of human diseases affecting the native kidneys, TEC produce detectable amounts of collagens even before they lose cytokeratins[17]. Of note, the fact that TEC are able to produce ECM is not surprising, since TEC must build their own basement membrane. Nevertheless, manufacturing significant amounts of ECM and modifying the cytoskeleton in the same way as mesenchymal cells, attests to a cell reprogramming which precisely mirrors mesenchymal function (and as such would help to "contain" the injured area). One last point should be highlighted: cell matrix interactions also regulate the epithelial phenotype, hence qualitative changes in the matrix also matter. For instance, the deposition of fibrillar collagen types Ⅰ and Ⅲ (but not type Ⅳ) might further divert TEC from a normal (epithelial) differentiation, thus creating a vicious circle[22,23].

Importantly, the intensity of EPC was found to be predictive of a more rapid progression of interstitial fibrosis and tubular atrophy in renal grafts undergoing sequential biopsies taken for immunological surveillance, and of a poorer allograft function in the long run[10,21]. To what extent TEC contribute to net fibrogenesis by the direct production of ECM is, however, unknown. EPCs may still serve as biomarkers to identify patients who have a high propensity for renal fibrosis, although the anti-fibrotic intervention required for these patients has yet to be developed. We have used two robust markers of EPC which resemble EMT, namely, the *de novo* expression of vimentin, and the translocation of beta-catenin into the cytoplasm, in the decision tree of the Certitem study, a prospective, multicenter trial performed in France. In this study, patients were stratified depending on the presence of EPC on a graft biopsy sample taken at three months' post-transplant, and then randomized either to a conventional immunosuppressive regimen to prevent graft rejection, or to discontinue cyclosporine A and replace it with a mammalian target of rapamycin (mTOR) inhibitor[24]. This strategy was chosen because at the time the trial was designed, calcineurin inhibitors were regarded as the main cause of graft fibrogenesis. The main results of the Certitem trial are that the conversion from cyclosporine to everolimus at 3 mo (a timepoint at which interstitial fibrosis was not present or was very mild) failed to protect EPC^+ grafts from fibrogenesis, since conversion to everolimus increased both clinical and infra-clinical graft rejection episodes. Any benefit that could have been expected from cyclosporine withdrawal was thus masked by inflammatory lesions. However, the predictive value of EPC was good, especially for patients who had a pristine kidney at three months' post-transplant, and this study may serve as a proof of concept that the epithelial phenotype can be used in everyday practice. Should an anti-fibrotic agent enter our materia medica in the future, these markers would undoubtedly be helpful.

TUBULAR EPITHELIAL CELLS SECRETE PRO-INFLAMMATORY AND PRO-FIBROTIC AGENTS

TEC placed under cellular stress may produce various cytokines and chemokines promoting the recruitment of leucocytes. Interstitial inflammation is frequently present in fibrotic areas, such that pathologists often

disregard this kind of inflammation. For obvious reasons, it is difficult to measure the respective contribution of each cell type in this production (a complex crosstalk probably exists between epithelial and inflammatory cells, and potentially between endothelial cells as well). Among factors that sustain the growth and the activation of fibroblasts, TGFβ is a powerful cytokine. TGFβ signals through its cognate receptor, ALK5, and induces Smad 2/3 phosphorylation. By doing so, TGFβ contributes to multiple tubular phenotypic changes in epithelial cells, including EMT and death by apoptosis, but conversely promotes activation and proliferation in fibroblasts[25]. Bechtel *et al*[26] elegantly demonstrated that durably exposed to TGFβ, fibroblasts will undergo epigenetic changes that will auto-perpetuate their proliferation. TEC were repeatedly found to be a source of TGFβ themselves, and thereby contribute to fibrosis progression[27]. CTGF is also an important molecule, since it can act as a positive trap for TGFβ (*i.e.,* facilitating its binding to ALK5) and as a negative trap for BMP7 (preventing its binding to ALK3)[28]. Of note, TGFβ increases the transcription of CTGF, and this positive feedback loop amplifies the process. In renal allografts, we have detected that TEC also produce CTGF, and that, unlike Banff acute or chronic scores, the intensity of CTGF staining in TEC correlates well with graft dysfunction and proteinuria at the time of allograft biopsy[21]. In observations made by others, tubular cells were also found to produce CTGF in diabetes mellitus nephropathy[29], IgA nephropathy[30] and renal allografts[31]. Type 1 plasminogen activator inhibitor (PAI-1) is another important target gene of TGFβ: by controlling the production of plasmin, PAI-1 regulates the activation of matrix proteases and of TGFβ itself, and is involved in inflammatory pathways[32]. Many studies have demonstrated that it may be secreted by renal epithelial cells during pathology[33].

The capacity for activated TEC to produce pro-fibrotic and pro-inflammatory agents directly can be enhanced by various circumstances, including renal hypoxia.

TUBULOINTERSTITIAL INJURY, INTRARENAL HYPOXIA AND FIBROSIS

Although renal blood flow represents 20% of the cardiac output, the kidney is physiologically at risk of hypoxia because of the presence of a complex arterio-venous oxygen shunt[34]. Hypoxia is instantly sensed by and in cells by the oxygen-dependent hypoxia inducible factor (HIF) pathway. HIF proteins (HIF-1 in epithelial cells, and HIF-2, also known as EPAS-1, in endothelial cells and fibroblasts) are heterodimeric transcription factors, composed of an α subunit and a common β subunit[35,36]. These two units only assemble under hypoxic conditions, because otherwise oxygen causes the ubiquitination of HIF-α through a complex system involving prolyl hydroxylases (PHDs) and Von-Hippel-Lindau (VHL) proteins. In the absence of oxygen, HIF-α heterodimerizes with HIF-1β, and the complex enters the nucleus to promote the expression of target genes. Of note, many growth factor stimulating fibroblasts, such as TGFβ, CTGF and PDGF, are also induced by HIF[37,38]. In addition, glycolytic enzymes which facilitate anaerobic production of ATP, and angiogenic factors including VEGF, are among HIF-target genes. In turn, VEGF promotes endothelial functions and survival. VEGF is constitutively and selectively expressed in podocytes and TEC in normal kidneys, whereas expression of the VEGF receptor (KDR/VEGFR2) is largely restricted to adjacent peritubular capillaries[39]. Transcription and translation of VEGF-A in TEC is up-regulated by hypoxia, and VEGF expression correlates with expansion or regression of peritubular capillaries[40]. To what extent is the epithelial secretion of VEGF important in the context of a renal injury? It has been found that the conditional knockout of VHL in tubular cells (artificially increasing HIF-α even in the absence of hypoxia) resulted in the enhancement of VEGF and PDGF-B expression, an increase in endothelial cell proliferation and an attenuation of the tubulointerstitial damage following ischemia/reperfusion injury[41]. Accordingly, the specific ablation of VEGF-A in tubules leads to a specific dropout of peritubular capillaries, and reflects the importance of an intimate tubulo-vascular crosstalk to maintain peritubular microvascularization. Conversely, inhibitors of PHD (and thus upregulation of HIF and hence of VEGF) were recently shown to exert a protective role in a model of diabetic nephropathy where carbonyl and oxidative stress are particularly high.

A loss of VEGF expression by TEC has been documented in progressive renal diseases[40,42]. This data is counterintuitive since interstitial fibrosis could theoretically alter oxygen supply. By increasing the distance between capillaries and TEC, accumulation of ECM probably impairs oxygen diffusion. However, tissue oxygenation is decreased early in chronic renal failure and this precedes the accumulation of ECM, suggesting causality the other way around, *i.e.,* a primary endothelial defect is probably there in the first place[35,37]. It could be speculated that the cell reprogramming that induces EPC also includes the decrease in secretion of VEGF, an important epithelial function. This would in turn promote capillary loss and, eventually, hypoxia[43]. Under hypoxia, TEC may either undergo apoptosis or survive with a mesenchymal phenotype[35].

TUBULAR CELL METABOLISM, RENAL TISSUE ACIDOSIS AND FIBROSIS

Proximal tubular cells, the predominant cell type in the interstitium, are notable in that they have a high

level of energy consumption because of multiple functions such as fluid and electrolyte homeostasis, active solute secretion and hormonal production[44]. They depend solely on aerobic oxidative metabolism[45] and, like cardiomyocytes, they use fatty acid oxidation (FAO) to produce energy. An abnormal accumulation of lipids was recently identified in epithelial cells in both mouse and human kidneys presenting fibrotic lesions, suggesting that β-oxidation is altered because of hypoxia. This accumulation might also alter epithelial functions and phenotype, and even lead to apoptosis[46].

In homeostasis, 80% of renal oxygen consumption is used for the tubular sodium reabsorption driven by Na-K-ATPase, which creates a negative membrane potential and a Na^+ gradient. Na^+-dependent co-transporters and counter-transporters use the energy of this gradient to promote the uptake of $HCO3^-$ and the secretion of H^+ which both ensure the systemic acid base balance[44,45]. Proximal TEC respond to acidosis by an increased bicarbonate reabsorption and transport into the blood and an increased extraction and catabolism of plasma glutamine, which allows for increased ammoniagenesis[44]. But the significant plasticity of intercalated cells eventually prevents acidosis in the collecting duct. They may alternatively secrete protons or bicarbonates, a phenotypic switch which is not due to EMT, but to a process of trans-differentiation[47]. However, how acidosis is sensed by cells from the collecting ducts remains unelucidated. Despite the fact that "systemic" metabolic acidosis usually appears at a late stage of chronic kidney disease[48], acid retention occurs earlier in the renal tissue. Thus, mice subjected to a 2/3 nephrectomy have H^+ retention, but without alteration of the renal function. Intrarenal acidosis, or even dietary H^+, can activate the renin angiotensin system, and increase intrarenal angiotensin 2 activity[49]. An oral alkali diet preserves GFR better than angiotensin 2 receptors or endothelin antagonists in experimental models of moderate chronic kidney disease in mice. In these models, H^+ renal retention is present but not sufficient to induce a metabolic acidosis in plasma[50,51]. Thus, a dysfunction of TEC metabolism, in particular of acid base regulation, probably contributes to renal fibrogenesis and reduction of GFR. Clinical studies are ongoing to determine whether an alkali diet or an increased fruit consumption (*i.e.,* a basic as opposed to acid dietary regimen) will affect the deterioration of GFR in patients with chronic kidney disease[52,53].

CONCLUSION

Preventing the progression of chronic kidney disease is still a major goal of modern medicine. It requires interventions that target and ideally reverse renal fibrogenesis. Of all the renal cell populations, whether resident and injured, or infiltrating and exacerbating injury, TEC are under closest scrutiny since they play a pivotal role in the process. They contribute directly to fibrogenesis by secreting aberrant amounts of extracellular matrix, and indirectly through the production of pro-fibrotic factors, which will act in a paracrine way and stimulate myofibroblasts and inflammatory cells. Progressively isolated by the surrounding matrix, and placed in a microenvironment where hypoxia and oxidative stress increase, they can no longer perform a protective function, including the promotion of endothelial cell survival and sufficient secretion of acid, in the absence of which fibrosis and inflammation increases. This circle is vicious on many levels, but also offers points of therapeutic intervention for the future.

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MINIREVIEWS

Early renal failure as a cardiovascular disease: Focus on lipoprotein(a) and prothrombotic state

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Abstract

Patients with renal failure are at increased risk of cardiovascular events even at the earliest stages of disease. In addition to many classic cardiovascular risk factors, many conditions that are commonly identified as emerging risk factors might contribute to occurrence of cardiovascular disease. Changes in circulating levels of many of these emerging risk factors have been demonstrated in patients with early stages of renal failure caused by different types of renal disease and have been associated with detection of cardiovascular complications. However, for most of these factors evidence of benefits of correction on cardiovascular outcome is missing. In this article, we comment on the role of lipoprotein(a) and prothrombotic factors as potential contributors to cardiovascular events in patients with early renal failure.

Key words: Early renal failure; Cardiovascular disease; Risk factors; Lipoprotein(a); Prothrombotic state

Core tip: Cardiovascular disease is the leading cause of morbidity and mortality in patients with chronic renal failure and even patients with moderate impairment of renal function have an increased risk to develop cardiovascular events. Traditional cardiovascular risk factors have a leading role in the pathophysiology of accelerated atherosclerosis of patients with renal failure, but emerging non-traditional factors might also be involved. Evidence of a possible contribution of lipoprotein(a) and prothrombotic state to cardiovascular outcomes of patients with early renal failure is discussed in this editorial.

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INTRODUCTION

Strong epidemiological evidence indicates that subjects with impaired renal function have shorter life-expectancy than subjects with normal renal function[1]. In subjects with renal failure, cardiovascular events are the leading cause of death and disability[2,3] and many mechanisms can contribute to increased cardiovascular risk. These mechanisms include, on one hand, conditions that are specific to renal failure, such as reduced hemoglobin and acid-base and electrolyte disturbances and, on the other hand, increased prevalence of classic traditional risk factors for atherosclerosis, such as age, diabetes, hypertension, and dyslipidemia[4]. In addition to classic cardiovascular risk factors, other conditions that are commonly identified as "emerging" risk factors[5], have been called into play because they can contribute to cardiovascular events occurring in subjects with relatively low cardiovascular risk as estimated by the current charts[6,7]. Many of these non-traditional emerging risk factors have been reported to be significantly increased in patients with end-stage renal failure, strongly suggesting a contribution to occurrence of cardiovascular events in these patients[8,9] (Table 1). It is clear, however, as also previously stated, that subjects with severely impaired renal function have a myriad of additional conditions that contribute to cardiovascular risk and can hide the relevance of some emerging risk factors. Therefore, the weight of these factors on cardiovascular outcomes of renal patients would be more appropriately examined in subjects at the initial stage of renal disease. Noteworthy, some conditions that increase the cardiovascular risk of end-stage renal patients have also been detected in the earliest stages of renal failure and could possibly contribute to cardiovascular outcomes also in patients with mild impairment of renal function[10]. Here we comment on the evidence supporting the view that early renal failure is a condition associated with high cardiovascular risk and focus on the possible contribution of lipoprotein(a) [Lp(a)] and prothrombotic state to the cardiovascular outcomes of these patients.

EARLY RENAL FAILURE AND CARDIOVASCULAR DISEASE

Evidence obtained in clinical studies demonstrates that the cardiovascular outcome is worse in subjects with initial impairment of renal function as compared to subjects with normal renal function. In a cross-sectional investigation of patients with primary hypertension and different degree of renal function impairment, prevalence of coronary heart, cerebrovascular, and peripheral artery disease was significantly higher in patients with glomerular filtration rate (GFR) comprised from 30 to 89 mL/min per 1.72 m^2 than in patients with a GFR of 90 mL/min per 1.72 m^2 or more[11]. In a first prospective cohort study with 10-year follow-up, incidence of myocardial and cerebral infarction in patients with GFR between 20 and 50 mL/min per 1.72 m^2 was three-times higher than in general population[12]. Patients who had cardiovascular events in this study also had elevated plasma levels of Lp(a), fibrinogen, and homocysteine. A subanalysis of the Hypertension Optimal Treatment study was conducted in hypertensive individuals to estimate the risk of patients with plasma creatinine of 1.7 mg/dL or more to have cardiovascular events or death over a 3.8-year period[13]. In this analysis, incidence of myocardial infarction and stroke resulted significantly greater in patients with high plasma creatinine levels. Moreover, increased plasma creatinine was associated with a risk of cardiovascular events that was higher than that attributed to other risk factors, including diabetes and previous myocardial infarction. In a post-hoc analysis of the Antihypertensive and Lipid Lowering Treatment to Prevent Heart Attack Trial, frequency of coronary events and end-stage renal failure was estimated in 3 groups of patients with hypertension who were stratified according to GFR in a 6-year follow-up[14]. Patients with a GFR < 60 mL/min per 1.72 m^2 had a six-fold higher probability to have a cardiovascular event than to require dialysis, clearly showing that patients with early renal disease are more likely to have cardiovascular disease than evolve to uremia. Conclusive evidence of a graded association between decreasing GFR and increasing rate of cardiovascular events, however, was reached after publication of two milestone studies that came out in 2004. First, in the Valsartan in Acute Myocardial Infarction Trial (VALIANT) 14527 subjects with myocardial infarction and decreased left ventricular ejection fraction were followed for 2 years[15]. In patients with a GFR lower than 81 mL/min per 1.72 m^2 relative risk of death and non-fatal cardiovascular events increased by 10% for each 10 mL/min per 1.72 m^2 reduction in GFR. Second, in a longitudinal prospective study, Go *et al*[16] followed more than one million adults who had been included in a health care system to examine the possible association of GFR, as estimated by the MDRD formula, with the risk of death, cardiovascular events and hospitalization. After a follow-up of 2.8 years, mortality progressively increased as the baseline GFR fell below 60 [hazard ratio (HR): 1.2], 45 (HR: 1.8), and 30 (HR: 3.2) mL/min per 1.72 m^2. Similarly, the HR for nonfatal cardiovascular events was progressively higher with decreasing GFR. Thus, these two important studies together with previous observations obtained in more limited investigation definitely demonstrate that even mild impairment of renal function increases significantly the risk of cardiovascular morbidity and mortality[17].

Table 1 Classic traditional and emerging non-traditional cardiovascular risk factors in chronic renal failure

Classic traditional cardiovascular risk factors	Emerging non-traditional cardiovascular risk factors
Older age	Proteinuria
Male sex	Left ventricular hypertrophy
Arterial hypertension	Anemia
Diabetes mellitus	Electrolyte abnormalities
Smoking	Acid-base imbalance
Increased LDL-cholesterol	Abnormal calcium/ phosphate metabolism
Decreased HDL-cholesterol	Extracellular fluid overload
Family history of cardiovascular events	Lipoprotein(a) and apolipoprotein(a) isoforms
Physical inactivity	Prothrombotic state
	Homocysteine
	Insulin resistance
	Oxidative stress
	Endothelial dysfunction
	Arterial stiffening

LDL: Low-density lipoprotein; HDL: High-density lipoprotein.

EARLY RENAL FAILURE AND EMERGING NON-TRADITIONAL CARDIOVASCULAR RISK FACTORS

As stated above, a variety of emerging non-traditional cardiovascular risk factors might contribute to increase the cardiovascular risk of patients in the early stages of renal failure (Table 1). Most of these conditions meet three of the requisites needed to define a risk factor, that is biological plausibility as to why it may cause cardiovascular disease, demonstration of a dose-response relationship with decreasing renal function, and demonstration of an independent association with cardiovascular disease and renal failure in observational studies. However, so far none of them has met the fourth and most important requisite, that is demonstration in controlled clinical trials that correction of the risk factor is beneficial for cardiovascular outcomes.

It is well known that some lipoproteins are fundamental to the atherosclerotic process and can increase the impact of renal failure on cardiovascular outcomes. Lp(a) is a heterogeneous low-density lipoprotein that incorporates the highly polymorphic apolipoprotein(a) [apo(a)][18]. The *apo(a)* gene is the major gene controlling Lp(a) concentrations[19] that vary over a broad range and are inversely related to the size of apo(a)[20]. In 160 hypertensive patients with early impairment of renal function (GFR 30-89 mL/min per 1.72 m^2) Lp(a) levels were significantly higher than those of 257 hypertensive patients with normal renal function (GFR 90 mL/min per 1.72 m^2 or more) and a significant inverse and independent relationship between Lp(a) levels and GFR was reported[21]. In a further study, it was shown that elevated serum Lp(a) concentrations are not related to the size polymorphism of apo(a) in patients with early renal failure, indicating that in these patients Lp(a) increase can not be ascribed to variations at the *apo(a)* gene locus[22] and strongly suggesting that Lp(a) increase is secondary to impairment of renal function. In this view, one possibility is that the kidney might have a catabolic function on Lp(a) as suggested by detection of degraded apo(a) fragments in urine in an amont that is correlated with GFR[23]. Furthermore, elevated Lp(a) levels and decreased GFR were significantly associated with increased prevalence of cardiovascular events[24], suggesting a contribution of this lipoprotein to cardiovascular outcomes in patients with early renal failure. The association of GFR with Lp(a) levels was investigated in the 7675 participants of the Third National Health and Nutrition Survey (NHANES)[25]. In this population study, a moderate impairment of GFR was associated with greater Lp(a) levels although this association was more prominent in non-Hispanic blacks and Mexican Americans than non-Hispanic whites. Despite this association of elevated Lp(a) levels with early decrease of GFR, other studies demonstrated that this lipoprotein does not contribute to progression of chronic kidney disease[26]. The mechanisms through which Lp(a) promotes atherosclerosis in patients with or without renal failure are not clearly understood. Proposed mechanisms include and increased Lp(a)-associated cholesterol capture in the arterial intima, inflammatory cell recruitment, and carrying of proinflammatory oxidized phospholipids[27].

In addition to the proatherogenic properties, prothrombotic effects of Lp(a) due to its structural homology with plasminogen might explain the contribution of this lipoprotein to cardiovascular events[24]. Also, elevated Lp(a) levels have been found to be frequently associated with hyperhomocysteinemia in patients with pre-dialysis renal failure[28]. Although an inverse relationship of Lp(a) levels with dietary alcohol[29] and omega-3 polyunsaturated acid[30] consumption has been reported and levels of Lp(a) were slightly decreased by use of nicotinic acid[31] and mipomersen[32], impact of either dietary or pharmacologic interventions on Lp(a) levels is minimally relevant. Thus, Lp(a) levels are inversely related with renal function and might contribute to cardiovascular outcomes in patients with early impairment of renal function, but lack of treatments that effectively decrease its levels limits this evidence.

Because a prothrombotic state has been demonstrated in patients with end-stage renal disease, research on emerging risk factors potentially contributing to cardiovascular disease in early renal failure has focused on the hemostatic system[33]. Assessment of the state of activation of the coagulation cascade can be obtained by measurement in plasma of prothrombin fragment 1 + 2 (F1 + 2) that is released when activated factor X converts prothrombin to thrombin, fibrin D-dimer, a breakdown fragment of fibrin, and fibrinogen. In 425 hypertensive patients, 172 of whom had GFR from 30 to 89 mL/min per 1.72 m^2, we measured hemostatic variables and assessed prevalence of cardiovascular events. After adjustment for confounders, GFR was significantly and inversely correlated with plasma

levels of F1 + 2, D-dimer, and fibrinogen, and for the latter two variables correlation was independent of demographic and anthropometric variables, blood pressure levels, plasma lipids, and urinary protein excretion[34]. This observation indicated that and activated hemostatic cascade can be detected also in subjects with mild-to-moderate renal failure, possibly leading to a prothrombotic state and increased incidence of atherothrombotic vascular complications. In these patients with early renal failure and activated coagulation system, prevalence of coronary heart disease, cerebrovascular disease, and peripheral arteriopathy was significantly higher than in patients with GFR of 90 mL/min per 1.72 m^2 or more and cardiovascular disease was independently predicted by both plasma D-dimer and fibrinogen levels. Consistently, in 50 patients with stage 2-3 renal failure plasma fibrinogen was significantly increased possibly contributing to the high cardiovascular morbidity of these patients[35]. In the 3758 patients with GFR of 20 to 70 mL/min per 1.72 m^2 of the Chronic Renal Insufficiency Cohort Study, a prothrombotic state was associated with increased prevalence of peripheral artery disease[36]. In a prospective study of 4029 men aged 60-79 years who were followed for an average period of 6 years, mild-to-moderate renal failure was associated with increased plasma levels of hemostatic markers and caused significantly increased cardiovascular mortality[37].

Thus, it is clear that changes in coagulation parameters suggesting a prothrombotic state occur early in the course of renal disease and could contribute to increase the cardiovascular risk[38]. Similar to Lp(a), in the case of hemostatic variables there is no evidence supporting possible benefits on the cardiovascular outcomes of these patients of treatments that may correct the prothrombotic state.

CONCLUSION

Cardiovascular disease is the leading cause of morbidity and mortality in patients with chronic renal failure and even patients in the early stages of renal disease have much greater risk to have cardiovascular disease than to require substitutive treatment with either dialysis or transplantation. It is clear that traditional cardiovascular risk factors play a major role in the pathophysiology of accelerated atherosclerosis typical of renal failure patients, but emerging non-traditional factors might be involved and contribute to cardiovascular disease. For some of these factors, contribution to cardiovascular outcomes might be relevant from the earliest stages of renal failure. However, conclusive evidence should be obtained from intervention trials with correction of these factors and this is currently missing. Therefore, evidence on whether these and other, as yet unidentified, factors contribute to cardiovascular morbidity and mortality in patients with early renal failure is not conclusive and is the subject of ongoing investigation.

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MINIREVIEWS

Erectile dysfunction in chronic kidney disease: From pathophysiology to management

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Abstract

Chronic kidney disease (CKD) is encountered in millions of people worldwide, with continuously rising incidence during the past decades, affecting their quality of life despite the increase of life expectancy in these patients. Disturbance of sexual function is common among men with CKD, as both conditions share common pathophysiological causes, such as vascular or hormonal abnormalities and are both affected by similar coexisting comorbid conditions such as cardiovascular disease, hypertension and diabetes mellitus. The estimated prevalence of erectile dysfunction reaches 70% in end stage renal disease patients. Nevertheless, sexual dysfunction remains under-recognized and under-treated in a high proportion of these patients, a fact which should raise awareness among clinicians. A multifactorial approach in management and treatment is undoubtedly required in order to improve patients' quality of life and cardiovascular outcomes.

Key words: Chronic kidney disease; Erectile dysfunction; Management; Quality of life; Hypertension; Diabetes mellitus; Phosphodiesterase-5 inhibitors

Core tip: Erectile dysfunction is highly prevalent among patients with chronic kidney disease in rates that reach even 70%, especially in those suffering from end stage renal disease. The rates of patients suffering from sexual dysfunction tend to be higher when additional risk factors, such as coronary artery disease, diabetes mellitus, hypertension or prescription of antihypertensive drugs, coexist. Integrated management of these patients through lifestyle measures, hormonal replacement, and use of drugs such as phosphodiesterase-5 inhibitors, is essential in order to improve sexual function among these patients, thereby maintaining a satisfactory quality of life.

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INTRODUCTION

Sexual dysfunction is highly prevalent in patients with chronic kidney disease (CKD) especially those receiving dialysis. Almost 70% of men with CKD report erectile dysfunction (ED) and these estimates are higher than in the general population[1]. The cause is generally multifactorial with psychological, neurological, endocrinological, vascular and iatrogenic factors acting in concert to increase the likelihood of ED[2]. A number of recent studies suggest an association between endothelial dysfunction and ED[3].

This review aims to analyze the pathophysiology of ED in patients with CKD, to present its prevalence rates in various stages of CKD, to highlight comorbid conditions and common risk factors, which both diseases share and, eventually, to discuss possible therapeutic options, which might improve sexual function of CKD patients.

We performed a systemic search of the literature using the PubMed, OVID, EMBASE and Cochrane Central Register databases from their inception to July 2014. The studies addressing the association between ED and CKD were identified by using the following terms in various combinations: CKD, erectile dysfunction, impotence, renal failure, end stage renal disease, kidney transplantation, phosphodiesterase (PDE)-5 inhibitors. In addition, we reviewed the reference lists of the identified original papers, the studies citing identified papers and review papers relevant to this topic.

Data were extracted by four independent members of our team (Papadopoulou E, Varouktsi A, Lazaridis A, Boutari C) and were discussed with the senior author of our paper (Doumas M). The following criteria were required for a study to be included in our review: observational studies with at least 20 participants, detailed description of a proper estimation of renal function and erectile function.

PATHOPHYSIOLOGY

Erectile dysfunction is defined as the persistent inability to attain and/or maintain penile erection sufficient for sexual intercourse[4]. The erectile process is a complex neurovascular event. In response to sexual stimulation, cavernous nerve terminals and endothelial cells release nitric oxide (NO) which is believed to be the main vasoactive mediator of penile erection. NO promotes penile vasodilation and blood flow, by activating soluble guanyl cyclase to produce 3, 5-cyclic guanosine monophosphate resulting in an enzymatic cascade that reduces intracellular calcium and induces relaxation of cavernosal smooth muscle[5].

Appropriate hormonal environment permits a successful erection with testosterone playing a primary role[6]. Disturbances in neurovascular control, abnormal hormone levels or psychological factors are responsible for the vast majority of ED that is broadly classified as psychogenic (generalized, situational), organic (vasculogenic, neurogenic, anatomic, endocrinologic) or mixed[7].

Psychological factors include primary lack of sexual arousal, chronic disorder of sexual intimacy, depression and performance anxiety. Physiological factors are a more common aetiology and neurological disorders such as Parkinson's disease, stroke, tumour, multiple sclerosis and spinal cord injury are noted to be associated with ED. In addition hormonal disorders such as hypogonadism, hyperprolactinemia and both hyperthyroid and hypothyroid states are known to result in ED. Arterial insufficiency associated with diabetes, hypertension, dyslipidemia, cigarette smoking, blunt perineal or pelvic trauma and pelvic irradiation, tend to be the most common cause of ED[8].

In men with chronic renal disease a combination of testicular failure and secondary disturbances in the pituitary-gonadal axis can be detected in the early stages of CKD and progressively worsen as the renal disease progresses[9]. Some authors have reported that successful kidney transplantation may improve sexual function with reference to the previous situation of haemodialysis[10]. Impaired spermatogenesis and testicular damage with decreased volume of ejaculate, either low or complete azoospermia, low percentage of motility and infertility were reported[11].

Histological changes in the testes revealed decreased spermatogenic activity especially in the later stages of spermatogenesis which are hormonally dependent[12]. Testicular biopsy is often performed to demonstrate reduced spermatogenesis[13]. Leydig and Sertoli cells show absence of hypertrophy or hyperplasia and the reduced levels of total and free testosterone presented in CKD, suggest a Leydig cell dysfunction[14].

Hypogonadism (low testosterone) defined as total testosterone below 300 ng/mL is a prevalent condition in men with CKD especially in those undergoing dialysis and can contribute to decreased libido, ED, oligospermia infertility and anaemia[15]. On the other hand total plasma estrogen concentration is often elevated. The plasma concentration of the pituitary gonadotropin luteinizing hormone (LH) is elevated probably as a result of the decreased release of testosterone from the Leydig cells and the consequent loss of normal negative feedback. In addition the metabolic clearance rate of LH is reduced and it is not corrected by dialysis[16].

In uremic subjects disturbances in LH secretion has been observed but it is not known whether this is the result of a change in GnRH release from the hypothalamus or a change in the responsiveness of the pituitary. However kidney transplantation seems to restore the secretory pattern of LH. Follicle-stimulating hormone (FSH) secretion is also elevated in men with CKD. A peptide

called inhibin produced by the Sertoli cells has a negative feedback on the release of FSH. Uremic patients with severe damage in seminiferous tubules and Sertoli cells tend to have higher plasma FSH concentrations as less inhibin is secreted[9].

Prolactin levels also appear substantially elevated in men with CKD, with a prevalence of hyperprolactinemia from 30%-65%, as a consequence of both reduced renal clearance and increased production. Again these abnormalities seem to resolve after kidney transplantation. Evidence indicates that hyperprolactinemia is associated with infertility, loss of libido, testosterone deficiency and increased risk of cardiovascular events and mortality in CKD. Bromocriptine treatment reduces prolactin levels with no significant side effects[14].

According to the "artery size hypothesis" atherosclerosis is more likely to develop first in the smaller arteries than in the larger ones. Since penile arteries are significantly smaller (1-2 mm diameter) than coronary arteries (3-4 mm), symptoms of ED occur several years before coronary artery disease (CAD) symptoms. ED is also found to be a stronger predictor of CAD than any of the traditional risk factors such as family history, hypertension, dyslipidemia and can be considered as a marker of ischemic heart disease in both CKD and non CKD patients[17,18].

NO is the primary neurotransmitter of penile erection. In chronic renal failure NO bioavailability is reduced. The expression of NO-synthase (NOS) has been shown to be alterned thus leading to a disturbance in sexual function[12]. Possible causes of NO deficiency are substrate limitation (L-arginine), as a result of disturbances in the renal biosynthesis of this amino acid and increased levels of circulating endogenous inhibitors of NOS especially asymmetric dimethylarginine (ADMA). Elevated levels of ADMA has emerged as an independent risk factor in end stage renal disease and reducing ADMA concentration might be a therapeutic goal[19].

Uremic polyneuropathy is an important contributor of ED. Patients undergoing haemodialysis are reported to have an abnormal response to Valsalva manoeuvre, impaired nocturnal penile tumescence and bulbocavernous reflex as evidence of autonomic and peripheral neuropathy, all correlated to sexual dysfunction[20].

CKD is associated with higher anxiety, higher distress, high depression and especially dialysis patients report interpersonal difficulties, lower employment, reduced social activity and low quality of life (QoL)[21]. Changes in body shape and image (catheter, fistula) also contribute to lack of desire and sexual dysfunction. The presence of higher depressive symptoms which are highly prevalent in patients undergoing haemodialysis are independently associated with sexual dysfunction and probably common factors are responsible for both[22].

Treatment of hypertension has also been associated with sexual dysfunction. B-blockers could cause ED by decreasing testosterone levels and potentiating α1-adrenergic activity in the penis. Patients taking thiazide diuretics report difficulty in gaining and maintaining an erection and difficulty with ejaculation. Spironolactone can cause gynecomastia, decreased libido and ED while drugs such as calcium antagonists, angiotensin converting enzyme (ACE) inhibitors and angiotensin II receptor blockers, are associated with lower incidence of these side effects. Other drugs commonly involved in the development of ED are cimetidine, tricyclic antidepressants and metoclopramide[9,23,24].

Available data point towards a detrimental effect of CKD on spermatogenesis[9]. Moreover, zinc deficiency caused by reduced dietary intake, malabsorption and possible loss during haemodialysis, has been implicated in the pathophysiology of reduced sperm motility in CKD patients[9,23,25].

PREVALENCE

Sexual dysfunction is a common feature in patients with CKD despite the fact that is often underestimated by clinicians. Existing comorbidities such as diabetes mellitus, hypertension, atherosclerosis, and certain medications (medications *e.g.,* antidepressants diuretics, beta-blockers and other antihypertensive drugs) as well as pathophysiological conditions such as peripheral vascular disease, peripheral neuropathy and uremia are associated with a decrease in erectile function of male patients[26].

Since 1997, Rosen *et al*[27] have developed the International Index of Erectile Dysfunction (IIEF), a questionnaire which includes all aspects of male sexual functions (erectile function, orgasmic function, sexual desire, intercourse satisfaction, overall satisfaction) and can evaluate as objectively as possible sexual function in male patients. Variable prevalence rates have been reported, mainly due to the study populations' diversity and the variety of sexual dysfunction assessment instruments. Bellinghieri *et al*[11] reported a direct correlation between IIEF and GFR, an inverse correlation between testosterone and cholesterol and an increased number of diabetic patients with ED in stage 3 of chronic renal failure.

The prevalence of erectile dysfunction is strongly age-dependent. The prevalence rises sharply with age. In particular, in the Massachusetts Male Aging Study (MMAS), erectile dysfunction is found in 8% in patients aged in their 40 s and rises up to 80% in patients over 70 years of age[28]. Messina *et al*[29] reported that men under 50 years old with CKD have a higher prevalence of ED than men over age 50 years, while in the MMAS the level of impotence and the prevalence of erectile dysfunction was positively associated with the subjects' age[30].

During the last decades the prevalence of end stage renal disease has significantly increased worldwide and due to the progress in renal replacement therapy. People with end stage renal disease (ESRD) appear to have a reduction in the QoL, which is associated with several factors such as age, therapy complications, psychological factors, and co-existing diseases[31]. Mesquita *et al*[32] reported that the prevalence of ED

was 76.5%, with 72.3% in stage 3 CKD, 81.5% in stage 4 and 87.5% in stage 5 CKD.

A study of 174 male HD patients (controls: 1133 healthy males) revealed that the prevalence of ED in men older than 40 years was higher than 80%, significantly higher than that described in control groups of the same age[33]. Espinoza *et al*[34] reported an ED prevalence of 48.9% in kidney transplant recipients in a study conducted among men with kidney transplantations. Rosas *et al*[26] reported that the prevalence of ED was 82% for all HD patients in a cross sectional study of 302 subjects treated with haemodialysis. ED was present in 90% of older HD patients (> 50 years) whereas its prevalence in younger subjects (< 50 years) was 63%[26].

A large systematic review and meta-analysis of observational studies (50 studies, 8343 patients) reported that the prevalence of any level of erectile dysfunction is approximately 70% (21 studies, 4389 patients) with no difference in prevalence rates among hemodialysis and peritoneal dialysis patients. However, in kidney transplant recipients the prevalence was lower (59% *vs* 75%)[35].

EFFECT OF COMORBIDITIES

Erectile and kidney dysfunction share common risk factors and are associated with diseases involving endothelial impairment such as diabetes mellitus, hypertension, dyslipidemia, coronary artery disease, smoking and obesity[32].

Cigarette smoking is an established modifiable risk factor of arteriosclerosis. As a result of the MMAS, Feldman *et al*[28] reported that current smokers presented an adjusted odds ratio of 1.97 for incident ED compared to non-smokers (P = 0.03). Other studies also report a higher prevalence of ED in the groups of smokers compared to non-smokers and a higher percentage of smoking in men with severe/complete ED but these results did not appear to be significant in multivariate analysis[36].

As far as obesity is concerned, the nine-year follow-up prospective study from the MMAS revealed that it had an independent effect on ED, as a BMI $\geqslant$ 28 kg/m^2 predicted incident ED (OR: 1.96, P = 0.01). Similar results were observed in the Rancho-Bernando Study, where the age-adjusted BMI appeared to be significantly higher in men with severe ED or men who were sexually inactive (P < 0.05)[28,37].

Dyslipidemia is associated with an increased risk of erectile dysfunction due to its effect on endothelium and smooth muscle cells of the corpus cavernosum. The prospective study of MMAS failed to indicate a link between serum lipids and prediction of ED[28]. On the other hand, in the Rancho-Bernando Study elevated serum cholesterol levels and triglyceride levels were associated with more severe ED, as men without ED had lower cholesterol levels compared to men with moderate ED (P < 0.05), and men with no sexual activity or severe/complete ED had higher triglyceride levels than men without ED (P < 0.05)[37]. In a prospective study among 2869 men, Ponholzer *et al*[38] reported that hyperlipidemia was independently and significantly correlated to the presence of erectile dysfunction with an OR of 2.29 (P = 0.04). Hyperlipidemia is common among men with ED at rates that may reach 40%[39]. There have been reports that lipid-lowering therapy and use of statins may have a negative impact on erectile function; nevertheless statins are not generally accepted as a cause of ED and through their pleiotropic effects, statins may increase vascular NO activity and improve endothelial function[40].

Diabetes mellitus is considered to be a risk factor for ED due to vasculopathy and autonomic neuropathy and, additionally, one of the most frequent causes of CKD. Several studies have shown that it is highly prevalent and independently associated with erectile dysfunction in general population. Giuliano *et al*[41] reported a prevalence of ED of 71% among patients with DM and noted a trend of association between decreased mean IIEF-5 score and an increased duration of type 1-diabetes, lack of glycemic control and existence of complications. In a study of arterial risk factors (diabetes, hypertension, hyperlipidemia and smoking) among 440 impotent men, diabetes was the only risk factor that in isolation significantly reduced the penile blood-pressure index[42]. In another large prospective study including 2869 men diabetes was associated with a three times higher risk for ED[38]. Diabetic nephropathy is one of the most common causes of CKD and highly prevalent among end stage renal disease patients. Diabetes mellitus is significantly associated and considered to be an independent risk factor for erectile dysfunction in these patients[29,33,43]. No association between ED and cause of CKD has been proven. Nevertheless, in a study including 119 men with CRF in hemodialysis program, the highest prevalence of ED was among men whose kidney disease was due to diabetes[36]. Rosas *et al*[26] found that men with diabetes had twice the odds of suffering from erectile dysfunction compared to non-diabetic men in a cohort study among 302 subjects in hemodialysis, whereas Mesquita *et al*[32] reported that among 81 patients with CKD, diabetic patients were 4 times more likely to have impaired erectile function compared to non-diabetic subjects (P = 0.048).

In the general population, hypertension is considered to be a risk factor for erectile dysfunction due to its contribution in the atherosclerotic process and the endothelial dysfunction in penile vessels. In a multicenter prospective study conducted in Spain among 2130 men with primary hypertension, erectile dysfunction was reported in 45.8% of them[44]. In a smaller study, which included 634 Greek patients with essential hypertension, erectile dysfunction was prevalent in 35.2% of them compared to a rate of 14.1% found in the normotensives subjects (P < 0.01) and was associated with the severity of hypertension[23]. Despite the fact that a

strong association between hypertension and the emerge of erectile dysfunction has been well established in several other studies[38,41,45], data occasionally remain uncertain if hypertension is an independent risk factor for[28,42]. Although the frequency of hypertension is high among patients with CKD in rates reaching even 70%-95%, its association with sexual dysfunction among these patients is not always statistically significant[29,32,36,43]. Erectile dysfunction observed in hypertensive patients may be associated with the disease itself or it may be caused by the antihypertensive therapy being administered to these patients. In CKD patients, the drug therapy prescribed is often multifactorial including many types of old-antihypertensive drugs such as central acting antihypertensive drugs, diuretics (*e.g.,* thiazide diuretics and spironolactone), and beta-blockers, especially nonselective ones, which have shown a major influence on erectile function. New-generation agents, which include calcium-channel blockers, nebivolol and renin-angiotensin system inhibitors seem to have less deteriorating effects in sexual activity[6,46]. Indeed, Giuliano *et al*[41] reported greater IIEF-5 scores in patients receiving ACE inhibitors or angiotensin II receptor blockers compared to other antihypertensive treatment, while Rosas *et al*[26] revealed a significant association between ACE inhibitors and a decreased prevalence of erectile dysfunction in hemodialysis patients (OR: 0.42). As far as CKD patients are concerned, other widely used drugs, which are traditionally associated with increased rates of erectile dysfunction such as antidepressants, H_2 antagonists and benzodiazepines, should be taken into account as their use may impair sexual function[46].

Atherosclerosis and vascular calcification are very common in the CKD population, thus contributing to higher rates of cardiovascular disease and mortality among these patients. These findings tend to be present even among young adults undergoing hemodialysis compared to healthy subjects of the same age[47]. In the uremic subjects with ESRD, cardiovascular disease (CVD) is responsible for 40%-50% of all deaths and CVD mortality rates in those patients are approximately 15 times higher than the general population[21]. In addition to typical cardiovascular disease risk factors, end stage renal disease patients have impaired calcium and phosphorus homeostasis, deficiency of calcification inhibitors and receive high doses of vitamin D treatment, factors which have shown to promote the vascular calcification process[48]. In the general population, it has been reported that erectile dysfunction is associated with silent coronary disease as patients experiencing sexual dysfunction symptoms tend to have higher coronary artery calcification scores even in the absence of angina symptoms[49,50], a finding which is additionally observed among hemodialysis patients with severe ED, who also tend to present greater coronary artery calcium scores[3]. In a retrospective cohort study of 12825 ED patients, it was reported that there was a two-fold increase in the risk of acute myocardial infarction among men with ED[51]. Due to the apparent link between erectile dysfunction and other vascular abnormalities, CKD patients suffering from erectile dysfunction are in high risk of CVD. Therefore, clinicians should perform a careful evaluation in order to assess coexisting comorbid conditions and modify possible risk factors.

Cardiovascular risk and its association with sexual activity should be evaluated in all men with indications or confirmed cardiovascular disease. According to the Second Princeton Consensus Conference algorithm, patients are classified as low, intermediate or high cardiac risk depending on their sexual activity and their management depends on which category they are integrated. Although the Princeton-Ⅱ algorithm is based on acknowledged cardiovascular risk factors such as hypertension, diabetes mellitus or history of myocardial infarction or angina symptoms, CKD is not included as a condition increasing the cardiovascular risk in men with erectile dysfunction. Considering the increased risk of cardiovascular disease among patients suffering from renal failure and sexual dysfunction, it becomes obvious that these patients should be likewise evaluated and managed[52].

MANAGEMENT OF ED

The improvement of sexual function in CKD patients through a multifactorial approach is associated with an increase in patients' QoL and improved cardiovascular outcomes.

Lifestyle and general measures

Treatment of erectile dysfunction should start with an assessment of general status, evaluation of possible covariates and adoption of lifestyle measures, such as quit smoking, decrease of alcohol consumption and regular physical activity. As far as dialysis patients are concerned, clinicians should focus on optimization of dialysis delivery and adequate nutritional intake of these patients. The medication profile of each patient should be reviewed, considering that many drugs such as diuretics, beta-blockers, antidepressants, and H_2-antagonists are related to erectile dysfunction. Moreover, drugs inducing hyperprolactinaemia such as metoclopramide, haloperidol, phenothiazine, chlorpromazine, and methyldopa should be taken into account[53].

"Curable" causes of erectile dysfunction

Psychogenic ED: Psychotherapy and psychoeducational interventions such as rational, emotive therapy, sex group therapy and sexual counseling should be recommended when depression and other psychogenic causes of erectile dysfunction are suspected or in cases, in which it is indicated[53].

Hormonal-endocrine approach: Therapy with recombinant human Erythropoietin (rHuEPO) has shown to improve many aspects of functional health, such as exercise tolerance, sexual function, and QoL

of patients with CKD[54]. This improvement is likely to be associated with the correction of anemia, induced by the introduction of rHuEPO. In addition, some studies have shown that rHuEPO therapy is associated with alterations on endocrine function, affecting the pituitary-gonadal feedback mechanism. There is evidence supporting that it is associated with reduced prolactin, FSH and LH levels and increased plasma testosterone levels[55,56], although some small studies support that the prolactin levels suppression remains controversial among rHuEPO recipients[57]. Testosterone deficiency is a recognizable contributing factor in the development of anemia in CKD patients. Testosterone replacement therapy may increase blood count, QOL and sexual function[56].

Testosterone replacement therapy has been associated with multiple benefits in men with late onset hypogonadism. However, its effectiveness in men with CKD remains controversial considering that the improvement noted in libido, sexual desire, mood and energy is more profound than in erectile dysfunction individually[58]. Testosterone treatment may be also beneficial in increasing muscle mass and strength and in enhancing erythropoiesis[56]. Derivatives of testosterone can be delivered as injectable, oral, buccal, transdermal and subdermal preparations. Potential side effects such as cardiovascular adverse events, prostate cancer or exacerbation of sleep apnea should be identified and carefully assessed by clinicians[56].

An additional potential therapeutic option affecting endocrine disorders in CKD patients is dopaminergic agonists such as bromocryptine, which normalize prolactin levels, elevate plasma testosterone levels and improve libido and potency[59]. It has been reported that oral zinc supplements improve testosterone levels but its effect on sexual function remains conflicting. Subsidiary administration of oral vitamin E has shown that it may decrease prolactin and plasma testosterone levels[56].

First line therapy-oral pharmacotherapy

Since their introduction in 1998, phosphodiesterase-5 inhibitors are considered first-line agents for erectile dysfunction treatment in the general population. Sildenafil which is the agent most widely used is metabolized mainly in the liver and excreted approximately 80% in the feces and 13% in the urine; therefore, its pharmacokinetics are not significantly different in mild to moderate renal disease compared to healthy men, although its bioavailability may be increased in patients with creatinine clearance < 30 mL/min[60]. In several RCTs for treatment of sexual dysfunction in patients with CKD, treatment with sildenafil and vardenafil is associated with improvement in the overall score of IIEF-5, an increase of the score of all individual IIEF-5 tool domains (erection frequency, erection quality, penetration ability, maintenance frequency of penetration, maintenance of erection after penetration and erection confidence) compared to placebo and an increase of the overall satisfaction score of the IIEF-15 sexual assessment tool. For the use of other agents such as tadalafil or mirodenafil in CKD patients data is limited[61]. Sildenafil citrate is also considered an important first-line therapeutic option among kidney transplant recipients with sexual dysfunction as it has no effect on renal function or immunosuppressive drug levels[62]. The frequency of adverse events in CKD patients is similar to the general population, with headaches, flushing, dyspepsia, myalgia, and back pain, nasal congestion being most commonly reported, while more serious adverse events such nonarteritic anterior ischemic optic neuropathy or cardiovascular events are extremely rare. Due to the possible emerge of hypotension these agents are contraindicated in patients receiving nitrates. In addition, PDE-5 inhibitors should not be administered with PDE-3 inhibitors, such as cilostazol which is used for the management of peripheral artery disease.

Other therapeutic options beyond PDE-5 inhibitors

Vacuum constriction devices are an alternative therapeutic option. They provide negative pressure to the penis, resulting in increased blood flow and thus, causing erection. However, the satisfaction rates among patients remain variable.

Intraurethral or intracavernosal delivery of alprostadil (prostaglandin E_1) individually or in combination with other drugs such as papaverine or phentolamine can be used as a second-line therapy in case of non-response to oral drugs. A penile prosthesis is another therapeutic option in case of previous therapeutic failure and is preferred by some patients as it provides more permanent results. Nevertheless, it is recommended to be delayed after renal transplantation, as a percentage of ESRD patients may improve their sexual function afterwards[53].

Erectile dysfunction in post-transplant patients

Kidney transplantation is considered to be the most effective therapeutic option for patients suffering from CKD. The majority of kidney transplantations are carried out in middle age, where sexual function and fertility remain important[62]. Several studies suggest that erectile dysfunction remains highly prevalent, reaching 50% after kidney transplantation[34,63]. Sexual function post-operatively may be limited by graft malfunction, preexisting comorbid conditions of diabetes mellitus, hypertension, smoking and dyslipidemia, duration of dialysis before transplantation, effects of immunosuppressive or hypertension therapy and is associated with the original cause of kidney insufficiency[10,64]. The influence of haemodialysis duration before kidney transplantation observed by Rebollo *et al*[10] may be owing to the longer duration of peripheral vascular disease, and thus, prolonged vascular damage and hormonal changes in dialysis patients. With regard to immunosuppressive treatment, Malavaud *et al*[63] reported no statistically significant

association between cyclosporine therapy and erectile dysfunction, while the study of Rebollo *et al*[10] showed no association between ED and use of tacrolimus as immunosuppressant. The combination of cyclosporine and prednisone may have a more beneficial effect than azathioprine in gonadal function after kidney transplantation[65]. Renal transplantation usually results in normalization of hormonal profiles of kidney transplant recipients, reducing high levels of prolactin and LH and elevating plasma testosterone[65-68]. Despite these alterations, recovery of sexual function is not present in all patients, as erectile dysfunction may be affected by various factors and thus, can be highly prevalent in patients with renal insufficiency even after kidney transplantation.

CONCLUSION

Sexual dysfunction and chronic renal failure share common pathophysiological pathways and are affected by similar comorbid conditions. Erectile dysfunction tends to be more frequent in patients with CKD. Its incidence is strongly associated with age and stage of renal failure. Despite the advances in therapeutic options, especially the emerge of PDE-5 inhibitors, and the potential relief they may offer, erectile dysfunction still remains highly prevalent and further studies are needed.

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MINIREVIEWS

Epidemiology, clinical characteristics, and management of chronic kidney disease in human immunodeficiency virus-infected patients

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Abstract

Antiretroviral therapy has markedly reduced acquired immune deficiency syndrome-related deaths and opportunistic infectious diseases. This has resulted in prolonged survival of individuals infected with the human immunodeficiency virus (HIV). However, this improvement in survival has been accompanied by an increase in the incidence of chronic kidney disease (CKD) and end-stage renal disease. CKD is now epidemic among HIV-infected populations in both Western and Eastern countries. Risk factors associated with CKD in HIV-infected populations include aging, hypertension, diabetes mellitus, co-infection with hepatitis C virus, a low CD4 cell count, and a high HIV viral load. Clinical experience has shown that HIV-infected individuals often have one or more concurrent risk factors for CKD. The cumulative effect of multiple risk factors on the development of CKD should be noted in this population. Glomerular disease directly related to HIV infection, so-called HIV-associated nephropathy, remains an important cause of CKD among a limited HIV population of African descent, but is less likely to be common among other urban HIV populations. The impact of exposure to nephrotoxic antiretroviral agents on the development of kidney disease is both an old and a new concern. In particular, the association of tenofovir with kidney tubular injury has been an area of great interest. The findings regarding tenofovir's adverse effect on long-term kidney function vary among studies. The early identification and treatment of CKD is recommended for reducing the burden of patients requiring dialysis in HIV-infected populations. Periodic monitoring of urinary concentrations of albumin, protein, and tubular injury markers such as low-molecular-weight proteins may be useful for the early diagnosis of patients at risk for incident CKD. This review focuses on recent epidemiology, clinical characteristics, and management of CKD in a contemporary HIV-infected population.

Key words: Antiretroviral therapy; Tenofovir; Human immunodeficiency virus-associated nephropathy; Albuminuria; Renal tubular biomarkers; Cystatin C; Diabetes mellitus; Hypertension

Core tip: Kidneys are affected by the human immunodeficiency virus (HIV) and its associated therapies. As HIV subjects now have longevity while they receive combination anti-retroviral therapy (cART), kidney disease has been prominent among the current HIV subjects on cART. HIV subjects often have several coexisting risk factors of kidney disease, including diabetes and hypertension. Measurements of albuminuria, proteinuria, urinary low-molecular weight proteins, and serum cystatin C are necessary for early detection of kidney disease. Collaborative discussions between HIV experts and nephrologists are warranted to achieve the good treatment of chronic kidney disease in HIV patients.

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INTRODUCTION

Although combination anti-retroviral therapy (cART) has contributed to the longevity in individuals affected by human immunodeficiency virus (HIV), the life-span extension is followed by the emergence of chronic kidney disease (CKD), leading to their high morbidity and mortality[1-9]. Now, nephrologists are faced with several problems related to CKD among HIV populations, including how to find out subclinical kidney insults, to identify incipient stage of kidney illness, and to collaborate with HIV healthcare staff to offer how to treat CKD. The frequency of CKD is increasing in HIV patients living in Asian countries[10,11] likewise in Western countries[12,13]. Generally speaking, as the early identification of kidney disease gives a chance to exert treatments that inhibit progression of kidney dysfunction[14-16], it could be most crucial to find out HIV patients at high risk of incident CKD as the first step in weakening the frequency of CKD in this population[17-19]. The 2012 KDIGO guidelines elaborated the identification and prognosis of CKD by combining albuminuria with estimated glomerular filtration rate (eGFR)[20,21]. The review attempted to summarize recent advances in the study on CKD in the current HIV individuals.

PREVALENCE OF CKD: PROTEINURIA, ALBUMINURIA, AND A LOSS IN RENAL FUNCTION IN HIV INDIVIDUALS

A simple and reliable biomarker of renal insult is persistent urinary excretion of protein or albumin. Whereas 7.2%-13.7% of HIV-infected subjects manifest proteinuria on a urine dipstick test[7,9,10,22-26], 8.7%-17.8% of those subjects have albuminuria, based on the urinary excretion of albumin[10,27,28]. The frequency of a persistent loss in renal function less than 60 mL/min per 1.73 m^2 varies between 3.5% and 9.7% in different HIV populations[9-12,26]. When both of the existence of urinary protein and a decline in glomerular function were considered, the frequency of CKD stages 1 to 5 ranged 15% to 24%[2,9,10,12,26]. Difference of the CKD prevalence across various countries has not been studied yet. Table 1 demonstrates the frequency of kidney disease in Japan, China, Europe, and the United States, as previously reported.

Numerous reports have shown that albuminuria seems to be one of independent risk factors of a poor prognosis among HIV-infected individuals[27,28]. A quite recent paper has shown that low-grade proteinuria is highly prevalent in a large HIV-infected white cohort on cART[29]. It is therefore reasonable to assume that the KDIGO classification would be more practical for the identification of CKD and for estimating prognosis in HIV-infected individuals than the conventional KDOQI staging. However, the measurement of albuminuria is expensive, with public health care insurance systems in most countries limiting the application to follow-up for diabetic nephropathy. Therefore, a total of 1447 HIV-infected Japanese (1351 males, 96 females; mean age, 44.4 ± 11.5 years) were classified using the 2012 KDIGO guidelines for estimating CKD risk: a combination of eGFR and dipstick proteinuria, as a convenient alternative to albuminuria[30]. Proteinuria was classified into 3 grades: [A1] $\leqslant$ +/-, [A2] 1+ to 2+, and [A3] 3+ $\leqslant$ eGFR was classified into 6 grades: [Gade 1] $\geqslant$ 90, [Grade 2] 60-89, [Grade 3a] 45-59, [Grade 3b] 30-44, [Grade 4] 15-29, and [Grade 5] < 15 mL/min per 1.73 m^2, using colored heat map zones. It was shown that the prevalence rates of individuals in the green, yellow, orange, and red zones were 85.9%, 11.0%, 2.1%, and 1.0%, respectively. The prevalence of individuals at high and very high risk for a poor prognosis in the KDIGO classification was nearly halved, compared with the risk for CKD $\geqslant$ stage 3 in the KDOQI system (3.1% *vs* 6.6%) (Figure 1).

GLOMERULAR AND TUBULAR DISEASES IN HIV-INFECTED PATIENTS

Glomerular and tubular diseases that are often identified in HIV-infected patients are summarized in Table 2. The traditional problems of HIV-associated nephropathy (HIVAN), HIVIC, and TMA are still crucial because of the delay in HIV diagnosis or the non-response to ART even in the contemporary cART years[31]. Patients at the earlier stage of HIVAN may manifest almost normal kidney glomerular function, albuminuria, or subclinical proteinuria. Their renal function often remains constant over some years after the start of cART[32,33]. HIV-

GFR grade	eGFR (mL/min per 1.73 m^2)	A1	A2	A3
G1	⩾ 90	G1A1 518 (35.8%)	G1A2 25 (1.7%)	G1A3 0 (0.0%)
G2	60-89	G2A1 725 (50.1%)	G2A2 79 (5.5%)	G2A3 4 (0.3%)
G3a	45-59	G3aA1 55 (3.8%)	G3aA2 21 (1.5%)	G3aA3 3 (0.2%)
G3b	30-44	G3bA1 5 (0.3%)	G3bA2 5 (0.3%)	G3bA3 1 (0.1%)
G4	15-29	G4A1 2 (0.1%)	G4A2 3 (0.2%)	G4A3 1 (0.1%)
G5	< 15	G5A1 0 (0.0%)	G5A2 0 (0.0%)	G5A3 0 (0.0%)

Figure 1 Distribution of human immunodeficiency virus-infected individuals determined by the KDIGO 2012 classification. The percentage of HIV-infected individuals in each category is expressed in each color box. The cohort includes 1447 HIV-infected patients. The prevalence of individuals in the green, yellow, orange, and red zone was 85.9%, 11.0%, 2.1%, and 1.0%, respectively. KDIGO: Kidney Disease: Outcomes Quality Initiative; CKD: Chronic kidney disease; eGFR: Estimated glomerular filtration rate; A1: No proteinuria (dipstick - or +/-); A2: Mild proteinuria (dipstick 1+ or 2+); A3: Heavy proteinuria (dipstick ⩾ 3+); HIV: Human immunodeficiency virus.

Table 1 Comparisons of prevalence of chronic kidney disease in human immunodeficiency virus-infected patients across previous studies

	Prevalence (%)	Countries	Ref.
Proteinuria			
	7.20	United States	[7]
	9.50	Japan	[11]
	13.70	China	[10]
Albuminuria			
	8.70	Norway	[25]
	11.00	United States	[26]
	17.80	Japan	[11]
CKD stages 1-5			
	15.40	Japan	[11]
	15.50	United States	[23]
	16.80	China	[10]
	23.70	United States	[13]
CKD stages ⩾ 3			
	3.50	EuroSIDA	[12]
	5.60	China	[10]
	5.90	United States	[23]
	9.70	Japan	[11]
	9.70	United States	[13]

CKD: Chronic kidney disease; EuroSIDA: European study of patients with HIV-1 infection including 93 centers across Europe.

infected individuals with African pedigree have been considered being at higher risk of HIVAN arising from podocyte proliferation and tubular dilatation with atrophy and flattening of the tubular epithelial cells[34,35].

RISK FACTORS OF CKD IN HIV SUBJECTS

Known risk factors of CKD in HIV patients are shown in Table 3. Epidemiologic investigations showed that variates associated with CKD in HIV-infected patients include traditional risks including elder age, hypertension, and DM[9-12,22-26]. This has been confirmed by a report from a prospective study with a 6-year median follow-up including a large white HIV cohort receiving antiretroviral treatment[36]. Lipids levels, decreased CD4 cell counts, and elevated HIV RNA load are perhaps specific risks for HIV-infected subjects[10,11,23-25]. Moreover, HCV infection contributes to renal insults in HIV people[11,25,31]. Nearly 30% of subjects with HIV are affected with HCV[37]. Liangpunsakul *et al*[38] performed a study to see the association between non-diabetic patients concurrently having HCV and albuminuria, based on a database from the NHANES Ⅲ. Adjusted for known variates, they demonstrated that HCV co-infection was independently involved in microalbuminuria in individuals without diabetes mellitus. Furthermore, Tsui *et al*[39] showed a significant relationship between albuminuria and HCV seropositivity in people who were classified by age.

ART AND CKD

Some antiretroviral agents are related to kidney disease, hyperlipidemia, diabetes mellitus, and hypertension which may intensify the risk of incidence of CKD[40]. Whereas HIVAN was the major renal involvement before the era of ART, comorbidities and adverse renal effects of various drugs for ART now complicate the landscape of kidney disease in HIV[41]. Drug-induced decrease in kidney function was shown in some NRTIs, TDF, and protease inhibitors (PIs). In those PIs, indinavir is predisposed to generate crystalline stones and it has been changed by PIs with safer agents with integrase inhibition. In addition, atazanavir (ATV) is likely associated with acute interstitial nephritis[42,43] and sub-acute or chronic renal insufficiency due to granulomatous interstitial nephritis characterized by the coexistence of crystalline deposition[44-46]. TDF is secreted from proximal renal tubules, and may be associated with its tubular damage representing mitochondrial dysfunction[47,48]. Although studies of the Gallant *et al*[49] did not show that tenofovir was responsible for renal failure, HIV-infected groups on TDF at the Johns Hopkins Clinical Cohort had a significant decrease in creatinine clearance for 3 years, as compared to patients not having tenofovir[50]. Nevertheless, another study using the same cohort

Table 2 Glomerular or tubular diseases in human immunodeficiency virus-infected patients

Diseases	Clinical characteristics
HIV-specific glomerular disease	
HIVAN	Detectable viral load, a high amount of proteinuria, albuminuria, RPGN
HIVIC	Proteinuria and/or hematuria, variable manifestation including AKI
TMA	AKI, proteinuria, hematuria with microangiopathic hemolytic anemia and thrombocytopenia
HIV-non-specific glomerular disease	
HCV-related MPGN/ cryoglobulinemia	Proteinuria and/or hematuria, nephritic syndrome, a decrease in serum complements
Diabetec nephropathy	Proteinuria (microalbuminuria to nephrotic syndrome), a decrease in GFR
Glomerular sclerosis	Older patients, hypertension, no or low amount of proteinuria, coexistence of atherosclerotic diseases
Membranous glomerulopathy	Nephrotic syndrome; idiopathic and secondary causes associated with HBV or cancers
Minimal change disease	Nephrotic syndrome, use of NSAIDs
IgA nephropathy	Hematuria and/or proteinuria with or without renal failure
Post-infectious glomerulonephritis	Hematuria and/or proteinuria with or without renal failure
ART-associated tubular injury	
Acute tubular necrosis	Use of TDF
Cristal nephropathy	Use of IDV and ATV
Acute or chronic interstitial nephritis	Use of ATV

HIVAN: HIV-associated nephropathy; HIVIC: HIV-associated immune complex kidney disease; TMA: Thrombotic microangiopathy; HCV: Hepatitis C virus; HBV: Hepatitis B virus; AKI: Acute kidney injury; GFR: Glomerular filtration rate; NSAID: Non-steroidal anti-inflammatory drug; ART: Antiretroviral therapy; TDF: Tenofovir disoproxil fumarate; IDV: Indinavir; ATV: Atazanavir.

showed that kidney function did not significantly change between HIV-infected subjects on cART with or without the regimen including tenofovir[51]. These differences between the two studies may be derived from the difference in the cumulative time for cART. The latter included only cART-naïve subjects, while the former included both cART-naïve and -experienced subjects. These disparate results on the TDF's nephrotoxicity remain conflicting, but a recent meta-analysis showed that the relevance of the adverse impact of TDF is mild, which may imply that restriction of "TDF use without regular monitoring of renal function" is not basically necessary[52]. Table 3 shows the known factors related to CKD in HIV-infected individuals.

HOW TO IDENTIFY HIV-INFECTED INDIVIDUALS AT HIGH RISK OF CKD

Measurement of albuminuria and proteinuria

The early diagnosis of renal illness in HIV patients is critical for preventing progression of prevalent renal injury and adding suitable treatment promptly. To help

Table 3 Traditional and human immunodeficiency virus-related factors associated with chronic kidney disease

Variables	Ref.
Black race	[34,35]
Older age	[9-12,22-26]
Low CD4 cell count	[10,11,23-25]
High HIV-RNA viral load	[10,11,23-25]
Diabetes mellitus	[9-12,22-26]
Hypertension	[9-12,22-26]
Hepatitis C virus coinfection	[11,25,31,37]
Proteinuria	[3,27,28,30]
Albuminuria	[3,29,55]
eGFR < 90 mL/min per 1.73 m^2	[10,12,23-25]
Elevation of urinary tubular markers	[56-64]
Use of TDF or ATV	[40-52]

eGFR: Estimated glomerular filtration rate; TDF: Tenofovir disoproxil fumarate; ATV: Atazanavir.

HIV experts with the identification of kidney disease, the IDSA guidelines suggest to conduct urinalysis and the evaluation of glomerular function at the diagnosis of HIV infection[3]. Although a dipstick test is a simple measure to use, it is unable to identify subclinical levels of urinary albumin. A comparison of a dipstick test and urinary protein concentration corrected for creatinine (PCR) in HIV-infected patients showed that the dipstick test could not detect individuals with mild or moderate proteinuria[53]. Therefore, the screening of proteinuria should be done according to PCR than dipstick test[54]. Ando *et al*[55] have found that a moderate to mild level of ACR (30 mg/g > ACR $\geqslant$ 10 mg/g) is an indicator of the incidence of CKD, likely emphasizing that the measurement of the ACR may be of higher relevance than that of the PCR for the detection of new CKD among HIV individuals.

Urinary low-molecular weight proteins for detection of tubular damage

The measurement of urinary biomarkers for identifying early tubular damage in HIV subjects, especially receiving cART has special importance. Some researchers measured urinary low-molecular weight proteins to examine whether patients on cART may have kidney tubular injury in the absence of renal dysfunction[56-62]. Approximately a quarter of HIV-infected patients on cART could have prevalent kidney tubular injury in the absence of renal dysfunction, probably resulting in a near future decrease in glomerular function and a higher emergence of urinary protein[63]. Also, Shlipak *et al*[64] indicated that novel urine biomarkers for tubular injury including KIM-1 and interleukin-18 identify risk for ensuing decrease in renal function in HIV-infected women in the Women's Interagency HIV Study cohort. Measuring urinary low-molecular-weight proteins could be helpful to the early detection of subjects, particular those who take tenofovir, who have high risk of definite CKD. In addition, Peralta *et al*[65] showed that some urinary indices of tubular damage are relevant to mortality in the Women's Interagency HIV Study.

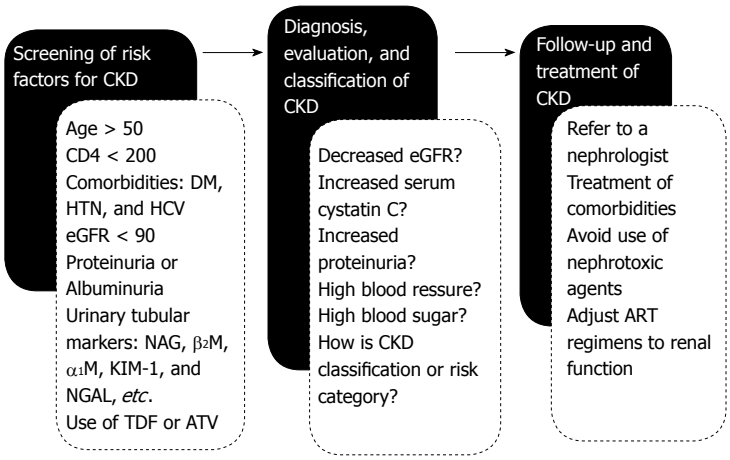

Figure 2 Flow chart for management of chronic kidney disease in human immunodeficiency virus-infected patients. This algorithm includes a clinical flow from screening of risk factors to identification, evaluation and follow-up care of patients for prevalent or incident CKD. CKD: Chronic kidney disease; HTN: Hypertension; DM: Diabetes mellitus; eGFR: Estimated glomerular filtration rate; NAG: N-acetyl-D-glucosaminidase; M: Microglobulin; ART: Antiretroviral therapy; ATV: Atazanavir; TDF: Tenofovir disoproxil fumarate.

Comprehensive assessment of risk factors

HIV-infected subjects usually possess several co-existing risks associated with renal illness, but the clinical impact of them on the emergence of chronic renal disease has remained unknown. A clinical model of predicting the incidence of CKD has been constructed. This model including age, CD4 cell count, diabetes, proteinuria, and a loss in glomerular function less than 90 mL/min per 1.73 m^2 were related to the incident CKD and predicted the development of CKD[66]. In addition, Scherzer *et al*[67] developed a point-based score to discriminate an HIV patient's risk of CKD over 5 years. Figure 2 shows a screening algorithm of the detection and practical management for patients infected with HIV.

CLINICAL SIGNIFICANCE OF CYSTATIN C FOR HIV-INFECTED PATIENTS

Cystatin C is an index for early glomerular dysfunction and may be a potential marker of chronic inflammation. Accordingly, cystatin C is something more than a marker of renal function. In fact, its elevation portends the incidence of heart and vessel diseases and all-cause mortality in the older people[68]. Moreover, it may be associated with a high likelihood of developing cancers[69,70]. However, serum cystatin C concentration are sometimes affected by non-renal factors including age, sex, race, and others[71]. The serum cystatin C level among HIV-infected patients could be greater in those with HIV infection than those without[72], as the serum cystatin C concentration is influenced by prevalent inflammatory diseases and the HIV viral replication[73]. Validation would be needed to confirm the utility of serum cystatin C level for assessing kidney function in HIV individuals.

MAHAGEMENT OF HIV-INFECTED INDIVIDUALS WITH CKD

A careful examination of the medical history and cumulative ART exposure is important for the past and further investigation of HIV individuals with CKD. The cART has beneficial effects on HIV-related diseases, such as HIVAN and HIVIC, but has adverse effects due to the long-term cumulative exposure. In addition to the metabolic changes of glucose and lipids induced by ART, some antiretroviral drugs may directly affect kidney function. Therefore, the detection of patients at high risk of CKD by the periodic measurements of ACR, PCR, and tubular biomarkers is most crucial with special reference to renal protection.

Further examination includes the follow-up of glomerular function, the test of urinary sediments, the ultrasonography of kidneys, and the pathological assessment of biopsied kidney tissues. Renal biopsy study is required for differentiating HIVAN from other glomerular nephtitis including diabetic nephropathy and HCV-related glomerulonephropathies, however, the risk of biopsy-related complications should be fully considered.

Adverse effects of cART on kidney are likely based on the overdosing of medications[74], and thus drug dosages have to be correctly altered according to eGFR. The major treatments for CKD may involve the strict control of high blood pressure, serum sugar and lipids. ART initiation in those having HIVAN is advocated, being independent on the control of $CD4^+$ cell count and HIV infection[75,76]. Prednisolone and ACE inhibitors could be useful for caring HIVAN[77,78].

KEY MESSAGE

The periodic examination of proteinuria or albuminuria combined with eGFR, serum cystatin C, and markers for renal tubular damage may enable the early detection of CKD in HIV-infected subjects.

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ORIGINAL ARTICLE

Retrospective Study

Urethral complications after tension-free vaginal tape procedures: A surgical management case series

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Author contributions: Larsson PG and Jarlshammar B performed the surgical management of all cases; Sergouniotis F performed the follow-up urethrocystoscopy; all authors contributed to the drafting and approval of the manuscript.

Ethics approval statement: No ethical approval was required for this case-report series after contacting the Gothenburg Regional Ethical Committee.

Informed consent statement: All study participants provided informed verbal consent prior to the study enrollment, which was obtained at the visits for postoperative follow-up urethrocystoscopy following the tape removal. The patients encouraged us to report the complications, to prevent others from suffering.

Conflict-of-interest statement: All the authors declare no conflict of interest.

Data sharing statement: No additional data are available.

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Abstract

AIM: To analyze the clinical features, diagnostic modalities, and the surgical management of urethral complications after tension-free vaginal tape procedures.

METHODS: This study encompasses a retrospective review of nine patients presented with urethral complications after midurethral sling procedures. The patients underwent the procedures during a period from 1999 to 2012 in three different regional hospitals in the southwest part of Sweden. The time from sling placement to diagnosis, the risk factors, clinical features, diagnosis, surgical management, and functional outcome are presented. The presenting symptoms were described as either early onset (< 12 mo) or late onset (> 12 mo) according to when they were first reported.

RESULTS: Eight cases of urethral erosion and one case of bladder-neck erosion were detected. The mean interval for diagnoses of the erosions ranged from 3 mo to 11 years. The most common presenting symptoms included *de novo* urgency with or without incontinence (7/9 patients), urinary retention/voiding dysfunction (4/9 patients), urethritis (4/9 patients), relapse of stress-incontinence (3/9 patients), recurrent urinary tract infections (5/9 patients), and hematuria (1/9 patient). In most cases, voiding dysfunction and urethritis occurred early after the operation. The surgical management applied in most cases was transurethral resection of the intraurethral part of the mesh. The removal of the intraurethral mesh resulted in improvement or complete cure of urgency symptoms

in 5/7 patients with urgency. Four patients were reoperated with a new stress-incontinence surgery, one with laparoscopic Burch, and three with retropubic tension-free vaginal tape procedures.

CONCLUSION: Urethral complications should be suspected in the case of *de novo* urgency and relapse of stress-incontinence. Transurethral excision of the intraurethral mesh is the recommended treatment.

Key words: Bladder neck erosion; Complications; Intraurethral mesh; Stress incontinence; Tension-free vaginal tape; Urethral erosion

Core tip: We present eight cases of urethral erosion and one of bladder neck erosion after tension-free vaginal tape procedures. The mean interval for complication diagnoses ranged from 3 mo to 11 years. The clinical profile of the complications included *de novo* urgency, voiding dysfunction, urethritis, relapse of stress incontinence, recurrent urinary tract infections, and hematuria. It is important to consider urethral complications in the postoperative follow-up if these symptoms occur. A control urethrocystoscopy is important for the diagnosis. The transurethral excision of the intraurethral part of the mesh is recommended as the treatment of choice.

Sergouniotis F, Jarlshammar B, Larsson PG. Urethral complications after tension-free vaginal tape procedures: A surgical management case series. *World J Nephrol* 2015; 4(3): 396-405 Available from: URL: http://www.wjgnet.com/2220-6124/full/v4/i3/396.htm DOI: http://dx.doi.org/10.5527/wjn.v4.i3.396

INTRODUCTION

Stress urinary incontinence (SUI) is a significant and common problem in women. SUI is defined as an involuntary leakage of urine on effort, straining, or coughing[1]. Some of the potential causes of SUI include childbirth, older age, obesity, chronic bronchitis, and chronic constipation[2]. Although relatively mild, symptoms often have a negative effect on the patient quality of life in terms of physical and social well-being.

In 1996, Petros and Ulmsten introduced a new, minimally invasive sling procedure for the treatment of SUI. The new procedure applied a tension-free vaginal tape (TVT) under the mid-urethra. The midurethral sling reinforced the weakened pubourethral ligaments and recreated the "hammock" support of the lax anterior vaginal wall and endopelvic fascia[3-5]. The advantage of the TVT procedure is that it is minimally invasive and can be performed under local or regional anesthesia as outpatient surgery.

The TVT procedure has undergone numerous modifications and improvements since its initial introduction. In 2001, De lorme *et al*[6] described a surgical approach where the polypropylene suburethral sling was placed between the two obturator foramina. The goal was to maintain the same position of the sling under the mid-urethra while reducing the risk of complications associated with the blind passage in the retropubic space, such as bladder, bowel, and iliac vessel injury. The procedure is an "outside-in" technique. The technique involves a blind percutaneous introduction of a curved trocar lateral to the vagina, around the inferior ischiopubic ramus, through the obturator foramen and into the anterior vaginal wall at the midurethral level.

A later modification of the Delorme technique was described by de Leval[7] in 2003. De Leval designed a trans-obturator inside-out procedure (TVT-O), a technically more convenient method than the outside-in technique. The surgeon does not need to use an index finger to guide the needle coming from the outside. Furthermore, a "wing-guide" is used during the dissection of the paraurethral tunnel in order to protect the urinary tract[5].

In 2006, a third generation of midurethral slings was introduced with the development of single incision mini-slings. The new method applied a shorter polypropylene mesh with a single suburethral incision. Because the technique avoided the need for blind passage through the retropubic or obturator spaces, it aimed to reduce the complications and increase the safety of the procedure[8-11]. An additional benefit of the procedure was that it could also be performed under local anesthesia.

Since 1996 when it was first introduced, the TVT technique has become the gold standard of minimally invasive surgery in the treatment of stress incontinence. The efficacy of the TVT procedure has been comprehensively documented in the literature. However, TVT-associated complications and their management are less well understood[12-15].

Perioperative perforation of the bladder is a common complication associated with retropubic TVT sling procedures. In a Swedish study that evaluated the results of over 700 patients treated with TVT, the frequency rate of bladder perforations was 1.7%[16]. Urethral erosion of the mesh is a rare complication after a sling operation and may present with various symptoms. The complication occurs when the sling placed outside the urinary tract gradually erodes into the urethra[17]. The first described case of urethral erosion after a TVT operation was published in 2001[18]. Since then, there have been a limited number of reports and case series published on this unusual complication[12,19-26].

The objective of this study was to evaluate the clinical features, physical findings, and diagnostic procedures of postoperative urethral complications. Furthermore, the effectiveness of our management approach in controlling the condition, as well as the outcomes after treatment, were addressed.

MATERIALS AND METHODS

Our first case of urethral complication was noted in 2006.

We had examined a woman *via* urethrocystoscopy due to postoperative urinary tract discomfort, including dysuria and frequency and voiding dysfunctions. The urethrocystoscopy revealed an intraurethral section of the displaced mesh. Following the initial case, all women with dysuria, frequency and voiding dysfunctions, and *de novo* urgency after TVT-procedures underwent urethrocystoscopy.

Herein, we performed an analysis of women with urethral complications after synthetic midurethral sling procedures. The patients' medical records were reviewed retrospectively. The time between sling placement to diagnosis, risk factors, presenting symptoms, diagnostic procedures, surgical management, and postoperative outcomes were recorded. A control urethrocystoscopy was performed on the patients with remaining symptoms.

Five different surgeons from three different regional hospitals in the southwest part of Sweden had performed the sling procedures over a 13-year period (1999-2012) in women diagnosed with intraurethral displaced tape. The presenting symptoms were described as either early onset (< 12 mo postoperatively) or late onset (> 12 mo postoperatively), based on when the symptoms were first reported.

The manufacturers' standard recommendations were followed when placing the mesh, so that the mesh laid tension-free under the urethra. All the surgeons were very experienced and had performed more than 200 TVT procedures. Vaginal sonography was applied as part of the routine postoperative control.

RESULTS

In the period from 1999-2012, nine cases of intraurethrally displaced mesh after midurethral sling procedures were identified. In all nine cases, the mesh was surgically removed at our clinic.

Case 1

A 46-year-old woman was operated upon with TVT-O for genuine SUI in May 2006. The patient was 2-para, had a hormone intrauterine device (IUD; Mirena®) and a body mass index (BMI) of 23.0. The patient also had a medical history of chronic cough, appendectomy, and her mother was operated on with Burch-plastic. During the procedure, the band was doubled with an Allis clamp in a little loop in order to avoid tension of the sling.

Postoperatively, the patient developed urinary retention and had to use intermittent catheterization. The patient complained of pain in the urethra and was treated for urinary tract infection (UTI). An urethrocystoscopy performed 19 d after the surgery showed swelling over the bladder neck; hence, a suprapubic catheter was applied. Two weeks later, the patient had spontaneous voiding, and the suprapubic catheter was removed.

Five months after the surgery, the patient complained of *de novo* urgency, voiding dysfunction, urethral pain, and dyspareunia. An urethrocystoscopy showed an erosion of the sling directly across the urethra. A second surgery, 5.5 mo after the primary TVT operation, was performed using a transvaginal urethroplasty, and the tape was removed to restore the urethra. A suprapubic catheter was applied for three weeks.

At the three-month short-term follow-up, the patient had improved voiding, no urgency, fewer frequency symptoms, and little vaginal pain. Over the long-term, the patient had a relapse of SUI and *de novo* urge incontinence. The former was first treated with two paraurethral silicon injections and then with laparoscopic Burch colposuspension. The latter was treated with anticholinergics.

Case 2

A 55-year-old postmenopausal woman underwent a TVT-O for mixed incontinence. The patient was 2-para, obese with a BMI of 40.7, and on medication for hypertension and type Ⅱ diabetes. The TVT-O procedure was performed without complications. Perioperatively, it was noticed that she had a short urethra. The mesh was placed approximately 1 cm from meatus.

During the first postoperative month, the patient experienced worsening of her urgency symptoms, together with urethral pain. The patient was treated with antibiotics for urethritis and had two urethrocystoscopy procedures without any signs of erosion. Due to voiding difficulties, the patient received urethral dilatations with a slight reduction in her symptoms, and she was scheduled for further dilatations. After urethrocystoscopy, 3 mo postoperatively, it was noticed that the patient had urethral erosion.

The patient was reoperated upon 3 mo and 9 d after the sling application. The sling was cut outside the urethra, excised transurethrally, and the defect in the urethra was closed. A suprapubic catheter was applied for three weeks.

During the short-term follow-up, the patient developed a local vaginal infection and UTI; however, the urgency symptoms improved. Over the long-term follow-up, the patient had a worsening of her urgency and slight relapse of SUI. The patient was treated with anticholinergics for her urgency. During this time, the patient underwent an operation for disc herniation in the lower spine and had a gastric bypass. The patient was later offered polyacrylamide injection treatment for the stress component of her incontinence but felt that she needed no further operative treatment.

Case 3

A 60-year-old woman, 3-para, with a normal BMI of 24.1, ulcerative colitis, and cardiac arrhythmia underwent a TVT-O in 2005 for genuine SUI with no complication. The cardiac arrhythmia was later treated with a pacemaker. The patient had voiding dysfunction shortly after the TVT-O operation and used intermittent catheters for 1 mo.

Twenty months after the TVT-O procedure, the patient developed *de novo* urge incontinence and local

vaginal pain, and was prescribed antibiotics for her UTI. An urethrocystoscopy showed a small part of the mesh in the urethra. A new operation was performed two years postoperatively, with transurethral excision of the intraurethral part of the mesh. No urethroplasty was needed. A suprapubic catheter was placed for 10 d, and antibiotics were given for two weeks.

In the long-term follow-up, the patient had no urgency symptoms, no vaginal pain, and no SUI.

Case 4

A 43-year-old woman, 2-para, with a normal BMI of 26.1 and no previous surgery, underwent a mini-sling TVT-Secur operation for genuine SUI in 2009. After the application of the sling, the perioperative urethrocystoscopy was normal.

A short time after the operation, the patient developed *de novo* urgency with leakage, urethral pain, local vaginal pain, and dyspareunia, but her SUI had been cured. An urethrocystoscopy was performed 2 mo postoperatively. The procedure showed a small erosion of two loops of the TVT mesh in the urethra at the 5 o'clock position. The patient was treated conservatively and received antibiotics for urethritis.

The mesh continued to erode into the urethra, and the next urethrocystoscopy, conducted 6 mo postoperatively, showed progression of the erosion and visible passing of the sling right through the urethra between the 3 o'clock and 9 o'clock positions. The patient was followed conservatively for 1.5 years, but the sling did not progress further through the urethra. A transurethral excision of the intraurethral part of the mesh was performed 20 mo postoperatively. The patient was then treated with antibiotics for 1 mo.

Three months after the operation the patient had no urethral pain, less dyspareunia and vaginal pain, and improved urgency symptoms. However, the patient's SUI relapsed. Therefore, a TVT-retropubic procedure was performed two years after the first sling placement. This dissection was somewhat more difficult due to the presence of the scar tissue.

In the long-term follow-up, the patient had no stress incontinence, slight vaginal pain, and began anticholinergic treatment.

Case 5

A 38-year-old woman underwent a retropubic TVT for genuine SUI in 1999. The patient was 5-para, with a BMI of 36.2, had been sterilized with a laparoscopic procedure, and then re-sterilized with a new laparoscopy because of an ectopic pregnancy. The TVT procedure was uncomplicated, but the patient did have a voiding dysfunction and irritative urinary symptoms after the operation. An urethrocystoscopy 3 mo postoperatively was normal.

In 2004, five years after the first operation, the patient had a relapse of her SUI, and a TVT-O operation was performed in 2005. No preoperative urethrocystoscopy was done at this procedure. With the exception of mild voiding problems, the patient was satisfied. The patient had also undergone a vaginal hysterectomy after the second sling procedure.

In 2010, the patient experienced a macroscopic hematuria. An urethrocystoscopy was performed 11 years and 1 mo after the first TVT. This procedure revealed a 1 cm horizontal erosion of the white TVT-classic mesh into the urethra. This tape used in 1999 was undyed, whereas the tape used in 2005 was dyed blue. A transurethral extirpation of the intraurethral part of the mesh was performed 12 years and 3 mo after the first TVT. The patient was treated with antibiotics for three weeks postoperatively.

In the long-term follow-up, the patient had no voiding problems, little urgency, and experienced improvement of her incontinence after all procedures.

Case 6

In 2011, a 47-year-old woman was operated upon with the MiniArc™ single-incision sling system for genuine SUI. The patient was 4-para, obese with a BMI of 35.3, had asthma, fibromyalgia, and irritable bowel syndrome. She was previously sterilized laparoscopically. The preoperative cystoscopy was normal, and the MiniArc™ procedure was uncomplicated. Postoperatively, the patient had voiding difficulties and did not experience any improvement in her incontinence.

In a follow-up, 8 mo after the operation, the patient had a worsening of her SUI and was treated with antibiotics because of a UTI. The urethrocystoscopy showed that the mesh had eroded 0.5 cm into the urethra. A reoperation was performed 10 mo after placement of the sling. Transurethrally, the intraurethral mesh was cut on both sides, a catheter was placed, and local antibiotics were applied in the urethra. The patient was treated with oral antibiotics postoperatively.

At the three-month follow-up, the patient had unchanged SUI and recurrent UTIs. However, the urethrocystoscopy was normal. The patient later had a successful retropubic TVT. The postoperative follow-up was uncomplicated with the exception of a UTI.

Case 7

A 44-year-old woman was operated upon with retropubic TVT for genuine SUI in 2002. The patient was 3-para, had a BMI of 29.7, and an appendectomy in her medical history. There were no preoperative complications. In the long-term, the patient complained of recurrent UTIs, *de novo* urgency without incontinence, nocturia, and minor voiding dysfunctions. An urethrocystoscopy, nine years after the primary operation, showed one part of the mesh eroded into the urethra at the right side near the bladder neck.

A reoperation was conducted 1 mo later, and the intraurethral mesh was cut and removed. The excision was performed transurethrally, and urethroplasty was not needed. Antibiotics were given for prophylaxis.

Three months after the reoperation, the patient had a slight relapse of her SUI. The urgency symptoms and

Table 1 Background factors for patients with urethral erosion after tension-free vaginal tape procedure

Case	Age (yr)	Parity	BMI	Menopause	Previous operations	Postoperative medical history	Primary operation
Case 1	46	2	23.0	Hormone IUD (Mirena®)	Appendectomy	No	TVT-O, Gynecare®
Case 2	55	2	40.7	Yes	No	Gastric by-pass	TVT-O, Gynecare®
Case 3	60	3	24.1	Yes	No	Pacemaker	TVT-O, Gynecare®
Case 4	43	2	26.1	No	No	No	TVT-Secure, Gynecare®
Case 5	38	5	36.2	No	Laparoscopic sterilization, Re-sterilization because of ectopic pregnancy	Hysteroscopy, vaginal hysterectomy	TVT-retropubic, Gynecare®
Case 6	48	4	35.3	No	Laparoscopic sterilization	No	MinArcTM, AMS®
Case 7	44	3	29.7	No	Appendectomy	No	TVT-retropubic, Gynecare®
Case 8	40	1	21.3	No	No	Abdominal total hysterectomy and bilateral salpingo-oophorectomy for ovarian mass, re-laparotomy because of ileus	TVT-retropubic, Gynecare®
Case 9	66	2	26.0	Yes	Abdominal hysterectomy, pelvic organ prolapse procedure	Laparoscopic operation of adhesions	TVT-retropubic, Gynecare®

BMI: Body mass index; IUD: Intrauterine device; TVT: Tension-free vaginal tape; TVT-O: Trans-obturator inside-out procedure.

voiding problems did improve, and the patient no longer had the nocturia. The urethrocystoscopy was normal at 3 mo. However, a year later during the follow-up urethrocystoscopy, a small section of threads from the mesh was noticed at the 3 o'clock position. As the patient was symptom free, it was handled conservatively, and an urethrocystoscopy was planned for the following year.

Case 8

A 40-year-old woman underwent a retropubic TVT for genuine SUI in 2004. The patient was 1-para, healthy, and had a BMI of 21.3. There were no perioperative complications, and the patient was asymptomatic for 6.5 years. She had also undergone an abdominal salpingo-oophorectomy and total hysterectomy due to a large ovarian cyst. Five days later, the patient was reoperated upon because of an adherent ileus of the small intestine.

Seven years and 8 mo after the sling placement, the patient had an examination due to a relapse of her SUI and minor *de novo* urgency. An urethrocystoscopy showed erosion of the tape into the urethra. A transurethral procedure was performed seven years and 9 mo postoperatively. Local antibiotics were applied in the urethra along with systemic antibiotic prophylaxis.

In the follow-up 5 mo later, the symptoms of SUI with minor urgency remained. The urethrocystoscopy was normal, and there was a slight detrusor contraction while filling the bladder. However, there was no need for anticholinergics. The patient was operated upon with a new retropubic TVT, and at the one-year follow-up she had no SUI or urgency.

Case 9

A 66-year-old woman, 2-para, with a BMI of 26.0, and a medical history of hypertension and gastritis underwent a TVT-retropubic procedure for SUI in 2010. She was operated upon at a perimenopausal age for an abdominal hysterectomy because of a fast-growing, but benign myoma. The patient had also previously undergone a vaginal operation for pelvic organ prolapse in the form of a rectocele and enterocele.

The patient developed SUI after her pelvic organ prolapse operation. Therefore, the previously mentioned TVT retropubic procedure was performed in 2010. The mesh was doubled with Allis forceps, and no leakage was observed. The perioperative urethrocystoscopy was normal.

Five months after the operation, the patient was examined for severe urgency symptoms. However, her SUI had been cured. The urethrocystoscopy showed that the mesh had eroded to the left side of the bladder neck.

A reoperation was performed 8 mo after the TVT procedure. The mesh had eroded from the bladder neck into the bladder making it difficult to cut the mesh transurethrally. Using a suprapubic trocar (laparoscopic trocar) from the abdomen to the bladder, the mesh was excised using laparoscopic scissors under the guidance of a cystoscope. The patient had a suprapubic catheter for 1 d and bladder catheter for 2 d. Antibiotics were given for one week.

In the long-term, the patient had an improvement in her urgency and no incontinence symptoms. An urethrocystoscopy performed 5 mo postoperatively was normal.

The patient had to undergo later a laparoscopic lysis of pelvic adhesions due to a chronic dyspareunia. These adhesions were caused by the previous hysterectomy.

Patient characteristics

Pertinent patient characteristics are presented in Table 1. The mean patient age at the primary operation was 48.9 years (range: 38-66 years). Six of the patients were premenopausal, and three were postmenopausal. The median BMI was 29.2 (range: 21.3-40.7), and three patients had a BMI > 30. Only one patient had a medical history of a pelvic organ prolapse operation

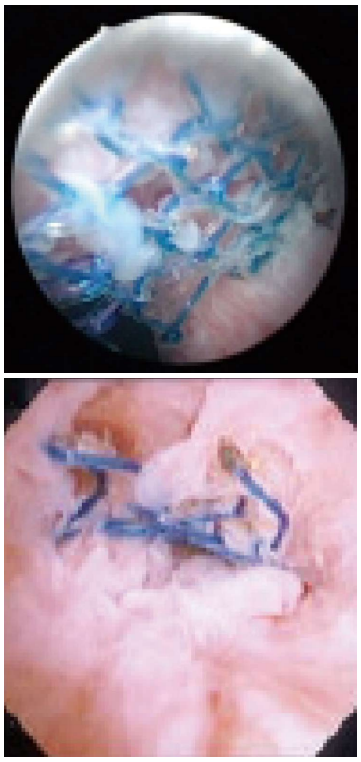

Figure 1 Mesh visible in the urethra.

before the primary incontinence procedure. Two patients had medical records of chronic asthma, and three patients had physically demanding work. Four of the patients had undergone other surgical procedures after the primary TVT operation. There were no obvious common predisposing factors for urethral erosion.

Primary procedure characteristics

Eight of the patients were operated upon for genuine SUI and one for mixed incontinence. The type of primary operation varied from TVT-retropubic to TVT-obturator and mini-slings (Table 1). Therefore, no obvious correlations with the types of operations were found. We do not have data on the number of different TVT operations performed since 1999 in the three hospitals. Therefore, the frequency of the urethral erosion by the mesh cannot be calculated.

In two cases, the sling was doubled preoperatively to make the sling tension-free. Finally, postoperatively, four of the nine patients used intermittent catheterization because of voiding difficulties.

Complication characteristics

Eight of the presented cases had urethral erosions, and one had erosion of the mesh in the bladder neck. The mean interval for diagnosing the erosions was 42 mo (range: 3-133 mo). Four patients were diagnosed within the first year after the operation and five later, up to 11 years after the primary procedure (Table 2). The presenting symptoms were divided into an early-onset (< 12 mo postoperatively) and late-onset (> 12 mo) group according to when they were first reported. It is not clear if all symptoms were directly related to the described complications, but they might be a useful indicator of suspected erosion. Seven patients had clear clinical symptoms less than 12 mo after their operation. The list of early-onset symptoms for each patient is listed in Table 2. Four patients had new symptoms reported after 12 mo postoperatively.

Overall, the majority of patients (77.7%) had some grade of *de novo* urgency with or without urge incontinence and recurrent UTIs (55.5%). The most common early-onset symptoms included urinary retention and voiding dysfunctions, urethritis, and vaginal pain. The late-onset symptoms were more often recurrent UTIs and recurrence of SUI.

Surgical treatment

The median interval between the diagnosis and surgical treatment was 1.5 mo (range: 10 d to 3 mo) for seven cases of urethral erosion. There were two cases with an interval of 13 mo and 18 mo, which were first treated conservatively.

The first case required transvaginal excision of the intraurethral mesh followed by transvaginal urethroplasty. This was a rather complicated and time-consuming operation, which led us to change our approach to transurethral resection of the intraurethral part of the mesh.

Best method for removing the intraurethral mesh based on our experience

The best position for identifying and removing the intraurethral mesh was to have the patient in the lithotomy position under general or regional anesthesia. Using a regular cystoscope [Karl Storz Gmbh, Tuttlingen, Germany; Charrière (Ch) 22, 0° or 30° optics, with a deflecting mechanism with Albarran lever, and a working channel with two ports] the intraurethral mesh was identified (Figure 1), and a 5 Ch "open-end" ureteral catheter was inserted into the urethra. A 0-0 monofilament thread was then introduced through a loop in the central part of the mesh, and a good portion of the thread was pushed through the mesh into the bladder. Then, the cystoscope was retracted, and the thread position in the urethra was secured by grasping it at the meatus. The ureteral catheter was removed from the cystoscope, which was reintroduced into the bladder, and the distal part of the thread was located and grasped with cystoscopic pincers and extracted through the urethra. This created a thread loop through the mesh allowing the application of tension on the mesh while cutting.

To avoid cutting the loop by mistake, it is recommended to use a monofilament thread that is a different color than the mesh. It can be technically challenging to insert the monofilament thread into one of the loops of the TVT mesh. The procedure is easier when using a child cystoscope (Ch 10, one port channel, with an optic of 0°). The small cystoscope is more easily handled in the urethra and does not require a

Table 2 Clinical symptoms of urethral erosion and outcomes after the intraurethral mesh was removed

Case	Interval for diagnosis	Early-onset symptoms (< 12 mo)	Late-onset symptoms (> 12 mo)	Interval for surgical management	Outcome after surgical management	Further treatment
Case 1	5 mo	Urinary retention/voiding dysfunction, UTI, urethral pain (urethritis), *de novo* urgency, dyspareunia	_	5 mo	Improved urgency, voiding dysfunction resolved, relapse of SUI	Anticholinergic, incontinence pad, electric stimulation, two macroplasty procedures, laparoscopic Burch
Case 2	3 mo	Urethral pain (urethritis), worsening of urgency	_	3 mo	Worsening of urge incontinence, relapse of SUI, recurrent urethritis	Anticholinergic, local antibiotics into the urethra, no further SUI treatments
Case 3	1 yr 8 mo	Urinary retention/voiding dysfunction	*De novo* urgency, recurrent UTI, vaginal pain	1 yr 11 mo	Urgency resolved, vaginal pain resolved	No further SUI treatments
Case 4	2 mo	*De novo* urgency, urethral pain (urethritis), vaginal pain, dyspareunia	_	1 yr 8 mo	Improved urgency, urethral pain resolved, relapse of SUI	Anticholinergic, retropubic TVT
Case 5	11 yr 1 mo	Urinary retention/voiding dysfunction, UTI, voiding pain (urethritis)	Relapse of SUI, hematuria after TVT-O	12 yr 2 mo	Recurrent minor SUI (patient already had second incontinence operation), improved urgency, urethral pain, voiding dysfunction resolved	No further SUI treatments
Case 6	8 mo	Urinary retention/voiding dysfunction, direct relapse of SUI, UTI	_	10 mo	Same as before the surgical management	Retropubic TVT
Case 7	9 yr 1 mo	_	Recurrent UTI, *de novo* urgency, nocturia	9 yr 2 mo	Slight relapse of SUI, improved urgency and voiding dysfunction	Local estrogens, no further SUI treatments
Case 8	7 yr 8 mo	_	Relapse of SUI, minor *de novo* urgency, nocturia	7 yr 9 mo	Same as before surgical management	Retropubic TVT
Case 9	4 mo	*De novo* urgency	_	7 mo	Improved urgency, cured SUI, no relapse after the surgical management	No further SUI treatments

SUI: Stress urinary incontinence; TVT: Tension-free vaginal tape; TVT-O: Trans-obturator inside-out procedure; UTI: Urinary tract infection.

ureteral catheter to pass the monofilament thread through the instrument and the mesh. After placing the thread, the procedure is continued as described above with a normal cystoscope. From our experience, the monofilament thread of 0-0 size is the most convenient to use.

The next step was to carefully dilate the urethra to Hegar pin number 10. A nephroscope (Storz S27092 AMA, 0° optic and an operating sheath 27093BN, Ch 28 with a working channel of 5 mm) (Figure 2) has been the most convenient instrument to use when cutting the mesh. The regular cystoscope was not the best choice, as the small scissors used through a cystoscope were too weak to cut the mesh and easily broke. With a video technique, it was possible for the assistant to manipulate the urethra with one finger in the vagina. Through the working channel of the nephroscope, laparoscopic scissors (Storz, Metzenbaum scissor 5 mm, 34210 MW or Hak scissor, 34210 EH) were used to cut the mesh. It was easy to cut the mesh at the first side, and with the monofilament suture, it was possible to keep the mesh tensioned while cutting the other side. Cutting of the mesh at the mucosal level did not require suturing.

As much as possible of the visible mesh should be cut. If some small part remains, it will probably disappear. Only one case had small remains of the mesh at the control cystoscopy performed a couple of months after surgery. This patient will be followed-up with a new urethrocystoscopy after one year.

Preoperative antibiotics and intraurethral antibiotics were given as prophylaxis. Additionally, we have postoperatively left a suprapubic catheter in place for 7 d. During this time, oral antibiotics were given.

Under the ideal circumstances, the procedure took 30 min or less.

Outcomes

Four patients had relapses of their SUI after the intraurethral mesh was removed (Table 2). For five patients, no further SUI treatment was necessary after the removal of the intraurethral tape. Five out

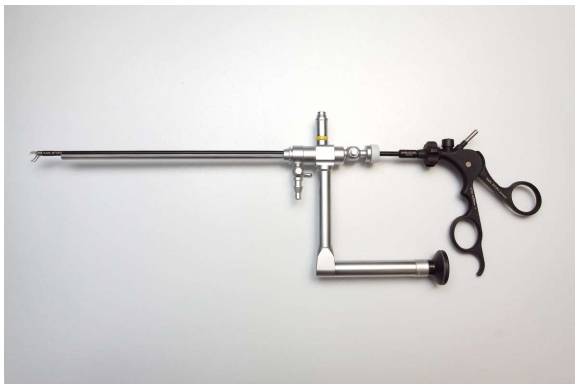

Figure 2 Operating nephroscope.

of seven patients patients with urgency experienced improvements or cures of their symptoms after the removal of the intraurethral mesh. Only three out of seven patients with *de novo* urgency were in need of anticholinergics after the last follow-up. Three patients also experienced improvements or cures of their voiding difficulties.

DISCUSSION

Risk factors

The exact pathophysiology behind erosion of the sling materials is not fully understood[3,17]. Various factors may predispose a patient to erosion. Factors associated with the pelvic anatomy include urogenital atrophy, poorly estrogenized tissue, previous pelvic radiation, previous vaginal surgery, or concomitant procedure and local infection, as well as a high body weight[13,17,27,28]. Our series had only one patient with a previous pelvic procedure, so these risk factors could not be verified.

Predisposing factors associated with the surgical technique include excessive tensioning of the sling and placement of the mesh too close to the urethra, which is the most feasible explanation, but it is not possible to extract this information from the medical records. When operating with mini-slings, it has been suggested to put the sling closer to the urethra than with traditional slings. This could explain our two cases of urethral complication after mini-sling procedures. Other possible explanations are inadequate vaginal tissue coverage and improper dissecting near the urethra, which might damage the urethral tissue and its vascularity[3,13,17,27]. Two of our cases had the sling doubled under the urethra perioperatively to ensure a tension-free application of the mesh. Four of our cases suffered from tight placement of the sling under the urethra; two patients had to use intermittent catheterization and two experienced voiding difficulties postoperatively.

The surgical manuals on retropubic TVT procedures recommend a rigid catheter guide inserted into the indwelling catheter for contralateral displacement of the bladder to minimize the risk of perforation. The urethra is then pulled towards the TVT needle, potentially increasing the risk of perforating the edge of the urethra. This might explain cases of urethral perforations and erosions in retropubic TVTs, but not in the other methods, as there was no displacement of the urethra during the operation.

More than one million TVT procedures have been performed worldwide since 1996 when the method was introduced[3,19]. In Sweden, about 4000-5000 women are operated on annually for SUI, most of whom receive the retropubic technique[16].

Urethral complications might be more common than reported, but that does not mean that the TVT procedures should be restricted. Urethral injury is still a rare complication, while the TVT procedures have a high success rate and a great improvement in the quality of life of the operated patients.

Symptoms

Urethral erosion may present with various symptoms. These include postoperative urinary retention, voiding dysfunction, hematuria, urethral or pelvic pain, recurrent UTIs, relapse of SUI, and *de novo* urgency[15].

There are only sporadic case reports referring to the clinical profile of erosions after sling procedures. As it seems in our study, the clinical profile of urethral complications might vary depending on the early or late onset of the symptoms after the operation. In the early period, we should expect voiding difficulties and urethritis symptoms, whereas in the later stage, it is more common with the relapse of SUI. *De novo* urgency (with or without leakage) and recurrent UTIs are also common symptoms that might appear early or late in the postoperative process.

In a review of 376 women with adverse events after suburethral sling procedures, Petri *et al*[28] analyzed the most common complications. *De novo* urgency with or without leakage was presented in 54% of the cases, voiding dysfunction in 48%, vaginal erosion in 19%, and urethral and vaginal pain in 14%. A total of 17 (4.5%) cases of urethral and bladder base perforation were found in their study, and most often the complications had occurred peroperatively. However, urethral and bladder base perforations might be associated with severe morbidity, and even lead to urethravaginal fistulae if undetected. Our study describes similar clinical symptoms secondary to urethral erosions.

A majority (77.7%) of our cases had *de novo* urgency. Postoperative *de novo* urgency has been reported in 10% of TVT procedures. If urethrocystoscopy is implemented postoperatively, the number of undiagnosed urethral erosions could considerably be reduced.

Treatment

There has been a variety of approaches to the surgical management of urethral complications after

sling procedures. The transvaginal excision of the intraurethral part of the sling with urethroplasty was the first approach to be used[18]. We used this method for our first case, but the transurethral excision of the mesh has become the preferred method for the majority of the patients in our series.

Another alternative is the conservative approach. In case 3, only one small loop of the mesh was seen in the urethra during the first cystoscopy. Cystoscopies were performed every 6 mo as a follow-up, and the mesh migrated directly across the urethra, but did not progress further after that.

We tried conservative treatment after receiving a report from colleagues who found a part of the mesh in the urethra but did not remove it. Therefore, we believe that urethral erosion might be a much common complication, at least in Sweden, where gynecologists perform TVT procedures, but do not routinely carry out urethrocystoscopy.

We also introduced preoperative urethrocystoscopy to all pubovaginal sling procedures. Including urethrocystoscopy in the preoperative investigation had many advantages. First, filling the bladder with 300 mL of saline solution without any detrusor contraction made cystometry unnecessary. Second, conducting a preoperative pad test with 300 mL in the bladder, with the patient exercising for 1 min and coughing ten times, would be much faster, if it is done at the same time as the urethrocystoscopy. Third, it would make urethrocystoscopy a standard and familiar procedure for the gynecologist.

Urethral complications after sling procedures might be more common than previously thought. One of the reasons is that the urethrocystoscopy is not included routinely in the postoperative follow-up of patients with residual or new symptoms from the urinary or vaginal tracts. This makes it difficult to identify complications because of the wide variation in the clinical profile and the timing of the presenting symptoms. It is important to suspect urethral complications if symptoms, such as urgency, voiding dysfunction, recurrent UTIs, or relapse of SUI occur after sling procedures.

We recommend the transurethral approach for the excision of the intraurethral mesh as the treatment of choice for urethral erosion.

ACKNOWLEDGEMENTS

We are grateful to all the patients who participated in the study.

COMMENTS

Background

Introduction of the tension-free vaginal tape (TVT) operations in 1996 to treat stress incontinence changed the surgical management of the condition. Instead of being an abdominal operation with one week of hospitalized postoperative care, TVT became a minimal invasive outpatient surgery, with same-day discharge. This meant many more women could be operated upon with this minimally invasive technique improving the patient's quality of life tremendously. However, even the minimally invasive procedures have associated complications with a unique set of symptoms.

Research frontiers

Mesh erosions are very serious complications, especially in prolapse surgery, with a high patient morbidity rate. In TVT operations, very few complications have been reported, mostly referring to perforation of the bladder. Urethral complications have been reported very sporadically.

Innovations and breakthroughs

The authors have performed routine urethrocystoscopy on women with some voiding difficulties after a TVT operation. Nine cases of urethral mesh erosions were identified. The initially used method required removal of the tape with an intravaginal approach, a rather long and complicated operation. The authors made a necessary modification with an intraurethral removal of the tape using a nephroscope. This device is normally used in kidney operations and not in vaginal procedures.

Applications

As much as 10% of reported cases of *de novo* urgency occur after a TVT operation. Many of these women might have urethral erosion that can be easily operated upon, if identified during urethrocystoscopy.

Terminology

Stress incontinence is leakage of urine during coughing, laughing, and running. Urge incontinence is leakage after a strong feeling of need to void. *De novo* urge is a symptom of urgency that develops after the stress incontinence procedure. TVT procedures are minimally invasive operations with placing of a small tape under the urethra. Cystoscopy is a diagnostic procedure where a small instrument with a camera is introduced into the bladder and identifies the inside. Urethrocystoscopy is the same procedure that also includes examining of the urethra. It is not always performed during a routine cystoscopy.

Peer-review

This article is very interesting for individuals involved in the treatment of stress incontinence. Authors present their experience in the treatment of urethral erosion after tension-free vaginal tape procedures. The diagnostic and therapeutic approach is clearly explained. Also, an unusual and rare but very interesting operation of transurethral excision of the intraurethral part of the mesh was performed.

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ORIGINAL ARTICLE

Clinical Trial Study

Albuminuria as a marker of arterial stiffness in chronic kidney disease patients

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Abstract

AIM: To access the association between albuminuria levels and arterial stiffness in non-diabetic patients with hypertension and chronic kidney disease (CKD) stages 1-2, treated with renin angiotensin blockade agents plus other hypertensive drugs when needed.

METHODS: One hundred fifteen patients [median age 52 years (68% males)] were consequently enrolled in the study. For each patient, we recorded gender, age, body mass index (BMI), peripheral systolic blood pressure (pSBP), peripheral diastolic blood pressure, peripheral pulse pressure, central systolic blood pressure (cSBP), central diastolic blood pressure (cDBP), central pulse pressure (cPP), hematocrit, hemoglobin, hsCRP, total cholesterol triglycerides, high-density lipoprotein-C, low-density lipoprotein-C, calcium, phosphorus, parathormone, and albumin, as well as 24 h urine albumin excretion. According to 24-h urine albumin collection, patients were then classified as those with moderately increased albuminuria (formerly called macroalbuminuria) ($\leqslant$ 300 mg/d) and those with severely increased albuminuria (formerly called macroaluminuria (> 300 mg/d). We considered aortic stiffness (AS) indices [carotid femoral pulse wave velocity (PWVc-f) and augmentation index (AIx)] as primary outcomes of

the study. We explored potential correlations between severely increased albuminuria and AS indices using a multiple linear regression model.

RESULTS: Fifty-eight patients were included in the moderately increased albuminuria group and 57 in the severely increased albuminuria. Blood pressure measurements of the study population were 138 ± 14/82 ± 1.3 mmHg (systolic/diastolic). There were no significant differences in age, sex, and BP measurements between the two groups. Patients with severely increased albuminuria had higher PWV and AIx than patients with moderately increased albuminuria ($P < 0.02$, $P < 0.004$, respectively). In addition these patients exhibited higher BMI ($P < 0.03$), hsCRP ($P < 0.001$), and fibrinogen levels ($P < 0.02$) compared to patients with moderately increased albuminuria. In multivariate linear regression analysis, severely increased albuminuria ($\beta = 1.038$, $P < 0.010$) pSBP ($\beta = 0.028$, $P < 0.034$) and Ht ($\beta = 0.171$, $P = 0.001$) remained independent determinants of the increased PWVc-f. Similarly, severely increased albuminuria ($\beta = 4.385$, $P < 0.012$), cSBP ($\beta = 0.242$, $P < 0.001$), cPP ($\beta = 0.147$, $P < 0.01$) and Ht levels ($\beta = 0.591$, $P < 0.013$) remained independent determinants of increased AIx.

CONCLUSION: These findings demonstrate an independent association between AS indices and severely increased albuminuria in non-diabetic, hypertensive patients with CKD stages 1-2 treated with renin angiotensin aldosterone system blockers.

Key words: Arterial stiffness; Pulse wave velocity; Augmentation index; Albuminuria

Core tip: Albuminuria heightened cardiovascular disease risk. Pulse wave velocity and augmentation index are markers of aortic stiffness (AS). However, whether severely increased albuminuria is a factor of AS elevation and its progressive deterioration in non-diabetic hypertensive patients treated with renin angiotensin aldosterone blockade agents (RAAS) has not be studied. In this study we aimed to access the association between albuminuria levels and AS, in chronic kidney disease (CKD) stage 1-2 non-diabetic patients with hypertension. All patients were already treated with RAAS blockade agents. Our findings demonstrate an independent association between AS indices and severely increased albuminuria in non-diabetic, hypertensive patients with CKD stages 1-2 treated with RAAS blockers.

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INTRODUCTION

The presence of albuminuria is associated with faster progression of renal failure and is also recognized as a marker of vascular dysfunction and heightened cardiovascular disease risk in hypertensive and chronic kidney disease (CKD) patients with or without diabetes[1,2].

Arterial stiffness (AS) assessment helps to predict cardiovascular events in patients with or without diabetes[3-5]. It is usually assessed with the aortic pulse wave velocity (PWV)[6,7] and peripheral pressure wave reflections, (Alx)[1,8]. These indices (PWV and AIx), as major determinants of the aortic pulse pressure, are early markers of atherosclerotic vascular changes and in CKD have been shown to be associated with renal micro vascular damage and kidney dysfunction[9]. On the other hand, AS was showed to be associated with incident albuminuria and the rate of decline in glomerular filtration rate (GFR)[10]. More recent information in patients with type 2 diabetes mellitus showed that levels of urinary albumin excretion, but not reduced estimated GFR, were associated with increased AS and atherosclerosis[11]. High normal albuminuria in the range (0-30 mg/g) is also associated with aortic stiffness[12] even in younger type 2 diabetic patients with shorter durations of disease[13], while in newly diagnosed patients with type 2 diabetes mellitus, moderately increased albuminuria was independently associated with AS and vascular inflammation[14].

However, limited data are available whether severely increased albuminuria is associated with AS in non-diabetic hypertensive patients already treated with renin angiotensin aldosterone blockade agents (RAAS)[15]. It has been suggested that endothelial dysfunction could be a possible mechanism involved in the remodeling of the arterial wall affecting AS and modifying glomerular permeability leading to increased albumin excretion[16,17]. In addition, AS could influence glomeruli function through an increased pulsatile stress, causing glomerular damage[18].

In this study we aimed to access the association between albuminuria levels and AS, in CKD stage 1-2 non-diabetic patients with hypertension. All patients were already treated with RAAS blockade agents plus other hypertensives when needed. RAAS blockers play an important role in the regulation of BP. These agents have demonstrated favorable effects beyond blood pressure control in situations, such as albuminuria, left ventricular hypertrophy and AS[16].

MATERIALS AND METHODS

We included 115 consecutive hypertensive patients with CKD stages 1-2 (Stage 1: kidney damage with normal or increased GFR, Stage 2: kidney damage with mild reduced GFR 60-89 mL/min). None of our patients had albuminuria > 1 g per 24 h. Albuminuria levels were stable for the past 6 mo. Patients with

Table 1 Demographic and aortic stiffness indices characteristics

Patients characteristics	Study population	Patients with moderately increased albuminuria	Patients with severely increased albuminuria	*P* value
No	115	58	57	-
Age (yr)	52.6 ± 14.6	51.4 ± 14.4	54.2 ± 15.07	NS
Sex (M/F), (*n*)	76/39	39/19	37/20	NS
BMI (kg/m^2)	30.9 ± 19.3	28.6 ± 4.5	33.9 ± 28.2	< 0.03
Smoking, *n* (%)	5	3	2	NS
pSBP (mmHg)	138.0 ± 14.2	139 ± 12.7	138.0 ± 15.5	NS
pDBP (mmHg)	84.2 ± 9.7	84.9 ± 10.7	82.6 ± 9.8	NS
pPP (mmHg)	57.9 ± 15.9	56.7 ± 15.1	59.4 ± 16.9	NS
cSBP (mmHg)	130.3 ± 14.6	130 ± 13.3	130.2 ± 16.5	NS
cDBP(mmH)	84.2 ± 9.7	84.9 ± 10.7	84.3 ± 8.6	NS
cPP (mmHg)	48.1 ± 17.6	47.9 ± 17.9	48.4 ± 17.8	NS
AIx (%)	21.1 ± 10.6	18.4 ± 10.0	24.5 ± 10.0	< 0.004
PWVc-f (m/sec)	8.7 ± 2.0	8.3 ± 2.0	9.1 ± 1.9	< 0.02
ACEIs, *n* (%) (ramipril or quinapril)	100 (86)	56 (96)	44 (77)	NS
AT1 RB, *n* (%) (valsartan or olmesartan)	20 (17.4)	9 (15.5)	11 (19.2)	NS
CCBs, *n* (%)	11 (9.5)	6 (10.3)	5 (8.7)	NS
Statins, *n* (%)	7 (6.08)	3 (5.1)	4 (7.01)	NS

Data are presented as mean value. M/F: Male/female; BMI: Body mass index; pSBP: Peripheral systolic blood pressure; pDBP: Peripheral Diastolic blood pressure; pPP: Peripheral pulse pressure; cSBP: Central systolic blood pressure; cDBP: Central diastolic blood pressure; cPP: Central pulse pressure; AIx: Augmentation index; AP: Augmentation pressure; PWVc-f: Pulse wave velocity carotid-femoral; ACEIs: Angiotensin converting enzyme inhibitors; AT1RB: Angiotensin type 1 receptor blockers; CCBs: Calcium channel blockers.

known glomerulopathy proven by biopsy were excluded. Most of our patients had hypertensive nephosclerosis with duration of hypertension for more than 10 years and none of them had diabetes mellitus. Furthermore we excluded patients with acute myocardial infarction, unstable angina, stroke, heart failure or transient ischemic attack within the past year. We recorded the demographic data of the patients including age, gender, body mass index (BMI), peripheral blood pressure measurements [(systolic, diastolic, pulse pressure (pSBP, pDBP, pPP) respectively] as well as central blood pressure measurements [(systolic, diastolic, pulse pressure (cSBP, cDBP, cPP) respectively].

All the patients were treated with RAAS agents (ACE inhibitors or angiotensin type 1 (AT1) receptor blockers) plus other antihypertensive drugs as needed. Our choice for the first agent was a RAAS blocker based on the favorable effects of these agents on albuminuria and AS reduction as well as for their anti-fibrotic and anti-inflammatory effects[17,18]. Furthermore, the reno- and cardio-protective effects beyond their hypotensive effects were also well established[19] and for our patients with moderately or severely albuminuria this treatment was already applied for at least 6 mo. Ramipril or quinapril were the ACE inhibitors used as well as valsartan or olmesartan were the AT1 receptor blockers, used in maximum doses. Ramipril and quinapril were prescribed at the doses of 20 mg OD and valsartan and olmesartan at the doses of 320 and 20 mg OD respectively. Six patients received valsartan 160 mg OD and 2 patients received quinapril 40 mg OD. A very small number of patients received amlodipine at a dose of 10 mg OD (Table 1).

Blood pressure measurements

The patient's representative peripheral BP levels at each visit, were the average of three consecutive BP measurements in a sitting position after 5 min rest, within 2 min intervals between them by an automated sphygmomanometer. Peripheral PP was defined as the difference between pSBP and pDBP.

Arterial stiffness measurements

For the assessment of the central aortic pressure were used the Sphygmocor system (Atcor, Sydney, Australia) and its software with an applanation tonometry (Millar tonometer, Millar Instruments, Houston, TX). The AIx was calculated as the increment in pressure from the first systolic shoulder to the peak pressure of the aortic pressure waveform expressed as a percentage[20]. Carotid-femoral pulse wave velocity (PWVc-f) was also calculated non-invasively by the aforementioned software of the Sphygmocor system (Atcor, Sydney, Australia) described elsewhere[20]. Primary outcomes of the study were considered arterial stiffness indices, AIx and PWVc-f.

Laboratory measurements

In all patients, blood samples were obtained after 12 h fasting. Serum samples were analyzed for haematocrit (Ht), haemoglobin (Hb), hsC-Reactive Protein (hsCRP), and serum lipids, such as total cholesterol (T-CHOL), triglycerides (TRG), and high-density lipoprotein-C (HDL-C). Low-density lipoprotein-C (LDL-C) was calculated using the Friedewald formula provided fasting TRG levels less than 400 mg/dL. In

patients with serum TRG values greater than 400 mg/dL, LDL-C concentrations were not determined. HsCRP was measured using a latex enhanced immunonephelometry assay on a Dade Behring BN Ⅱ nephelometer. We also measured serum levels of calcium, phosphorus, parathormone and albumin, as well as 24 h urine albumin excretion, using common commercial serological kits. Albuminuria was the mean value of 2 separate 24 h urinary collections. Patients were then classified as those with moderately increased albuminuria (formerly called macroalbuminuria) ($\leqslant$ 300 mg/d) and those with severely increased albuminuria (formerly called macroaluminuria (> 300 mg/d). Smoking status was defined as current or past smoker *vs* non-smoker.

All patients were received RAAS blockers (ACE inhibitors or AT1 receptor blockers and 5 patients (4.34%) were received double RAAS blockade agents (ACE inhibitor and AT1 receptor blocker). Our hospital Ethics Committee approved this study protocol. Informed consent was obtained from all patients.

Statistical analysis

We presented data separately for patients with moderately increased albuminuria and patients with severely increased albuminuria. Data were presented as absolute numbers and frequencies for binary and categorical variables, and as mean ± SD for continuous variables. Between the two groups, comparisons for binary variables were performed using χ^2 or Fisher's exact test. Comparisons for continuous variables were performed using Mann-Whitney *U* test. To investigate whether there was a potential relation between AS and patient characteristics, we first performed univariate linear regression analyses for each variable using the AS indices, *i.e.,* PWVc-f, and AIx as dependent variables.

We considered as independent covariates the following: albuminuria category (moderately *vs* severely increased albuminuria), age, sex, BMI, pSBP, pDBP, Ppp, cSBP, cDBP, cPP, anti-RAAS agents, Ht, Hb, T-CHOL, TRG, HDL-C, LDL-C, fibrinogen, and hsCRP.

Variables with a *P*-value < 0.1 from the univariate analysis were evaluated further in a multivariate regression analysis. For each variable beta (β) co-efficient with the corresponding confidence interval (CI) were calculated in the multivariate model. A two-sided P-value <0.05 was considered as statistically significant. The SPSS, version 16 (SPSS Inc.) statistical package was used for the statistical analysis. Statistically significant was considered a *P*-value < 0.05.

RESULTS

One-hundred fifteen hypertensive non diabetic patients were consequently enrolled in the study. The mean age of the patient's population was 52 years, and 68% of them were males. All enrolled patients exhibited estimated glomerular filtration rate (eGFR-MDRD) > 60 mL/min per 1.73 m^2. Fifty-eight patients were included in the moderately increased albuminuria group and 57 in the severely increased albuminuria group. Demographic and AS indices characteristics are reported in Table 1.

Patients with severely increased albuminuria compared to patients with moderately increased albuminuria had higher BMI ($P < 0.03$), and higher PWV and AIx values ($P < 0.02$, $P < 0.004$, respectively) (Table 1). No differences were found in the parameters of peripheral and central BP measurements between the two groups (Table 1).

Biochemical characteristics of the study populations are reported in Table 2. Patients with severely increased albuminuria compared to patients with moderately increased albuminuria had significantly lower values of haematocrit ($P < 0.01$) and haemoglobin ($P < 0.02$), as well as increased levels of fibrinogen ($P < 0.01$), hsCRP ($P < 0.001$), and phosphorus levels ($P < 0.01$), and lower eGFR-MDRD values ($P < 0.001$) (Table 2).

Univariate linear regression analyses for the association of the absolute values of PWV with other parameters are shown in Table 3. In multivariate linear regression analysis, only severely increased albuminuria ($\beta = 1.038$, $P < 0.010$), pSBP ($\beta = 0.028$, $P < 0.034$) and Ht ($\beta = 0.171$, $P = 0.001$) remained independent determinants of increased PWVc-f (Table 4).

Univariate linear regression analyses for the association of the absolute values of AIx with other parameters are shown in Table 5. Similarly, severely increased albuminuria ($\beta = 4.385$, $P < 0.012$), cSBP ($\beta = 0.242$, $P < 0.001$), cPP ($\beta = 0.147$, $P < 0.01$) and Ht levels ($\beta = 0.591$, $P < 0.013$) remained independent determinants of increased AIx values (Table 6). No other variables correlated significantly to AS indices.

DISCUSSION

The findings of our study demonstrate an independent association between AS indices (PWV and AIx) and severely increased albuminuria in hypertensive non-diabetic patients with moderate kidney dysfunction, CKD stages 1-2, treated with RAAS blockers.

Albuminuria is recognized as a marker of vascular dysfunction and heightened cardiovascular disease risk[1,2]. The presence of albuminuria is also an indicator of the underlying kidney disease with an increased probability of progressive kidney loss[18,21]. It is worth mentioning that the level of albuminuria and not the current level of GFR is the most relevant variable to predict CKD progression in those with a GFR of at least 30 mL/min[22].

In recent years, great emphasis has been placed on the role of AS in the development of cardiovascular diseases while this parameter has been used in the assessment of patients with hypertension and/or CKD, since AS measurement seems to have an additive value beyond traditional risk factors, including Framingham risk score[23]. Increased AS

Table 2 Biochemical characteristics of the study population

Patients characteristics	Study population	Patients with moderately increased albuminuria	Patients with severely increased albuminuria	*P* value
No	115	58	57	-
Ht (%)	42.3 ± 3.7	43.2 ± 3.7	41.4 ± 3.5	0.01
Hb (g/dL)	13.6 ± 1.3	14.1 ± 1.5	13.3 ± 1.5	0.02
Fe (μg/dL)	89 ± 35.5	90.2 ± 30	84.2 ± 38.5	NS
Ferritin (ng/L)	127.4 ± 90.1	127.8 ± 87.9	131.3 ± 96.5	NS
Fibrinogen (mg/dL)	336 ± 82.9	339 ± 86.5	394 ± 133.9	< 0.01
hsCRP (mg/dL)	0.19 ± 0.06	0.18 ± 0.05	0.23 ± 0.10	< 0.001
T-CHOL (mg/dL)	203 ± 37.5	208 ± 39.2	209 ± 46.8	NS
TRG (mg/dL)	126 ± 45.7	123 ± 46.3	145 ± 70.2	0.05
HDL-C (mg/dL)	53.4 ± 13.3	53 ± 12.9	54.2 ± 14.9	NS
LDL-C (mg/dL)	119 ± 32	120 ± 3.7	121 ± 3.6	NS
Ca^{2+} (mg/dL)	9.7 ± 0.4	9.7 ± 0.5	9.8 ± 0.4	NS
PO_4^{3-} (mg/dL)	3.42 ± 0.4	3.3 ± 0.4	3.5 ± 0.5	0.01
Ca^{2+} x PO_4^{3-}	33.7 ± 4.8	32.6 ± 4.5	33.9 ± 8.6	NS
PTH (pg/mL)	42.4 ± 15.4	39.8 ± 13.6	45.4 ± 29.9	NS
sAlb (gr/dL)	4.5 ± 0.2	4.5 ± 0.3	4.3 ± 0.5	0.03
eGFR-MDRD (mL/min per 1.73 m^2)	92 ± 0.5	91.5 ± 20.5	79.1 ± 15.3	< 0.001
CKD-EPI(mL/min per 1.73 m^2)	73.4 ± 1.25	73.1 ± 13.7	73.6 ± 11.2	NS

Data are presented as mean value. Ht: Haematocrit; Hb: Haemoglobin; Fe: Serum iron; Ferritin: Serum ferritin, hsCRP: High sensitive C-reactive protein; T-CHOL: Total cholesterol; TRG: Triglycerides; HDL-C: High density lipoprotein cholesterol; Ca^{2+}: Calcium; PO_4^{3-}: Phosphorus; PTH: Parathormone; sAlb: Serum albumin; eGFR-MDRD: Estimated-glomerular filtration rate-modification of diet in renal disease; RAAS-blocker: Renin angiotensin aldosterone system blocker; Alb: Albuminuria.

Table 3 Univariate linear regression analysis of the parameters associated with the absolute values of pulse wave velocity

Covariates	β	*t*	β (95%CI)	*P* value
Alb	0.841	2.271	0.107-1.574	< 0.025
Age	0.026	2.047	0.001-0.051	< 0.043
BMI	0.009	0.880	0.011-0.028	0.381
pSBP	0.032	2.511	0.007-0.058	< 0.013
pDBP	0.005	0.285	-0.032-0.042	< 0.776
pPP	0.029	2.277	0.004-0.053	< 0.025
cSBP	0.031	2.298	0.004-0.508	< 0.024
cDBP	-0.004	-0.173	-0.045-0.038	0.963
cPP	0.014	1.257	0.008-0.037	0.212
Ht	0.103	2.077	0.005-0.202	0.040
Hb	0.227	1.812	0.020-0.476	0.070
T-CHOL	0.021	0.456	-0.011-0.007	0.649
TRG	0.002	0.752	0.009-0.004	0.454
HDL-C	-0.006	-0.417	-0.033-0.022	0.677
LDL-C	0.023	0.445	0.013-0.477	0.674
Fbrinogen	0.0001	0.524	-0.004-0.002	0.602
hsCRP	-0.011	0.046	-0.442-0.422	0.964
RAAS-blocker	0.081	-0.103	-1.650-1.487	0.918

Alb: Albumin; BMI: Body mass index; pSBP: Peripheral systolic blood pressure; pDBP: Peripheral diastolic blood pressure; pPP: Peripheral pulse pressure; cSBP: Central systolic blood pressure; cDBP: Central diastolic blood pressure; cPP: Central pulse pressure; Ht: Haematocrit; Hb: Haemoglobin; T-CHOL: Total cholesterol; TRG: Triglycerides; HDL-C: High density lipoprotein cholesterol; RAAS-blocker: Renin angiotensin aldosterone system blocker; LDL-C: Low density lipoprotein cholesterol; hsCRP: HsC-reactive protein.

provides prognostic information above traditional CV risk factors, such as BP itself, gender, age, smoking diabetes, and cholesterol[24,25]. It is an independent predictor of fatal stroke in patients with essential hypertension[3,4,26] and a powerful predictor of mortality in both diabetes mellitus and glucose-tolerance-tested multi-ethnic population samples[27]. In addition, PWVc-f is independently associated with a faster decline of kidney function in patients with type 2 diabetes mellitus[10]. The relationship between AS and events is continuous,

Table 4 Multivariate linear regression analysis of the parameters associated with the absolute values of pulse wave velocity

Covariates	β	*t*	β (95%CI)	*P* value
UAlb	1.038	2.638	0.257-1.820	< 0.010
pSBP	0.028	2.149	0.002-0.053	< 0.034
Ht	0.171	3.319	0.069-0.273	< 0.001

pSBP: Peripheral systolic blood pressure; Ht: Haematocrit.

Table 5 Univariate linear regression analysis of the parameters associated with absolute values of AIx

Covariates	β	*t*	β (95%CI)	*P* value
UAlb	6.201	2.977	2.065-10.337	< 0.004
Age	0.236	3.427	0.099-0.373	< 0.0001
BMI	0.036	0.667	0.070-0.142	0.507
pSBP	0.291	4.427	0.161-0.422	< 0.0001
pDBP	0.001	0.002	-0.216-0.216	< 0.002
pPP	0.278	4.536	0.156-0.400	< 0.0001
cSBP	0.349	5.518	0.223-0.474	< 0.0001
cDBP	-0.045	-0.401	0.265-0.176	0.689
cPP	0.265	4.912	0.558-0.372	< 0.0001
Ht	-0.848	0.002	-1.382-0.314	< 0.002
Hb	-0.599	-0.826	-2.040-0.841	0.411
T-CHOL	0.050	1.836	-0.041-0.042	0.069
TRG	0.021	1.164	0.015-0.057	0.247
HDL-C	0.104	1.318	-0.534-0.035	0.262
LDL-C	0.047	1.265	-0.045-0.052	0.243
Fbrinogen	0.007	0.749	-0.122-0.273	0.456
hsCRP	0.015	0.732	-1.738-3.768	0.466
RAAS-blocker	1.043	0.139	13.881-15.966	0.890

Alb: Albumin; BMI: Body mass index; pSBP: Peripheral systolic blood pressure; pDBP: Peripheral diastolic blood pressure; pPP: Peripheral pulse pressure; cSBP: Central systolic blood pressure; cDBP: Central diastolic blood pressure; cPP: Central pulse pressure; Ht: Haematocrit; Hb: Haemoglobin; T-CHOL: Total cholesterol; TRG: Triglycerides; HDL-C: High density lipoprotein cholesterol; RAAS-blocker: Renin angiotensin aldosterone system blocker; LDL-C: Low density lipoprotein cholesterol; hsCRP: HsC-reactive protein.

Table 6 Multivariate linear regression analysis of the parameters associated with the absolute values of AI

Covariates	β	*t*	β (95%CI)	*P* value
UAlb	4.385	2.557	1.023-8.146	< 0.012
cSBP	0.242	3.563	0.107-0.376	< 0.0001
cPP	0.147	2.623	0.036-0.259	< 0.0001
Ht	0.591	2.536	1.055-0.128	< 0.013

cSBP: Central systolic blood pressure; cPP: Central pulse pressure; Ht: Haematocrit.

however, a threshold of > 12 m/s has been suggested as a significant marker of vascular alterations and aortic dysfunction in middle-aged hypertensive patients[28]. A more recent expert consensus statement adjusted this threshold value to 10 m/s[29]. In fact in our study patients had mean PWV values < 10 m/s (Table 1).

Several cross-sectional studies demonstrated a relationship between AS and moderately increased albuminuria in the general population, in individuals with hypertension[30] and/or type 2 diabetes mellitus[5,14]. Additionally, epidemiologic evidence showed an independent association between AS, moderately increased albuminuria and other indices of subclinical target organ damage in non-hypertensive, non-diabetic individuals[28].

In our study we expanded this relationship in hypertensive patients with stage 1-2 CKD without diabetes already treated with RAAS blockers. Our results are in accordance with the results from the Framingham cross-sectional analyses in patients with moderate CKD that showed that PWVc-f was associated with both urinary albumin-to-creatinine ratio and moderately

increased albuminuria ($P < 0.0001$)[31]. In the study by Munakata *et al*[32] each 400 cm/s increase in brachial-ankle PWV, increased the incidence of new-onset moderately increased albuminuria about 2.4 times at 2-year follow-up, suggesting that higher brachial-ankle PWV could be an independent risk factor for the future development of moderately increased albuminuria in patients with hypertension[32]. A study by Kim *et al*[15] showed that AS is independently associated with moderately increased albuminuria, irrespectively of various covariates, in non-hypertensive, non-diabetic individuals. In our study we showed that these results are expanded to the patients with severely increased albuminuria.

The mechanisms linking AS and albuminuria are not fully established. However, it has been suggested that endothelial dysfunction could be a possible mechanism involved in the remodeling of the arterial wall causing structural and functional changes in the target vessels, resulting in the increase of the AS. On the other hand, endothelial dysfunction modifies glomerular permeability and as a consequence leads to increased albumin excretion[33,34]. Alternatively, an increased pulsatile stress mediated by an increased AS causes a pressure load on the glomeruli and could lead to their damage[35].

In our study the optimal BP control in our patients is believed to play a substantial role and contributed to a lower AS indices as well as lower levels of albuminuria. Furthermore all patients in our study were treated with a RAAS blocker.

In patients with arterial hypertension and albuminuria blockage of the RAAS is the treatment of choice[18]. These agents offer a cardio-renal protection which may be mediated, at least in part, by their beyond blood pressure control drug-specific effects. In the past, we showed that these agents improve AS and decrease significantly moderately increased albuminuria[36]. In the present study we showed that patients with severely increased albuminuria had a higher AIx which remained an independent determinant of increased AS. No significant differences in central aortic pressures between the groups were observed. It is known that AIx and central aortic pressures reflect different arterial wall properties compared to PWV. The latest, reflect changes in pressure wave reflections from the large arteries at the distal sites. In contrast, AIx reflect functional properties from the small arteries. In the Framingham study AIx was not associated with urinary albumin excretion[31]. In contrast, in our study, the association of AIx with severely increased albuminuria remains significant in multivariate analysis despite the administration of RAAS blockade agents.

In patients with severely increased albuminuria lower Ht levels were found, which were independently associated with PWV and AIx. In this group of patients, despite the near normal degree of Ht levels along with increased inflammatory indices, such as fibrinogen and hsCRP, Ht might be related to the kidney dysfunction as suggested by Hiramoto *et al*[37]. Of note, in hypertensive patients, increased levels of inflammatory biomarkers, such hs-CRP, are associated with AS indices (*i.e.*, PWV and AIx)[38]. Furthermore, these markers are also increased with the deterioration of renal function[38]. Thus, our results, emphasize the possibility of a common pathophysiologic mechanism affecting renal dysfunction, anemia and AS deterioration: inflammation and endothelial dysfunction may play a prominent role in the interrelation of these entities[37,39].

The cross sectional design, which does not allow to establish cause-effect relationships as well as the small number of patients involved are potential limitations of our study. Subsequently, further prospective studies are required to verify whether albuminuria is a contributing factor and/or a consequence of the increased AS independently of the RAAS blockade and BP control.

Our findings suggest an independent association between AS and severely increased albuminuria in non-diabetic, hypertensive patients, already treated with RAAS agents, who exhibited early renal dysfunction.

COMMENTS

Background

Albuminuria is associated with higher cardiovascular risk. Pulse wave velocity (PWV) and augmentation index (AIx) are early markers of vascular changes and aortic stiffness (AS) in patients with chronic kidney disease (CKD).

Research frontiers

The current research hotspot is the association between albuminuria levels and arterial stiffness. Patients participated in the study were non-diabetic with hypertension and CKD stages 1-2. All patients were treated with renin angiotensin blockade agents plus other hypertensive drugs for a rational period of time. Limited data are available whether severely increased albuminuria is associated with AS in non-diabetic hypertensive patients already treated with renin angiotensin aldosterone blockade agents.

Innovations and breakthroughs

Previous studies showed that in patients with type 2 diabetes mellitus the levels of urinary albumin excretion, but not reduced estimated glomerular filtration rate, were associated with increased AS and atherosclerosis. Even high normal albuminuria in the range (0-30 mg/g) is also associated with aortic stiffness even in type 2 diabetic patients. Limited data are available whether severely increased albuminuria is associated with AS in non-diabetic hypertensive patients already treated with renin angiotensin aldosterone blockade agents. This association even in treated patients with agents that could reduce albuminuria or could reduce arterial stiffness still exists.

Applications

The study results suggest an independent association between AS indices and severely increased albuminuria in non-diabetic, hypertensive patients with CKD stages 1-2 treated with renin angiotensin aldosterone system (RAAS) blockers. Despite the treatment of these patients with RAAS blockers still an association between arterial stiffness and severe increased albuminuria exist.

Terminology

Albuminuria is associated with faster progression of renal failure and is also recognized as a marker of vascular dysfunction and heightened cardiovascular disease risk. Arterial stiffness assessment helps to predict cardiovascular events in patients with or without diabetes. It is usually assessed with the aortic PWV and peripheral pressure wave reflections. These indices (PWV and AIx), as major determinants of the aortic pulse pressure, are early markers of atherosclerotic vascular changes and in CKD have been shown to be associated with renal micro vascular damage and kidney dysfunction.

Peer-review

This is a cross sectional study in which the authors demonstrate an independent association between AS indices and severely increased albuminuria in non-diabetic, hypertensive patients with CKD stages 1-2 treated with RAAS blockers. The study is interesting because all the patients were treated with

RAAS agents [ACE inhibitors or angiotensin type 1 (AT1) receptor blockers] plus other antihypertensive drugs as needed. The choice for the first agent was a RAAS blocker based on the favorable effects of these agents on albuminuria and AS reduction as well as for their anti-fibrotic and anti-inflammatory effects and this treatment was already applied for at least 6 mo. The results suggest an independent association between AS indices (PWV and AIx) and severely increased albuminuria in hypertensive non-diabetic patients with moderate kidney dysfunction, CKD stages 1-2, even though these patients were treated with RAAS agents (ACE inhibitors or AT1 receptor blockers) for this period of time and the BP levels were well controlled.

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ORIGINAL ARTICLE

Prospective Study

Low T3 syndrome and long-term mortality in chronic hemodialysis patients

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Informed consent statement: All involved patients gave a verbal informed consent for the legal use of their blood samples and handling of their personal data before their enrollment.

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Abstract

AIM: To investigate the predictive value of low freeT3 for long-term mortality in chronic hemodialysis (HD) patients and explore a possible causative role of chronic inflammation.

METHODS: One hundred fourteen HD patients (84 males) consecutively entered the study and were assessed for thyroid function and two established markers of inflammation, high sensitivity C-reactive protein (hsCRP) and interleukin-6 (IL-6). Monthly blood samples were obtained from all patients for three consecutive months during the observation period for evaluation of thyroid function and measurement of inflammatory markers. The patients were then divided in two groups based on the cut-off value of 1.8 pg/mL for mean plasma freeT3, and were prospectively studied for a mean of 50.3 ± 30.8 mo regarding cumulative survival. The prognostic power of low serum fT3 levels for mortality was assessed using the Kaplan-Meier method and univariate and multivariate regression analysis.

RESULTS: Kaplan-Meier survival curve showed a negative predictive power for low freeT3. In Cox regression analysis low freeT3 remained a significant predictor of mortality after adjustment for age, diabetes mellitus, hypertension, hsCRP, serum creatinine and albumin. Regarding the possible association with inflammation, freeT3 was correlated with hsCRP, but not IL-6, and only at the first month of the study.

CONCLUSION: In chronic hemodialysis patients, low plasma freeT3 is a significant predictor of all-cause mortality. Further studies are required to identify the underlying mechanisms of this association.

Key words: C-reactive protein; Hemodialysis; Inflammation; Interleukin-6; Low T3 syndrome; Mortality

Core tip: Monthly blood samples were obtained from 114 patients for three consecutive months during the observation period for evaluation of thyroid function and measurement of inflammatory markers high sensitivity C-reactive protein (hsCRP) and interleukin-6 (IL-6). Patients were then followed-up for 7-years. Low mean freeT3 (< 1.8 pg/mL) emerged as a significant predictor of all-cause mortality after adjustment for age, diabetes mellitus, hypertension, hsCRP, serum creatinine and albumin. However, freeT3 was correlated with hsCRP, but not IL-6, and only at the first month suggesting that further studies are required to identify the underlying pathogenetic mechanisms of the association between thyroid function and survival.

Fragidis S, Sombolos K, Thodis E, Panagoutsos S, Mourvati E, Pikilidou M, Papagianni A, Pasadakis P, Vargemezis V. Low T3 syndrome and long-term mortality in chronic hemodialysis patients. *World J Nephrol* 2015; 4(3): 415-422 Available from: URL: http://www.wjgnet.com/2220-6124/full/v4/i3/415.htm DOI: http://dx.doi.org/10.5527/wjn.v4.i3.415

INTRODUCTION

Despite the significant advances in hemodialysis (HD) techniques during the last decades, morbidity and mortality of HD patients remains unacceptably high and it is attributed mainly to the excess prevalence of cardiovascular disease (CVD)[1]. Beyond traditional risk factors (Framingham risk factors), which are highly prevalent in end-stage renal disease (ESRD) patients, patients on renal replacement therapy appear to be subject to the deleterious effect of a number of other harmful factors, including uremic milieu, anemia, bone-mineral disorders and hyperhomocysteinemia[2]. Moreover, recent studies suggested that oxidative stress, endothelial dysfunction and inflammation, also exacerbate cardiovascular disease[3,4]. So far, there is a lack of a strong marker of disease severity in ESRD, easy to perform and with high availability and low cost, that could guide treatment or even serve as a prognostic indicator of mortality[5]. Presumably, a multifactorial approach of traditional markers combined with novel biomarkers could fulfill this necessity, though this hypothesis needs to be confirmed in large prospective trials[6,7].

Low free triiodothyronin (freeT3) has emerged as a potent biomarker in ESRD in several studies and represents the main finding of non thyroidal illness syndrome (NTIS) in renal disease[2,8-10]. This syndrome, has been a debate for several years, as changes in hormone levels have been considered either a laboratory pitfall or an adoptive response to chronic stress aimed to spare calories[11,12]. It is important to emphasize the significance of values range, especially in differentiating incidents of subclinical hypothyroidism that could be mistaken as low T3 syndrome cases[13]. More recently, a narrower range of TSH values was proposed, although not widely accepted[14,15].

The exact pathogenetic mechanisms of NTIS are not yet fully elucidated but iodine retention, alterations to protein binding, derangements to deiodinases activity and dysregulation at the hypothalamic level appear to play a major role[16]. In addition, some studies demonstrated a significant negative correlation between interleukin-6 (IL-6) and serum T3 levels and suggested that NTIS is an acute phase response, yielded by activation of a cytokine network[17,18]. However, a causal role of chronic inflammation in the development of low T3 syndrome remains to be identified.

The aim of this prospective study was to investigate the predictive power of low freeT3 for long-term mortality in chronic hemodialysis patients; moreover the correlation between thyroid function and inflammation, assessed by measurements of high sensitivity CRP (hsCRP) and IL-6, was studied.

MATERIALS AND METHODS

Design

Prospective, observational study in 118 clinically stable chronic hemodialysis patients.

Patients

One hundred eighteen chronic hemodialysis patients from two large dialysis centers in Northern Greece, the Renal Unit at "G. Papanikolaou" General Hospital of Thessaloniki and the Department of Nephrology at University Hospital of Alexandroupolis, consecutively entered the study. Both centers had similar methods regarding hemodialysis and treatment strategies in ESRD, which are in accordance with the Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines[19]. All patients had been stabilized on renal replacement therapy for > 3 mo prior to enrollment (mean HD duration 113.4 ± 65.1 mo, range 15-427 mo) and were clinically stable and free of active infection.

Patients with known history of thyroid disorders or taking medications with possible effect in thyroid hormone values, like amiodarone or lithium, were excluded from the study[20]. None of the patients was receiving antibiotics at the time of the study or had required hospitalization up to 3 mo prior to study entry. All patients were on a 4hr three times weekly hemodialysis schedule. The dialysis modalities were on-line hemodiafiltration (OL-HDF) or conventional hemodialysis (C-HD) with high-flux or low-flux dialyzer membranes respectively. Both dialysis centers used semisynthetic membranes (polyamide or polysulphone) with surface ranging from 1.6-2.1 m^2. The dialysis solution consisted of standard bicarbonate preparations ($HCO3^-$: 32-35 mmol/L, Na: 138 mmol/L, K: 1-3 mmol/L, Mg: 0.5-0.75 mmol/L, Ca: 1.25-1.75 mmol/L). Low-molecular-weight or unfractionated heparin were used as standard anticoagulation. Dialysis prescription was guided by a goal of achieving a value of $\geqslant$ 0.65 for the urea reduction ratio and a value of Kt/V $\geqslant$ 1.2. The above indices of adequacy of dialysis were calculated by the formula [(pre-dialysis urea)-(post-dialysis urea)/predialysis urea], and by the second generation Daugirdas equation, respectively.

Body mass index (BMI) was calculated by dividing the dry weight in kilograms by the square of the height in meters. Blood pressure (BP) was measured in a supine position after 15 min of recumbency before a routine midweek dialysis session with standard mercury sphygmomanometers. Patients were considered hypertensive if they had pre-dialysis BP $\geqslant$ 160/90 mmHg or if they were receiving one or more anti-hypertensive drugs at the time of the study. History of cardiovascular disease was defined as history of myocardial infarction, coronary artery bypass or clinical signs of angina pectoris, stroke or transient ischemic attack or peripheral vascular disease.

The study protocol was conformed to the ethical guidelines of the Declaration of Helsinki and was approved both by the institutional review board and the ethics committee of each participating centre. Furthermore, all patients gave informed consent for the legal use of their blood samples and handling of their personal data before their enrollment.

Laboratory methods

Monthly blood samples were taken before a midweek routine hemodialysis session, for three consecutive months. Blood was drawn from the "arterial" lumen of fistula needle or the arterial port of the central hemodialysis catheter after extracting heparin lock in order to avoid artifactual alterations of measured freeT3 and the other studied parameters[21]. All samples were centrifuged for 15 min at 3500 rpm and the supernatant was immediately separated into vials (SST Ⅱ BD Vacutainer). Blood samples from Alexandroupoli were transferred to General Hospital "G. Papanikolaou" in Thessaloniki in dry ice. We were tied in with all appropriate scientific measures and legislation about transportation of biologic fluids. Samples from both institutions were eventually gathered and stored at G.H. "G. Papanikolaou" at -70 ℃.

All laboratory parameters were measured in the Central Biochemical Department of General Hospital "G. Papanikolaou". Complete blood cell count, urea, creatinine, total cholesterol, triglyceride, total protein and albumin, were determined by routine techniques using an automated analyser. Serum thyroid parameters (freeT3, freeT4, TSH), were measured by chemiluminescent, immunometric assay, according to the routine laboratory methods, using an IMMULITE-2000 analyzer, Laboratory reference values were 0.4 to 4.0 mIU/mL for TSH, 1.8 to 4.2 pg/mL for freeT3 and 0.89 to 1.76 ng/dL for freeT4.

Serum CRP levels were measured by high sensitivity nephelometrry ("Beckman Coulter" Ireland Inc). The detection limit was 0.1 mg/dL, with intra-assay and inter-assay coefficient of variation of 5% and 6.5%, respectively. Values above the threshold of 0.15 mg/dL were considered to be abnormal.

Serum IL-6 concentrations were measured by sandwich ELISA immunoassay using commercially available standard kits (AMS Biotechnology, United Kingdom). The concentrations of IL-6 were calculated by reference to standard curves performed with the corresponding recombinant molecule. All serum samples were tested in duplicate. The detection limit was 0.92 pg/mL, with intra-assay and inter-assay coefficient of variation of 3.4% and 5.2%, respectively.

Seven-year follow-up study

After the initial assessment and the determination of laboratory parameters for three consecutive months, all patients were followed-up for up to 7 years. During follow-up deaths were recording accurately by reviewing patient's hospital records.

Statistical analysis

Normality of variable distribution was tested using Kolmogorov-Smirnov test. Data are reported as mean ± SD (normally distributed data), median and interquartile range (non-normally distributed data) or as percentage frequency, as appropriate. Friedman's test was used to detect significant variations between non-parametric variables during the three month blood sampling period. According to the conducted power analysis each group had to include a minimum of 30 subjects in order to test differences in survival after a 7 year follow-up period. The significance of differences in means between the two groups was assessed by Student's *t* test or Mann-Whitney test. Differences in proportions were tested with the use of χ^2 test. Correlations were tested by Pearson's r or Spearman test for parametric and non-paramertric data analysis respectively. Non-normally distributed variables were log-transformed before entering regression analysis. For the survival analyses, patients were divided in low and normal freeT3 groups according to mean 3 mo freeT3. Patients included in the low freeT3 group had mean 3 mo values below the

Table 1 Baseline demographic, hemodynamic and clinical characteristics of low and normal freeT3 patient groups

Variable	Normal freeT3 (n = 79)	Low freeT3 (n = 35)	P value
Age	59.96 ± 14.74	66.84 ± 13.11	0.019
Female gender	18 (22.78%)	12 (34.28%)	0.237
BMI (kg/m^2)	25.65 ± 2.92	26.49 ± 4.02	0.622
HD duration (mo)	66.42 ± 59.69	56.49 ± 41.08	0.302
HD morning shift	75 (94.93%)	32 (91.42%)	0.418
OL-HDF	22 (27.84%)	11 (31.42%)	0.484
CVC access	10 (12.65%)	9 (25.71%)	0.160
Kt/V	1.27 ± 0.39	1.29 ± 0.42	0.822
Smoking	16 (20.25%)	9 (25.71%)	0.606
History of CVD	24 (30.37%)	17(48.57%)	0.048
Diabetes	16 (20.25%)	16 (45.71%)	0.017
Hypertension	60 (75.94%)	23 (65.71%)	0.488
SBP (mmHg)	131.06 ± 19.94	130.50 ± 21.94	0.819
DBP (mmHg)	68.42 ± 13.87	71.22 ± 14.47	0.357

Values are expressed as means ± SD or numbers (%) as appropriate. BMI: Body mass index; CVC: Central venous catheter; CVD: Cardiovascular disease; DBP: Diastolic blood pressure; HD: Hemodialysis; OL- HDF: On-line hemodiafiltration; SBP: Systolic blood pressure.

Table 2 Baseline laboratory parameters of low and normal freeT3 patient groups

Variable	Normal freeT3 (n = 79)	Low freeT3 (n = 35)	P value
Creatinine (mg/dL)	9.50 ± 2.75	8.44 ± 2.35	0.041
Urea (mg/dL)	146.59 ± 36.73	140.33 ± 51.06	0.556
Cholesterol (mg/dL)	180.67 ± 47.3	175.8 ± 52.52	0.176
Triglycerides (mg/dL)	183.86 ± 94.69	171.20 ± 130.36	0.215
Hematocrit (%)	37.41 ± 3.74	36.63 ± 3.86	0.860
Hemoglobin (g/dL)	12.04 ± 1.33	11.52 ± 1.31	0.262
WBC (/mm^3)	7.33 ± 2.20	7.61 ± 2.18	0.406
Albumin (g/dL)	3.99 ± 0.34	3.74 ± 0.497	0.002
TSH (μIU/mL)	2.77 ± 8.45	1.87 ± 1.02	0.354
FreeT4 (ng/dL)	1.13 ± 0.20	1.11 ± 0.3	0.170
IL-6 (pg/mL)	13.73 ± 13.29	16.49 ± 16.85	0.948
hsCRP (mg/dL)	1.56 ± 3.37	1.99 ± 2.73	0.156

Values are expressed as means ± SD or interquartile range as appropriate. hsCRP: High sensitive C-reactive protein; freeT4: Thyroxine; IL-6: Interleukin-6; TSH: Thyroid-stimulating hormone; WBC: White blood count.

lower laboratory's normal range (1.8 pg/mL). Time was calculated from the end of the 3 mo observation and blood sampling period until death or censoring. The data were censored if a patient underwent renal transplantation, switched from hemodialysis to peritoneal dialysis during the study period, died and at the end of follow-up. Furthermore, patients were censored if they were diagnosed with thyroid disease, primary or as a complication of treatment. Survival curves according to mean freeT3 values were calculated using the Kaplan-Meier method. Differences in survival were assessed with the use of log-rank test. Survival analyses were also made with the Cox proportional hazards model. The relative risks for mortality were determined by univariate and multivariate Cox regression analysis and presented as hazard ratio (HR; 95%CI). Covariates tested in the Cox model were age, diabetes mellitus, hypertension, hsCRP, creatinine, history of CVD, duration of hemodialysis and hemoglobin. Variables were included in the multivariate analyses if they had a P value < 0.05 in the univariate analysis or if they were clinically important confounders. The calculations were performed using SPSS 19.0 (Statistical Package for Social Sciences, IBM SPSS, Chicago, Ill). Statistical review of the study was performed by a biomedical statistician. A two-tailed P value < 0.05 was considered to be statistically significant.

The statistical review of the study was performed by a biomedical statistician.

RESULTS

During the blood sampling period four patients that required hospitalization or died were excluded from the analyses. Thus finally 114 patients (84 males) consecutively entered the study and were followed-up for 7 years (mean ± SD: 55.3 ± 2.9 mo). Mean age was 62.3 ± 14.3 years and mean HD duration 114.3 ± 91 mo (range 15-427 mo). Primary disease was diabetic nephropathy in 23.9% of the patients, glomerulonephritis in 19.7% and unknown in 27.4%. Three-month mean freeT3 was 2.17 ± 1.25 pg/mL, freeT4 was 0.956 ± 0.19 ng/dL, TSH was 1.94 ± 0.65 mIU/mL, hsCRP was 1.57 ± 2.98 mg/dL and IL-6 was 14.12 ± 11.52 pg/mL. Patients were divided into two groups based on the mean freeT3 values; the low freeT3 group consisted of thirty five patients (30.7%) with freeT3 below the lower normal laboratory range (1.8 pg/mL) and the normal freeT3 group consisted of 79 patients with freeT3 ⩾ 1.8 pg/mL).

Baseline (at the beginning of follow-up period) demographic, hemodynamic and clinical characteristics of low and normal freeT3 groups are shown in Table 1 and laboratory parameters in Table 2. Compared with patients that had normal freeT3, patients with low freeT3 were significantly older (P = 0.019) and had higher prevalence of diabetes mellitus (P = 0.017) and history of cardiovascular disease (P = 0.048). Gender, HD duration and morning shift, dialysis modality, Kt/V, type of vascular access, prevalence of smoking and hypertension and systolic and diastolic blood pressure did not differ significantly between the two groups (Table 1). Moreover, compared with patients with normal freeT3, patients with low freeT3 had at baseline significantly lower serum creatinine (P = 0.041) and albumin (P = 0.002). Low freeT3 group had also higher hsCRP and above the upper normal range (1.5 mg/dL) but the difference failed to reach statistical significance (P = 0.156). Hemoglobin and hematocrit, white blood cell count, blood urea, lipid levels, IL-6, freeT4 and TSH did not show significant differences in the two patient groups (Table 2).

Correlations of freeT3 levels with clinical and laboratory parameters

No statistically significant variation was observed in

Table 3 Demographic, clinical and laboratory significantly associated with survival in univariate Cox regression analyses

Variable	Spearman's rho	*P* value
Age (yr)	0.345	< 0.001
Diabetes mellitus (yes/no)	0.199	0.050
Hemodialysis vintage (mo)	-0.435	0.017
Hemoglobin (g/dL)	-0.329	0.001
Hematocrit (%)	-0.270	0.007
Hypertension (yes/no)	0.275	0.006
Free T3 (pg/mL)	-0.257	0.011
Previous CVD events (yes/no)	0.305	0.002
hsCRP (mg/dL)	0.240	0.017
Creatinine (mg/dL)	-0.400	< 0.001

hsCRP: High sensitive C-reactive protein; freeT3: Free triiodothyronine; CVD: Cardiovascular disease.

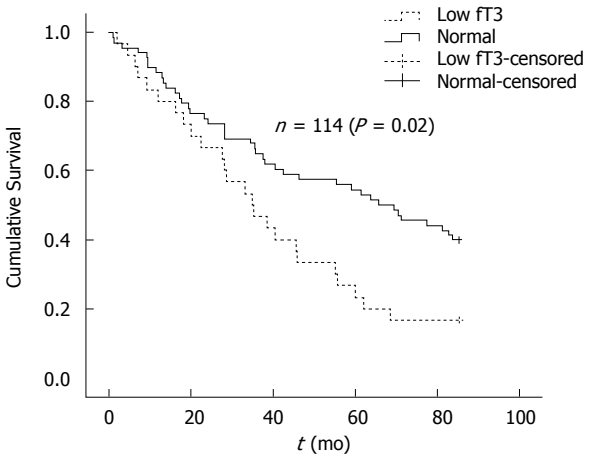

Figure 1 Kaplan-Meier estimate of survival in patients with low freeT3 (*n* = 35) and normal freeT3 (*n* = 79) levels.

hsCRP or IL-6 values within the 3 monthly samples (Friedman test, $P = 0.088$ and $P = 0.168$ respectively). In contrast, freeT3 levels showed a statistical significant variation during the three months ($P < 0.001$).

A linear statistically significant association was noted between age and mean freeT3, which resulted in the following algorithm: Mean freeT3 = 2.80-0.01*Age. In addition, serum albumin showed a strong correlation with freeT3 in all three consecutive measurements (meanAlb - meanfreeT3: Spearman's rho = 0.318, $P = 0.001$). No statistically significant correlations were observed between hsCRP or IL-6 with the other studied clinical parameters.

In the first month, significant correlations were found between TSH and freeT4 (Spearman's rho = -0.259, $P = 0.010$), hsCPR and freeT3 (Spearman's rho = -0.323, $P = 0.001$) and hsCRP and IL-6 (Spearman's rho = 0.312, $P = 0.001$). These associations remained significant in the following 2 mo except from the correlation between hsCRP and freeT3.

Correlation of freeT3 with mortality

During the 7 year follow-up period, 69 patients (60.5%) died. In addition, 11 patients were transplanted, one switched from hemodialysis to peritoneal dialysis and three patients were lost to follow-up. During follow-up 41 deaths (59.4%) were recorded in the normal freeT3 group and 25 deaths (83.3%) in the low freeT3 group ($P = 0.036$). Also, compared with patients that had normal freeT3 levels, patients with low freeT3 had a lower survival (54.9 ± 3.4 mo *vs* 39.8 ± 2.7 mo, $P = 0.019$). Furthermore, Kaplan-Meier survival curves differed significantly between patients with low and normal freeT3 levels [Log Rank (Mantel-cox), $P = 0.02$] (Figure 1).

In univariate (unadjusted) Cox regression analysis low freeT3 was a significant predictor of mortality [HR = 1.89 (1.146-3.124), $P = 0.011$]. Other parameters associated significantly with mortality were age, HD duration, history of CVD, hypertension, hemoglobin and hematocrit, serum creatinine and hsCRP. Moreover, diabetes mellitus showed a correlation of borderline significance with mortality (Table 3). In contrast, no association was observed between serum albumin and survival ($P = 0.178$). In addition, in a Cox Regression model, low freeT3 remained a significant independent predictor of mortality after adjustment for age, diabetes, hypertension, hsCRP, serum creatinine and albumin. However, in a multivariate stepwise Cox Regression analysis, including also hemoglobin, dialysis vintage and history of CVD, plasma freeT3 values failed to retain their predictive power for all-cause mortality. In the fully adjusted model, factors independently associated with mortality were age (3.2% higher odds of death for every year) and history of CVD (83.5% likelihood of death). Moreover, for every 1 g/dL of raise in hemoglobin levels there was a risk reduction for mortality of 43%. Diabetes mellitus and hsCRP values were not also associated with mortality in the fully adjusted model (Table 4).

DISCUSSION

Chronic uremia, similar to other chronic illnesses, may cause a variety of nonspecific wasting syndromes including protein loss, accumulation of fat stores, hyperglycemia and insulin resistance, hypoproteinemia, and hypertriglyceridemia[22]. Thyroid abnormalities are also very often in ESRD patients, with low freeT3 levels observed in the majority of cases[23,24]. The latter represents the main finding of euthyroid sick syndrome or non thyroidal illness syndrome which has been a debate for several year. Interestingly, recent studies in other models of metabolic derangements, investigated the causal link between circadian misalignment and metabolic homeostasis using a controlled simulation of "shift-work" in the clinical laboratory[25]. Circadian dysregulation caused decreased leptin levels and resulted in hyperglycemia and hyperinsulinemia. Moreover, daily cortisol excretion was reversed, arterial pressure was increased and sleep efficiency decreased[26]. Thus, it was hypothesized that neuroendocrine derangements should

Table 4 Adjusted and unadjusted relative risks of low freeT3 for all cause mortality

Variables	All cause mortality relative risks, (95%CI), *P* values	
	Model 1 (unadjusted)	Model 2 (fully adjusted)
LowT3_1.8 (pg/mL)	1.89 (1.146-3.124) *P* = 0.013	1.61 (0.88-2.92) *P* = 0.115
Age (1 yr)	-	1.031 (1.00-1.05) *P* = 0.008
DM (yes)	-	1.22 (0.68-2.18) *P* = 0.50
History of CVD (yes)	-	1.878 (1.09-3.21) *P* = 0.021
Mean_Hb (1 g/dL)	-	0.561 (0.426-0.73) *P* = 0.000
hsCRP (0.1 mg/dL)	-	1.076 (0.91-1.27) *P* = 0.388
HD duration (1 mo)	-	0.990 (0.98-0.99) *P* = 0.004

DM: Diabetus mellitus; CVD: Cardiovascular disease; Hb: Hemoglobin; hsCRP: High sensitive C-reactive protein; HD: Hemodialysis.

be crucial in ESRD patients and might play an important role in NTIS while also explain the possible association with inflammation and cardiometabolic syndrome[27].

In the present study, the prognostic value of NTIS for long-term mortality in chronic hemodialysis patients and its probable association with low-grade inflammation, a common feature of chronic uremia, was investigated[5]. Our results showed no association of freeT4 or TSH with either hsCRP or IL-6. Moreover, freeT3 had significant negative correlation with hsCRP but only at baseline. Some studies previously examined the latter association but the number is limited and their results are rather inconsistent. Zoccali *et al*[8] first reported that freeT3 had a significant negative correlation with CRP and IL-6. Meuwese *et al*[9] investigated the trimestal variation of thyroid hormones and IL-6; in their study, IL-6 was positively associated with TSH and negatively with T3 whereas CRP levels were positively associated with T4 but only at baseline**.** Finally, and in accordance with our results, in the study of Carrero *et al*[28], only T3 and not freeT3 showed a correlation with CRP and IL-6. The above discrepant results could be, at least partially, explained by differences in patient's characteristics, exclusion criteria and laboratory methods used. It should be noted that in our study patients with recent infection or chronic inflammatory diseases were carefully excluded as well as patients who presented with infection during the 3 mo observation-sampling period. However, based on the above it appears reasonable to conclude that in HD patients, chronic low grade inflammation does not play a major role in the development of NTIS.

During the 7 year follow-up period, 60.5% of patients died, mostly from cardiovascular causes. Compared with patients who remained alive, patients who died had significantly lower freeT3 values. Moreover, Kaplan Meier survival curves differed significantly between patients with low and normal freeT3. In univariate Cox regression analysis survival was also associated with freeT3. Other factors significantly affecting outcome were age, HD duration, diabetes mellitus, history of CVD, presence of hypertension, hemoglobin, serum creatinine and hsCRP. In contrast, no association was observed between serum albumin and survival either in univariate or multivariate analysis. However, it should be mentioned that although the low T3 group had significantly lower serum albumin compared to normal T3 group, only 8 patients (14%) of the former group had serum albumin below the lower laboratory normal range and none had albumin lower than 3 g/dL. Given the well known association between malnutrition, inflammation and atherosclerosis in uremia, the above findings are consistent with the presence of a very low grade of inflammation in our patient population and relatively good nutritional status. Interestingly, in multivariate Cox Regression analyses, low freeT3 remained a significant independent predictor of mortality after adjustment for some traditional and uremia-related risk factors including age, diabetes, hypertension, hsCRP, serum creatinine and albumin. Of note, compared with patients with normal freeT3, those with low freeT3 had increased incidence of fatal and non-fatal CVD events during follow up (data not shown). However, in the fully adjusted model including also mean hemoglobin, history of CVD event, and HD duration, low freeT3 lost its power as a predictor of mortality. Several previous studies have also suggested a correlation of NTIS with mortality although they have important differences with our report. Zoccali *et al*[10] observed an independent association between freeT3 and mortality during an average follow-up of 42 mo; however, they included both incident and prevalent HD patients whereas freeT3 values were significantly higher compared to those in our patients. Horáček *et al*[29] in unselected HD patients found that survival curves differed between patients with low and normal freeT3 values during a five-year follow-up; however, more detailed survival analyses are not reported. In their study in incident dialysis patients, Carrero *et al*[28] found an independent association between only T3, but not freeT3, with survival during a 20 mo median follow-up. Fernández-Reyes *et al*[30] were unable to detect any association between freeT3 and mortality in patients who survived at least 12 mo on dialysis during an almost 34 mo follow-up. Finally, in the study of Ozen *et al*[31], in chronic HD patients, freeT3 was also found to be correlated with survival only in unadjusted analysis. Taken together, the above studies argue for a role of thyroid function in the outcome of hemodialysis patients although in some cases the above association could be confounded by other factors which are commonly present in uremia, including inflammation and malnutrition. Nevertheless, further studies are needed to identify the exact nature of the association between low freeT3 and mortality in ESRD patients. Moreover, it remains to be investigated whether or not

routine measurement of freeT3 in hemodialysis would add significantly greater predictive power for mortality to models based on traditional and uremia-related risk factors including inflammation. Nevertheless, although the use of thyroid hormone therapy in NTIS is controversial, design of interventional studies in hemodialysis patients aimed to investigate whether normalization of T3 values would actually reduce mortality is a tempting idea[32-34]. However, a study in ESRD patients with NTIS, showed that administration of T3 resulted in excess protein turnover, therefore increasing the need for dialysis[35].

Our study has some strengths. Firstly, only one previous study examined thyroid hormone variation. Secondly, patients were carefully examined to rule out any occult infection before study entry and moreover, patients were excluded if they presented with an acute infection during the three month observation-sampling period. Finally, the present report has the longest follow-up. However, to properly address the implications of the present study some limitations should be considered. It is known that thyroid hormone production follows a circadian rhythm and blood samples were taken at two different time points according to the patient's shifts. However, only a very small percentage of the patients (6%) underwent hemodialysis in the evening and moreover, no association was observed between HD shift and either freeT3 or freeT4 values. Nevertheless, we believe that circadian rhythms may present alterations in patients on hemodialysis similar to other incidents of biological clock disarrangements like shift workers, although time of sampling was not an issue in relevant studies[29]. These metabolic changes could actually be interpreted as an adaptive mechanism to the high energy expenditure during hemodialysis treatment. In addition, our study included only chronic hemodialysis (prevalent) patients and the results would not necessarily apply to incident dialysis patients.

In conclusion, in this prospective study in chronic hemodialysis patients, low freeT3 levels had emerged as a significant predictor of mortality independently of traditional and some uremia-related risk factors including age, diabetes mellitus, hypertension, serum albumin and hsCRP. However, an association between NTIS and inflammation could not be documented and the exact mechanisms underlying the above association remain to be identified in future mechanistic and interventional trials.

COMMENTS

Background

Non thyroidal illness syndrome (NTIS) has been associated with several chronic severe illnesses. This syndrome has been a debate for several years, as alterations in thyroid hormone levels have been considered either a laboratory pitfall or an adoptive response to chronic stress. In end stage renal disease the main finding of the syndrome is low free triiodothyronin (freeT3) which recently has emerged as a potent biomarker of cardiovascular risk and a predictor of mortality.

Research frontiers

Previous studies have suggested a probable association between low freeT3 and inflammation in end stage renal disease and a link between NTIS and cardiorenal syndrome. However, the predictive power of NTIS for long-term mortality and the mechanisms underlying the above associations remain unclear.

Innovations and breakthroughs

In the present study, the predictive value of low NTIS for long-term mortality in chronic hemodialysis patients and its link with low-grade inflammation was investigated. Our results showed that low freeT3 was a significant predictor of mortality independently of traditional and some uremia-related risk factors including inflammatory markers. However, an association between NTIS and inflammation was not documented and the exact mechanisms underlying the above association remained unclear. In this regard, there is a limited number of previous relevant studies and their results are rather inconsistent. This study has several strengths. Firstly, only one previous study examined trimestral thyroid hormone variation. Secondly, patients were carefully examined to rule out any occult infection before their enrollment and moreover, patients were excluded if they presented with an acute infection during the three month observation-sampling period. Finally, the present report has the longest follow-up.

Application

So far, there is a lack of a strong marker of cardiovascular disease in end-stage renal disease, easy to perform and with high availability and low cost, that could guide treatment or even serve as a predictor of mortality. Presumably, a multifactorial approach of traditional markers combined with novel biomarkers could fulfill this necessity. Among the latter, low freeT3 has emerged as a potent predictor of adverse clinical outcome, which is easy to perform, inexpensive and widely available.

Terminology

Euthyroid sick syndrome or non thyroidal illness syndrome refers to patients with severe chronic illnesses like starvation, sepsis, end stage renal disease, myocardial infarction and others, in whom a decrease in serum thyroid hormone levels is observed without any identifiable primary thyroid disease.

Peer-review

It is a very good study well conducted.

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ORIGINAL ARTICLE

Randomized Controlled Trial

Changes in urinary excretion of water and sodium transporters during amiloride and bendroflumethiazide treatment

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Abstract

AIM: To quantify changes in urinary excretion of aquaporin2 water channels (u-AQP2), the sodium-potassium-chloride co-transporter (u-NKCC2) and the epithelial sodium channels (u-ENaC) during treatment with bendroflumethiazide (BFTZ), amiloride and placebo.

METHODS: In a randomized, double-blinded, placebo-controlled, 3-way crossover study we examined 23 healthy subjects on a standardized diet and fluid intake. The subjects were treated with amiloride 5 mg, BFTZ 1.25 mg or placebo twice a day for 4.5 d before each examination day. On the examination day, glomerular filtration rate was measured by the constant infusion clearance technique with ^{51}Cr-EDTA as reference substance. To estimate the changes in water transport *via* AQP2 and sodium transport *via* NKCC2 and ENaC, u-NKCC2, the gamma fraction of ENaC (u-ENaCγ), and

u-AQP2 were measured at baseline and after infusion with 3% hypertonic saline. U-NKCC2, u-ENaCγ, u-AQP2 and plasma concentrations of vasopressin (p-AVP), renin (PRC), angiotensin Ⅱ (p-ANG Ⅱ) and aldosterone (p-Aldo) were measured, by radioimmunoassay. Central blood pressure was estimated by applanation tonometry and body fluid volumes were estimated by bio-impedance spectroscopy. General linear model with repeated measures or related samples Friedman's two-way analysis was used to compare differences. Post hoc Bonferroni correction was used for multiple comparisons of post infusion periods to baseline within each treatment group.

RESULTS: At baseline there were no differences in u-NKCC2, u-ENaCγ and u-AQP2. PRC, p-Ang Ⅱ and p-Aldo were increased during active treatments (P < 0.001). After hypertonic saline, u-NKCC2 increased during amiloride (6% ± 34%; P = 0.081) and increased significantly during placebo (17% ± 24%; P = 0.010). U-AQP2 increased significantly during amiloride (31% ± 22%; P < 0.001) and placebo (34% ± 27%; P < 0.001), while u-NKCC2 and u-AQP2 did not change significantly during BFTZ (-7% ± 28%; P = 0.257 and 5% ± 16%; P = 0.261). U- ENaCγ increased in all three groups (P < 0.050). PRC, Ang Ⅱ and p-Aldo decreased to the same extent, while AVP increased, but to a smaller degree during BFTZ (P = 0.048). cDBP decreased significantly during BFTZ (P < 0.001), but not during amiloride or placebo. There were no significant differences in body fluid volumes.

CONCLUSION: After hypertonic saline, u-NKCC2 and u-AQP2 increased during amiloride, but not during BFTZ. Lower p-AVP during BFTZ potentially caused less stimulation of NKCC2 and AQP2 and subsequent lower reabsorption of water and sodium.

Key words: Amiloride; Thiazide; Sodium-potassium-chloride co-transporter; Aquaporin2; Epithelial sodium channels; Sodium; Water; Sodium transporters; Hypertonic saline; Urine

Core tip: Measurements of urinary sodium-potassium chloride co-transporter (NKCC2), epithelial sodium channel (ENaC) and aquaporin2 (AQP2) can be used as biomarkers of water- and sodium transport in the nephron. However, it has never been studied to what extent the function of NKCC2, ENaC and AQP2 is simultaneously affected in response to diuretics. The present study showed that infusion of 3% saline increased u-NKCC2 and u-AQP2 during amiloride and placebo, while u-NKCC2 and u-AQP2 remained unchanged during bendroflumethiazide. Therefor, in contrast to amiloride, bendroflumethiazide caused the absence of a compensatory reabsorption of sodium *via* NKCC2 and water *via* AQP2.

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INTRODUCTION

During normal conditions, approximately 60% of filtered sodium is absorbed in the proximal tubules and 30% of sodium is absorbed in the kidneys *via* the sodium-potassium chloride co-transporter (NKCC2) in the thick ascending limb of Henle's loop (TAL). The distal convoluted tubules are responsible for 5%-10% of sodium reabsorption *via* the sodium chloride co-transporter (NCC)[1]. Thiazides inhibit NCC in distal tubules and decrease sodium reabsorption[2]. In the collecting duct the epithelial sodium channel (ENaC) is responsible for the reabsorption of 3%-5% of filtered sodium[1]. Amiloride is a potassium sparing selective inhibitor of ENaC channels[3]. Water is predominantly reabsorbed in the proximal tubules and thin descending limb of Henle's loop[1]. In the collecting ducts water absorption depends on passive transport *via* AQP2 water channels and is regulated by vasopressin (AVP)[4]. AQP2 can be excreted into urine[5,6], and may be used as a biomarker of collecting duct water transport[7-9]. Similarly, urinary excretion of beta ENaC correlates with changes in urinary sodium excretion[10]. Recently, our group documented changes in transport of water *via* AQP2 and sodium *via* ENaC in healthy subjects after infusion of isotonic glucose or hypertonic and isotonic saline, by measurements of urinary excretion of AQP2 (u-AQP2) and gamma ENaC (u-ENaCγ)[11] and abnormal urinary excretion of NKCC2 (u-NKCC2) and u-AQP2 in patients with chronic kidney disease[12].

In the present randomised, placebo-controlled study present study in healthy young subjects, we hypothesize that excretion of NKCC2 will not be effected but compensatory increases in distal transporter activity will occur during thiazide treatment but not during amiloride. This is compared to placebo both at baseline and in response to a saline load. Therefore, the aim was to quantify changes in urinary excretion of NKCC2, u-ENaCγ and u-AQP2 as estimates of tubular water and sodium handling at baseline conditions and after 3% saline infusion, during treatment with bendroflumethiazide (BFTZ), amiloride and placebo. In addition, changes in renal tubular function, vasoactive hormones, body fluid volumes and blood pressure were measured. The novelty of this study is due to; measurements of u-NKCC2 and the interplay with ENaC, AQP2 and the regulating mechanisms involved in water and sodium homeostasis, while simultaneously antagonizing NCC with BFTZ and ENaC with amiloride. Quantification of sodium- and water channel proteins in urine during different conditions may provide important information of the mechanisms involved in water and

sodium balance in the nephron.

MATERIALS AND METHODS

Design

The trial was conducted as a randomized, double-blinded, placebo-controlled, 3-way crossover study. Subjects were randomized to tablet BFTZ, amiloride or placebo for 4.5 d. Each treatment period was followed by an examination day, separated by at least 3 wk.

Participants

Eligible participants were healthy non-smoking men and women aged between 18-45 years. Exclusion criteria were clinical signs or history of heart, lung, kidney, endocrine or malignant disease; abnormal findings in ECG, urine dipstick or biochemistry [blood cell count, plasma concentrations of glucose, bilirubin, alanine aminotransferase, alkaline phosphatase, sodium, potassium, creatinine, albumin, cholesterol and haemoglobin; arterial hypertension (24 h ambulatory BP > 130/80 mmHg); medical treatment (except oral contraceptives); alcohol and substance abuse, *i.e.*, more than 21 alcoholic drinks per week for males and 14 drinks for females]; current smoking; pregnancy, breast feeding; donation of blood within one month prior to the study and obesity (BMI > 32 kg/m^2). Withdrawal criteria were, development of conditions given in exclusion criteria during the study, withdrawal of informed consent and poor compliance.

Participants were recruited through advertisement at public institutions in Holstebro, Denmark.

Study settings

The study took place at Department of Medical Research, University Clinic of Nephrology and Hypertension, Regional Hospital Holstebro, Denmark, from 1st of August 2012 until 13th of September 2013.

Ethics

This study was reviewed and approved by the Regional Committees on Health Research Ethics, Skottenborg 26, Viborg, Denmark (j.no 1-10-72-178-12) and was carried out in accordance with the Helsinki Declaration. All study participants provided informed written consent prior to study enrolment.

Effect variables

The main effect variable was u-NKCC2. Secondary effect variables were: u-AQP2, u- ENaCγ, glomerular filtration rate (GFR), free water clearance (C_{H2O}), urine output (UO), urinary excretion of sodium (u-Na) and potassium (u-K), fractional excretion of sodium (FE_{Na}) and potassium (FE_K), plasma sodium (p-Na) and potassium (p-K), plasma osmolality (p-osm) and plasma albumin (p-alb), plasma concentration of renin (PRC), angiotensin Ⅱ (p-AngⅡ), aldosterone (p-Aldo), vasopressin (p-AVP), extracellular fluid volume (ECV), intracellular fluid volume (ICV), total body water (TBW), brachial systolic- (bSBP) and diastolic blood pressure (bDBP), pulse wave velocity (PWV) and central systolic- (cSBP) and diastolic blood pressure (cDBP).

Number of subjects

Using a significance level of 5% and a power of 80% it was calculated that the number of subjects should be 16, when the minimal relevant difference in u-NKCC2 was 0.3 ng/min and SD was 0.3 ng/min. In this study, incomplete voiding during study days was expected in some subjects; therefore, 20 subjects were included as a minimum.

Randomisation

Subjects were randomized to treatment using block randomization conducted at www.randomization.com. Aarhus Hospital Pharmacy, Denmark, generated the randomization sequence into five blocks of six from 01-30 and labeled the bottles. Five days prior to each examination day, participants received a numbered bottle containing BFTZ, amiloride or placebo tablets. BFTZ, amiloride and placebo were capsulated in grey DB Caps® size B (Capsugel) with click effect to obtain blinding. The randomization code was kept at Aarhus Hospital Pharmacy during the trial. Individual randomization codes were kept in sealed envelopes at Department of Medical Research if necessary for the investigator to know the given treatment. Investigators, participants and other study personnel were blinded to treatment assignment for the duration of the study.

Experimental procedures

Experimental procedure prior to the study day: Four days prior to each study day, subjects consumed a standardized diet regarding calories, sodium and fluid. The diet consisted of 11000 (kJ/d) with an energy distribution of 55% carbohydrates, 30% fat and 15% protein in accordance to general dietary guidelines. The sodium content was approximately 120-150 mmol pr. day. The subjects were asked to drink 2500 mL/d. No alcohol or soft drink consumption was allowed while on the standardized diet. A maximum of two cups (6 oz.) of coffee or tea was allowed daily. Subjects were instructed to keep their usual physical activity during the experiments but to abstain from hard training the day prior to the examination. A 24-h urine collection from 7:00 AM to 7:00 AM on the examination day was used to assess water and sodium balance. A 24-h ambulatory BP measurement was performed to evaluate the effect of the intervention on blood pressure (Table 1).

Interventions

During the four-day diet and the morning of the examination day, participants were randomized to capsules containing either 1.25 mg BFTZ, 5 mg amiloride or matching placebo twice daily at 7 AM and 6 PM.

Table 1 Experimental procedures

	Before the study day				On the study day											
	Day-4	Day-3	Day-2	Day-1	6:00-8:00	8:00-08:30	8:30-09:00	9:00-9:30	9:30-10:00	10:00-10:30	10:30-11:00	11:00-11:30	11:30-12:00	12:00-12:30	12:30-13:00	13:00-13:30
Periods										Baseline			Infusion		Post Infusion	
Time									0	30	60	90	120	150	180	210	240
Diet	x	x	x	x													
Study drug	xx	xx	xx	xx	x												
24-h BP				x------	-----x												
24-h urine				x------	-----x												
IV access						x											
Weight						x					x		x			x	
Water load						x----------					175 mL every 30 min		----------				x
Urine sample							(x)	x	x	x	x		x	x	x	x	
Blood samples								x	x	x	x		x	x	x	x	
Blood pressure						x		x	x	x	x		x	x	x	x	
^{51}Cr-EDTA								x	----------								x
IV. fluid												x----------					
App.Ton											x		x				
BIS						x					x		x			x	

24-h BP: 24-h ambulatory blood pressure measurements; 24-h urine: 24-h urine collection; App.Ton: Applanation tonometry; BIS: Bioimpedance spectroscopy.

Experimental procedure on the study day

Table 1 shows the time points of study interventions. Following an overnight fast, subjects arrived at our research facility at 8:00 AM. Two indwelling catheters for blood sampling and administration of ^{51}Cr-EDTA and fluid were placed in both cubital veins. Every 30 min, starting at arrival, participants received an oral water load of 175 mL. Urine was collected in standing or sitting position. Otherwise, subjects were kept in a supine position in a quiet temperature-controlled room (22 ℃-25 ℃). At 9:00 AM a priming dose of ^{51}Cr-EDTA was administered, followed by sustained infusion. Three 30-min baseline clearance periods were obtained from 9:30 AM to 11:00 AM. The baseline periods were followed by an infusion period from 11:00 AM to 12:00 PM during which a sustained infusion of 3% hypertonic saline was administered. The post infusion period consisted of three 30-min periods from 12:00 PM to 1:30 PM. Blood and urine samples were collected every 30 min from 8:30 AM to 1:30 PM.

Blood samples were drawn and analyzed for ^{51}Cr-EDTA, p-sodium, p-potassium, p-albumin and p-osmolality. Analysis of PRC, p-Ang Ⅱ, p-Aldo and p-AVP were conducted from blood samples drawn at 11:00 AM, 12:00 PM and 1:30 PM.

Urine samples were analyzed for u-^{51}Cr-EDTA, u-sodium, u-potassium, u-creatinine and u-osmolality. Analysis of u-AQP2, u-NKCC2 and u-ENaCγ was conducted from the 24-h urine collection and clearance period 10:30-11:00 AM (basal); 11:00-12:00 AM (fluid infusion), 12:00-12:30 PM (30 min after cessation of fluid infusion) and 1:00-1:30 PM (90 min after cessation of fluid infusion). For data analysis, the 30-min periods from 9:30 AM to 1:30 PM were subdivided into: baseline (0-90 min), infusion period (90-150 min) and three post infusion period 150-180 min, 180-210 min and 210-240 min).

Measurements of PWV, augmentation index (Aix) and cBP were performed at 11:00 AM (before infusion) and 12:00 AM (after infusion). Body composition was measured at 8:30 AM, 11:00 AM, 12:00 PM and 1:30 PM (end of examination day).

Measurements

Renal function: Glomerular filtration rate was measured by the constant infusion clearance technique with ^{51}Cr-EDTA as reference substance. More than 15% variation in GFR between the three baseline periods led to the exclusion of clearance related analysis.

Fractional excretion of sodium and potassium was calculated as: [Sodium/potassium clearance ($C_{Na/K}$)/GFR x 100%]. Free water clearance was calculated as: [Urine output (UO) - osmolar clearance (C_{OSM})]. C_{OSM} was calculated as: [Urine osmolality/plasma osmolality x UO].

Blood samples: were centrifuged for 10 min at 2200 x *g* at 4 ℃. Plasma hormone samples were kept frozen at -20 ℃ (Ang Ⅱ) and -80 ℃ (PRC, Aldo, and AVP) until assayed. Renin in plasma was determined using an immunoradiometric assay (CIS Bio International, Gif-Sur-Yvette Cedex, France). Minimal detection level was 1 pg/mL. The coefficients of variation were 14.5% (interassay) and 4.5% (intra assay). Aldosterone in plasma was determined by radioimmunoassay (Demeditec Diagnostics Systems Laboratories Inc., Webster, TX, United States). Minimal detection level was 22 pmol/L. The coefficients of variation were 8.2% (inter-assay) and 3.9% (intra-assay). Arginine vasopressin and Angiotensin Ⅱ were extracted from plasma with C_{18} Sep-Pak (Water associates, Milford, MA, United States) and subsequently measured using radioimmunoassay as previously described[13]. The antibody against angiotensin Ⅱ was obtained from the Department of Clinical Physiology, Glostrup Hospital, Glostrup, Denmark. Minimal detection level was 2 pmol/L. The coefficients of variation were 12% (inter-assay) and 8% (intra-assay). The antibody against AVP was a gift from Professor Jacques Dürr (Miami, FL, United States). Minimal detection level was 0.2 pmol/L. The coefficients of variation were 13% (inter-assay) and 9% (intra-assay).

Generation of NKCC2 specific antibody: A novel rabbit polyclonal antiserum against human NKCC2 (*Slc12a2*) was generated against the following peptide: CNITKTTPKKDGSIN by Genscript® (New Jersey, United States). The N-terminal cysteine was added for conjugation to carrier protein and for attaching the peptide to the affinity purification column. The immune serum from two rabbits (#593 and #594) was affinity purified using immunizing peptides, resulting in NKCC2-specific antibodies. NKCC2 antibody characterization has previously been described[12].

Urine sample immunoassays: Urines were stored frozen at -20 ℃ until assayed.

U-NKCC2 was measured in urine by a newly developed radioimmunoassay[12]. Antibodies were raised in rabbits against human NKCC2 (Slc12a2) against the peptide CNITKTTPKKDGSIN. The N-terminal cysteine was added for conjugation to carrier protein and affinity purification. Minimal detection level was 0.5 ng/tube. The coefficients of variation were 14% (inter-assay) and 6.8% (intra-assay).

U-AQP2 was measured by radioimmunoassay as previously described[9,14]. Antibodies were raised in rabbits to a synthetic peptide corresponding to the 15 COOH-terminal amino acids in human AQP2 to which was added an NH_2-terminal cysteine for conjugation and affinity purification. Minimal detection level was 34 pg/tube per tube. The coefficients of variation were 11.7% (inter-assay) and 5.9% (intra-assay).

U-ENaCγ was measured by radioimmunoassay as previously described[15,16]. Antibodies were raised against a synthetic ENaCγ peptide in rabbits and affinity purified[17]. Minimal detection level was 48 pg/tube. The coefficients of variation were 14% (inter-assay) and 6.7% (intra-assay).

Blood pressure measurement: Twenty-four hours BP was measured using Kivex TM-2430 (Kivex, Hoersholm, Denmark). Measurements were taken every 15 min during daytime and every 30 min overnight. Brachial blood pressure was recorded using a semiautomatic oscillometric device (Omron 705IT, Omron Matsusaka, Japan).

Plasma and urine: Concentrations of sodium, potassium, creatinine and albumin were measured using routine methods at the Department of Clinical Biochemistry, Holstebro Hospital.

Plasma and urine osmolality was measured by freezing point depression (Advanced Model 3900 multisampling osmometer).

Bioimpedance spectroscopy: Was performed at 50 frequencies, from 5 to 1000 kHz using the Fresenius Body Composition Monitor and the Fluid Management Tool, version 3.

Applanation tonometry: Recordings of PWA and carotid-femoral PWV were obtained by applanation tonometry (SphygmoCor® CPV system®, AtCor Medical, Sydney, Australia) as double-recordings by a trained observer. Only duplicate recording meeting the quality requirements were included in the final analysis. An operator index of 80 or more was required to accept recordings of a peripheral pulse-wave form[18].

Study drug

Bendroflumethiazide [Tablet Salures 2.5 mg (1/2 tablet)] were obtained from Pfizer AB, Sollentuna, Sweden. Amiloride (Tablet Amilorid Mylan 5 mg) were obtained from Mylan AB, Stockholm, Sweden *via* Tjellesen Max Jenne A/S, Medilink A/S, Roedovre, Denmark.

Statistical methods

Statistical analyses were performed by the authors using IBM SPSS statistics version 20.0.0 (IBM Corp.; Armonk, NY, United States).

As clearance data from the three baseline periods were very similar, single baseline values were obtained by taking the average of the measurements from the three baseline periods. Parametric data are presented as

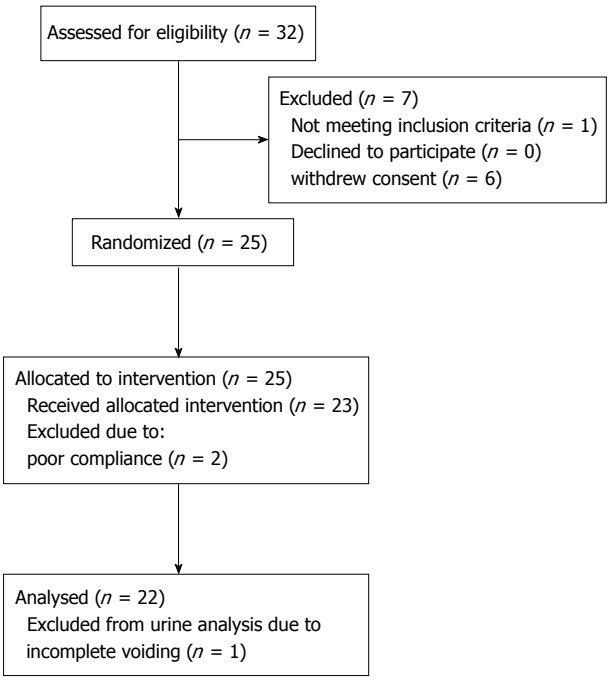

Figure 1 Flow chart.

means ± SD and nonparametric data as medians with 25th and 75th percentiles. General linear model (GLM) with repeated measures was performed, with time as within-subject factor and intervention as between subject factor, to test for differences within and between groups. One-way ANOVA was used for comparison of means between groups when differences were found. For non-parametric data, related samples Friedman's two-way analysis was used. Post hoc Bonferroni correction was used for multiple comparisons of post infusion periods to baseline within each treatment group. Statistical significance was defined as $P < 0.050$ in all analyses.

RESULTS

Demographics

Thirty-two healthy women and men were assessed for eligibility. Nine were excluded due to: withdrawal of informed consent (6), non-compliance (2) or 24-h BP above 130/80 mmHg (1). Thus, 23 were initially allocated to and completed the study. One was not able to void satisfactorily during baseline clearance experiments and was excluded from urine analysis (Figure 1).

The 23 subjects (8 males; 15 females) who completed the trial had a mean age of 26 ± 4 years, BMI 24 ± 3 kg/m^2, 24-h BP 117/69 ± 7/4 mmHg. Screening blood values were b-haemoglobin 8.4 ± 0.7 mmol/L, p-sodium 141 ± 1 mmol/L, p-potassium 3.7 ± 0.4 mmol/L, p-creatinine 79 ± 14 μmol/L, eGFR 93 ± 16 mL/min, p-albumin 43 ± 3 g/L, p-glucose 5± 1 mmol/L, p-alanine transaminase 25 ± 19 U/L and p-cholesterol 4.4 ± 0.8 mmol/L. Baseline values did not differ between males and females, apart from p-crea (males: 91 ± 15 μmol/L *vs* females: 73 ± 7 μmol/L, $P < 0.012$), b-haemoglobin (males: 8.9 ± 0.6 mmol/L *vs* females: 8.2 ± 0.5 mmol/L, $P < 0.016$) and p-albumin (males: 45 ± 2 g/L *vs* females: 42 ± 3 g/L, $P < 0.003$).

Effects of BFTZ and amiloride on 24-h urine and ambulatory BP

UO, u-osm, C_{H2O}, Creatinine-Clearance, u-Na, u-AQP2 and ENaCγ in 24-h urine were not significantly different between treatments. During BFTZ treatment u-NKCC2 and u-K were significant higher than both amiloride and placebo treatment (Table 2). Twenty-four hour ambulatory bSBP did not differ between treatments, however there was a small but significant lower bDBP during amiloride treatment (Table 2).

Effects of BFTZ and amiloride on u-NKCC2, u-ENaCγ and u-AQP2

Figure 2 shows the changes in urinary excretion of AQP2, NKCC2 and ENaCγ during basal, infusion and post-infusion periods.

At baseline, u-NKCC2 did not differ between groups. U-NKCC2 decreased during the infusion period and increased during the first post infusion period in all three treatments. U-NKCC2 increased further during amiloride (6% ± 34%; $P = 0.081$) and placebo (17% ± 24%; $P = 0.010$), whereas u-NKCC2 declined in the BFTZ treated group (-7% ± 28%; $P = 0.257$), during the two last post infusion periods. By the end of the examination day there was a significant difference between BFTZ *vs* amiloride ($P < 0.001$) and *vs* placebo ($P = 0.033$). There was no significant difference between amiloride and placebo groups ($P = 0.407$).

At baseline, u-ENaCγ was similar. In response to 3% saline, u-ENaCγ increased significant to a maximum after the first post infusion period. Although u-ENaCγ tended to be lower during amiloride treatment, there was no statistical difference between the three treatment groups throughout the examination day.

There was no significant difference in u-AQP2 at baseline. In response to 3% saline, u-AQP2 increased significantly and similarly during amiloride (31% ± 22%; $P < 0.001$) and placebo treatment (34 ± 27%; $P < 0.001$), but did not change during BFTZ (5% ± 16%; $P = 0.261$). By the end of the examination day there was a significant difference between BFTZ *vs* amiloride and placebo ($P < 0.001$), but there was no difference between amiloride and placebo.

Divided by gender, the creatinine adjusted excretion of u-AQP2, u-NKCC2 and u-ENaCγ tended to be higher in females compared to males in all three treatment groups, but the difference is attributed to a lower urinary excretion of creatinine in females (data not shown).

Effects of BFTZ and amiloride on GFR and tubular function

Table 3 shows the absolute values of C_{H2O}, UO, FE_{Na},

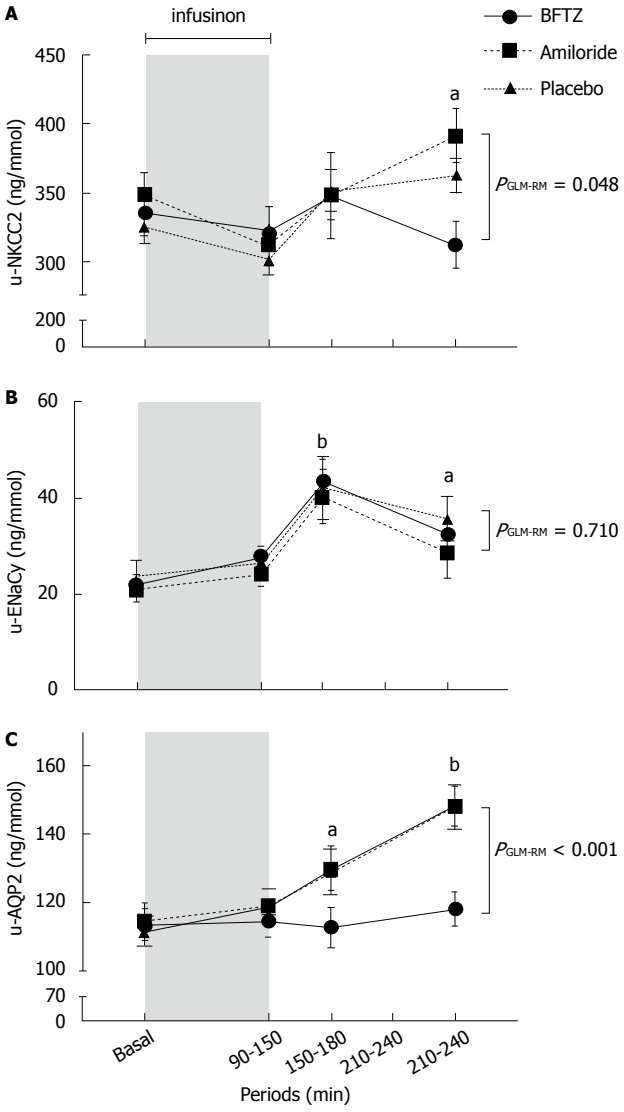

Figure 2 Effects of 3% hypertonic saline on urinary excretion of sodium-potassium-2chloride co-transporter (A), gamma fraction of epithelial sodium channels (B) and aquaporin2 (C) in 22 healthy subjects treated with bendroflumethiazide, amiloride or placebo. Values are means ± SEM. General linear model with repeated measurements (GLM-RM) was performed to test for differences between groups. A: U-NKCC2 increased during amiloride (P = 0.081) and placebo (P = 0.010) treatments. The increase in u-NKCC2 was however only significant during placebo. U-NKCC2 did not change during BFTZ; B: U-ENaCγ increased significantly and to the same extent during all three treatments; C: U-AQP2 increased significantly during amiloride and placebo (P < 0.001), but not during BFTZ. Paired t-test was used for comparison of post-infusion periods *vs* baseline. [a]P < 0.050; [b]P < 0.001. AQP2: Aquaporin2; U-NKCC2: Urinary excretion of sodium-potassium-2chloride co-transporter; ENaC: Epithelial sodium channels; BFTZ: Bendroflumethiazide.

u-Na, FE_K, u-K and ^{51}Cr-EDTA clearance.

C_{H2O} and UO decreased significantly in all three treatments. At baseline, C_{H2O} was lower during BFTZ and showed an attenuated decrease at post infusion period 210-240 min compared to amiloride (P = 0.207) and placebo (P = 0.005).

At baseline, FE_{Na} and u-Na were higher during amiloride compared to BFTZ and placebo. After 3% saline infusion there was a significant increase in u-Na and FE_{Na} in all three treatments, but less pronounced during BFTZ (P = 0.001).

There was no difference in u-K at baseline. In response to 3% saline, u-K and FE_K decreased during BFTZ and increased during amiloride compared to placebo. There was a significant difference between all three treatments (P = 0.001). GFR did not change significantly.

Effects of BFTZ and amiloride on plasma hormones

Figure 3 shows the changes in PRC, AngⅡ, p-Aldo and p-AVP during the examination day. PRC, AngⅡ and p-Aldo were significantly increased during active treatment compared to placebo. PRC and p-AngⅡ were highest during BFTZ treatment (P < 0.001), whereas p-Aldo was highest during amiloride treatment (P < 0.001). PRC, AngⅡ and p-Aldo declined significantly in response to 3% saline, in all three treatments, with no relative differences between treatments.

P-AVP was similar at baseline. P-AVP increased in all three groups, in response to 3% saline. Although, p-AVP was lower during BFTZ at 150 min (P = 0.048), the relative increase in p-AVP, after 3% saline, was not significantly different between BFTZ *vs* placebo (82% ± 100% *vs* 116% ± 67%; P = 0.072)

Effects of BFTZ and amiloride on plasma

Table 4 shows the absolute values of p-Na, p-K, p-Osm and p-Alb during basal-, infusion- and post infusion periods. During baseline conditions p-osm and p-Na were significantly lower during BFTZ and amiloride compared to placebo. P-K was higher in the amiloride group compared to placebo and BFTZ, and p-K was lower during BFTZ compared to placebo.

In response to 3% saline infusion, p-Na and p-osm increased to the same extent in all three treatments, but remained highest in the placebo group. P-K decreased significant in the amiloride group compared to BFTZ and placebo. P-alb decreased significantly in all three treatments, in response to 3% saline.

Effects of BFTZ and amiloride on blood pressure

Table 5 shows the absolute values of bSBP, bDBP, pulse rate, cSBP, cDBP, PWV and AIx. At baseline there was no difference in bSBP or bDBP. In response to 3% hypertonic saline, bSBP increased and bDBP decreased. At the end of the day the decrease in bDBP was more pronounced during BFTZ (-6% ± 6%) compared to amiloride (-2% ± 6%; P = 0.030) and placebo (-2% ± 5%; P = 0.021).

There was no difference in cSBP at baseline or in response to 3% saline between treatments. At baseline cDBP was the same in all three treatments, however cDBP decreased significant in the BFTZ group (P < 0.001) but not during amiloride and placebo. PWV followed the same pattern, however the decrease during BFTZ treatment was not significant.

Effects of BFTZ and amiloride on body fluid volumes

Figure 4 shows the changes in ICV, ECV and TBW during the examination day.

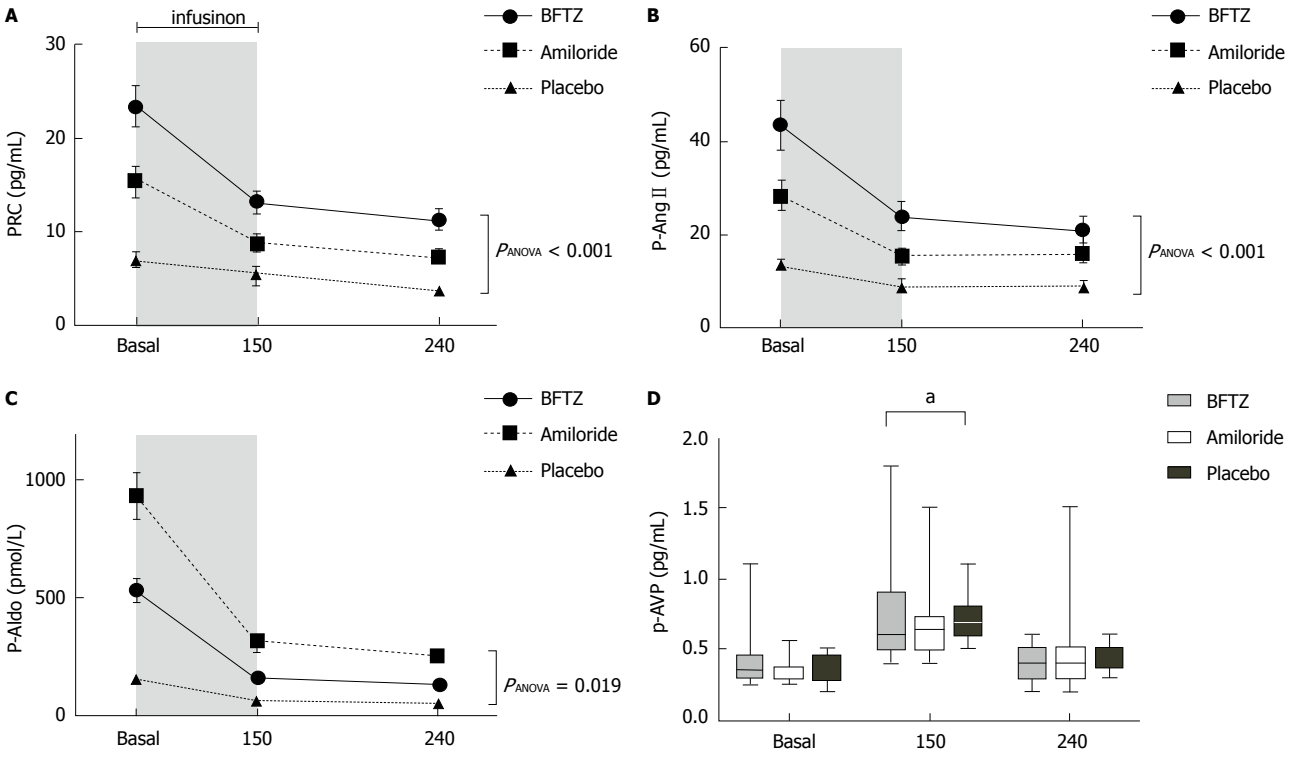

Figure 3 Effects of 3% hypertonic saline on plasma concentrations of renin (A), angiotensin II (B), aldosterone (C) and arginine vasopressin (D) in 23 healthy subjects pre-treated with bendroflumethiazide, amiloride or placebo. A-C: There was a significant difference between PRC, p-Ang II and p-Aldo plasma levels throughout the study day. Values are means ± SEM. One-way ANOVA was used to test for differences between treatments; D: P-AVP increased significantly at 150 min with a borderline significant difference between treatments ($^{a}P = 0.048$). Values are medians with upper and lower limits. Friedman's test was used to test for differences between treatments. BFTZ: Bendroflumethiazide; PRC: Plasma renin concentration.

Table 2 Twenty-four hours brachial blood pressure and urine collection with fluid deprivation (12 PM to 8.00 AM) in 23 healthy subjects

	Examination day			*P* (ANOVA)
	Thiazide	Amilorid	Placebo	
Urine output (mL/24 h)	2527 ± 728	2418 ± 469	2316 ± 700	0.481
u-osm (mosm/24 h)	865 ± 158	835 ± 176	761 ± 187	0.087
C_{H2O} (mL/min)	-0.40 ± 0.33	-0.37 ± 0.47	-0.24 ± 0.53	0.534
Cr.Cl (mL/min per m^2)	113 ± 25	118 ± 32	110 ± 26	0.549
u-NKCC (ng/mmol)	0.35 ± 0.07	0.30 ± 0.05	0.32 ± 0.06	0.025
u-AQP2 (ng/mmol)	113.2 ± 39.2	103.3 ± 25.5	98.5 ± 17.4	0.244
u-ENaCγ (ng/mmol)	37.8 ± 26.7	30.8 ± 17.3	32.7 ± 15.6	0.867
u-Na (mmol/24 h)	108 ± 34	121 ± 27	106 ± 37	0.263
u-K (mmol/24 h)	80 ± 20	64 ± 21	60 ± 19	0.002
bSBP (mmHg)	119 ± 6	114 ± 7	116 ± 7	0.213
bDBP (mmHg)	72 ± 4	69 ± 3	70 ± 4	0.034

Values are means ± SD. One-way ANOVA was used for comparison between groups. *P*-values represent the possibility of a difference between groups. u-osm: Urine output, urine osmolality; C_{H2O}: Free water clearance; Cr.Cl: Creatinin clearance; u-NKCC2: Urinary NKCC2; u-AQP2: Urinary excretion of AQP2; u-ENaCγ: ENaCγ excretion adjusted for creatinin; u-Na: Urinary excretion of sodium; u-K: Potassium; bSBP: Brachial systolic blood pressure; bDBP: Brachial diastolic blood pressure.

At baseline, ECV and TBW tended to be lower during amiloride ($P = 0.515$) and BFTZ ($P = 0.951$) compared to placebo. However, it did not reach statistical significance. ICV did not differ between treatments. As expected, after administering 3% saline, ICV decreased while ECV and TBW increased reaching a maximum at the end of the study day. Although there was a tendency towards a lower ECV and TBW in the two diuretic groups there were no statistically significant differences in volume status between the three treatments.

DISCUSSION

In the present study, the aim was to investigate the

Table 3 Effect of 3% hypertonic saline on urinary parameters in 22 healthy subjects treated with bendroflumethiazide or amiloride

Periods	Baseline	Infusion	Post infusion			$P_{GLM\ RM}$
	0-90 min	90-150 min	150-180 min	180-210 min	210-240 min	
C_{H2O}						< 0.001
BFTZ	3.1 ± 1.6[a]	-0.2 ± 0.5[d]	-1.6 ± 0.6[d]	-1.6 ± 0.7[d]	-0.6 ± 1.6[d]	
Amiloride	3.7 ± 0.9	-0.6 ± 0.7[d]	-2.1 ± 0.6[d]	-2.4 ± 0.8[d]	-1.4 ± 1.7[d]	
Placebo	4.4 ± 1.1	-0.6 ± 0.6[d]	1.8 ± 0.7[d]	-2.5 ± 1.0[d]	-2.0 ± 0.5[d]	
$P_{GLM\ between\ subjects}$			0.061			
P_{ANOVA}	0.006	NS	NS	0.001	0.007	
UO (mL/min)						0.029
BFTZ	6.1 ± 1.6	2.6 ± 0.6[d]	1.4 ± 0.5[d]	1.7 ± 1.0[d]	2.7 ± 1.9[d]	
Amiloride	6.8 ± 1.3	2.5 ± 0.6[d]	1.6 ± 0.5[d]	2.0 ± 0.7[d]	3.2 ± 1.2[d]	
Placebo	7.3 ± 1.2	2.3 ± 0.9[d]	1.7 ± 0.8[d]	2.2 ± 1.2[d]	2.4 ± 1.1[d]	
$P_{GLM\ between\ subjects}$			0.245			
P_{ANOVA}	0.019	NS	NS	NS	NS	
u-Na (mmol/min)						< 0.001
BFTZ	1.4 ± 0.4	1.7 ± 0.4[b]	1.9 ± 0.7[b]	2.1 ± 0.6[d]	2.0 ± 0.6[b]	
Amiloride	1.6 ± 0.5	2.0 ± 0.7	2.5 ± 1.0[b]	2.9 ± 1.2[d]	3.0 ± 1.0[d]	
Placebo	1.3 ± 0.3	1.9 ± 1.1[a]	2.5 ± 1.3[b]	3.3 ± 1.7[d]	3.0 ± 1.0[d]	
$P_{GLM\ between\ subjects}$			0.020			
P_{ANOVA}	0.028	NS	NS	0.007	<0.001	
FENa (%)						< 0.001
BFTZ	1.5 ± 0.4	1.8 ± 0.5[a]	2.0 ± 0.6[b]	2.2 ±0.6[d]	2.1 ± 0.6[d]	
Amiloride	1.8 ± 0.6	2.1 ± 0.6[a]	2.6 ± 1.0[b]	2.9 ± 1.2[d]	3.0 ± 1.1[d]	
Placebo	1.4 ± 0.4	2.1 ± 1.0[b]	2.7 ± 1.2[d]	3.0 ± 1.1[d]	3.1 ± 1.1[d]	
$P_{GLM\ between\ subjects}$			0.036			
P_{ANOVA}	0.022	NS	NS	0.019	0.001	
u-K (mmol/min)						
BFTZ	20.3 ± 6.7	17.7 ± 5.3	15.8 ± 4.4[b]	14.2 ± 5.6[b]	13.3 ± 6.3[b]	< 0.001
Amiloride	18.5 ± 8.4	15.7 ± 9.3	18.6 ± 11.1	22.4 ± 12.0	23.3 ± 10.2[a]	
Placebo	22.3 ± 9.3	15.6 ± 6.9[d]	16.4 ± 8.5[a]	22.3 ± 12.3	20.4 ± 7.9	
$P_{GLM\ between\ subjects}$			0.255			
P_{ANOVA}	NS	NS	NS	0.015	0.001	
FEK (%)						
BFTZ	21.7 ± 7.4	20.0 ± 6.6	16.1 ± 4.8[b]	15.0 ± 5.9[b]	14.4 ± 6.9[b]	< 0.001
Amiloride	20.8 ± 9.8	18.7 ± 10.8	20.5 ± 12.0	23.6 ± 12.4	25.0 ± 11.0[a]	
Placebo	23.7 ± 9.3	18.1 ± 8.2[b]	17.5 ± 8.9[a]	20.6 ± 9.9	21.4 ± 8.8	
$P_{GLM\ between\ subjects}$			0.254			
P_{ANOVA}	NS	NS	NS	0.018	0.001	
^{51}Cr-EDTA (mL/min per 1.73m^2)						0.271
BFTZ	92.1 ± 10.8	91.3 ± 11.9	96.0 ± 16.7	96.9 ± 16.8	96.5 ± 21.2	
Amiloride	92.7 ± 13.7	93.2 ± 12.8	94.1 ± 12.1	96.7 ± 14.1	100.2 ± 15.6	
Placebo	96.5 ± 9.5	90.0 ± 13.3	94.7 ± 15.1	102.4 ± 14.3	98.1 ± 15.9	
$P_{GLM\ between\ subjects}$			0.887			

Free water clearance (C_{H2O}), urinary output (OU), excretion of sodium (u-Na) and fractional excretion of sodium (FENa), urinary excretion of potassium (u-K) and fractional excretion of potassium (FEK) and ^{51}Cr-EDTA clearance in a randomized, placebo-controlled, crossover study of 23 healthy subjects. Values are mean ± SD. General linear model (GLM) with repeated measures was performed for comparison within the group and intervention as between subjects factor. One-way ANOVA was performed when differences were found between interventions. Post hoc Bonferroni correction was used for multiple comparisons of post infusion periods to baseline within each treatment group. [a]$P < 0.05$; [b]$P < 0.01$; [d]$P < 0.001$.

effect of five days BFTZ and amiloride treatment on the urinary excretion of NKCC2, ENaCγ and AQP2 during baseline conditions and after an acute intravenous volume load of 3% hypertonic saline in healthy subjects. To our knowledge, this study is the first randomized, placebo-controlled trial that measured the changes in u-NKCC2, u-ENaCγ and u-AQP2 during inhibition of the NCC cotransporter with BFTZ and ENaC with amiloride in humans.

This study showed that, in response to 3% saline, u-NKCC2, u-ENaCγ and u-AQP2 increased to the same extent during amiloride and placebo treatment, but neither u-NKCC2 nor u-AQP2 changed significantly during BFTZ.

Sodium and tubular sodium transporters

Thiazides predominantly inhibit NCC along the distal convoluted tubules[2]. Animal studies have shown that when thiazide was administered chronically, urinary sodium returned to normal within 2-3 d[19]. This is in accordance with our findings in 24 h urine collection. Further, a study documented that longer term NCC

Table 4 Effect of 3% hypertonic saline on plasma in 23 healthy subjects treated with bendroflumethiazide or amiloride

Time	Baseline	Infusion	Post infusion			$P_{GLM\text{-}RM}$
	0-90 min	150 min	180 min	210 min	240 min	
p-Na						0.281
BFTZ	137 ± 2	141 ± 2[d]	141 ± 2[d]	139 ± 2[d]	139 ± 2[d]	
Amilorid	137 ± 2	141 ± 2[d]	141 ± 2[d]	140 ± 2[d]	139 ± 2[d]	
Placebo	139 ± 1	43 ± 2[d]	142 ± 1[d]	141 ± 1[d]	140 ± 1[d]	
$P_{GLM\ between\ subjects}$		0.003				
P_{ANOVA}	0.001	0.019	0.004	0.007	0.005	
p-K						0.001
BFTZ	3.35 ± 0.22	3.32 ± 0.23	3.43 ± 0.26	3.42 ± 0.21	3.40 ± 0.20	
Amilorid	4.32 ± 0.30	4.17 ± 0.23[d]	4.26 ± 0.27	4.27 ± 0.25	4.20 ± 0.20[a]	
Placebo	3.89 ± 0.18	3.83 ± 0.22	3.97 ± 0.23	3.94 ± 0.22	3.92 ± 0.20	
$P_{GLM\ between\ subjects}$		< 0.0001				
P_{ANOVA}	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	
p-Osm						0.600
BFTZ	281 ± 5	288 ± 4[d]	288 ± 6[d]	286 ± 5[d]	284 ± 4[d]	
Amilorid	283 ± 4	290 ± 4[d]	290 ± 3[d]	287 ± 3[d]	285 ± 3[a]	
Placebo	286 ± 3	294 ± 4[d]	292 ± 4[d]	290 ± 4[d]	289 ± 3[b]	
$P_{GLM\ between\ subjects}$		< 0.0001				
P_{ANOVA}	< 0.001	< 0.001	0.005	0.002	< 0.001	
p-Alb (g/L)						0.007
BFTZ	40.7 ± 3.1	35.5 ± 2.2[d]	35.9 ± 2.7[d]	36.0 ± 2.8[d]	36.0 ± 2.7[d]	
Amilorid	40.9 ± 2.9	35.5 ± 2.3[d]	36.4 ± 2.6[d]	36.4 ± 2.7[d]	36.3 ± 2.5[d]	
Placebo	39.0 ± 2.4	34.5 ± 2.0[d]	35.1 ± 2.2[d]	35.1 ± 2.3[d]	35.3 ± 2.4[d]	
$P_{GLM\ between\ subjects}$		0.203				

Plasma concentrations of sodium (p-Na), potassium (p-K) and albumin (p-Alb) and plasma osmolality (p-osm). Values are mean ± SD. General linear model (GLM) with repeated measures was performed for comparison within the group and intervention as between subjects factor. One-way ANOVA was performed when differences were found between interventions. Bonferroni correction was used for multiple comparisons between study-periods *vs* baseline. [a]$P < 0.05$; [b]$P < 0.01$; [d]$P < 0.001$.

inhibition might cause a structural adaption, which will activate ENaC and cause increased distal sodium reabsorption and kaliuresis[20]. Twenty-four hours urine collections, demonstrated a small, but significantly increased u-K, and increased u-ENaCγ during BFTZ compared to amiloride and placebo; which supports the theory of a compensatory increase in sodium reabsorption *via* ENaC during longer-term thiazide treatment.

In this present study, 3% hypertonic saline induced an increase in u-NKCC2 when subjects were treated with amiloride and placebo. It was probably related to a counter regulatory mechanism to compensate for temporarily impaired lower fractional sodium reabsorption in proximal tubules during volume expansion[21-23]. It has previously been described in healthy humans that u-NKCC2 decreased after 3% saline[12]. However, the subjects' average age was approximately 35 years older in the aforementioned study. Tian *et al*[24] showed a blunting in the up regulation of sodium transport proteins in response to water restriction in aged rats, which seemed to be particularly apparent with regards to NKCC2. The age difference might explain the discrepancy in the u-NKCC2 response between the two studies.

During BFTZ treatment, u-NKCC2 ceased to increase. In rats, chronic thiazide treatment produces a compensatory fractional increased reabsorption of sodium in proximal tubules[19], which might explain why u-NKCC2 ceased to increase in the late post infusion periods during BFTZ. Thus, during BFTZ, there was no need of a compensatory reabsorption *via* NKCC, which is supported by the relative lower increase in FE_{Na} during BFTZ compared to both amiloride and placebo, in response to 3% saline. In animals, AVP has been demonstrated to increase NKCC2 activity, mediated by V2 receptors *via* adenylate-cyclase-6 to facilitate phosphorylation and trafficking of NKCC2 to the apical membrane[25,26]. As p-AVP was lower during BTFZ treatment, it cannot be excluded that the decline in u-NKCC2 might also reflect a lack of stimulation from AVP.

Thus, our findings reflect a more profound change in glomerular tubular balance during BFTZ treatment, than the more distal acting diuretic, amiloride.

In the collecting ducts, sodium transport occurs *via* the ENaC located in the luminal membrane of principal cells[27,28]. ENaC can be regulated by aldosterone[29,30], but is also regulated by AVP, that binds to the V2 receptors and induces a rapid change in channel activity *via* ENaC opening[31-36]. Recently our group demonstrated an increased u-ENaCγ after hypertonic saline infusion in healthy young subjects. The increase in u-ENaCγ was explained by an increased sodium load to the distal tubules caused by a decrease in renal sodium absorption in the proximal tubules[11,21,22]. In the present study, we measured a similar increase during all three treatments. As amiloride inhibits

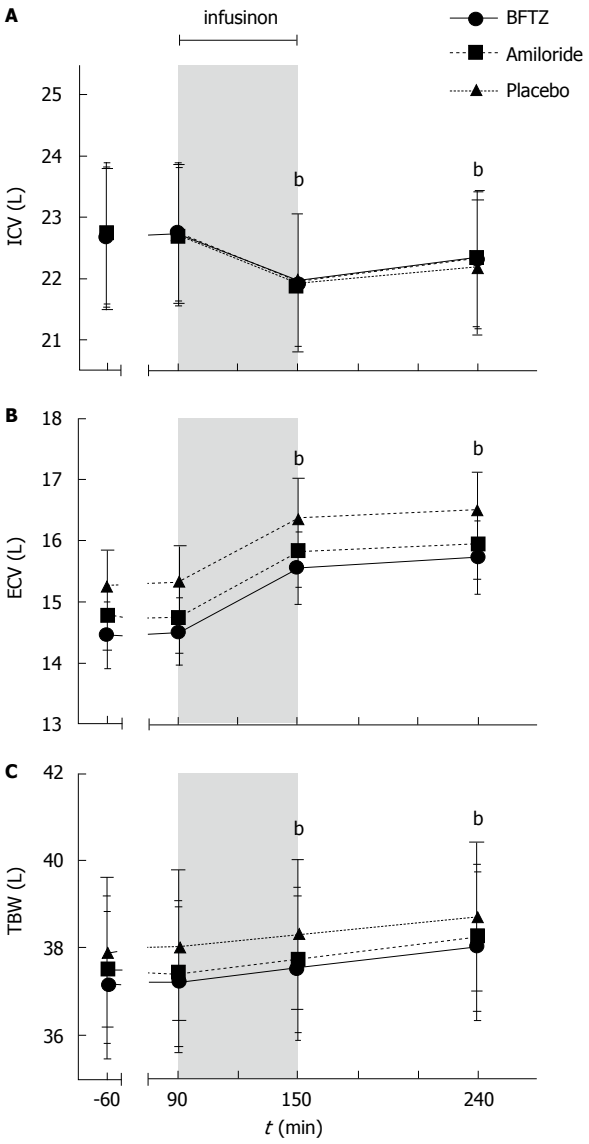

Figure 4 Effects of 3% hypertonic saline on (A) intracellular and (B) extracellular volume and (C) total body water in 23 healthy subjects pretreated with bendroflumethiazide, amiloride or placebo. Values are means ± SEM. General linear model with repeated measures was non-significant between treatments. Paired *t*-test was used for comparison of post infusion periods *vs* baseline $^{b}P < 0.001$. BFTZ: Bendroflumethiazide; ECV: Extracellular volume; ICV: Intracellular volume; TBW: Total body water.

ENaC, we expected to find a decrease in u-ENaCγ both at baseline and in response to 3% saline treatment during amiloride treatment, especially as we had also found an increased FE_{Na} at baseline. There are several possible explanations: Firstly, as ENaC only controls as little as 2%-5% of sodium reabsorption, perhaps a small decrease in the fractional reabsorption of sodium *via* ENaC would cause a significant rise in excretion of sodium. Secondly, p-Aldo was highest during amiloride treatment and its stimulation on the principal cells might have increased the amount of ENaC within the apical membrane, and thus antagonized the effect of amiloride. Thirdly, amiloride treatment has been shown to increase whole cell channel abundance caused by an intracellular sodium feedback mechanism[37,38]. These intracellular counter regulatory mechanisms might also be involved.

As expected, during the acute hypertonic sodium load, PRC, p-AngⅡ and p-Aldo decreased and urinary sodium excretion increased[9,11,39]. The increase in urinary sodium excretion is preceded by a decrease in ENaC expression and activity[40]. Meanwhile, p-AVP increased due to increased p-osm, and likely caused ENaC channels to be inserted in the apical membrane and thus increased reabsorption of sodium[41].

Thus, despite increased FE_{Na} at baseline during amiloride treatment, u-ENaCγ did not differ significantly between treatments neither at baseline nor after 3% saline. These findings do not appear to be dependent on aldosterone, but more to reflect a compensatory role of ENaC to adjust for the decreased reabsorption of sodium in proximal parts of the nephron during an acute sodium load.

Water and AQP2

Vasopressin (AVP) regulates AQP2 function by binding to V2 receptors in the basolateral membrane of principal cells, increasing the delivery of intracellular vesicles containing AQP2 to the apical membrane and thus increasing water reabsorption[42,43]. AQP2 is also excreted into urine[5-9,44-46]. Volume expansion with 3% hypertonic saline increases plasma osmolality, p-AVP, reabsorption of water and u-AQP2[11,14,47]. In the present study, there was an increase in u-AQP2, in response to 3% saline, during amiloride and placebo treatment, but not during BFTZ. The changes in u-AQP2 during BFTZ correspond to the attenuated decrease in CH2O. Different explanations include: (1) an increased water load to the collecting tubules due to inhibition of NCC in distal collecting ducts, resulting in higher water excretion; (2) Decreased p-osm and p-AVP during BFTZ treatment and thus a reduced effect on V2R in the collecting ducts; and (3) A reduced need for at counter regulatory water reabsorption in the collecting ducts due to the lower reabsorption of sodium *via* NKCC2.

Potassium

Potassium is freely excreted in glomerulus and it is reabsorbed and secreted across the nephron[48]. Intracellular signalling networks, volume status, p-K status and aldosterone tightly regulate the balance of potassium excretion[49,50]. Thiazides do not affect potassium transport directly, but induce adaption primarily along the connecting and collecting tubules where enhanced sodium reabsorption stimulates potassium secretion *via* renal outer medullary K+ (ROMK) and large-conductance K+ (BK) channels[20,51].

It has recently been shown that angiotensinⅡ directly inhibits ROMK in potassium-depleted animals, and thereby contributes to potassium conservation[52,53]. In this present study, p-Ang Ⅱ was highest during BFTZ treatment, and may have inhibited ROMK, and explains the increased sodium reabsorption during 3% saline

Table 5 Effect of 3% hypertonic saline on brachial blood pressure, central blood pressure and pulse wave velocity in 23 healthy subjects treated with bendroflumethiazide or amiloride

Periods	Baseline	Infusion	Post infusion			$P_{GLM\ RM}$
	0-90 min	150 min	180 min	210 min	240 min	
bSBP						
BFTZ	112 ± 9	118 ± 9[d]	116 ± 10	116 ± 11	117 ± 13	0.825
Amilorid	110 ± 8	115 ± 9[d]	114 ± 10[d]	114 ± 10[b]	114 ± 10[b]	
Placebo	113 ± 10	117 ± 10[d]	115 ± 9	115 ± 8	115 ± 10	
$P_{GLM\ between\ subjects}$			0.679			
bDBP						
BFTZ	66 ± 7	63 ± 7[b]	62 ± 6[d]	62 ± 7[d]	62 ± 7[b]	0.055
Amilorid	64 ± 4	62 ± 5	60 ± 5[b]	61 ± 5	62 ± 6	
Placebo	64 ± 6	63 ± 5	63 ± 5	62 ± 6	63 ± 6	
$P_{GLM\ between\ subjects}$			0.695			
Pulse Rate						
BFTZ	58 ± 10	61 ± 11[a]	61 ± 11[b]	62 ± 10[b]	62 ± 10[b]	0.782
Amilorid	57 ± 10	61 ± 11[d]	60 ± 10[d]	61 ± 11[d]	61 ± 11[d]	
Placebo	55 ± 10	59 ± 12[a]	59 ± 12[b]	59 ± 12[b]	59 ± 13[a]	
$P_{GLM\ between\ subjects}$			0.712			
cSBP						
BFTZ	99 ± 7	98 ± 7				NS
Amilorid	96 ± 5	97 ± 7				NS
Placebo	98 ± 6	98 ± 8				NS
P_{ANOVA}	NS	NS				
cDBP						
BFTZ	67 ± 5	63 ± 6				< 0.001
Amilorid	65 ± 5	65 ± 6				NS
Placebo	65 ± 5	64 ± 5				NS
P_{ANOVA}	NS	NS				
PWV						
BFTZ	5.5 ± 0.6	5.3 ± 0.4				0.055
Amilorid	5.3 ± 0.5	5.3 ± 0.5				NS
Placebo	5.3 ± 0.7	5.3 ± 0.6				NS
P_{ANOVA}	NS	NS				
AI						
BFTZ	-2.2 ± 14.6	-5.9 ±17.7				NS
Amilorid	0.4 ± 12.5	-1.4 ± 13.4				NS
Placebo	-1.6 ± 14.6	-4.9 ± 18.6				0.034
P_{ANOVA}	NS	NS				

Values are mean ± SD. General linear model (GLM) with repeated measures was performed for comparison within the group and intervention as between subjects factor for brachial systolic (bSBP), brachial diastolic blood pressure (bDBP) and pulse rate. Bonferroni correction was used for multiple comparisons between study-periods *vs* baseline. [a]$P < 0.05$; [b]$P < 0.001$; [d]$P < 0.001$; Paired *t*-test was used for comparison between post infusion *vs* baseline for central systolic (cSBP), central diastolic (cDBP), pulse wave velocity (PWV) and augmentation index (AIx).

while potassium secretion decreased.

During amiloride treatment there was a decrease in u-K at baseline. Amiloride exerts a direct effect on potassium excretion due to the blocking of ENaC. If the influx of sodium does not occur, there will be no lumen negative potential to drive potassium excretion[48]. In response to 3% hypertonic saline however, the excretion of potassium increased most during amiloride. This phenomenon might have been due to prolonged effect of aldosterone to increase sodium reabsorption *via* ENaC and secretion *via* ROMK, despite current amiloride blockage.

Blood pressure

Thiazide decreases ECV and peripheral vascular resistance[2,54]. Amiloride is an antihypertensiva that exhibits its effects by significant natriuresis[55]. In this study the lack of difference in 24 h ambulatory blood pressure might partly be explained by the fact that the subjects were young and healthy with normal BP before entering the study. Moreover, BFTZ and amiloride are relatively weak antihypertensives and a very negligible blood pressure lowering effect was expected in these normohypertensive subjects.

Data showed that bDBP decreased significant in all three treatments, but bDBP decreased relatively more during BFTZ treatment compared to amiloride and placebo. As brachial office BP was also used to calibrate the SphygmoCor cDBP this may explain the reduction in cDBP during BFTZ. A negative augmentation index (AIx) has been reported in healthy young subjects, but is of limited use due to normal cardiovascular elasticity in this age group[56].

Body fluid volumes

The determination of body fluid volumes *via* bioimpedance

spectroscopy (BIS) is an accurate method for estimating total body water and the distribution of water between the intracellular and extracellular spaces[57]. In this present study, we measured no statistical difference between the groups, but as expected TBV was lower during both diuretic treatments compared to placebo, due to a decrease in ECV followed by sodium deficit. This reduction in ECV, during BFTZ treatment, is in agreement with current knowledge[2]. We did not expect a major decrease in ECV after amiloride, being a weak diuretic agent[55]. However the decrease in ECV was very similar to BFTZ. A significant difference in body fluid volumes between treatment groups was not detected in the present study, possibly due to the small number of subjects in each group.

Strengths and limitations

The major strength of this study was the design as a randomized, placebo controlled, double-blinded crossover study with a homogenous group of healthy young men and women. The test conditions were very well defined regarding diet, sodium and fluid intake. Thus, the results are not confounded by differences in sodium or water intake. However, as the study group was healthy humans the conclusions is limited to this population group and may not be extracted to patients with disturbances in water and sodium balance. Also the excretion of NCC was not measured, which would have provided us with even more information on renal handling of sodium.

Conclusion

In this study of healthy humans, amiloride and placebo clearly increased u-NKCC2, u-ENaCγ and u-AQP2 in response to 3% hypertonic saline, while u-NKCC2 and u-AQP2 were unchanged during BFTZ. In contrast to amiloride, BFTZ treatment seemed to have changed glomerular-tubular balance, which caused the absence of a compensatory reabsorption of sodium *via* NKCC2 after hypertonic saline. It is possible that the lower p-AVP during BFTZ treatment resulted in a relatively less stimulation of NKCC2 and AQP2, with subsequent reduced transport of sodium and water *via* the transporters. During all three treatments, the increase in u-ENaCγ might reflect a compensatory reabsorption to adjust for the decreased reabsorption of sodium in the proximal part of the nephron.

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COMMENTS

Background

The discovery that urine contains proteins from renal epithelia of proximal tubule, Henle's loop, distal convoluted tubule and the collecting ducts has provided us with urinary biomarkers as a tool to investigate physiological and pathophysiological processes in renal sodium and water homeostasis.

Research frontiers

Urinary excretion of the aquaporin2 water channel (u-AQP2) is a biomarker that has been investigated in numerous studies of various water-balance disorders. It has also been demonstrated that the urinary excretion of epithelial sodium channels (u-ENaC) can be used as biomarkers of sodium transport *via* excretion of epithelial sodium channels (ENaC). However the exact physiological mechanisms are still unknown, and studies are needed to address the complete physiological handling of sodium and water in humans.

Innovations and breakthroughs

In animals, volume expansion and diuretics changes proximal water and sodium reabsorption and the expression of AQP2 and sodium transporters along the nephron. In addition, changes in transport activity of the sodium-potassium-2chloride cotransporter (NKCC2); ENaC and AQP2 may also be involved in the abnormal tubular function in patients with chronic kidney disease. However, it has never been studied to what extent the function of NKCC2, ENaC and AQP2 is simultaneously affected in response to amiloride and bendroflumethiazide (BFTZ) in humans. In the present study, the u-NKCC2 and u-AQP2 increased during amiloride and placebo, while u-NKCC2 and u-AQP2 remained unchanged during BFTZ, in response to infusion of 3% saline.

Applications

Thus, measurements of water- and sodium transporters in urine, as biomarkers of water-and sodium transport *via* NKCC2, ENaC and AQP2 may provide important information of the mechanisms involved in water and sodium balance in the kidney.

Terminology

AQP2, NKCC2 and ENaC are transporters in the nephron that play essential roles in regulating water and sodium homeostasis, extracellular volume and controlling blood pressure by reabsorbing water and sodium. BFTZ is a diuretic that inhibit the sodium-chloride co-transporter and amiloride is a diuretic that block the ENaC channels. These diuretics, which were developed empirically to treat patients with edema and hypertension, can be used as tools to characterize sodium transport pathways.

Peer-review

This is an interesting paper.

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CASE REPORT

Unexpected hypercalcemia in a diabetic patient with kidney disease

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Author contributions: Lupica R, Buemi M and Santoro D designed the report; Santoro D performed the biopsy analyses; Trimboli D, Canale V, Cernaro V and Campennì A collected the patient's clinical data; Lupica R analyzed the data and wrote the paper.

Ethics approval statement: The study was reviewed and approved by the Institutional Review Board.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrolment.

Conflict-of-interest statement: The authors declare that they have no relevant financial interests.

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Abstract

We report a case of a diabetic patient with progressive chronic kidney disease and unexplained hypercalcemia. This unusual presentation and the investigation of all possible causes led us to perform a renal biopsy. The systemic sarcoidosis diagnosis was confirmed by the presence of interstitial multiple granulomas composed of epithelioid and multinucleated giant cells delimited by a thin fibrous reaction, and by pulmonary computed tomography finding of numerous lumps with ground-glass appearance. Sarcoidosis most commonly involves lungs, lymph nodes, skin and eyes, whilst kidney is less frequently involved. When it affects males it is characterized by hypercalcemia, hypercalciuria, and progressive loss of renal function. Early treatment with steroids allows for a gradual improvement in renal function and normalization of calcium serum values. Otherwise, the patient would quickly progress to end stage renal disease. Finding of hypercalcemia in a patient with renal failure must alert physicians because it may be a sign of several pathological entities.

Key words: Biopsy; Granulomatous; Hypercalcemia; Kidney; Sarcoidosis

Core tip: This report not only describes a case of kidney sarcoidosis, but also explains the diagnostic algorithm that led to the correct diagnosis of a case wrongly labelled

as chronic kidney disease (CKD) secondary to diabetic nephropathy. The absence of other microangiopathic alterations such as retinopathy and secondly the presence of hypercalcemia with hypoparathyroidism in a patient with CKD need to be further explored.

Lupica R, Buemi M, Campennì A, Trimboli D, Canale V, Cernaro V, Santoro D. Unexpected hypercalcemia in a diabetic patient with kidney disease. *World J Nephrol* 2015; 4(3): 438-443 Available from: URL: http://www.wjgnet.com/2220-6124/full/v4/i3/438.htm DOI: http://dx.doi.org/10.5527/wjn.v4.i3.438

INTRODUCTION

Sarcoidosis is a multisystem granulomatous disease with unknown etiology. The diagnosis requires histological confirmation by the discovery of noncaseating granulomas, the exclusion of other diseases with similar symptoms and the clinical evidence of multiple organ involvement[1]. It is often subclinical or self-limited or the symptoms are shaded[2]. The presentation can be various: respiratory symptoms are not specific and may be mistakenly attributed to other lung diseases, for which empiric therapy may be attempted before other diagnostic tests are performed. Moreover we can find dermatological or ocular abnormalities and totally nonspecific systemic symptoms (fever, weight loss, night sweats). It usually has a benign course with spontaneous resolution in up to two-thirds of cases. However, in one third of cases it is a progressive disorder with significant organ impairment[3].

CASE REPORT

In October 2013, a caucasian 59-year-old man was admitted to Nephrology division. The history was notable for non-insulin diabetes mellitus (ten years), with poor glycemic control (glycosylated haemoglobin A1c = 10.1%). Two months before he had been hospitalized at the internal medicine ward and insulin therapy had been started. Because of increased creatinine levels with lower limb oedema, the patient was referred to the Nephrology Unit. On admission, physical examination revealed normal blood pressure (120/70 mmHg) and heart rate (100 beats/min), moderate leg oedema; laboratory data showed serum creatinine equal to 2.9 mg/dL, calcium 10.4 mg/dL, proteinuria 156 mg/24 h, calculated creatinine clearance (cCrCl) 30.69 mL/min per 1.73 m^2 (other blood tests are shown in Table 1); electrocardiogram and echocardiogram were normal. Renal ultrasound (Figure 1) and Colour Doppler showed normal kidneys [antero-posterior diameter of right kidney = 11.17 cm, left kidney = 11.48 cm; normal cortico-medullary representation; resistance index (RI) = 0.70 to right, 0.73 to left]. Fundus oculi was negative for typical lesions of diabetic retinopathy. The patient was discharged with diagnosis of "stage Ⅲ chronic kidney disease (CKD)" and insulin and antibiotic therapy was recommended. After approximately 1 mo, the patient was hospitalized again due to the worsening of his general conditions and hypercalcemia. He complained of loss of appetite and weakness, with decrease in body weight of about 2 kg. General physical examination was normal except for the presence of right eye hyperaemia, more represented peripheral oedema and reduced breath sounds over the entire pulmonary area. The laboratory tests on the admission are shown in Table 1.

The patient was studied for other secondary causes of hypercalcemia by using an appropriate algorithm[4]. Because of the non-PTH mediated further increase in serum calcium (PTH = 5.5 pg/mL), normal level of 25D (21.73 ng/mL) and absence of vitamin D therapy, hematologic screening was performed to exclude cancer. Chest X-ray (Figure 2) detected a parenchymal consolidation in the right hilum, two pseudo nodular thickenings in the base with a diffuse micronodular pattern. Pulmonary computed tomography (CT) (Figure 3) showed multiple ground-glass like lumps involving both lungs, but not in the basal regions, associated with a thickening of the central and peripheral interstitium and multiple reactive mediastinal lymph nodes. Finally, since data were not suggestive for diabetic nephropathy we decided to perform a renal biopsy. Formalin-fixed and paraffin embedded tissue was processed using standard techniques. Light microscopy showed the presence of interstitial multiple granulomas composed of epithelioid and multinucleated giant cells delimited by a thin fibrous reaction (Figures 4 and 5). Edema was present in the interstitium with mild to moderate lymphocyte-monocyte infiltration and tubular atrophy affecting about 15%-20% of the core. Some tubules showed detachment of necrotic cells in the tubular lumen and in some cases rupture of the basement membrane. Arteriolar hyalinosis and arterial intimal fibrosis were also present. Immunofluorescence staining of frozen sections was negative for IgG, IgM, IgA, C3, C1q, fibrinogen, kappa, and lambda light chains.

Diagnosis

Interstitial Granulomatous nephritis secondary to Sarcoidosis. Arteriosclerosis and arteriolosclerosis.

Clinical follow up

The patient was treated with oral steroids at the dose of 1 mg/kg per day that was progressively tapered.

Normalization in calcium values and improvement of renal function were observed after the first month of corticosteroid therapy (Table 1). There was also resolution of peripheral oedema and asthenia, and improvement of appetite with progressive body weight increase. At the same time, chest CT showed amelioration of pulmonary alterations and disappearance of lumps.

DISCUSSION

Sarcoidosis most commonly involves lungs, lymph

Table 1 October 2013: laboratory data on first hospitalization; December 2013: laboratory data on second hospitalization before kidney biopsy; January 2014: laboratory data after 1 mo of treatment; February 2014: laboratory data after 2 mo of treatment

Serum chemistries	October 2013	December 2013	January 2014	February 2014
Sodium (mmol/L)	141	136	139	140
Potassium (mmol/L)	38	4.4	4	4.6
Glucose (mg/dL)	139	140	117	145
HbA1c (%)	10.1	9	6.30	7
Proteinuria (mg/24 h)	156	408	328	394
Creatinine (mg/dL)	2.9	3.6	1.83	1.82
eGFR (mL/min per 1.73 m^2)	30.69	20.69	42	64
Calcium (mg/dL)	10.05	13.01	8.3	8.1
Phosphorus (mg/dL)	4.6	4.2	3.9	4
PTH (pg/mL)	3.50	5.50	59.76	61.38
VIT D3 (ng/mL)	30.67	21.73	20.50	19.83
AST (U/L)	12	15	17	12
ALT (U/L)	9	8	24	9
ALP (U/L)	89	80	79	86
Hematologic studies				
WBC count (x 10^3/μL)	10.4	13.6	11.2	10.90
Hemoglobin (g/dL)	10	10.6	13.60	12.2
Platelet count (x 10^3/μL)	448	456	432	

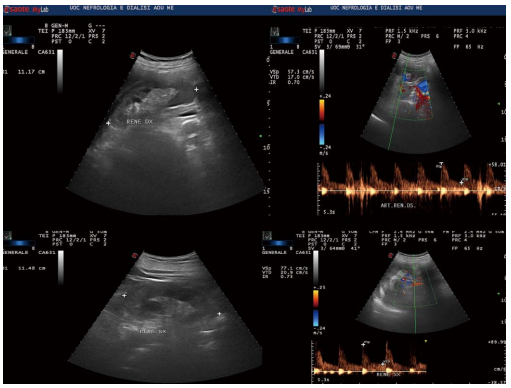

Figure 1 Renal ultrasound.

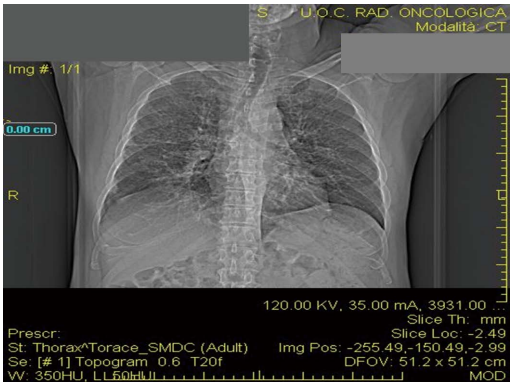

Figure 2 Chest X-ray.

nodes, skin and eyes, whilst kidney is less usually affected[5]. The disease typically occurs in young and middle-aged black race women. Clinical presentation is different depending on the race. Pulmonary involvement covers 90% of patients, of which 61%-63% are women[6]. Among black subjects besides lungs, eyes and skin are often affected. Curiously, calcium metabolism abnormalities are more frequent and more severe in caucasic men with renal interstitial granulomatosis[6] The incidence of renal involvement remains unclear: in autoptic studies, granulomatous infiltrate is found in up to 23% of kidneys, and even up to 48% in small series of biopsies[7]. In our case, the characteristic lesions were related to the presence of multiple granulomas consisting of epithelioid and multinucleated giant cells, delimited by a thin fibrous reaction, interstitial oedema with a mild to moderate lymphocyte and monocyte infiltration and tubular atrophy, involving about 15%-20% of the renal tissue. Our patient was initially classified as a case of CKD secondary to diabetic nephropathy in a context of poor glycemic control. However, first of all the absence of other microangiopathic alterations such as diabetic retinopathy and secondly the presence of hypercalcemia with hypoparathyroidism in a patient with CKD needed to be further explored[8]. On the second admission, the persistence of hypercalcemia despite the suspension of vitamin D therapy, weight loss, anorexia and asthenia suggested for a tubular damage. Differential diagnosis for hypercalcemia at that time was betweeen malignant disease and systemic sarcoidosis. In particular, hypercalcemia characterizes paraneoplastic syndromes[9-12] secondary to bronchial carcinoma, small cell lung cancer, breast, gastric and uterus cancer, myeloma, lymphoma. It could be the first clinical manifestation of a parathyroid adenoma; in an autonomous and independent way, neoplastic tissue produces parathyroid-related hormone (PTHrP), which stimulates bone turnover and calcium reabsorption in the kidney, thus increasing serum calcium values[13]. However, in these situations not only calcium but also PTH is elevated, while in our patient PTH was suppressed. We then considered other causes of hypercalcemia, especially an inappropriate intake or increased activation of vitamin D. In the first hypothesis, suspension of oral vitamin D should have led to a normalization of calcium values, but it was not our case. Moreover, the subject was also suffering from CKD, a condition characterized by reduced production of 1-α-hydroxylase and consequently low concentrations of 1,25 dihydroxycholecalciferol[8] due to progressive replacement of renal parenchyma with fibrotic tissue. We therefore concluded that hypercalcemia was sustained by an abnormal production of 1-α-hydroxylase from granulocyte monocyte-macrophage cells, secondary to a chronic granulomatous disease without any feed-back mechanism of control: this may lead to calcium retention, hypercalciuria and nephrocalcinosis[14]. These situations may be consistent with a diagnosis of sarcoidosis.

Our patient was wrongly categorized as chronic renal failure secondary to diabetes mellitus, whilst it was a case of acute renal injury secondary to sarcoidosis, the identification and treatment of which led to the improvement of renal function. The

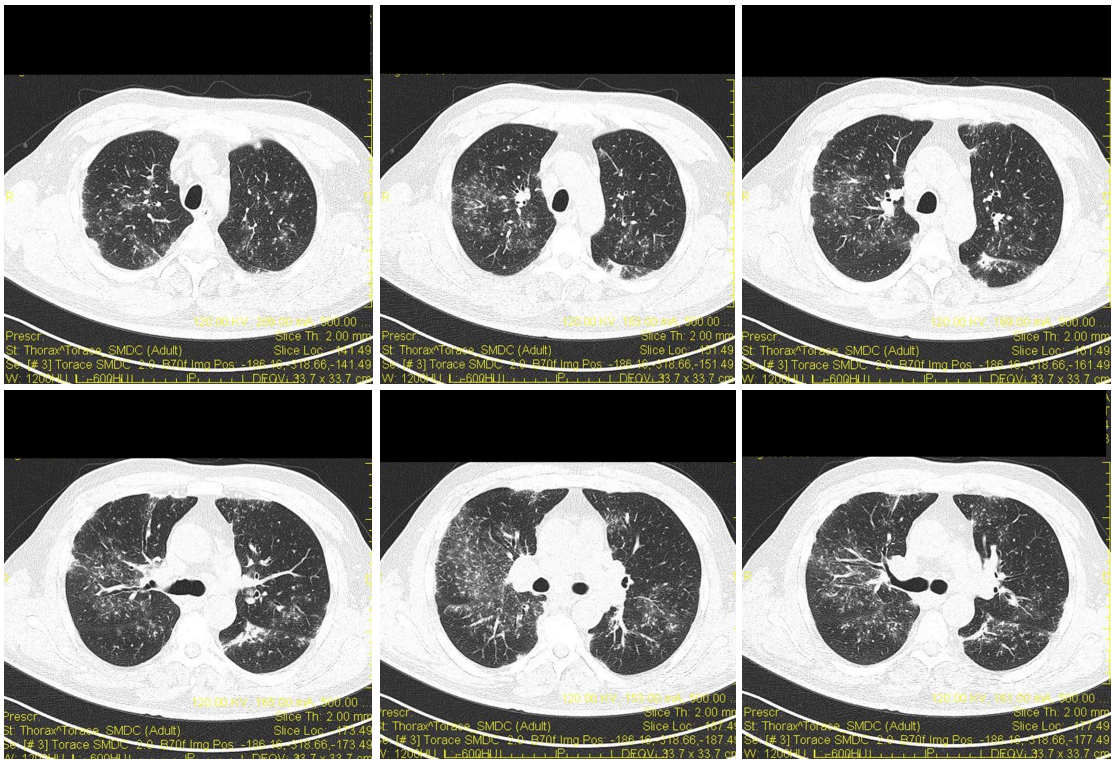

Figure 3 Pulmonary computed tomography.

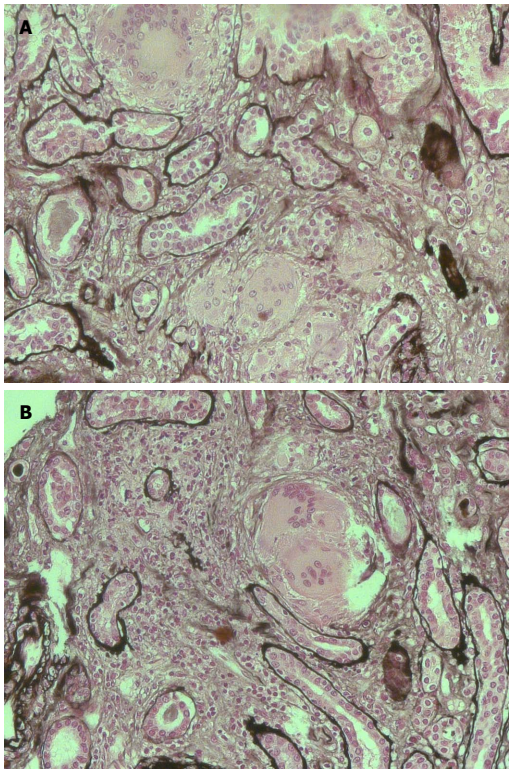

Figure 4 Histological findings. A: Presence of interstitial multiple granulomas composed of epithelioid cells and moderate lymphocyte and monocyte infiltration (silver stain); B: Multinucleated giant cells (silver stain).

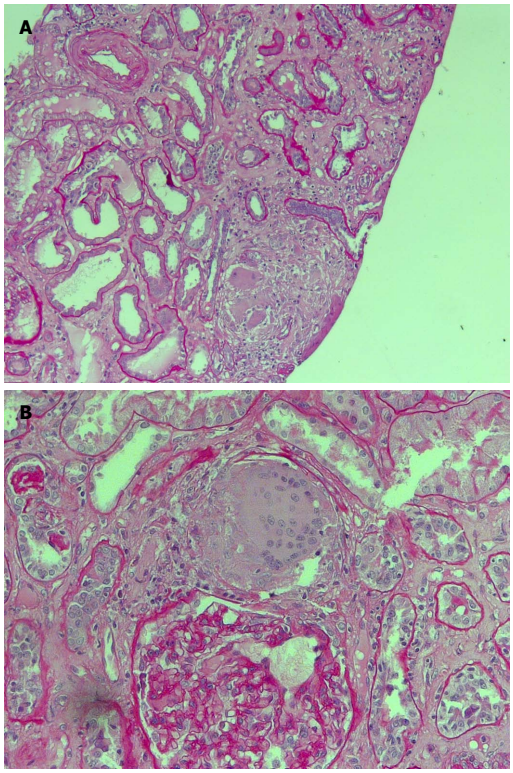

Figure 5 Histological findings. A: Moderate lymphocyte and monocyte infiltration; B: Multinucleated giant cells [periodic acid schiff (PAS) stain].

investigation of all possible causes of "hypercalcemia" allowed to diagnose systemic sarcoidosis confirmed by the evidence of granulomas in renal biopsy. A comprehensive evaluation should be performed in all patients with suspected sarcoidosis, including history, physical examination, chest radiography, pulmonary

function tests, peripheral blood counts, complete serum chemistries. Pulmonary evaluation starts with function tests (such as spirometry, diffusing capacity, *etc.*) reveal a restrictive defect with reduced gas exchange and reduced functional status; chest X-ray. Bilateral hilar lymphadenopathy is the most typical sign at chest radiography. Computed tomography (CT) is essentially face for atypical manifestations of the disease,in order to avoid confusion with differential diagnoses and, sometimes, comorbidities. CT typically shows diffuse pulmonary perilymphatic micronodules, with a perilobular and fissural distribution and upper and posterior predominance, even when an atypical CT pattern is predominant. CT allows deciphering pulmonary lesions in cases of pulmonary fibrosis, pulmonary hypertension, and airflow limitation[15]. Gallium-67-citrate scintigraphy is another imaging method that can be employed both in diagnosis, staging and in the follow-up. Use of fluorine-18-fluorodeoxyglucose positron emission/computed tomography (18F-FDG-PET/CT) seems to have higher diagnostic accuracy with respect to Gallium-67-citrate but the high fee of the method limits its employ. Diagnosis relies on compatible clinical and radiological presentation, evidence of noncaseating granulomas and exclusion of other diseases with a similar presentation or histology. However, there are important variations in diagnostic work-up due to diverse expressions of sarcoidosis and differences in clinical practices among physicians. In our case for example, Gallium-67-citrate scintigraphy, 18F-FDG-PET/CT and serum angiotensin-converting enzyme (ACE) dosage were not made as the result of histological examinations performed on renal tissue was diagnostics. On the other hand, rapid worsening of renal function has been made it necessary to perform biopsy.

In conclusion, we can assume that clinical, laboratory and instrumental data should not be ignored because they are all pieces of a greater puzzle that once rebuilt allows to reach the correct diagnosis and give the right treatment. Like in our case, in a diabetic patient, the presence of hypercalcemia with CKD and the absence of other microangiopathic alterations needs to be further explored because the diagnosis of cancer, paraneoplastic syndrome or parathyroid adenoma has to be excluded as the cause of hypercalcemia.

COMMENTS

Case characteristics

A caucasian 59-year-old man with non-insulin diabetes mellitus and poor glycemic control, increased serum creatinine level and hypercalcemia.

Clinical diagnosis

The patient was initially classified as a case of chronic kidney disease (CKD) secondary to diabetic nephropathy; however, the absence of other microangiopathic alterations in a diabetic patient with CKD needed to be further explored.

Differential diagnosis

Differential diagnosis for hypercalcemia was with cancer, paraneoplastic syndromes and parathyroid adenoma.

Laboratory diagnosis

Laboratory data showed serum creatinine 2.9 mg/dL, calcium 10.4 mg/dL, proteinuria 156 mg/24 h, cCrCl 30.69 mL/min per 1.73 m^2, PTH 5.5 pg/mL, 25D 21.73 ng/mL.

Imaging diagnosis

Ultrasound showed normal kidneys. Pulmonary computed tomography revealed multiple ground-glass like lumps involving both lungs.

Pathological diagnosis

Histological examination showed the presence of interstitial multiple granulomas composed of epithelioid and multinucleated giant cells delimited by a thin fibrous reaction. Oedema was present in the interstitium with mild to moderate lymphocyte and monocyte infiltration and tubular atrophy affecting about 15%-20% of the core.

Treatment

The patient was treated with oral steroids at the dose of 1 mg/kg per day that was progressively tapered. Normalization in calcium values and improvement of renal function were observed after the first month of corticosteroid therapy.

Related reports

Sarcoidosis most commonly involves lungs, lymph nodes, skin and eyes, whilst kidney is less frequently affected. The incidence of renal involvement remains unclear: in autoptic studies, granulomatous infiltrate is found in up to 23% of kidneys, and even up to 48% in small series of biopsies.

Experiences and lessons

The patient was wrongly categorized as chronic renal failure secondary to diabetes mellitus, whilst it was an interesting case of acute kidney injury secondary to sarcoidosis, the identification and treatment of which led to the improvement of renal function.

Peer-review

It is a good article.

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EDITORIAL

Pediatric primary urolithiasis: Symptoms, medical management and prevention strategies

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Abstract

In the past few decades pediatric urolithiasis has become more frequent. The reason for this increase is not completely clear but has been attributed to changes in climate, nutritional habits and possibly other environmental factors. Although less frequent than adult stone disease, urolithiasis in the pediatric age group is also related to significant morbidity, particularly since stones tend to recur, and, thus, should not be underestimated. Most children with idiopathic stone disease have an underlying metabolic abnormality substantiating the importance of metabolic evaluation already following initial diagnosis of urolithiasis. Identification of the metabolic abnormality allows for more specific prescription of non pharmacological and pharmacological interventions aimed at preventing recurrent stone formation. A better understanding of the causes of kidney stone disease will provide better strategies for stone prevention in children.

Key words: Urolithiasis; Hypercalciuria; Cystinuria; Hyperoxaluria; Treatment; Prevention

Core tip: In the past few decades pediatric urolithiasis has become more frequent. The reason for this increase is not completely clear. Although less frequent than adult stone disease, pediatric urolithiasis is also related to significant morbidity, particularly since stones tend to recur. Most children with idiopathic stone disease have an underlying metabolic abnormality. Identification of the metabolic abnormality allows for more specific prescription of non pharmacological and pharmacological interventions aimed at preventing recurrent stone formation. A better understanding of the causes of kidney stone disease will provide better

strategies for stone prevention in children.

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INTRODUCTION

Urolithiasis (UL) is a worldwide problem and is the end product of a multifactorial process. It affects children of all ages and recurrence is a striking feature. No technique of calculi removal diminishes or alters this recurrence morbidity that in pediatric patients is directly related to surgical interventions and morphological changes resulting from possible obstructions of the urinary tract as well as to their clinical manifestations.

The incidence, composition and clinical characteristics of urinary calculi in children vary in relation to geographical location and historical periods. This variation is related to climate, genetic and dietary factors and socio-economical factors[1-3].

Recent decades studies have shown an increased incidence of kidney stones in adults[4-8]. This same trend has also been observed in children[9-14], and possibly results from increased attention to the diagnosis of UL, the routine evaluation with ultrasonography (USG) in children with specific or nonspecific symptoms, and changes in socio-economic conditions and dietetic habits of the pediatric population. However, the true incidence of pediatric UL remains unknown due to the multiplicity of etiopathogenic factors, unspecific clinical picture and lack of studies with appropriate epidemiological design. Studies conducted in different areas of the globe showed variation regarding gender and age. Sas *et al*[11] in South Carolina, United States, showed that the incidence of UL in children under 18 years was 7.9/100000 in 1996 and 18.5/100000 in 2007, higher in girls *vs* boys, and more prevalent in adolescents. In Japan, Yasui *et al*[5] showed an incidence of 17.7/100000 in males and 12.4/100000 in females in children and adolescents between 10 and 19 years of age. In Iceland, Edvardsson *et al*[15] reported that the incidence in patients younger than 18 years was 5.6/100000 on the basis of 26 new diagnoses of UL during a 6-year period among a national population of approximately 78000 children. VanDervoort *et al*[9] demonstrated that pediatric UL increased almost five times over the last decade in United States. Dwyer *et al*[13] reported that the incidence of pediatric UL in Minnesota, United States, increased from 13/100000 between 1984-1990 to 36/100000 between 2003-2008. Even in the United States, 1/685 pediatric hospitalizations are motivated by urinary calculi and over 50% are under 13 years-old individuals[10]. In 2013, Penido *et al*[14] demonstrated that the annual incidence of primary pediatric UL *per* 1000 renal clinic visits tripled from 1999 to 2010 in a children's hospital in the Midwestern United States. Data from Croatia showed that UL was responsible for 2.5/1000 pediatric hospitalizations, and its overall incidence rate in children under 18 years in 2011 was 6.5/100000[16].

UL is multifactorial and different factors are involved in its genesis, working in an interrelated way: infectious, anatomical, epidemiological, climatic, socioeconomic, dietary, genetic and metabolic. Some medications are also associated with higher risk for stone formation and among them sulfadiazine, ceftriaxone, topiramate, indinavir, triamterene, furosemide, steroids and vitamin D[17]. These risk factors, along with the physical and physiological changes in urine alter the balance between promoter elements, aggregation inhibitors and growth of crystals, resulting in the formation of stones. The evaluation of risk factors and calcium oxalate calculi formation may be evaluated through methods such as the BONN-Risk Index. This index reflects an individual balance between the promoters and inhibitors of the crystallization processes ongoing in the whole native urine[18,19]. This method is simple, cost-effective and provides accurate results. Porowski *et al*[20] showed that an increased Bonn-RiskIndex reflects the risk of calcium oxalate crystallization and may indicate early metabolic disorders leading to urolithiasis in children and adolescents.

Although various aspects of the UL have not yet explained, it is known that hypersaturation of urine is indispensable for the formation of urinary stones. Therefore, crystallization starts when the urine is supersaturated for a particular solute. If the solution is unsaturated, crystals are not formed[3]. The supersaturation depends on the ionic strength, abnormalities of the urinary pH, decreased urine volume, inability of crystallization inhibitors (citrate, magnesium, pyrophosphate, nephrocalcin, glycosaminoglycans, *etc*.) and states of hyperexcretion of calcium, uric acid, phosphorus, oxalate and cystine[3].

At this point, is necessary to mention the Randall plaque. It initially forms at the basement membrane of the thin loops of Henle before expanding to the interstitium. Randall plaque's formation has been established as an integral part of idiopathic calcium oxalate stone disease[21]. Bouchireb *et al*[22] described 25 pediatric cases of urolithiasis and Randall plaques, pointing to a prevalence of approximately 3%.

UL in children and adolescents is associated with metabolic abnormalities identified in 30% to 84% of the cases[14,23-25]. Idiopathic hypercalciuria is the most prevalent metabolic disorder in pediatric patients[9-12,14,23-25]. Besides hypercalciuria, hypocitraturia is also common and is the second most prevalent metabolic disorder in childhood UL[9,26-29]. Idiopathic hypocitraturia may present as isolated or in association with hypercalciuria, secondary to chronic diarrhea, diuretic-induced hypokalemia, renal tubular acidosis and predisposes to UL[29,30]. Less often is hyperuricosuria,

absorptive hyperoxaluria, cystinuria, and hypomagnesiuria[14,23,24].

The association between idiopathic hypercalciuria and reduced bone mineral density has been described in adult patients[31-35] and in children[36-46]. The loss of bone mass or unsuitable gain can be harmful to growth of children, because the peak bone mass occurs in childhood and at a highest rate during adolescence[47,48]. This process should occur without interference for an individual to achieve his/her optimal bone mass. Anything affecting continually a child's bone metabolism could increase the possibility of osteoporosis and fractures during adulthood[49-51]. However, alterations in childhood bone mass acquisition may not affect bone mass many decades later in late adulthood because there is a homeostatic system that tends to return to a set point after any transient perturbation[52]. Thus, workup of idiopathic hypercalciuria necessarily involves the investigation of bone mineral metabolism and the characterization of the profile of bone changes, so the physician can act objectively in prevention and treatment[40,42,43,53].

Obesity associated with metabolic syndrome is a known risk factor for UL in adults, however, this association is not well established in pediatric patients. Kieran *et al*[54] collected obesity related data from 134 patients with urinary calculi. No difference regarding stone properties was observed when BMI was considered. Another study (by Dwyer *et al*[13]) confirmed that no tendency towards obesity was associated with stone formers. This tendency was also described by Routh *et al*[12], where no different pattern of nutritional status in both pediatric stone forming and the normal population was observed. A reasonable explanation for the different nutritional trends between lithiasic pediatric patients and adults rely probably on the distinct lithogenic profiles. Uric acid stones are more common in obese adults, whereas this etiology is relatively scarce in children[7]. Stones due to hypercalciuria are not linked to obesity and can therefore explain this particularity[13,14].

Epidemiological studies have shown that diet has a major role in the pathogenesis of UL[24,30]. Diets low in animal protein but rich in cereals contribute to formation of endemic bladder stones in children[30,55]. Moreover, a high intake of animal protein predisposes hyperexcretion of uric acid, calcium, oxalate, a hypoexcretion of citrate and reduces urinary pH, all favoring the formation of calcium oxalate calculi[30]. The association between urinary sodium concentration and the calcium excretion and, consequently, the relationship between the sodium content of the diet and hypercalciuria has been described[17,56-59]. In developed countries, high consumption of processed foods far exceeds the physiological sodium needs[57]. A study showed that chloride sodium intake induces mild metabolic acidosis and may impair bone mass, as could be a risk factor for the formation of calculi[60]. On the other hand, the high potassium intake has an inverse effect on urinary calcium, *i.e.*, reduces the excretion of urinary calcium[17,56].

The clinical and metabolic pattern of pediatric UL has changed in recent years. Thus, specific and detailed diagnostic tests are required for each child or adolescent presenting renal calculus, even if unique. Considering that every pediatric patient is metabolically active, diagnostic steps should be directed to elucidate the pathophysiology of UL in order to prevent recurrence and reduce morbidity.

SIGNS AND SYMPTOMS

A pediatric patient can be considered acute due to a stone in the ureter, or may be diagnosed as an incidental finding of an intrarenal or intravesical stone, during workup imaging in the abdomen for any other reason. In adult patients, the most frequent clinical presentation is the classical renal colic caused by displacement of calculi or clots in the urinary tract. This clinical presentation is also observed in adolescents, however, abdominal pain is the main complaint in school children[61]. Lack of specificity related to localized pain is typical of lithiasic infants and preschool children[61]. Gross or microscopic hematuria and uncharacteristic abdominal pain are much more prevalent than the classic renal colic, which appearing in only 10% to 14% of all pediatric cases[62]. General manifestations such as nausea, vomiting, anorexia and malaise may be present.

As aforementioned, hematuria, flank or abdominal pain as well as urinary tract infection (UTI) are the most common clinical presentations. Gross or microscopic hematuria appears in 30% to 55% of all pediatric UL[9,63,64] and may remain for some time before the stone appears. Recurrent UTI or unexplained sterile pyuria should raise the level of suspicion for UL and generally should lead to the suspicion of urolithiasis in younger children[64-66]. Some authors have reported that about 10% of pediatric UL have signs and symptoms of lower urinary tract dysfunction (nocturnal and/or diurnal enuresis, urgency and/or urinary incontinence, suprapubic or urethral pain)[9,67,68]. Although the pediatric UL could have many different signs and symptoms at clinical onset, long time intervals without urinary complaints may be observed in these patients. Authors refer that 15% to 25% of children with UL, specially the younger ones, are asymptomatic and require more attention[9,63].

Lower urinary tract symptoms, *i.e.*, dysuria, urine retention, enuresis, urinary incontinence and polakiuria may be associated with distal displacement of calculi. Excessive manipulation of genitalia in preschool children may be an early sign of urethral lithiasis. Urethral obstruction due to calculi migration may be even palpable in infants. This may not allow the urine flux, resulting in pain[64].

MANAGEMENT OF ACUTE PEDIATRIC UL

Laboratory and imaging tests are needed to confirm

the diagnosis. The tests performed in the acute phase are: urine routine, bacterioscopy of uncentrifuged urine, urine culture and antibiogram, plain abdominal radiography (Rx) and kidney and urinary tract USG. Usually, blood tests are not required, however, in cases with suspected acute pyelonephritis, a complete biochemical evaluation should be performed to appropriate patient monitoring and evaluation of the severity of this clinical condition.

USG of the urinary tract usually suffices regarding diagnosis. Its main advantages include lack of exposure to radiation and potential adverse effects of contrast media, *i.e.*, in computed tomography (CT) and intravenous pyelography. These, however, are indicated in specific cases in which USG was not sufficient for a clinical decision concerning the intervention. The need of sedation is another disadvantage of CT in pediatric patients[64,69]. Calculi migration may also be followed by sequential USG, which is another advantage of this method[64]. Although the noncontrast CT scanning is considered the gold standard test for the UL diagnostic, it is a costly procedure and not always available. When obstruction is a concern or if anatomic details are necessary, the use of contrast agents may be used.

Type and stone dimension are directly related to success in diagnosing urinary stones and their position within the urinary tract. Diagnosis of small calculi depends on operator experience, but even a lithos with a diameter of just 2 mm can be observed and its position correctly described by experienced professionals[64].

Urinary stones migrating within the renal collecting system can cause pain or infection in a partially or completely obstructed urinary tract. Pain is intense and requires immediate and effective care. It is due to stimulation of receptors during dilatation of the urinary system and release of pain mediators through to local irritation and swelling of the wall of the renal pelvis or ureter. The use of nonsteroidal anti-inflammatory drugs (NSAIDs) may be indicated as first choice, due to their higher benefits in this situation. Ureteropelvic consequences of the acute attempt to eliminate stones, such as ureteral oedema, increased peristalsis and pelvic pressure, may be effectively alleviated by nonsteroidal anti-inflammatory drugs through inhibition of prostaglandin synthesis. Hospital readmissions and new pain episodes may be avoided through these drugs, but time until complete elimination or even the likelihood of stone passing appears to be unaffected[70]. During its use, renal function should be monitored due to the risk of nephrotoxicity. Other antispasmodic and/or analgesic drugs that could be used for this acute pain control are: n-scopolamine butylbromide, amitriptyline, calcium channel blockers, steroids, morphine and analogues used in cases of intractable pain and alpha-1 blockers (*e.g.*, tamsulosin). The direct effect of alpha-1 blockers on pain is still controversial, but it is probably related to relief of ureteral spasm and promotion of stone expulsion[71].

During the acute phase, hydration should be increased after the diagnosis of a migrating calculus, considering it may be eliminated. The increased urine flow will be guaranteed by oral or parenteral hydration in cases with severe vomiting, diarrhea or lack of oral acceptance.

Adequate urinary flow is essential to prevent supersaturation of calcium oxalate and phosphate as well as uric acid. Urine flow equal to or higher than 1 mL/kg was shown by Lande *et al*[72] to be efficacious as protector against kidney stone formation. This water intake should be distributed throughout the 24 h, and should not exceed two liters. Clinical, laboratory and imaging evaluation should be done systematically at the patient with a migrating calculus. This interval depends on the severity of the clinical picture. The patient should be instructed to observe the elimination of the stone, because it may occur even without pain. About 60%-70% of calculi will be spontaneously eliminated and the size and characteristics of the surface limit their passage. The waiting period for stone migration without affecting the kidney function is six weeks[73,74]. After this period, it is advisable the referral to an urologist.

UL's presence does not necessarily imply surgical removal and there are criteria that help the decision. A surgical approach may be considered in cases of intractable pain, obstruction or associated infection. Indications for calculi removal in the proximal ureter include: calculi with a diameter > 5 mm; calculi with diameter < 4 mm associated with complete obstruction, urosepsis, solitary kidney, renal function deterioration, intractable symptoms, and no migration of the calculus for six weeks. In cases with involvement of the distal ureter, the indications for surgical removal of the calculus are: calculi with diameter > 7 mm; calculi with diameter < 6 mm associated with complete obstruction, urosepsis, solitary kidney, renal function deterioration, intractable symptoms, and no migration for six weeks. Therefore, the management of the stone is related to its location and its effect on the kidneys. Therapeutic options for stones that do not progress include: extracorporeal shockwave lithotripsy (ESWL), endoscopic lithotripsy with ultrasound, and percutaneous nephrolithotomy and open pyelolithotomy. ESWL can be used for treatment of children with stones and is safe with minimal complications[75,76].

Recently, Long and Srinivasan showed a significant improvement in management of pediatric UL with the miniaturization of both ureteroscopes and percutaneous nephrolithotomy equipment. These new technology possibilities have facilitated the access to the entirety of the urinary tract and have made ureteroscopy a first-line therapy option along with shock-wave lithotripsy for kidney and ureteral pediatric stones[77]. Nevertheless, larger studies with long follow-up time are required.

MANAGEMENT OF NON-ACUTE PEDIATRIC UL

After resolution of the acute phase, considering stone

elimination or removal by any technique, the pediatric patient will be conducted to the metabolic evaluation. All pediatric patient is metabolically active and, as already mentioned, rates of metabolic abnormalities in pediatric stone formers have been quoted as 30% to 84% of all cases[14,23-25]. The high recurrence rate is considered a major issue in pediatric urolithiasis. Lack of treatment results in a 50% recurrence rate within 7 years after the first colic episode[71,78]. Milliner and Murphy reported that 221 children have developed one or more kidney stones in mean follow-up of 59 mo[79]. Schwarz *et al*[80] showed a recurrence rate of UL in children equal to adults. Whereas all pediatric patients are metabolically active and that the recurrence rate is high, the metabolic study is always indicated in the pediatric UL.

All evaluations should be performed at least one month after diagnosis of the stone(s) while participants were asymptomatic and on their usual diet, normal fluid intake and physical activities[14,64]. In order to preserve the 24-h urinary sample should be used[81,82]. Pediatric metabolic testing should consist of: two 24-h urine collections analyzed for total volume, creatinine, calcium, phosphate, citrate, sodium, potassium, uric acid, oxalate; one venous blood sample analyzed for creatinine, calcium, phosphorus, uric acid, magnesium, sodium, chlorine, potassium, bicarbonate and blood gases; one random urine for urinalysis and pH. This criterion is similar to "The American Urological Association Guideline for medical management of kidney stones in adults"[83].

An adequate 24-h urine collection may be impracticable in patients without sphincter control. Random urine quantification and its proportion per mg of urine creatinine may allow the identification of the metabolic abnormality[64]. The following analytes should be quantified: oxalate, sodium, potassium, magnesium, uric acid, phosphate, citrate and calcium[64]. Qualitative determination of cystine through the nitroprusside test is acceptable, since the sensibility of the test is near to the level accepted as the limit for cystinuria. Amino acids chromatography remains, however, the gold standard for the diagnosis. When a stone is available, clinicians should obtain a stone analysis at least once. Stone composition of a single element is the exception, leading to the need of determination of the multiple components of the calculus. Despite the possibility of quantification of small amounts of a constituent (less than 1 mg, *i.e.*,) through infrared spectroscopy or X-ray diffraction, the exact stone analysis is prone to errors[64].

The metabolic diagnosis will enable appropriate treatment. Therefore, this will result in preventing the formation of new and growth of existing stones, inducing the patient to metabolic inactivation. A small percentage of pediatric patients forming urinary stones presents no metabolic abnormality[14]. Table 1 shows abnormal values for the excretion of various substances[17,81]. Interruption of the growth process involving preexisting calculi as well as development of new ones should be the goal of the medical treatment. Identification of the underlying metabolic cause, adequate treatment with supplements (potassium citrate), drugs (thiazides) and dietary modification mean prevention, and all these measures together are assigned as metaphylaxis.

To date there is no known medical treatment to determine the healing of UL. Those existing are directed to restore the biochemical and urinary physical chemistry. The UL treatment consists of long-term general measures (hydration, nutrition, physical activity) and specific measures (pharmacological intervention). Free urinary flux and adequate hydric ingestion compose the mainstay of urine supersaturation avoidance. It must be ensured a urinary flow at least 1.0 mL/kg per hour to reduce the urinary concentration[72] but ideally 2.0 to 3.0 mL/kg per hour. If there are higher expenses (insensitive and sweating loss), there should be an increase of this intake. The amount of liquid intake should be distributed throughout the day for good and constant urinary flow maintenance. About half of net quantity must be water and the other half, can be chosen by the patient (juices, teas, *etc*.). Hydric ingestion is well bellow the desired range in the vast majority of children with urolithiasis[56]. Beverage constituents should be monitored, since they can act as pro-lithiasic beverages (apple and grapefruit juice)[84] or anti-lithiasic (coffee, tea and alcoholic beverages)[84,85]. The reason for those associations is unknown.

The use of soda based beverages and urolithiasis is controversial[84,85]. Studies in adult populations showed no relation, but the discontinuation of this kind of drink was described as protective against stone recurrence in others, particularly those containing phosphoric acid[86]. For children it would be appropriate to allow the use of soda drinks only in special occasions. Severe dietary restrictions are contraindicated. First because they can hinder adherence to treatment; second, because they can determine nutritional deficiencies that may be more significant than the UL *per se* (reduced bone mineral density, height and weight loss, multiple vitamin deficiency, other). The diet should be corrected and appropriate to the child or adolescent's needs and recommended normal diet for calcium, calories and proteins according to RDA.

The ideal daily intake of sodium varies according to age: 1.2 g for 4-8 years old children, 1.5 g for those aged 9-18 years. The corresponding upper limits are 1.9 g and 2.3 g, above which health risk may be attributable[87]. Potassium is mostly provided as dairy products, vegetables and fruits. Its optimal recommendations also vary according to age: 3.8 for 4-8 years old children and 4.5 g for those between 9 and 18 years[87]. This is roughly equivalent to 3 units a day.

Monitoring of adequate ingestion of these elements can be achieved through determination of urine Na/K ratio, which should be under 2.5[88]. Higher ingestion of sodium-containing food is associated with increased natriuria, which can determine hypercalciuria, a stone predisposing condition[88]. All patients with hypercalciuria should have the Na/K ratio checked and

Table 1 Normal values for random urine and 24-h urine factors for children and adolescents

	24-h urine	Random urine corrected by creatinine		Random urine factored for GFR
Volume	⩾ 1.0 mL/kg per hour			
Creatinine	2 to 3 yr: 6 to 22 mg/kg > 3 yr: 12 to 30 mg/kg			
Calcium	< 4.0 mg/kg (0.10 mmol/kg)	Age 0-6 mo 6-12 mo 1-2 yr 2-18 yr	mg/mg; mmol/mmol < 0.80; < 2.24 < 0.60; < 1.68 < 0.40; < 1.12 < 0.21; < 0.56	< 0.10
Citrate	⩾ 400 mg/g creatinine	⩾ 0.28 (mmol/L/mmol/L)		> 0.18 (mg/L/mg/L)
Calcium/Citrate	< 0.33	< 0.33		
Na/K	⩽ 3.5	⩽ 3.5		
Uric acid	< 815 mg/1.73 m^2 BS	< 0.65		< 0.56 mg < 0.03 mmol
Cystine	< 60 mg/1.73 m^2 BS	< 0.02 (mg/mg) < 0.01 (mmol/mmol)		
Magnesium	> 88 mg/1.73 m^2 BS			
Oxalate	< 50 mg/1.73 m^2 BS < 0.49 mmol/1.73 m^2 BS	Age 0-6 mo 7 mo-4 yr > 4 yr	(mg/mg) < 0.30 < 0.15 < 0.10	
Phosphate		TP/GFR: > 2.8 and < 4.4 mg/dL[1]		

[1]Phosphate tubular reabsorption by glomerular filtration rate. GFR: Glomerular filtration rate; BS: Body surface.

natriuria considered as an important dietary factor to be modified, in case of an inadequate urinary finding. Another possible dietary intervention is the reduction of animal derived protein intake (such as red meat)[85-87]. Protein metabolism end-products result in increased acidity, which should be buffered by bone-released bicarbonate[89-91]. When the bone resorption is excessive, decreased bone mineral density and hypercalciuria may appear[89-91]. Stone formation is also associated with ingestion of other sugars (sucrose, fructose), vitamins (vitamin C), while magnesium and phytate may impair calculus formation[92].

Fats and sugars need to be avoided, because they may predispose to obesity, lead to increased incidence of hypercalciuria and hyperoxaluria associated stones. Some errors in dietary guidelines are very common as the elimination of tomatoes, dairy products, chocolate, teas, *etc*. These are held beliefs in the population and difficult to change.

Exercise must be regular, since the incidence of stones is directly proportional to physical inactivity and obesity (in adults). However, we must emphasize the care with fluid replacement after exercise so as not to encourage the concentration and urinary saturation.

PHARMACOLOGICAL INTERVENTION

Idiopathic calcium stones

Hypercalciuria: The initial approach to hypercalciuric children consists of adequate high fluid intake, low sodium diet and the recommended ingestion (RDA) of protein and calcium[46,87,93-95]. Dietary compliance is particularly difficult in children and adolescents, leading to usage of pharmacotherapy[56]. Pharmacological therapy is typically added if dietary treatment fails[93-95].

A randomized controlled trial pointed to beneficial effects of citrate use in adults with Urolithiasis[96]. Improvement of bone mineral density was also described by Pak *et al*[97] in adults with calcium oxalate stones after long-term use of potassium citrate. Modifications regarding increased urine pH as well as citrate and potassium levels were described during treatment. Urolithiasis in the pediatric age group had the prognosis definitely changed by citrate, due to a decrease in recurrence rate, growth of residual lithiasic fragments after lithotripsy and in children with hypocitraturia[98,99]. In hypercalciuric osteopenic adults, both thiazide diuretics and potassium citrate were previously demonstrated to be effective in simultaneously reversing hypercalciuria and improving reduced BMD[100,101].

The first line therapy in pediatric urolithiasis consists of potassium citrate. The main reason is the fact of being considered as a supplement, but studies with more detailed information on its effects in the pediatric population are lacking[102]. Studies by Reusz *et al*[103] and Schwaderer *et al*[104] demonstrated the beneficial effects of thiazides and/or potassium citrate on bone mineral density in children with IH. According to Srivastava *et al*[102], drug therapy should be reserved for children with symptomatic hypercalcuria and/or rare monogenic disorders. In 2012, Moreira Guimarães Penido *et al*[46] demonstrated an improvement of bone mineral density Z-score in 84 pediatric hypercalciuric patients after treatment with potassium citrate and thiazides, suggesting a beneficial effect and potential need for treatment. The use of thiazides in adult patients, albeit normocalciuric patients in many cases, still remains a

prevalent option of drug treatment. The absorption of calcium in the proximal tubule is enhanced, probably due to volume contraction[105].

Hypocitraturia: The choice of potassium citrate over the alkaline preparation is warranted because the sodium load may interfere with calcium excretion, minimizing the impact of urine citrate increase[71]. Compared to placebo, administration of citrate in hypercalciuric stone formers led to significative reduction in stone forming[106,107].

Treatment of calcium stones should include not only citrate, which may raise urinary pH and propitiate calcium stone formation, but also maintain adequate fluid intake. Initial dose for children is 0.25-0.5 mEq/kg two times a day in order to increase urinary levels to a minimum of 180 mg/g creatinine (Table 1)[17,81]. Urinary acidity should be monitored and should not exceed 6.5[108]. An important side effect of citrate is stomach pain, which can sometimes disrupt the treatment adherence[71]. Increased ingestion of citrus (*i.e.*, orange and lemon juices) may modify the profile of citrate excretion, acting as an alternative to citrate preparations[71].

Hyperoxaluria: Increased urinary levels of oxalate may be due to primary hyperoxaluria. Different mechanisms, resembling distinct enzymatic defects, lead to classification of this genetic entity into 3 forms, namely primary hyperoxaluria Ⅰ (PH1), Ⅱ (PH2) and Ⅲ (PH3). Deficient production of the enzyme alanine-glyoxylate aminotransferase by the liver is responsible for the more serious form of the illness, leading to end-stage renal disease[71]. High fluid intake, thiazides diuretics, citrate, pyrophosphates and magnesium oxide compose the mainstay treatment[17]. Liver-kidney or sequential liver and kidney transplantation are the best medical options after diagnosis is confirmed. Discussion upon the most appropriate moment of transplantation still remains.

The hepatic enzymatic defect is the hallmark of hyperoxaluria and restriction of dietary oxalate rich-food does not play a pivotal role in the treatment. Ingestion of food with high oxalate content, *i.e.*, spinach, rhubarb, brown rice, berries and dark teas should be avoided, as well as ascorbic acid. Adequate calcium intake must be encouraged[17]. It is also recommended, reducing fat intake and avoid use of vitamin C.

Hepatocytic peroxisomes are dysfunctional, leading to an increased synthesis of oxalate due to impaired glyoxylate metabolism. Vitamin B6 (piridoxine) is a cofactor of AGTX and its supplementation on a minimal pharmacological dose of 30 mg twice a day is recommended in order to achieve reduction of urinary oxalate levels (possible in up to 30% of PHI patients)[109]. PH 2 is linked to glyoxylate reductase/hydroxypyruvate reductase defficiency. PH3 is a more benign form of disease and is caused by mutations in the 4-hydroxy-2-oxoglutarate aldolase 1[110].

Another therapeutic option to enhance colonic secretion of oxalate involves probiotics. Studies with a naturally occurring bacterium, *Oxalobacter formigenes*, showed an inverse association with the presence of calcium oxalate stones. Nevertheless, degradation of intestinal oxalate also acts sinergystically with the colonic secretion, reducing blood and urine oxalate levels[111-113]. Colonization or intestinal recolonization with Oxalobacter formigenes would be an attractive therapeutic or prophylactic strategy to prevent or limit the formation of calcium oxalate stones, however, more studies are necessary[113].

Absorptive hyperoxaluria may also be idiopathic or secondary to malabsorptive disorders, *i.e.*, pancreatic insufficiency and small bowel resection. Under these circumstances, the absorption of ingested oxalate is augmented as well as the renal excretion. Another situation (which is rare in children and adolescents) that may lead to a similar physiological behavior of the gut is bariatric surgery. Restriction of oxalate intake in the forementioned conditions is primordial[17,71].

Lactic acid bacteria (LAB) are Gram-positive organisms that produce lactic acid as a final product of carbohydrate fermentation. This group includes Lactobacillus, Enterococcus, Lactococcus among others. Experimentally, LAB can degradate oxalate. However, in vivo results are contradictory. Goldfarb *et al*[114] found that lactic acid bacteria are ineffective in patients with absorptive hypercalciuria. Effective reduction in urine oxalate excretion was described by Lieske *et al*[115] in patients with secondary absorptive hyperoxaluria associated with fat malabsorption. Drugs that act primarily as phosphate binders, such as sevelamer hydrochloride, were unsuccessful in reducing oxalate absorption[116,117].

Uric acid stones: A combination of diverse factors, *i.e.*, low urine output, hyperuricosuria and abnormal reduced urine pH leads to uric acid (UA) stone formation. Notwithstanding, the main determinant of uric acid precipitation remains low pH. This factor is remarkable in adult patients (which are mainly not hyperuricosuric) and may be a biochemical manifestation of insulin resistance[71]. Alkalinization is the pillar of treatment of UA stones. Potassium citrate preparations are preferred due to a possible increased calcium excretion secondary to sodium load in sodium citrate.

Treatment of children with uric acid stones is complex due to the need of multiple interventions. The initial dose of potassium citrate is 0.5 to 1.5 mEq/kg per day and urine pH should be between 6.0-6.5. Dietary purine restriction is also indicated (seafood, small fish - especially sardines, beans, peas, chicken liver, heart, guts) when urinary urate excretion is high. When the hyperuricosuria is refractory to these measures, attempt with xantine oxidase inhibitors, *e.g.*, allopurinol may be tried (50 mg daily for children younger than 10 years

and 100 mg for older patients)[71].

Cystine stones: The transport of dibasic amino acids (*i.e.*, cystine, lysine, arginine and ornithine) is essential to maintain adequate solubility of these compounds. Defective tubular and intestinal transport of cystine leads to cystinuria, a cause of recurrent urolithiasis in up to 4% of pediatric urinary stone formers. In areas where consanguinity is high, this proportion may be even higher[14].

Cystinuric patients produce stones with a high degree of cystine content. Infrequently, mixed content with calcium salts may occur[118]. Daily excretion of 250 to more than 1000 mg leads to a permanent need for urine dilution, alkalinization and chelation. Cystine should stay in a urine suspension with particular chemical conditions: concentration under 250 mg/L and urine pH around 7. There is an apparent correlation between urine volume and cystine excretion: in order to excrete 750 mg of cystine, 3 L of urine output are necessary. Fluid intake must be constant and well distributed along the day. Potassium citrate (1.0-3.0 mEq/kg) is recommended to raise pH up to 7.0. In case of stone recurrence despite these measures, cysteine-binding compounds may be added. Modification of the chemical structure of cystine is possible through re-arrangement of disulfide bonds with thiol-binding drugs, *i.e.*, D-penicillamine and tiopronin (alpha-mercaptopropionylglycine_alpha-MPG). Resulted compounds are 50-times more soluble than original cystine. D-penicillamine as well as alpha-MPG proved to be efficient in decreasing stone formation in cystinuric patients in whom hydration and the use of alkalis showed to be ineffective[17,71].

The use of D-penicillamine must be judicious, regarding its potential side effects, such as its anti-pyridoxine effect[119]. Supplementation with pyridoxine (vitamin B6) 25-50 mg, daily, is advocated. Despite the better availability of D-penicillamine, tiopronin carries a better profile regarding incidence as well as severity of adverse reactions. Conflicting results with ACE-inhibitors (Captopril), which is a sulfhydryl agent, were already reported. The potential hypotensive effect of this drug resulted in an indication of "rescue therapy", where other measures failed[17].

The development of new techniques allowed the conceivement future of alternative treatments for cystinuria. Inhibition of the cystine transporter[120] and of the cystine crystal growth (L-cystine dimethyl ester_L-CDME) are promising measures to prevent cystine Urolithiasis[121,122]. They appear to be effective even at low concentrations, improving the safety profile of this sort of treatment. Dietary modifications, such as sodium and protein-restriction (0.8-1.0 g/kg per day), may lead to a modest decrease of cystine excretion. Once eliminated, stone analysis in cystinuric patients should be performed. Admixture with calcium salts is possible when urine pH is above 7.0[71].

CONCLUSION

The belief that pediatric urolithiasis is rare has lead to delayed etiological diagnosis in the past. The complete metabolic investigation of every infant or child with stones is mandatory. General measures involving adequate fluid intake and dietary modifications are considered general metaphylaxis for all kind of stones. Novel treatment modalities are scarce and the challenge of treating certain types of stone-forming diseases, *i.e.*, cystinuria, still remains.

Additionally, hypercalciuria has been evaluated in many studies during the last decade. Emphasis was laid mainly on the effects of dietary modification, alkali use (particularly potassium citrate) and thiazides, regarding calcium stone formers. However, more studies are warranted to compare pharmacotherapy and dietary changes, single *vs* combination therapies, among others. New approaches such as the use of probiotics like *Oxalobacter formigenes*, which act as oxalate-degraders, appear to be promising in calcium oxalate stone formers. However, the results are not consistent[114,115]. Future alternative treatments of hyperoxaluria involve upregulation of intestinal secretion through the increase of the anion transporter activity (S1c26a6)[118]. Studies on the pathogenesis of pediatric urolithiasis and the potencial pathogenic role of Randall's plaque and the tubular retention of crystals are currently on the way[108].

Individualized approaches to stone forming conditions will be available in a near future and will allow the start of early and adequate treatment to prevent recurrence, reduce morbidity and prevent progression to end-stage kidney disease[2,3,17].

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FRONTIER

Update on immunoglobulin A nephropathy, Part I: Pathophysiology

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Abstract

Immunoglobulin A (IgA) nephropathy is one of the most common glomerulonephritis and its frequency is probably underestimated because in most patients the disease has an indolent course and the kidney biopsy is essential for the diagnosis. In the last years its pathogenesis has been better identified even if still now several questions remain to be answered. The genetic wide association studies have allowed to identifying the relevance of genetics and several putative genes have been identified. The genetics has also allowed explaining why some ancestral groups are affected with higher frequency. To date is clear that IgA nephropathy is related to auto antibodies against immunoglobulin A1 (IgA1) with poor O-glycosylation. The role of mucosal infections is confirmed, but which are the pathogens involved and which is the role of Toll-like receptor polymorphism is less clear. Similarly to date whether the disease is due to the circulating immunocomplexes deposition on the mesangium or whether the antigen is already present on the mesangial cell as a "lanthanic" deposition remains to be clarified. Finally also the link between the mesangial and the podocyte injury and the tubulointerstitial scarring, as well as the mechanisms involved need to be better clarified.

Key words: Immunoglobulin A; Immunoglobulin A galactosylation; Genome-wide association studies; Auto antibodies; Complement in renal diseases; Mesangial linked growth factors

Core tip: For few glomerular diseases a new pathogenetic pathway has been recognized in the recent years as happened for the immunoglobulin A (IgA) nephropathy. Finding in the genetics allowed identifying several loci putative for the disease progression. Spectrometry mass studies and 3 dimension studies have allowed bettering clarifying the molecules involved at glomerular level. Molecular studies of the mesangium allowed identifying new receptors responsible for the IgA immune complexes deposition and for the binding to the mesangial structure. Finally molecular and cellular studies opened new ways to understanding the

link and the cross-talk between the glomeruli and the tubulointerstitial structure.

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INTRODUCTION AND EPIDEMIOLOGY

The immunoglobulin A (IgA) nephropathy (IgAN) is represented by a proliferation of glomerular mesangial cells jointed with the presence of IgA deposition in the mesangial area. The IgAN represents the most frequent glomerulonephritis and is represented by extremely different clinical signs and histopathologic features[1]. Because of the typical histopathological and immunological picture and the heterogeneous clinical aspects, the diagnosis of IgAN is principally based on the pathologic picture at the renal biopsy. As a consequence, the IgAN frequency is probably underestimated and the disease is the most prevalent glomerulonephritis in those countries where renal histology is more frequently used in the diagnostic algorithm. Its estimated frequency is approximately 2.5 cases/year per 100000 adults[2]. IgAN is worldwide diffused even if with different frequencies. IgAN has prevalence for male sex in the Caucasian race[3-5]. It is more frequent in some races (Asians, Hispanics, whites, American Indians) while is less frequent in other races as African and American blacks[6]. Recently new epidemiological findings, supported by the genetics, have allowed confirming that the disease incidence varies greatly according the geographical location. Indeed the IgA nephropathy is found in up to 40% of native kidney biopsies in Eastern Asia, but in less than 5% of native biopsies in central Africa[7]. In addition to the different criteria in performing renal biopsy, genetics is thought to play a significant role in explaining some geographical differences[8]. In addition, a diagnostic difficulty is represented by the huge difference in clinical presentation. Indeed, clinically, IgAN may present an asymptomatic course. Such patients are occasionally diagnosed during the work up for other diseases as hypertension or reduced glomerular filtration rate (GFR). Other patients present with macroscopic hematuria, often related to upper respiratory tract infections. In addition, some patients may present with rapidly progressive disease. Generally, the progression of the disease is related to the presence of well-known clinical risk factors that impact on the evolution of most glomerulonephritis as hypertension, proteinuria above 1 g/d, impaired renal function, smoking and obesity[9-11].

It is now clear that IgAN may be present in subjects apparently health as documented by biopsies of kidneys suitable for transplantation, or by data of autopsies not related to renal diseases[12,13]. This is further documented by the fact that most persons affected by IgAN may have a benign course or spontaneous resolution as documented by subjects followed for 10 years after diagnosis in China and in Spain[14,15]. Moreover, after transplantation of an IgAN kidney into a non IgAN recipient, disappearance of the glomerular changes has been documented, suggesting that the defect leading to IgAN is not related to the kidneys[16]. In addition, the high recurrence rates following kidney transplantation confirm that the key pathogenetic alteration in IgAN might reside outside the kidney[17].

PATHOGENESIS

As aforementioned, the IgAN is characterized by mesangioproliferative changes in the glomeruli with typical IgA deposits in the mesangial area. Deposits of IgG and C3 are also frequently present. The glomerular IgAs eluted from biopsies of patients affected by IgAN belong almost always to the IgA1 subclass and are principally polymeric. Interestingly, they are poorly glycosylated. In particular, these abnormal IgA1s exhibit a defect of galactose molecules that are normally linked to O-glycans in the hinge region. Defective glycosylated IgA1s exhibit higher blood levels in patients affected by IgAN than in normal subjects. However, this high circulatory level of galactose-deficient IgA1s (Gd-IgA1) *per se* is not able to determine the renal disease. Different steps or processes are needed for the clinical development and manifestation of the IgAN. New findings concerning these processes have been recently discovered, they are under the genetic control and genetics and immunobiology of IgAN are strictly linked[7,18].

A genome-wide association study (GWAS) performed by Gharavi *et al*[19] recently found five susceptibility loci for IgAN and allowed to identify the molecules responsible for the above mentioned steps.

These are represented by: (1) abnormal IgA1 glycosylation; (2) antibody production towards the abnormal IgA1; (3) binding of the anti-glycan antibodies to the abnormal IgA1 and consequent production of immune-complexes; and (4) deposition of the immune-complexes in the mesangial area and induction of the renal damage.

INSIGHTS FROM GENETICS

After the GWAS finding already mentioned[19], more recently a GWAS again has identified more candidate genes offering new views on the hits involved into the IgAN pathogenesis. The hits involved are represented by the antigen elaboration and presentation, the immunity mucosa-related, and the complement activation.

Antigen elaboration and presentation

The GWAS mentioned identified three different candidate loci that might impact on this pathway. They all have

Table 1 Common genetic determinants for immunoglobulin A nephropathy

Genetic locus	Genes	Function
6q21	*HLA-DRB1, HLADQA1* and *HLA-DQB1*	Class II major histocompatibility complex
	PSMB8/9 and *TAP/2*	Regulators for antigen processing and presentation
1q32	*CFHR1/3*	Modulators for complement activation and inflammation
22q12	*HORMAD2*	Unknown
17q13	*TNFSF13*	Important for B cell development and IgA isotype switching
8p23	*DEFA1*	Encoding a-defensins as a type of endogenous antimicrobial mediators
1p13	*VAV3*	Regulators for lymphocyte development and antigen presentation
9q34	*CARD9*	Participant in antigen-induced signalosome formation (CARD9-BCL 10-MALT1) and NF-κB activation
16p11	*ITGAM* and *ITGAX*	Mediators for immune cell adhesion and phagocytosis

IgA: Immunoglobulin A.

been identified on chromosome 6p21, are located on major histocompatibility complex (MHC) and are called: HLA-*DRB1/DQB1*, HLA-*DPB1/DPB2* and *TAP1/PSMB9*.

A strength link was identified in a different HLA region that includes the HLA-*DRB1-DQA1* genes[19]. The same region had been previously associated with several autoimmune diseases[20-27]. Another MHC locus has been found in the region that includes the *HLA-DPA1*, *BPB1* and *DPB2* genes.

Immunity mucosa-related: The clinical association of hematuria and infective episodes related to mucosal sites allowed to suspect that abnormalities in the IgA production might be responsible for the IgAN[28].

GWAS identified three loci involved in the mucosal pathogenesis of the IgAN. A locus is located on chromosome 17p13. This locus contains the gene *TNFS 13* that codes a proliferation-inducing ligand (APRIL). APRIL might determine the proliferation of IgA-producing cells[29,30] and APRIL serum levels may be higher in subjects affected by IgAN[31].

A second locus on chromosome 22q12 affects the circulatory IgA load and the susceptibility to develop IgAN[19]. This locus includes several genes among which the genes *LIF* and *OSM*, that encode cytokines[32]. The cytokines encoded by these genes belong to the interleukin 6 (IL-6) family and influence the immuno-regulation[33,34].

On the *DEFA* gene cluster located on the chromosome 8p23, another locus related to IgAN has been identified. It encodes the small peptides secreted by the mucosal cells with antimicrobial properties called α-defensin[35]. While α-defensin 1, 3 and 4 are secreted by neutrophil, α-defensin 5 and 6 are secreted into the gut by the Paneth cells.

Complement activation

On chromosome 1q32 is located the locus that contains the gene encoding complement factor H (CFH). GWAS found that the deletion of two CFH related genes (*CFHR3* and *CFHR1*) is a protective genetic factor for IgAN[19].

Indeed, the deletion of *CFHR3* and *CFHR1* is associated with the lacking of their products and CFHR1 is a competitive antagonist of CFH in regulating the complement activity[36]. As a consequence, the association of elevated CFH levels with the absence or low levels of CFHR1 determines a strong inhibition of complement activation. In addition, the relationship between mesangial C3 deposits and different CFH, CFH and CFHR1 levels suggests that these proteins are related to the pathogenic IgA-IC deposition[37].

In addition to the above mentioned pathways, recently performing a GWAS in 20612 patients affected by IgAN, other relevant possible genes have been found; four in *ITGAM-ITGAX*, *VAV3* and *CARD 9* and two in the HLA-*DQB1* and *DEFA* loci. Most loci carry genetic risk correlation with local intestinal pathogens, supporting the possibility that host pathogens might favor the IgAN in genetically predisposed patients[38]. All the candidate genes and their function are summarized in Table 1.

FOUR HITS FOR GENERATION OF RENAL INJURY

Several authors[7,18] have formulated the so called "four hits" pathogenesis of the IgAN. According several studies the IgAN pathogenesis is multivariate and implies the co-existence of several factors. Indeed, after an increase in galactose-deficient circulating IgA1 (Gd-IgA1), an antibody production against these IgA1 is essential for the disease initiation. Later on IC are formed that may deposit in the kidney and activate an inflammatory response (Table 2).

Step 1: Regulation of IgA1 glycosylation and genetic impact on galactose-deficient circulating IgA1

The IgA1 hinge region is located between the constant region domains CH1 and CH2 of the α 1 heavy chains. This region contains O-glycosylation sites composed of serine/threonine (Ser/Thr) and Proline residues. In normal condition only some of these sites are glycosylated[39,40]. A key role in the IgA O-glycosylation is exerted by core 1 β 1, 3 galactosyl-transferase (C1β3Gal-T) and its molecular chaperone core 1-1-phosphateuridyltransferase (Gal-T)-specific chaperone (Cosmc). Patients affected by IgAN have

Table 2 Summary of the four hits involved in the pathogenesis of immunoglobulin A nephropathy with distinction of the pathogenetic process (putative environmental factors involved, putative genetic factors involved, potential clinical biomarkers and potential novel therapeutic approaches)

Hit	Pathogenic process	Putative environmental factors involved	Putative genetic factors involved	Potential clinical biomarkers	Potential novel therapeutic approaches
1	Hereditary increase in circulating galactose-deficient IgA1	Potential role of mucosal exposure to infectious of dietary antigens	Strong evidence for high heritability of serum galactose-deficient IgA1 level Potential role of chromosome 22q12.2	Serum galactose-deficient IgA1 level (HAA-based ELISA)	Suppression of synthesis of galactose-deficient IgA1 Enzymatic boost of galactose transfer to IgA1 hinge- region O-glycans Suppression of sialylation of galactose-deficient O-glycans
2	Circulating antibody directed against galactose-deficient IgA1	Potential role of mucosal exposure to infectious or dietary antigens	Potential role of three MHC-II loci in antigen presentation and humoral response to galactose-deficient IgA1 O-glycans	Serum anti-glycan antibodies (dot blot assay)	Alteration of processing and presentation of galactose-deficient IgA1 O-glycopeptides Specific B-cell depletion therapy
3	Formation of pathogenic IgA1-containing immune complexes	Unknown	Unknown	Circulating and/or urinary immune complexes	Competitive blockade of immune complex formation by non-cross-linking anti-glycan antibodies or specific glycopeptides
4	Mesangial deposition of IgA1 containing immune complexes, cell activation and initiation of glomerular injury	Unknown	Protective effect of common deletion in CFHR1 and CFHR3	Circulating and/or urinary complement degradation products, or novel markers of glomerular injury	Suppression of the alternative complement pathway Targeted CFHR1/3 depletion Blocking mesangial cell signaling induced by nephritogenic IgA1-containing immune complexes

HAA: Helix Aspersa; ELISA: Enzyme-linked immunosorbent assay; CFHR3: Complement factor H receptor 3; IgA: Immunoglobulin A.

more elevated blood levels of IgA1 with the O-glycans poorly galactosylated and having either GalNac as terminal molecule or GalNac containing the secretory J component (Figure 1)[41]. A recent study, using cell lines from a human B-lymphoma documented that the T 2 helper cytokine IL-4 may control the glycosylation of the IgA[42]. Indeed, the IL-4 stimulation decreased the messenger RNA (mRNA) levels of both core the enzyme (C1β3Gal-T) and its molecular chaperone. Studies on animals confirmed the relevance of cytokines on IgA glycosylation[43,44]. O-glycan specific lectin initially allowed identifying the defective IgA1 O-glycosylation in IgAN[45]. More recently, other techniques as the liquid chromatography and the 3D mass spectrometry have allowed us to an improved understanding of the O-glycosylation process and the molecules involved[46,47].

The origin of the poor galactosylated IgA1s is still a not resolved question. Several studies have documented a significant difference in IgA1s generated in the systemic compartment with respect to the IgA1s generated on the mucosal surface[48]. Mucosal IgAs are predominantly polymeric (pIgA), while systemic IgAs are monomeric. Interestingly, in the IgAN the pathogenic IgA immune complexes (IgA-IC) principally contain poor galactosylated pIgA with the J secretory component[49].

The high serum level of mucosal-type IgAs in the IgAN patients might suggest that mucosal sites are the origin of poor galactosylated IgA1s. In contrast, in IgAN either systemic pIgAs directed against antigens typical of the mucosa and systemic pIgA plasma cells in systemic sites have been described[50,51]. Hence the hypothesis that the overproduction in the serum of poor galactosylated IgA1 might be the result of the movement of mucosal IgA1 committed B cells from the mucosa to the systemic compartment. A mucosal B cell mis-homing to systemic sites is the most likely mechanism[52].

The abnormal activity of Toll-like receptors (TLRs) might be another factor that contributes to the increased response to mucosal antigens in IgAN. Indeed, the association of increased Toll-like receptor 4 in circulating cells and signs of renal diseases have been reported by several studies[53]. Other studies examining the single nucleotide polymorphism, found an association between the TLR-9 polymorphism and the IgAN progression. This suggests that the involvement of TLR-9/MyD88 might exert its effect over the progression of IgAN[54].

Step 2: Synthesis of antibodies directed against GdIgA1

The synthesis of abnormally glycosylated IgAs is not "*per se*" enough to justify the mesangial lesions that characterize the IgAN. Indeed, comparing the IgA glycosylation of IgAs eluted from serum with those eluted from biopsy specimens we may observe that Gd-IgAs eluted from kidney biopsies are less glycosylated when compared to the glycosylation rate of serum IgAs from the same IgAN patients[55,56]. This fact highlights a GdIgA1 tropism for the mesangium which might contribute to explain the recurrence of IgA deposits on kidney allograft. Moreover, a study documented that in families affected by IgAN an abnormal glycosylation may be present both in IgAN patients and in asymptomatic relatives[57]. The latter observation confirms that the

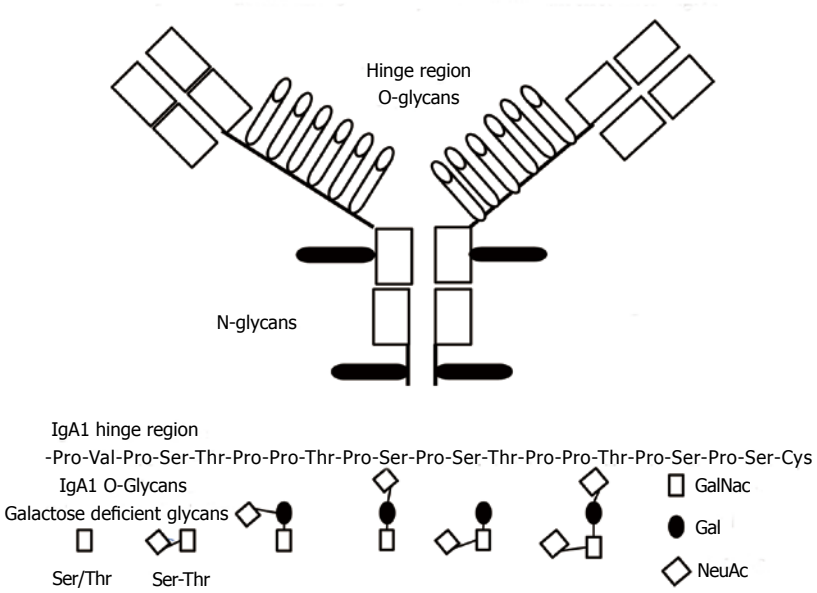

Figure 1 Immunoglobulin A1 and its hinge region with O-linked glycans (white) and N-linked glycans (black). Under the aminoacids chain of the hinge region. Sites of attachment are in bold. IgA: Immunoglobulin A.

presence of abnormally glycosylated IgAs does not "*per se*" justify the mesangial lesions and that other factors should be associated.

Recent studies suggest that auto antibodies recognizing the abnormally glycosylated IgA1s are essential in the pathogenesis of the disease[58,59]. These findings document that IgAN is an autoimmune disease due to the mesangial deposition of immunocomplexes containing GdpIgA1. Other molecules such as sCD89, fibronectin, collagen and laminin are also found in IgA1 containing immune complexes, even if their role remains to be determined[60].

Circulatory auto antibodies (IgG and/or IgA) bind to Gd-IgA1s and form large pathogenetic immune complex[61]. A better understanding of these antibodies is provided from an elegant experiment that used Epstein Barr virus (EBV-immortalized) lymphocytes from subjects affected by IgAN[62]. These B cells are able to product IgG that bind Gd-IgA1 and a subsequent analysis of the cloned chains of these IgG auto antibodies identified as their unique feature the complementary-determining region (CDR) 3 of the heavy chains[61]. In particular the third portion in CD3 is typically serine in patients with IgAN. Whether bone marrow or mucosal tissues are at the origin of IgA1s in circulating immune complexes is still now a matter of controversy. The acute onset of the disease is often accompanied by a concurrent infection of the upper respiratory tract and in this site cells are present able to produce polymeric IgA1s, typical of the pathogen IgA[29]. However, in other studies, polymeric IgA1s and J chain producing cells have been found in the bone marrow of subjects affected by IgAN[63-65]. The aforementioned mis-homing with transposition of plasma cells from the mucosa to systemic sites might explain this finding[52].

Recently Barratt *et al*[66,67] postulated the so called hypothesis of the "right antibodies in the wrong place at the wrong time". According this hypothesis, the "right" antigen represented by the mucosal derived Gd-IgA is in the systemic compartment that is the wrong place. Later on, when a large quantity of Gd-IgA1 is in circulation, a large quantity of the right antibody anti Gd-IgA1 is secreted at the wrong time.

The stimuli leading to the production of these auto antibodies remains to be explained.

A hypothesis might be that these antibodies are secreted against pathogen cell surface GalNac-containing glycoconiugates cross-reacting with GdIgA1, realizing a molecular mimicry[68]. A prevalence of IgA1 autoantibody response[61] anti GdIgA1 may justify the fact that some patients have only IgA1 antibody in the glomeruli[69].

In conclusion, a portion of the IgA1 molecules secreted by the plasma cells in patients affected by IgAN is Gal-deficient and is identified by the anti-glycan IgG (or IgA1) antibodies[70]. The formed IC, due to their size, cannot reach and bind the asialoglycoprotein receptor (ASGP-R) in the liver, and be metabolized. Moreover, the terminal GalNac residues, which might interact with the ASGP-R, are covered by specific antibodies that prevent such interaction[71]. As a consequence, a larger fraction of the IgA-IC may reach the glomerular capillaries overlying the mesangium.

Summarizing the two steps above described in the IgA pathogenesis, in the first time a high quantity of under-galactosylated IgA1 is present in the blood. At this regard several points remain to be clarified. The plasma cell defect is inherited or acquired? In addition, to justify the systemic presence of GdIgA1 high quantity, a plasma cell mis-homing from mucosa to systemic sites is needed or not?

In the second step we have the IgG auto antibodies production anti the GdIgA1. At this regard several questions remain to be answered.

Are these antibodies the result of a molecular mimicry triggered by infections?

In addition, are these antibodies the result of a Toll-like receptor polymorphism?

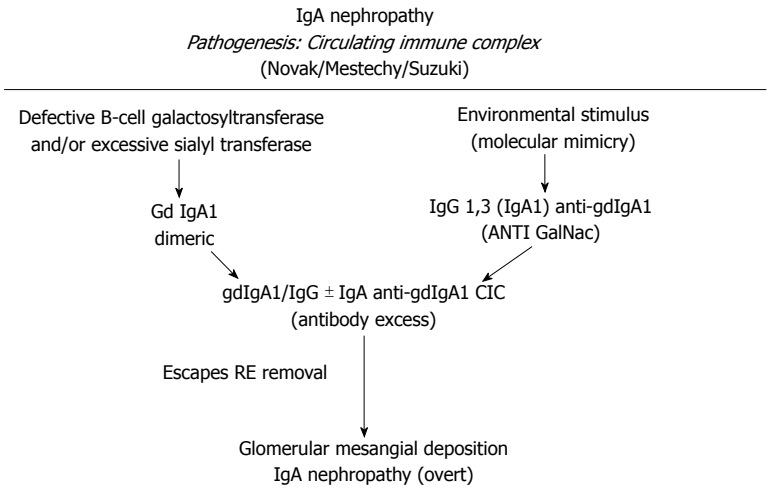

Figure 2 Schematic representation for the possible pathways involved in the generation of a circulating immune complex pathogenesis for the immunoglobulin A nephropathy. CIC: Circulating immuno complex; Gd IgA1: Galactose deficient immunoglobulin A1.

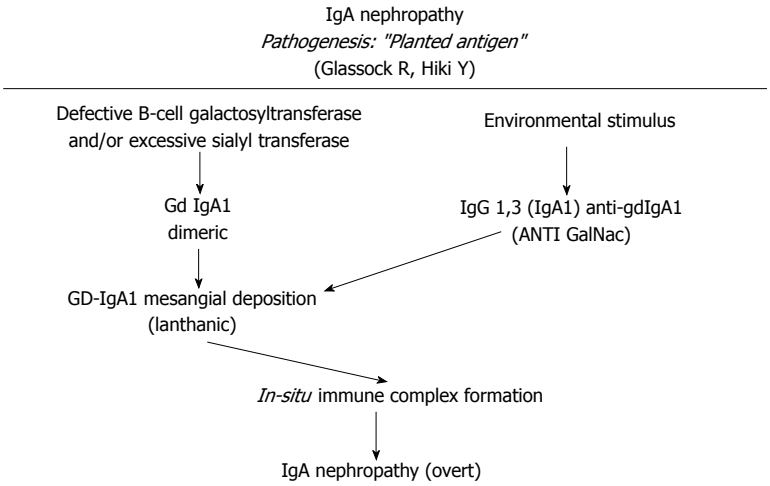

Figure 3 Schematic representation for the possible pathways involved in an *in situ* pathogenesis for the IgA nephropathy. IgA: Immunoglobulin A; Gd-IgA1: Galactose deficient immunoglobulin A1; ANTI GalNac: ANTI N-acetylgalactosamine.

Finally, is there a genetically determination induced by somatic mutations in the IgG heavy chains?

Step 3: Formation of pathogenic immune complexes containing IgA and their mesangial deposition

Circulating anti-glycan auto antibodies recognize Gd-IgA1 and pathogenic immune complexes are formed as a consequence. IgA1s must be within an immune complex to activate the mesangial cell proliferation; indeed not complexed GdIgA1s do not stimulate the proliferation of mesangial cells[72-74]. Additional components from serum need to be present to form stimulatory complex[72].

Several models have been proposed to explain the IgA1 immune complex depositions on the mesangial cells. Immune complexes containing IgA1/IgG or IgA could directly deposit on mesangial cell[61,62,75]. Another hypothesis is that poor glycosylated IgA1 might be present in the mesangial area as lanthanic deposits and later newly generated anti-glycan antibodies might bind and realize immune complexes in situ capable to activate mesangial cells[3] (Figures 2 and 3). In addition, a further theory is that self-aggregated Gd-IgA1s might deposit or bind to specific receptors in the mesangial area realizing "planted" depositions that are not pathogenic "*per se*". When an exposure to similar environmentally derived antigens occurs, an auto-antibody production and the involvement of several mediators cascade lead to the disease[76,77]. Further studies led to identify the relevance of IgA receptors (IgA-R) in the deposits. Several IgA-Rs have been recognized[78-80]. In the IgAN pathogenesis, two IgA-R expressing cells have been principally involved: (1) mesangial cells which have been documented to be implicated in kidney injury; and (2) myeloid cells (essentially kidney infiltrating macrophages) which have been documented to modulate the extent of the inflammatory response. Studies from several groups have ruled out that the mesangial expression of receptors as ASGP-R, CD 89 and pIgAR might have a role[81]. In addition, although on the mesangial cells is located the Fc alpha mu receptor (Fcα/μR), neither IgM nor the recombinant Fcα/μR inhibit the IgA1 binding to mesangium[82]. The transferrin receptor (TfR1 or CD71) has been identified as the mesangial IgA1 receptor implicated and the pIgA1 binding to TfR1 induces mesangial cells activation[83,84]. Moreover, TfR1 co-localizes with deposited IgA in the IgAN biopsies[85]. The same group documented that both glycosylation and size of IgA1 are relevant factors for the TfR-IgA1 interaction. This probably is the first step of the

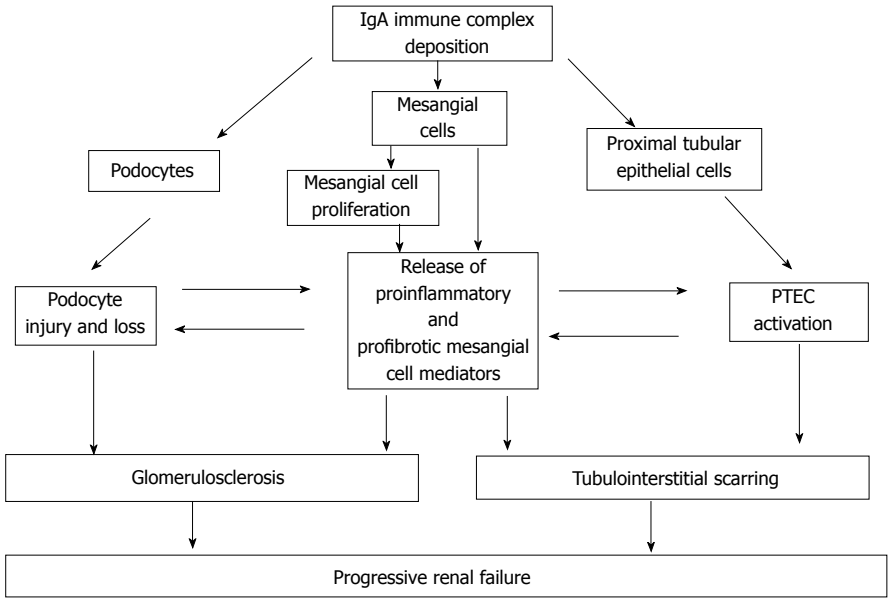

Figure 4 Pathways to glomerular damage and tubulointerstitial injury in Immunoglobulin A nephropathy. Deposition of IgA-ICs in the mesangium leads to activation of mesangial cells, triggering mesangial cell proliferation and release of proinflammatory and profibrotic mediators. Podocyte loss accentuates glomerular scarring and filtered mesangial cell-derived mediators and IgA-ICs stimulate PTEC to adopt a proinflammatory and profibrotic phenotype, which in turn drives tubulointerstitial scarring. IgA: Immunoglobulin A; PTEC: Proximal tubule epithelial cells.

IgAN injury[86]. Finally, as an alternative hypothesis, has been proposed that the soluble form of the Fcα receptor (sCD89) might "*per se*" generate complexes with Gd-IgA1[87]. The formation of circulating Gal deficient pIgA1 immune complexes (IgA1-CIC) induces an alteration in the interaction between IgA and CD89 that are found in the mesangial deposits and is implicated in the diseases exacerbation through the activation of pro-inflammatory cytokines and the secretion of chemokines[88,89].

Step 4: Mesangial and glomerular cells activation, glomerular injury and fibrosis

The renal damage after mesangial cell deposition of immune complexes may be distinguished into three phases: (1) mesangial cell activation; (2) podocyte injury; and (3) tubulo-interstitial scarring. All these kinds of renal injuries may be mediated by different pathways as the complement activation, the innate immunity activation, and the non-complement mediators of IgA nephropathy (Figure 4).

Mesangial cells are activated by IgAs and may transform into inflammatory and fibrotic cells after the exposure to IgA-IC. Indeed, the mesangial cells binding to IgA-IC containing poorly galactosylated IgA1 triggers the proliferation and the programmed death of the mesangial cells. In addition a reduced synthesis of vascular endothelial growth factor (VEGF), an abnormal integrin production and an abnormal production of extracellular matrix increase the renal damage[86,90-92].

The role of complement in the activation of the mesangial cells in IgAN has been recently reviewed by Maillard *et al*[93]. In IgAN, polymeric or aggregated IgA1s, principally Gd-IgA1s, may stimulate the alternative complement pathway, determine the C3 deposition and the production of the complement terminal complex (C5b-C9)[94-97]. Similarly the same pathways might be locally stimulated on the mesangial cells by polymeric Gd-IgA1s as well as by the secretory IgAs (SIgA)[97]. In addition, the complement involvement by the alternative and the mannan binding lectin (MBL) pathways may be activated by the polymeric IgA1, thus participating to the pathogenesis of the IgAN and characterizing a more severe disease.

Components of the innate immunity are similarly involved in the pathogenesis of the IgAN. Some studies have documented that the TLRs are able to induce the IgA production by the B cells[98]. It has also been documented a link between the TLRs stimulation, the overproduction of Gd-IgA1s, the more aggressive aspects of IgAN and the activation of the enzyme and molecules involved in the IgA glycosylation[54,99,100].

Mesangial injury: IgA1-ICs containing secretory IgA with a high sialic acid content and anionic IgA-IC stimulate mesangial cells, may stimulate the p42/p44 mitogen activated protein kinase, activator protein 1, and NF-kB signal transduction. Similarly other chemokines and cytokines are up-regulated, among which the IL-6, the transforming growth factor β (TGFβ), the tumor necrosis factor α (TNFα) and the monocyte chemo attractant protein (MCP-1)[101-103].

The platelet derived growth factors (PDGFs) are among the growth factors most involved in the mesangial cell proliferation[104]. The PDGFs are five potent mitogens and chemoattractants that play important roles in the mesangio-proliferative diseases, principally in the IgA nephropathy. The PDGF-BB and PDGF receptors (PDGFR-β) are over expressed in the experimental

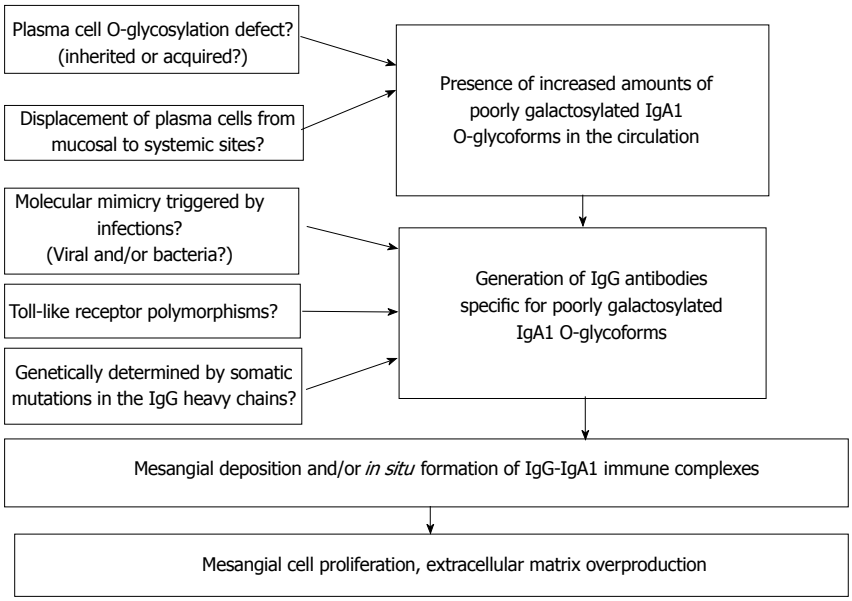

Figure 5 Doubts and different possibilities in generating the first two steps. IgA: Immunoglobulin A; IgG-IgA1: Immunoglobulin G-Immunoglobulin A1.

mesangio-proliferative nephritis and in the human IgAN[104].

The pathogen IgA1s are also able to activate the renin-angiotensin-aldosterone system (RAAS) intrarenally[105]. This system too is involved in the IgAN injury.

Finally a protective affect against mesangial cell activation by IgA-IC is exerted by bone morphogenetic protein 7 (BMP-7)[106]. BMP-7 suppresses TNFα induced synthesis of proinflammatory cytokines, and has an anti-fibrotic effect through antagonism of the cellular actions of TGF β[107,108].

Podocyte injury: Podocyte necrosis and detachment from the glomerular basal membrane has been reported in the IgAN and the degree of podocytopenia is closely related to the increasing severity of glomerular lesions[109]. Nephrin is a key component of the podocyte slit diaphragm and is essential for the maintenance of an intact glomerular filtration barrier. Interestingly, nephrin mRNA and extracellular nephrin expression are reduced in the IgAN[110,111]. In addition, evidence from in vitro studies suggests that the podocyte injury in the IgAN is likely to be mediated by both the mesangial cell derived soluble mediators and by the direct contact with filtered IgAs[112,113].

It has also been documented that IgA1-IC in IgAN patients might up regulate the production of CXCL1 and TGF beta from the mesangial cells. CXCL1 and TGF beta might exert a synergistic effect upon the podocytes, inducing podocyte dysfunction and podocyte death[114].

Tubulointerstitial scarring: It has until recently been thought that the mechanisms of the subsequent tubulointerstitial injury were generic and common to all forms of chronic kidney diseases. Recent studies have documented that specific mechanisms are operating in the IgAN.

Among the factors that are up regulated after stimulation of mesangial cells, the TGFβ is the most involved in generating fibrotic tissue related to the cell damage. It is generated by growth factors involved in the connective tissue generation[115,116]. In addition to producing fibrotic tissue, TGFβ also acts favoring the transformation of the tubular cells into a fibrotic phenotype.

With an increasing damage to the perm selective barrier, increasing amounts of high-molecular weight IgA-ICs enter the urine. In IgAN these IgA-ICs are enriched by GdIgA1s that reflects their localization within the mesangium[117]. Therefore the proximal tubule epithelial cells (PTEC) are constantly exposed to filtered IgA-ICs because of the impairment of the glomerular size barrier.

Recent studies suggest that there may be a direct and specific interaction between filtered IgA-ICs, mesangial cell-derived mediators, and PTEC. Indeed, when the mesangial cells are activated by the IgAs, they release mediators, which subsequently lead to up-regulation of angiotensin Ⅱ production, inflammatory changes and apoptosis of PTECs[118]. Similarly, the mesangial cell derived TNFα is known to activate the tubular cells inducing pro-inflammatory mediators, thus establishing an IgAs specific glomerular-tubular cross-talk[119]. There is also convincing in vitro evidence that the filtered mesangial cell-derived mediators also may determine a proinflammatory and profibrotic transformation of PTEC[118-121]. As a consequence, an IgA specific pathogenetic effects might exist, which further accelerate the progressive lost of renal function. In addition, a recent study documents that[122] IgAN might be associated to the Epithelial-mesenchimal

transition and the apoptosis of renal tubular epithelial cells favoring the renal scarring.

CONCLUSION

Figure 5 summarizes the four steps along which the IgAN pathogenesis develops. Every step is far to be completely understood. From one side several questions remain to be answered, from the other side new disease specific therapeutic approaches might be opened.

The first step is the presence in circulation of elevated levels of Gd-IgA1. This step is under the control of several putative genetic factors. Still opened questions are whether the IgA under-glycosylation defect is genetically or environmentally generated and whether there is an abnormal plasma cell mis-homing from mucosal to systemic areas.

Second step is represented by the production of auto antibodies against Gd-IgA1. GWAS has identified the possible role of MHC-Ⅱ loci involved either in the process of antigen elaboration or in the antibody response. Open questions at this regard are whether a molecular mimicry is triggered by infections and which is the role of Toll-like receptors and their polymorphisms. In addition, is not clear whether a somatic mutation genetically determined for the IgG heavy chains structure does exist.

The third step is the production of pathogenic immune complexes containing IgA and their following localization on the mesangium. Open questions are the exact composition of the immune complexes and whether there is a circulating immune complexes deposition or the Gd-IgA1 are already present in the mesangium as a lanthanic deposition followed by binding of auto antibodies.

The fourth step is represented by the renal injury IgA induced and caused by the mesangial IC deposition. The role of podocyte injury in determining the renal lesions is far to be clarified and similarly the tubulointerstitial scarring pathogenesis seems to be peculiar of IgAN, but still not completely clarified.

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REVIEW

Current management of autosomal dominant polycystic kidney disease

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Abstract

Autosomal dominant polycystic kidney disease (ADPKD), the most frequent cause of genetic renal disease affecting approximately 4 to 7 million individuals worldwide and accounting for 7%-15% of patients on renal replacement therapy, is a systemic disorder mainly involving the kidney but cysts can also occur in other organs such as the liver, pancreas, arachnoid membrane and seminal vesicles. Though computed tomography and magnetic resonance imaging (MRI) were similar in evaluating 81% of cystic lesions of the kidney, MRI may depict septa, wall thickening or enhancement leading to upgrade in cyst classification that can affect management. A screening strategy for intracranial aneurysms would provide 1.0 additional year of life without neurological disability to a 20-year-old patient with ADPKD and reduce the financial impact on society of the disease. Current treatment strategies include reducing: cyclic adenosine monophosphate levels, cell proliferation and fluid secretion. Several randomised clinical trials (RCT) including mammalian target of rapamycin inhibitors, somatostatin analogues and a vasopressin V2 receptor antagonist have been performed to study the effect of diverse drugs on growth of renal and hepatic cysts, and on deterioration of renal function. Prophylactic native nephrectomy is indicated in patients with a history of cyst infection or recurrent haemorrhage or to those in whom space must be made to implant the graft. The absence of large RCT on various aspects of the disease and its treatment leaves considerable uncertainty and ambiguity in many aspects of ADPKD patient care as it relates to end stage renal disease (ESRD). The outlook of patients with ADPKD is improving and is in fact much better than that for patients in ESRD due to other causes. This review highlights the need for well-structured RCTs as a first step towards trying newer interventions so as to develop updated clinical management guidelines.

Key words: Autosomal dominant polycystic kidney disease; Native nephrectomy; Cyst decortication; Kidney transplantation; Hypertension; Drug therapy; End stage renal disease; Extrarenal manifestatation; Total kidney volume

Core tip: Autosomal dominant polycystic kidney disease

(ADPKD), the most frequent cause of genetic kidney disease affecting approximately 4 to 7 million individuals worldwide (7%-15% of patients on renal replacement therapy), is a systemic disorder mainly involving the kidney but cysts can also occur in other organs such as the liver, pancreas and arachnoid membrane. This paper discusses radiological evaluation of ADPKD, necessity for screening for intracranial aneurysms and current treatment strategies include reducing: cyclic adenosine monophosphate levels, cell proliferation and fluid secretion. It further discusses the role of surgery in managing ADPKD patients and highlights areas of new research.

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INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is a monogenetic disorder characterised by bilateral renal cysts and possibly kidney pain, urinary tract infection, haematuria, nephrolithiasis, hypertension and progressive renal failure due to progressive enlargement of cysts and fibrosis[1,2]. It is a leading cause of end-stage renal disease (ESRD) and the most common inherited kidney disease[3,4]. ADPKD is a systemic disorder mainly involving the kidney but cysts can also occur in other organs such as the liver, pancreas, arachnoid membrane and seminal vesicles[5]. In contrast to ADPKD, autosomal recessive polycystic kidney disease produces kidneys which are hugely enlarged due to multiple cysts, hypertension, and congenital hepatic fibrosis characterised by dilated bile ducts and portal hypertension[6]. Autosomal recessive polycystic kidney disease and other cystic lesions of the kidneys present a different set of challenges and will not be discussed further in this review.

The work of Thong *et al*[3] suggest that the age of diagnosis of ADPKD and mean kidney length can be used to predict ESRD at least 10 years in advance and thus enable patients at higher risk of developing it to be identified early for treatment. The quality of life (QOL) of patients with ADPKD is indirectly linked to the total kidney and liver volume by virtue of its close correlation with abdominal distention that exerts an important influence on QOL. Other associated symptoms of ADPKD such as pain, sleep disturbance, heartburn, fever, gross hematuria and anorexia (though not always correlated with total liver and kidney volumes) affected QOL[7]. Improving these symptoms and reducing abdominal distention can enhance the QOL of patients.

ADPKD remains a therapeutic challenge as effective treatment to retard the growth of kidney and liver cysts has not been achieved despite decades of basic and clinical research[4,8]. The Spanish Working Group on Inherited Kidney Diseases, in the absence of good evidence, only made recommendations relating to management of hypertension, pain, cyst infections and bleeding, extra-renal involvement including polycystic liver disease, intracranial aneurysms, ESRD, and management of children with ADPKD; but none on specific ADPKD therapies[9]. There are no clinical guidelines on management of this common cause of ESRD. The aim of this review is to present a concise account of the current status of managing patients with ADPKD including the surgical options.

EPIDEMIOLOGY

ADPKD is the commonest cause of genetic renal disease affecting approximately 4 to 7 million individuals worldwide and accounting for 7%-11% of patients on renal replacement therapy (RRT) in Europe[5,10-13] and about 10%-15% of patients requiring dialysis in the United States[14].

According to the Danish National Registry on Regular Dialysis and Transplantation, 693 patients with ADPKD reached ESRD between 1990 and 2007. Analysis of the data showed that progression to ESRD increased from 6.45 per million people in 1990-1995 to 7.59 per million people in 2002-2007. In addition, the mean age at onset of ESRD increased by 4.7 years and patient survival improved by 38%[15]. In a study of the Catalan registry (1984-2009), Martínez *et al*[11] found 1586 (7.9%) of 20033 ESRD patients with ADPKD. The survival rate of ADPKD patients on renal replacement therapy was significantly higher than that of non-ADPKD patients. Review of the United States Renal Data System shows that of the 375152 patients initiated on ESRD therapy between 1992 and 1997, 5799 (1.5%) had polycystic kidney disease. As with the Catalan registry, patients with polycystic kidney disease had lower mortality compared to patients with other causes of ESRD[16].

In a retrospective comparison of clinical characteristics of 837 patients with ADPKD between 1961-1990 and 1991-2011, Helal *et al*[17] reported an earlier age of disease diagnosis (29 years *vs* 35 years), lower mean blood pressure (129/82 mmHg *vs* 142/91 mmHg), better estimated glomerular filtration rate (eGFR) (63.6 mL/min *vs* 44.6 mL/min), and more use of renin-angiotensin-aldosterone system (RAAS) inhibitors (42.5% *vs* 13.6%) during the later period.

PATHOLOGICAL CONSIDERATIONS

In 85%-90% of cases, ADPKD results from a mutation in the *PKD1* gene, and the other 10%-15% of cases are accounted for by mutations in *PKD2*. *PKD1* and *PKD2* encode for polycystin-1 and polycystin-2 proteins (polycystin signaling complex) which regulate different signals including 3',5'-cyclic adenosine monophosphate (cAMP), mammalian target of rapamycin (mTOR) and

epidermal growth factor receptor pathways. Abnormal activation of these signals causes an increased cell proliferation which is an important component of this disease[18]. ADPKD is characterized by the progressive development of cysts in renal tubular epithelial cells that gradually compress the parenchyma and compromise renal function. There is considerable interest in the primary cilia as a site of the proteins that are involved in renal cystogenesis in ADPKD[19,20]. Research on primary cilia has increased significantly during the last decade[21]. Cyst enlargement is thought to result from increased fluid secretion; and abnormal cell replication by the epithelium lining the cyst[22]. The processes underlying the decline in renal function include disruption of glomerular filtration and urine concentrating mechanisms, coupled with compression of adjacent nephrons in the cortex, medulla and papilla. Cyst-derived chemokines, cytokines and growth factors cause fibrosis that is similar to development of other progressive ESRD[23]. This concept that attributes important roles to tubular cell ciliary functioning, cell proliferation and fluid secretion, alterations in levels of intracellular calcium, cAMP and activation of cellular kinases, including mTOR[12] is the basis of potentially effective treatments discussed below.

Animal studies indicate that excessive activation of the alternative complement pathway is associated with ADPKD progression, probably mediated through cyst-lining cell proliferation, tubulointerstitial inflammatory cell infiltration and fibrosis. Regulating activation of the complement system might represent a new treatment strategy for ADPKD[24]. Cyst expansion causes ischemia within the kidney and activation of RAAS leading to the development and/or maintenance of hypertension. The features of disease progression in ADPKD include increasing total kidney volume (TKV), hypertension, cardiovascular complications, proteinuria and progression to ESRD[25].

Extrarenal manifestations

Apart from renal cysts, patients often have extra-renal disease encompassing cysts in the liver (94%), seminal vesicle (40%), pancreas (9%), arachnoid membrane (8%), and spinal meninges (2%); and connective tissue abnormalities such as mitral valve prolapse (25%), intracranial aneurysms (8%), diverticular disease (20%-25%) and abdominal hernia (10%); hypertension and left ventricular hypertrophy[26-28]. Recognition of extrarenal manifestations (ERM) reduces diagnostic uncertainty and may influence choice of treatment option[29].

Cardiovascular system

Other cardiovascular abnormalities include aortic aneurysms, arachnoid aneurysms, cerebral artery dolichoectasia, mitral regurgitation, aortic insufficiency, and tricuspid regurgitation. There is evidence to suggest that ADPKD is associated with an increased incidence of coronary aneurysms and dissection[30,31]. Cardiovascular complications are responsible for 80% more deaths in ADPKD than ESRD. Furthermore, intracranial aneurysms affect 4%-41.2% of ADPKD patients, with a risk of rupture about five times higher than in the general population[2,32].

Hypertension: Hypertension develops in about 50%-70% of patients with ADPKD and is associated with an increased risk of progression to ESRD. Stimulation of RAAS plays a significant role in the development of hypertension. The presence of cardiovascular changes such as carotid intima-media thickness, and arterial stiffness in young normotensive patients with ADPKD suggests that cardiovascular involvement starts early in these patients. Early diagnosis and treatment of hypertension with RAAS inhibitors, has the likely benefit of reducing the cardiovascular complications and slowing the progression of kidney dysfunction[33].

Left ventricular hypertrophy: Left ventricular hypertrophy (LVH) has been recognised as an early complication in patients with ADPKD. LVH is associated with arrhythmias, congestive heart failure, and increased cardiac mortality. Observational studies using echocardiography have estimated the prevalence of LVH in adults to range from 20%-40%[34]. The recently observed decline in the incidence of LVH may be as a result of earlier detection, treatment and more rigorous control of blood pressure including the increasing use of RAAS antagonists.

Miscellaneous

Another cited ERM is thoracic aortic dissection, which can cause high mortality and morbidity rates[35]. Also pulmonary dysfunction should be recognised as one of the extrarenal complications of ADPKD due to the demonstrable improvement in lung function following renal transarterial embolism[36].

Complications of ADPKD

Complications in ADPKD usually result from kidney involvement and include cyst bleeding and cyst infection. However, serious extrarenal features such as subarachnoid haemorrhage can also occur[5].

Cyst infection/Urinary tract infection Idrizi *et al*[37] studied 180 patients with ADPKD (2003 to 2008) and reported urinary tract infections caused by gram negative enteric organisms in 60% (108 patients). The episodes of isolated cyst infections (negative urine culture and no urinary white blood cell casts) were more frequent than those of acute or chronic pyelonephritis (urinary sediment containing white blood cell casts). The key challenge is how to distinguish between cyst infection and acute or chronic pyelonephritis. Hepatic pyocyst is an uncommon but potentially life-threatening complication of ADPKD. With extensive hepatic cystic disease, localization of a pyocyst and targeted aspiration or drainage is often a diagnostic challenge. Two ADPKD patients with recurrent gram-negative sepsis were

investigated with ^{67}Ga SPECT/CT to look for the source of infection - with accurate localisation in both cases[38].

Screening/surveillance

Ultrasonography remains the first choice imaging modality for diagnosing ADPKD[26]. However, computed tomography (CT) scanning is particularly useful in assessing pain, complex renal or hepatic cysts and in cyst aspiration[39]. New magnetic resonance imaging (MRI) methods developed by the Consortium for Radiologic Imaging for the Study of Polycystic Kidney Disease allow accurate estimates of change in TKV over time in ADPKD patients with intact renal function. PKD1 status, male sex, hypertension, reduced renal blood flow, and proteinuria are associated with increased renal volume and change in renal volume over time[25]. MRI has advantages when there is suspicion of malignancy and similar to CT, is useful in the assessment of living kidney donors[39].

Following a comprehensive literature review of articles published from 1998 to 2013, Ellimoottil *et al*[40] concluded that CT and MRI with/without contrast enhancement remained the gold standard for evaluating cystic lesions of the kidney. However, diffusion-weighted MRI and contrast enhanced ultrasound have surfaced as new tools for assessment of complex cysts. In a retrospective analysis, Israel *et al*[41] reported that findings on CT and MRI were similar in 81% of lesions. MRI may depict septa, wall thickening or enhancement leading to upgrade in cyst classification that can affect management.

Kawano *et al*[42] explored urinary biomarkers in ADPKD in human and in an animal model using gene expression analysis of the kidney from DBA/2FG-pcy mice (ADPKD model animals) to identify prospective biomarkers. Their study suggests that NGAL, M-CSF, MCP-1 are potential candidates for urinary biomarkers in ADPKD.

Intracranial aneurysms

Though the prevalence of IA is higher in patients with ADPKD than the general population (4%-41.2% *vs* 0.4%-6%), the mortality rate of aneurysm rupture is similar. Levey *et al*[43] showed that routine arteriographic screening for cerebral aneurysms in patients with ADPKD was not of significant benefit. Butler *et al*[44] reexamined this question by comparing an MRI screening strategy with a non screening strategy. Aneurysms detected by MRI screening were managed neurosurgically whereas the patients in the non screening arm received cerebrovascular care only in the event of subarachnoid hemorrhage. Taking into consideration a host of factors including the prevalence of asymptomatic aneurysms in ADPKD patients (15%); the annual incidence of aneurysmal rupture (1.6%); the morbidity and mortality rates associated with subarachnoid haemorrhage (70% and 56%, respectively); and the life expectancy of patients with ADPKD, the model predicted that the screening strategy would provide 1.0 additional year of life without neurological disability to a 20-year-old patient with ADPKD. Furthermore, a financial analysis showed that a screening strategy is likely to be more cost effective. Rozenfeld *et al*[10] performed a critical appraisal of the estimated value of screening for IA in the setting of ADPKD noting the variable length of the preclinical phase of aneurysm development and the fact that the clinical phase (symptoms to haemorrhage or death) can be quite short, they recommended only screening patients who have a family history of aneurysm or subarachnoid haemorrhage, high risk occupation, undergoing major surgery, exhibiting severe anxiety about the issue or if anticoagulation is contemplated for any reason.

Jiang *et al*[45] screened and followed up unruptured intracranial aneurysms (UIAs) and concluded that 3.0 T 3D-TOF (time of flight) MRA was feasible for UIAs follow-up in ADPKD patients. However, the risk of enlargement and rupture of UIAs in ADPKD patients was not higher than in the general population. The jury is therefore out on whether to screen ADPKD patients or not. A pragmatic way forward may be to define the population at risk and screen those.

Sixty-eight adults, pre-dialysis ADPKD patients underwent both screening for intracranial aneurysms with MRI of the brain and ambulatory blood pressure monitoring with a view to establishing an association between these in ADPKD. Ten of the 68 patients had intracranial aneurysms while 58 had none. The night time maximum diastolic blood pressure, maximum increase in diastolic BP from measurement to measurement at night, and the standard deviation of the daytime mean arterial pressure were significantly higher in patients with aneurysm. Additionally, those over 45 years of age with aneurysm had significantly worse parameters. They concluded after a series of analyses that hypertensive ADPKD patients with substantial fluctuations in BP assessed by automated blood pressure monitoring, especially those after 45 years-of-age, should become candidates for screening for intracranial aneurysms[46].

TREATMENT OF ADPKD

There is presently no effective treatment for ADPKD and management measures are focused mainly on managing the complications of the disease, not on slowing cyst development or preventing progression to kidney failure. Current treatment strategies include: lowering cAMP levels; inhibiting cell proliferation; and reducing fluid secretion[47]. Many clinical trials have been undertaken to study the effect of diverse drugs on the growth of renal and hepatic cysts, and on deterioration of renal function. The drug classes that have been tested in randomised clinical trials (RCT) include mTOR inhibitors (sirolimus and everolimus), somatostatin analogues (octreotide, lanreotide, pasireotide), and most recently, vasopressin V2 receptor antagonist,

tolvaptan. Other drugs being tested include bosutinib [sarcoma proto-oncogene Abelson murine leukaemia oncogene (SRC-ABL) tyrosine kinase inhibitor] and triptolide, a traditional Chinese herbal medication. Additional therapeutic strategies to retard cyst growth aim at blood pressure control *via* inhibition of RAAS and the sympathetic nervous system[8]. Also, targeting up or down regulated molecules in the renal epithelial cells are being tested[5].

Overactivity of both mTOR and cystic fibrosis transmembrane conductance regulator is thought to contribute to the progressive expansion of renal cysts in ADPKD. Recent research has established that AMP-activated kinase can suppress the activity of each of these proteins. Clinical AMP kinase activators such as metformin and berberine may thus have potential in the clinical management of ADPKD. The use of berberine in diarrhea may be due to the inhibitory impact of AMPK on chloride extrusion by small intestinal enterocytes[48].

Drug therapy for ADPKD

Rapamycin: He *et al*[49] conducted a meta-analysis of 4 RCTs (564 patients) regarding mTOR inhibitor therapy in patients with ADPKD investigating changes in patients' GFR, urinary protein, TKV, cyst volume, parenchymal volume, lipid profile and the frequency of adverse events. Their main findings were that though mTOR inhibitor therapy was associated with a smaller TKV than the control group, it did not slow down the decline of renal function. This agrees with the findings of a randomised, crossover study (The SIRENA Study)[50]. However, another meta-analysis of RCTs (5 studies, 619 patients) that used mTOR inhibitors to halt the progression of ADPKD failed to demonstrate any significant reduction in TKV or GFR between the TORI-treated and control groups[51]. These findings, in addition to a significantly higher level of proteinuria in the mTOR inhibitor-treated group than in the control group, were similar to those of another meta-analysis[52].

The above studies of mTOR inhibitor treatment of ADPKD showed no clear benefit on the primary endpoint of TKV or eGFR. Another trial evaluated two levels of rapamycin on the 12-mo change in (125)I-iothalamate GFR (iGFR) as the primary endpoint and TKV secondarily[53]. In a study of 30 adult patients with ADPKD randomised to low-dose rapamycin (trough level, 2-5 ng/mL; n = 10), standard-dose rapamycin trough level (> 5-8 ng/mL; n = 10), or standard care (SC group, n = 10), Braun *et al*[53] showed that patients receiving low dose rapamycin demonstrated a significantly better iGFR but without a significant effect on TKV after 12 mo.

Somotatostatin analogues: Therapy with somatostatin analogues is meant to regulate the activity of the tubular epithelial lining the cysts *via* secondary chloride transport thereby shrinking the renal cysts. A randomised, cross-over, placebo-controlled trial compared the risk/benefit profile of a 6-mo treatment with long-acting somatostatin (octreotide-LAR, 40 mg intramuscularly every 28 d) or placebo in ADPKD patients with mild-to-moderate renal insufficiency showed a significantly slower increase in TKV for patients on somatostatin compared to placebo[54]. The work of Hogan *et al*[55] agrees with this. In another study involving long term treatment with octreotide, Caroli *et al*[56] assessed the effect of 3 years of octreotide-LAR treatment on kidney and cyst growth and renal function decline in participants with ADPKD. They performed a multicentre, randomised, single-blind, placebo-controlled, parallel-group trial in five hospitals in Italy between 2000 and 2008 on adult (> 18 years) patients with eGFR of 40 mL/min per 1.73 m^2 or higher who were randomly assigned on a 1:1 ratio to 3 year treatment with two 20 mg intramuscular injections of octreotide-LAR (n = 40) or 0.9% sodium chloride solution (n = 39) every 28 d. The mean ± standard error of mean increase in TKV in the treatment group (220.1 ± 49.1 mL) was lower than in the placebo group (454.3 ± 80.8 mL) but the difference was not statistically significant. They reported four cases (10%) of cholelithiasis or acute cholecystitis in the octreotide-LAR group that were probably treatment-related.

Vasopressin 2 receptor antagonist: Blockade of vasopressin V2 receptor is thought to limit cyst growth, thereby delaying progressive renal dysfunction. Vasopressin antagonists and somatostatin analogues lower intracellular cAMP levels and though associated with limited clinical benefits, they have significant side effects[28]. Torres *et al*[57] conducted a large (1445 patients between 2007 and 2009) well-structured prospective study of Tolvaptan, a selective vasopressin 2 receptor antagonist in young patients (⩽ 50 years) with ADPKD with reasonably good kidney function (eGFR > 60 mL/min), and with MRI-measured TKV > 750 mL. They compared TKV, kidney function, albuminuria, kidney pain and vital signs. The TEMPO (Tolvaptan Efficacy and Safety in Management of Autosomal Dominant Polycystic Kidney Disease and Its Outcomes) trial showed that tolvaptan was effective in slowing the expansion of kidney volume and deterioration of kidney function[58]. Tolvaptan has been reported to prolong the median age at ESRD onset by 6.5 years and increase life expectancy by 2.6 years. Even if the benefits of tolvaptan persist in the longer term, it would still not be cost effective treatment[59]. Tolvaptan has significant adverse effects including polyuria, nocturia, polydipsia and elevation of aminotransferase enzyme concentrations with the potential for acute liver failure. In the TEMPO 3:4 trial, 8.3% of patients in the treatment arm had severe tolvaptan-related aquaresis leading to drug discontinuation[28]. Appropriate patient selection is critical to optimize long-term benefits while minimizing adverse effects and hepatotoxic risk factors[4].

Combined drug treatment such as the use of low doses of rapamycin, tolvaptan, and AEterna-Zentaris

slows the progression of PKD with limited side effects, suggesting the use of combined therapies also in clinical trials[60]. Although not targeting the causative mechanisms of cyst formation and growth, the HALT-PKD study examined the effects of dual blockade of the RAAS and aggressive blood pressure control on the rate of progression of ADPKD[61]. In summarising the various trials of drug therapy for ADPKD, Myint *et al*[62] called for further well-designed and suitably powered trials of longer duration using new biomarkers or therapeutic agents with better tolerance are required.

Hypertension

Patch *et al*[63] undertook a cohort study of 2085 patients with ADPKD between 1991 and 2008 to determine the association between antihypertensive therapy and mortality in patients with ADPKD. The proportion on antihypertensive drugs increased from 32% in 1991 to 62% in 2008. Also, use of drugs acting on the RAAS increased from 7% of participants to 46% by 2008. These changes were associated with a reducing mortality. Effective BP control prevents an increase in LVM index and reduces urinary albumin excretion, indicating the relative importance of good BP control in slowing cardiac and renal organ damage in ADPKD[64]. RAAS inhibitors cause regression of LVH and play an important role in the cardiovascular risk management of ADPKD patients[34].

Chronic pain

Chronic pain defined as pain existing for > 4-6 wk, is a significant cause of morbidity in patients with ADPKD. Chronic pain in ADPKD patients is often severe, impacting physical activity and social relationships and frequently difficult to manage[65]. Analysis of 171 questionnaires completed by patients with polycystic kidney disease of varying levels of renal function showed the order of frequency of pain as: low back pain, abdominal pain, headache, chest pain and leg pain. The severity of pain, documented by the visual analogue scale was 4 to 5/10 in the majority of patients[66]. MRI will differentiate between mechanical low back pain caused by cyst enlargement from cyst rupture or infection. Also, the increased incidence of uric acid nephrolithiasis as a factor in producing renal colic must be considered when evaluating acute pain in the population at risk. If stone disease is suspected, then abdominal CT scan and/or ultrasound should be the method of investigation.

Approaches to chronic pain management must include measures that help patients to adapt to chronic pain thereby limiting its interference with their life style[67]. Management ranges from non pharmacologic therapy to high-dose opioid therapy and more invasive procedures, including surgical intervention. Celiac plexus neurolysis and intercostal nerve radiofrequency ablations offer temporary respite. Dorsal column neurostimulation is a more permanent step, affording superior analgesia with better QOL[65,68]. The use of open or laparoscopic cyst decortication procedures for control of pain and infection in those with preserved renal function does not result in further renal dysfunction[14].

ESRD/dialysis

The key issues relating to peritoneal dialysis in patients with ADPKD are: a higher incidence of abdominal wall hernias, the increased risk of diverticulitis; and peritoneal space problems due to enlarged kidneys[69]. However, the little evidence available showed no real difference between ADPKD and non ADPKD patients[69,70].

SURGICAL OPTIONS

Cyst procedures

Anecdotal report of successful intracystic infusion of ciprofloxacin that achieved a sufficiently high antibiotic level in infected renal cysts so as to completely eradicate *S. choleraesuis* in a 52-year-old male with ADPKD refractory renal cyst infection with multiple pyocysts[71] has highlighted a potential salvage therapy for refractory renal cyst infection especially when surgery is contraindicated. Transcatheter renal artery embolisation is performed to reduce kidney volume in ADPKD patients with nephromegaly and improve lung function by reducing the splinting effect on the diaphragm[36]. Open transperitoneal bilateral renal cyst reduction surgery in patients with symptomatic ADPKD has been shown to be a relatively safe and effective treatment for individuals in whom more conservative therapies have failed[72].

Cyst decortication is highly effective in the management of disease-related chronic pain for the majority of patients with ADPKD and may alleviate hypertension and preserve renal function[73]. The technique of retroperitoneoscopic decortication as described by Hemal *et al*[74] is preferred in the presence of infected cysts so as to prevent intraperitoneal contamination.

Transplantation

When patients with ADPKD are assessed for renal transplantation, the key issues relate to native nephrectomy, liver cysts, screening for intracranial aneurysms and living-related kidney donation. Prophylactic native nephrectomy is indicated in patients with a history of cyst infection or recurrent haemorrhage or to those in whom space must be made to implant the allograft[75]. Other issues include anaemia management, the potential benefits of select immunosuppressive agents, the role for combined kidney-liver transplantation and complications of ADPKD after transplantation[76].

Few studies have investigated whether the TKV and liver volume in patients with ADPKD decrease after renal transplantation. Yamamoto *et al*[77] analysed changes in the volume of native kidney (bilateral: n = 28; unilateral: n = 5) and liver (concomitant polycystic disease: n = 18) in 33 patients with ADPKD, who underwent renal transplantation. Volumetry was retrospectively conducted using simple CT scan data 6

mo before transplantation, at the time of transplantation, and one, three, and five years after transplantation. Kidney volumes were significantly reduced in all but one patient after renal transplantation, decreasing by 37.7% and 40.6% at 1 and 3 years, respectively. In contrast, 16 of 18 patients showed significant increase of liver volumes after renal transplantation with the mean rates of increase of 8.6% and 21.4% at 1 and 3 years, respectively. In the light of these findings, native nephrectomy would be unnecessary if the space for an allograft is available in the absence of infection, bleeding, or malignancy. When ADPKD is combined with polycystic liver disease, the possibility of intolerable symptoms caused by growing liver cysts should be considered[75,77].

Nephrectomy

More recent data suggests that about a fifth of ADPKD patients undergoing renal transplantation would require native unilateral or bilateral nephrectomy[78-80]. Brazda *et al*[81] reported a higher rate of native nephrectomy (35.4%) and advocated that if native nephrectomy is needed, it would be better before transplantation than after.

Indication/timing: As highlighted above, the indications for nephrectomy include pain/discomfort, space for transplantation, ongoing haematuria, recurrent infections, and gastrointestinal pressure symptoms (early satiety)[82,83]. Another argument in favour of nephrectomy in those with complex cysts is the risk of malignancy as exemplified by two cases of renal cell carcinomas in 157 ADPKD patients undergoping nephrectomy before or after transplantation - an incidence of 1.3%[79]. Fuller *et al*[78] evaluated the indications for and outcome of pre-transplant, concomitant and post-transplant native nephrectomy in patients with ESRD due to ADPKD. Between 1992 and 2002, 32 (18.7%) of 171 patients with ESRD due to ADPKD who received a kidney transplant underwent native nephrectomy - 25 bilateral and 7 unilateral. They observed that the predominant indication for native nephrectomy depended on its timing - haematuria, a renal mass and chronic pain in the pretransplant group; lack of space in the concurrent group; and urinary tract infection in the posttransplant group[78]. Bilateral nephrectomy performed either before or during transplantation has the advantage of removing future complications of ADPKD while not significantly increasing immediate general complications[80]. Nunes *et al*[84] studied 159 renal transplants in patients with ADPKD divided into two groups according to the need for a unilateral native nephrectomy owing to enlarged kidneys (n = 143) *vs* those not needing it at the time of transplantation (n = 16). They reported no differences in rates of delayed graft function, acute rejection and chronic allograft dysfunction.

Song *et al*[85] assessed the transplant outcome of ADPKD patients who underwent concurrent bilateral nephrectomies during kidney transplantation. Their study compared 31 patients undergoing concurrent bilateral nephrectomywith 32 patients without and reported a significantly longer operation time (300 ± 30.85 min *vs* 120 ± 20.78 min, $P < 0.01$), higher need for blood transfusion (4.31 ± 1.05 U *vs* 1.35 ± 0.23 U, $P < 0.01$) and higher rate of adjacent organ injury (22.58% *vs* 0%, $P < 0.01$) during operation in the concurrent bilateral nephrectomy group. This was hardly surprising.

Tyson *et al*[86] examined population level data on 2368 patients with ADPKD and performed unadjusted, multivariable and propensity score adjusted analyses of postoperative outcomes of 271 patients (11.4%) who underwent simultaneous kidney transplantation and bilateral native nephrectomy compared to bilateral native nephrectomy alone. They concluded that except for increased rates of intraoperative bleeding, blood transfusion and urological complications there were no significant differences in postoperative adverse outcomes[86]. In patients with ADPKD native nephrectomy of massively enlarged kidneys may be safely performed during the transplant procedure with no repercussions on the length of hospital stay, graft short- and long-term function and patient survival. Concomitant native nephrectomy of enlarged kidneys at the time of renal transplantation is reasonable and safe for patients with ESRD due to ADPKD[87,88]. It must be bourne in mind however, that native nephrectomy in ADPKD is a major undertaking associated with significant morbidity and mortality. Kirkman *et al*[82] reported that two of 20 patients in the bilateral nephrectomy pre-transplant group and one in the bilateral nephrectomy post-transplant group died in the immediate post-operative period.

Nephrectomy technique: Historically, nephrectomy for ADPKD was performed by an open technique. Eng *et al*[89] performed a study to compare outcomes (operative time, complications, transfusion requirement, and length of stay) in hand-assisted laparoscopic nephrectomy (n = 56) with open nephrectomy (n = 20). Overall complication rates were similar but patients undergoing open bilateral nephrectomy were more likely to receive transfusion, and the length of stay was longer in the open group [5.9 d *vs* 4.0 d for unilateral (P = 0.013) and 7.8 d *vs* 4.6 d for bilateral]. The most frequent complications associated with hand-assisted laparoscopic nephrectomy were incisional hernia at the hand-port site and thrombosis of arteriovenous fistulae. Compared to open bilateral nephrectomy, the laparoscopic approach resulted in significantly shorter hospital stay, decreased morbidity and quicker recovery. With an average weight of 3 kg, these were really only moderately large kidneys[90,91]. For patients considering renal transplantation, avoidance of transfusion is important to prevent sensitisation which limits access to compatible organs. Laparoscopic nephrectomy is technically safe and feasible in patients with ADPKD but progressive cyst aspiration is a critical step, facilitating the identification of vital structures and the creation of

enough abdominal cavity space to operate[90].

Nephrectomy/transplant outcome

Jacquet *et al*[92] reported the outcome of a longitudinal study on renal transplantation in patients with ADPKD comparing 534 ADPKD patients with 4779 non-ADPKD patients. This comprehensive French study performed using DIVAT (Donnees Informatisees et VAlidees en Transplantaion) demonstrated that renal transplantation is associated with better graft survival but patients had more thromboembolic and metabolic complications, and an increased incidence of hypertension. And from Italy, Mosconi *et al*[93] analysed the results of 1800 patients with ADPKD and 12505 ESRD patients from other causes during 2002-2010. Among patients with long term follow-up, ADPKD patients had better graft survival compared with other kidney diseases (86% *vs* 82% at 5 years; $P < 0.01$); and mortality was not different (92% *vs* 79% at 1 year). ADPKD is a risk factor for the development of new onset diabetes after transplantation (OR = 2.41, $P = 0.035$)[94].

Dinckan *et al*[88] compared the outcome of renal transplantation in ADPKD patients undergoing concurrent unilateral ($n = 38$) or bilateral ($n = 125$) native nephrectomy with 161 randomly selected controls. Despite additional surgery and a higher complication rate, the long-term results of patients with complications were not affected negatively and graft survival was similar in the two groups. Following bilateral native nephrectomy, hypertension control was better and the incidence of lower urinary tract infection was lower postoperatively[85]. Overall one year patient and graft survival were 94%-97% and 92%-96% respectively[81,88,95]. Surgical complications, which might be associated with simultaneous nephrectomy requiring re-operation, occurred in 12% of patients[95]. One wonders whether the outcome of the 38% who received kidneys from living donors might have been different if they had pre-transplant native nephrectomy.

RESEARCH POINTERS

Interventions to halt progression of ADPKD

The potential role of glucose metabolism in the pathogenesis of ADPKD may provide a new perspective for the understanding of the pathobiology of ADPKD and open potential new avenues for therapeutical interventions[96].

Treatment aimed at preventing or reducing cyst formation or slowing cyst growth is a reasonable strategy for prolonging useful kidney function in patients with ADPKD[23]. The findings of Caroli *et al*[56]'s study provide the background for large randomised controlled trials to test the protective effect of somatostatin analogues against deterioration in kidney function and progression to ESRD. Advances in research into molecular mechanisms of cystogenesis will help develop new targeted ADPKD therapies[28].

Meijer *et al*[97] have designed DIPAK 1 study (Developing Interventions to Halt Progression of ADPKD 1) to examine the efficacy of the somatostatin analogue lanreotide on preservation of renal function. The DIPAK 1 study is a multicenter, randomised controlled, clinical trial designed to show whether subcutaneous administration of lanreotide every 4 wk slows down disease progression in patients with ADPKD.

Vitamin D is increasingly being recognised for a number of other important physiological functions, including reducing blood pressure and proteinuria as well as kidney inflammation and fibrosis. Vitamin D deficiency is associated with proteinuria, increased mortality and may mediate the progression to kidney failure. Based on the prediction that cholecalciferol will attenuate hypertension, proteinuria and reduce the urinary excretion of a biomarker, monocyte chemoattractant protein-1 (MCP-1, a surrogate inflammatory marker of progression in ADPKD). Rangan *et al*[98] have designed a study to provide evidence as to whether a simple intervention such as vitamin D repletion, in either deficient or insufficient states, is a treatment to prevent kidney failure in ADPKD.

QOL with ADPKD

Particular emphasis needs to be placed on performing clinical trials with the goal of improving outcomes and QOL of patients with ADPKD[76].

OUTCOME

ADPKD patients have good graft and patient survival[13]. Haynes *et al*[99] performed a retrospective cohort study of all patients with ADPKD who received RRT between 1971 and 2000 at the Oxford Kidney Unit. Age at start of RRT and presence of a functioning transplant were associated with improved survival in unadjusted analyses. After adjustment for age the period of treatment also became a significant predictor of overall survival. Survival on RRT appears to have improved and exceeds that observed in the general population, such that RRT now provides almost two-thirds of the life expectancy of the general population, compared to about half in earlier decades.

Data were retrieved from three Danish national registries (1993-2008) on about 823 patients of whom 431 had died during the study period. A multivariate competing risk model comparing the two 8-year periods, adjusted for age at ESRD, gender and treatment modality, showed that deaths from cardiovascular disease decreased by 35% and deaths from cerebrovascular disease decreased by 69% from the first to the second time period[100].

LIMITATIONS

The absence of large RCT on various aspects of the disease and treatment, and the preponderance of case series and observational studies is a significant limitation. Though these reports are valuable, there

still remains considerable uncertainty and ambiguity in many aspects of ADPKD patient care as it relates to ESRD. To a large extent our knowledge is based on small numbers in various trial, single centre retrospective data and numerous review articles.

CONCLUSION

The outlook of patients with ADPKD is improving and is in fact much better than that for patients in ESRD due to other causes. This review highlights the need for a well-structured RCT as a first step towards trying newer interventions so as to develop updated clinical management guidelines.

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EDITORIAL

Changing picture of renal cortical necrosis in acute kidney injury in developing country

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Abstract

Renal cortical necrosis (RCN) is characterized by patchy or diffuse ischemic destruction of all the elements of renal cortex resulting from significantly diminished renal arterial perfusion due to vascular spasm and microvascular injury. In addition, direct endothelial injury particularly in setting of sepsis, eclampsia, haemolytic uremic syndrome (HUS) and snake bite may lead to endovascular thrombosis with subsequent renal ischemia. Progression to end stage renal disease is a rule in diffuse cortical necrosis. It is a rare cause of acute kidney injury (AKI) in developed countries with frequency of 1.9%-2% of all patients with AKI. In contrast, RCN incidence is higher in developing countries ranging between 6%-7% of all causes of AKI. Obstetric complications (septic abortion, puerperal sepsis, abruptio placentae, postpartum haemorrhage and eclampsia) are the main (60%-70%) causes of RCN in developing countries. The remaining 30%-40% cases of RCN are caused by non-obstetrical causes, mostly due to sepsis and HUS. The incidence of RCN ranges from 10% to 30% of all cases of obstetric AKI compared with only 5% in non-gravid patients. In the developed countries, RCN accounts for 2% of all cases of AKI in adults and more than 20% of AKI during the third trimester of pregnancy. The reported incidence of RCN in obstetrical AKI varies between 18%-42.8% in different Indian studies. However, the overall incidence of RCN in pregnancy related AKI has decreased from 20%-30% to 5% in the past two decades in India. Currently RCN accounts for 3% of all causes of AKI. The incidence of RCN in obstetrical AKI was 1.44% in our recent study. HUS is most common cause of RCN in non-obstetrical group, while puerperal sepsis is leading cause of RCN in obstetric group. Because of the catastrophic sequelae of RCN, its prevention and aggressive management should always be important for the better renal outcome and prognosis of the patients.

Key words: Acute kidney injury; Hemolytic uremic syndrome; Renal cortical necrosis; Postpartum hemorrhage; Septic abortion; Puerperal sepsis; Eclampsia

Core tip: Acute kidney injury (AKI) due to renal cortical necrosis (RCN) is rare in developed countries with reported incidence of less than 2% of all cases of acute renal failure. In contrast, its incidence is higher in developing countries ranging between 6%-7% of all causes of acute renal failure (ARF). Pregnancy related complications are the most common cause of RCN. With improved health care, wider availability of dialysis, and marked decline in septic abortion, the incidence and severity of RCN has decreased in developing countries in recent years. RCN accounts for 3% of all causes of AKI in our recent study. The current incidence of RCN in obstetrical AKI was 1.44% in 2003-2014. The most common cause of RCN is haemolytic uremic syndrome among non-obstetric patients and puerperal sepsis is the leading cause of RCN in pregnant patients. The strategy involving prevention and effective management of haemorrhagic and septic complications of pregnancy will further reduce the RCN incidence in pregnant patients in developing countries.

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INTRODUCTION

Renal cortical necrosis (RCN) is a potentially fatal variety of kidney disease with adverse and serious outcomes. The total ischemic necrosis of allthe element (glomeruli, blood vessel and tubule) of the affected area of renal cortex is a typical histological feature of RCN. RCN is irreversible lesion leading to total loss of kidney function and end stage kidney failure in complete variety of cortical necrosis. However, recovery of renal function is variable in the incomplete type of cortical necrosis depending upon the amount of necrosed nephron in the kidney. RCN result from severe degree of renal ischemia secondary to significantly reduced renal tissue perfusion usually on account of intravascular coagulation, microvascular injury or extreme vascular spasm. The following two types of cortical necrosis have been identified on the basis of renal histology: (1) diffuse cortical necrosis: Confluent global cortical destruction extends into the columns of bertin. Thin rim of subcapsular and Juxtamedullary tissue is preserved. Irreversible renal failure leading to end stage renal diseases (ESRD) is the final outcome of diffuse cortical necrosis; and (2) patchy cortical necrosis: Contiguous area of cortical necrosis involve up to one-third to half of the entire cortical tissue. Partial recovery of renal function is known to occur in patchy cortical necrosis.

Renal histology of 113 patients with acute renal cortical necrosis (ACN) revealed complete and patchy cortical necrosis in 62.8% and 37.2% patients, respectively[1]. In our series of 57 patient with RCN, diffuse cortical necrosis was observed in 41 (72%) cases while, patchy cortical necrosis was noted in 16 (28%) patients[2]. Complete and patchy cortical necroses were reported in 80% and 20% of cases from another Indian study[3]. Diffuse and patchy cortical necrosis were noted in 84.2% and 15.8% of patient respectively from our centre earlier[4].

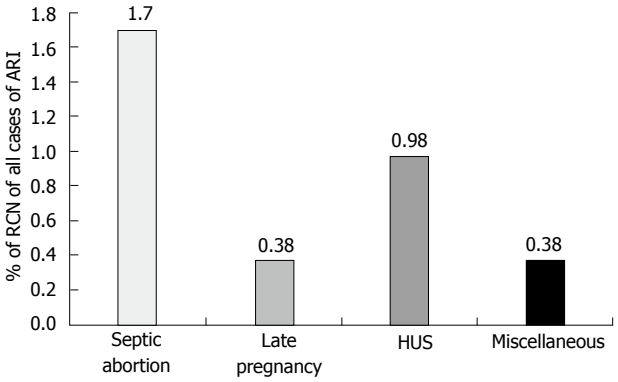

Figure 1 Causes of renal cortical necrosis of all cases of acute renal failure (*n* = 1822); 1984-2005. Adapted from Prakash *et al*[2]. HUS: Hemolytic uremic syndrome; RCN: Renal cortical necrosis.

EPIDEMIOLOGY OF RCN

RCN account for less than 2% of all cases of acute renal failure (ARF) in developed country and is rarely reported[5-7]. In contrast to developed country, the incidence of RCN is higher in developing country ranged between 6%-7% of all causes of acute kidney injury (AKI)[4,8-9]. In our previous study the incidence of RCN was 6.3% in patients with AKI[4]. Another Indian study from Chandigarh observed RCN in 7.1% of patients dialyzed for ARF[3,9]. However, decreased incidence of septic abortion related obstetric AKI resulted in reduced incidence of RCN in Indian patients in recent years[2]. To other previous study from India also reported a declining trend (3.8%-4.6%) in the incidence of RCN in patients with AKI similar to our study[1,2,10]. In a series of 1822 patients with ARF, RCN was observed in 57 (3.12%) patients in our most recent study[2]. The overall incidence of RCN decreased to 1.6% (of total cases of acute renal failure) in 1995-2005 from 6.7% in 1984-1994[2]. Septic abortion in obstetric group and haemolytic uremic syndrome (HUS) in non-obstetric group contributed to RCN in 1.7% and 0.98% of cases respectively, of total ARF cases (Figure 1). RCN was reported in one case (0.13%) of 46 patients undergoing kidney biopsy in a series of 748 cases of acute renal failure[11]. Thus, in contrast to developed world (Europe and North America) the incidence of RCN is still high in developing country. The septic complication of pregnancy such as puerperal sepsis and septic abortion is a major cause of higher incidence of RCN in developing countries including

India[1,2,4]. The causes of RCN are divided in two group: (1) obstetrical; and (2) non-obstetrical causes. Pregnancy related complications are the most common (50%-70%) causes of RCN and 20%-30% of total cases of RCN are due to non-obstetrical condition. RCN of non-obstetrical origin have higher incidence in male than female[4,6,12]. The various non-obstetrical causes of RCN include; extensive burns, sepsis, HUS, pancreatitis, snake bite, and diabetic ketoacidosis[1,3,13].

RCN of obstetric origin

Septic abortion, abruptio placentae, puerperal sepsis, eclampsia, obstetric haemorrhage, intrauterine death, and thrombotic microangiopathy of pregnancy (P-TMA) are the causes of RCN in a pregnant women[5,14-17]. Overall, obstetrical causes are the dominant causes of RCN account for 56%-61% of cases[1,2,18,19]. The RCN is reported to occur in 10%-30% of all cases of pregnancy related AKI compared with approximately in 5% of non-pregnant women[20]. We reported RCN in 25% of obstetric ARF in our previous study[19]. Of 57 patients with RCN; pregnancy-associated complication and non-pregnant condition were causative factor for RCN in 32 (52.2%) and 25 (43.8%) respectively[2]. We reported RCN due to pregnancy related complications in 15.2% of obstetric ARF; with higher (11.9%) incidence in post-abortal AKI compare to lower (3.3%) incidence in late pregnancy-AKI[2]. The incidence of RCN was 9% among patient with obstetric AKI in a study from Pakistan[21]. The RCN incidence has decreased from 17% in 1982-1991 to 2.4% in 1992-2002 in obstetric ARF in our recent publication[15]. Thus, the overall incidence of RCN in pregnancy associated AKI has decreased from 20%-30% to 5% in the last two decades in developing countries[2]. The current (2003-2014) incidence of RCN is 1.44% (1/69) in obstetric AKI in our study[22]. Post-abortal sepsis is a common cause of RCN in obstetric AKI in developing countries while, abruptio placentae is responsible for RCN in 50%-60% of case in pregnancy in developed countries[13,23]. Thus, septic abortion is common cause of RCN in developing countries but rarely reported from developed world[14,16,24]. The abortion is commonly conducted by unskilled persons mostly under unhygienic condition which leads to higher incidence of post-abortal sepsis. This possibly may explain higher incidence of RCN in post-abortal AKI in developing world. It is postulated that endothelial injury due to endotoxin may cause endovascular damage and vascular thrombosis with consequent renal ischemia in patient with sepsis and septic abortion.

RCN of non-obstetric origin

The various non-obstetrical causes of RCN include; extensive burns, snake bite, sepsis, pancreatitis, HUS, infancy and childhood dehydration, malaria and drugs and toxin[1,2,25-29]. Non-pregnancy associated complication accounted for RCN in 34.8% of total ARF cases in our earlier study[16]. RCN was due to pregnancy and non-pregnancy related complications, in 56.2% and 43.8% cases respectively in our recent publication[2]. HUS was the most common cause 18/25 (72%) of cortical necrosis in the non-obstetrical group[2]. Severe sepsis, extensive burns (80%), massive gastrointestinal haemorrhage, acute pancreatitis and diarrhoea associated shock are other causes of RCN in non-pregnant group[2]. The changing clinical feature of RCN was analysed and compared in 28 patients in English literature before and after 1980 from two countries; France (F) and India (I). This analysis revealed that pregnancy related cortical necrosis decrease to 28% after 1980 from 68% (F) and 71% (I) before 1980, while non-pregnancy related cortical necrosis increased to 72% after 1980 from 32% (F) and 29 (I) before 1980. The RCN was due to sepsis in 4/12 (F) and snake bite 6/14 (I) cases before 1980 but drug associated cortical necrosis was observed in 4/21 patient after1980 among the non-obstetrical causes of cortical necrosis[30]. RCN was reported in 19.9% of patient among 131 cases with post-surgical ARF from Japan in an autopsy study[31]. Despite a bit increasing trends in non-obstetrical cause of RCN, obstetrical complication is still remains the dominant cause of RCN in developing country. The development of RCN in live kidney donor and malaria was noted in Indian literature[32,33]. Figure 2 shows the RCN in live kidney donor. Donor was on maintenance haemodialysis for 4 mo and she eventually died of severe sepsis related to pneumonia[33]. RCN developed in a congenital solitary kidney following Road Traffic accident (Figure 3) in a child aged 11 years.

PATHOGENETIC MECHANISMS OF RCN

The pathophysiological events leading to RCN are poorly understood. However, significantly diminished renal arterial perfusion is the final common pathway resulting in ischemic necrosis of renal cortex. The exact pathogenetic mechanism of RCN is not completely known. The vasospasm of small vessel and liberation of toxin with consequent endothelial injury seems to be initiating event in the process of cortical necrosis[34,35]. The vasculature in pregnancy is more sensitive to vasoconstrictors, possibly related to sex hormone[35]. ACN and the generalised Schwartzmann reaction induced by endotoxin in rabbit have similar clinical feature[36-38]. Two small doses of endotoxin given at interval of 24 h may cause generalised Schwartzmann reaction in non-pregnant animal while only one dose is sufficient to produce this phenomenon in pregnant rabbits[37]. Intravascular coagulation was considered as the initial event in pathogenesis of RCN. However, available evidences does not support the role of intravascular coagulation in the genesis of RCN[6].

The role of endothelium-derived vasoactive substance particularly endothelin-1 has been suggested in the pathogenesis of ischemic ARF. The endothelin-1 is one of the most potent vasoconstrictor substance known[39], and renal vasculature appears to be 10 times more sensitive to this effect of endothelin-1 compared

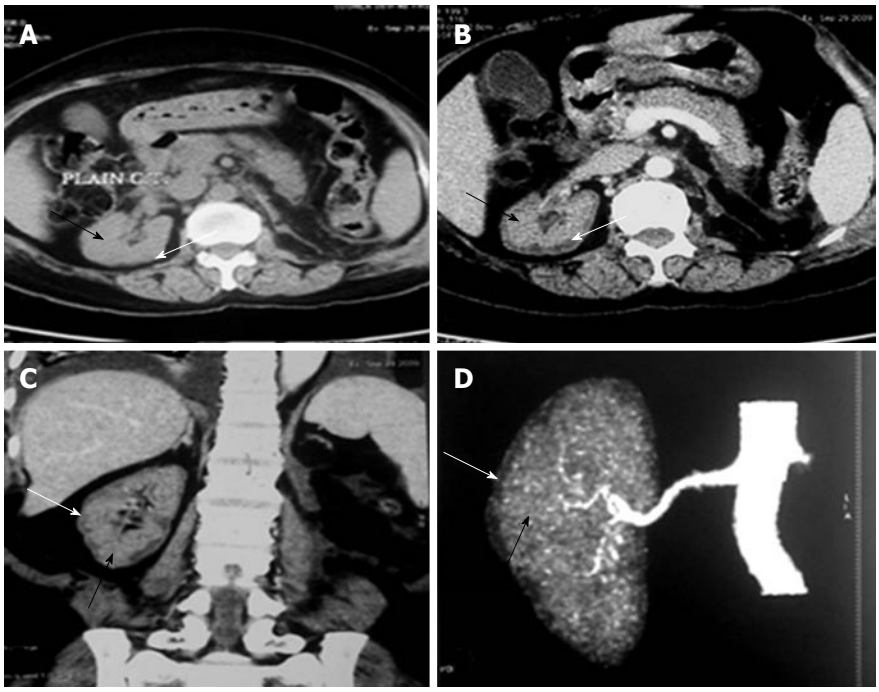

Figure 2 Renal cortical necrosis in a living kidney donor. Adapted from Prakash *et al*[32]. Non-contrast (A) computed tomography (CT) scan of abdomen at the level of right hilum showing hypoattenuating peripheral cortical rim (white arrow) as compared to the inner isoattenuating parenchyma (black arrow), On contrast-enhanced axial (B) and coronal (C) scans the central viable parenchyma enhances (black arrow) while the peripheral necrosed cortex does not show any significant enhancement (white arrow). Overall picture is suggesting renal cortical necrosis. The corresponding appearance is also well noted CT Angiogram (D) showing absent uniform nephrogram (white arrow).

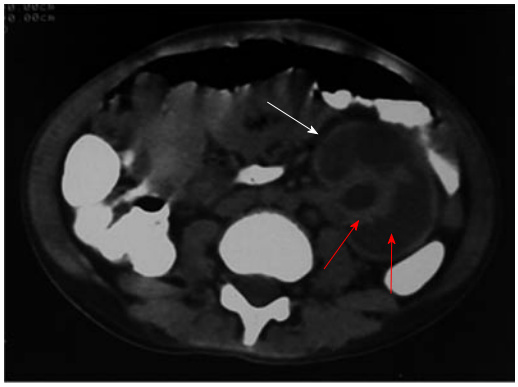

Figure 3 Contrast enhanced computed tomography showing diffuse cortical necrosis in solitary left kidney after road traffic accident in a child aged 11 years. This image shows isoattenuated peripheral cortical rim (white arrow) and hypoattenuated parenchyma (red arrow).

to all other vascular organ[40]. It is suggested that endotoxin initiate endothelial injury with subsequent development of intravascular thrombosis and reduce renal perfusion causing cortical necrosis in patient with sepsis. The endothelial injury seems to be a primary event in development of TMA[41]. In patient with HELLP syndrome, endothelial damage may progress to endovascular thrombosis leading to lumen occlusion, hypoperfusion and ischemic necrosis of renal cortex[42]. Two possible pathogenetic factors may contribute the development of RCN: (1) renal hypo-perfusion resulting from blood loss or hypotension such as in postpartum haemorrhage; and (2) vascular endothelial injury either through direct mechanism (HUS, eclampsia and snake bite) or indirect mechanism *via* release of circulating substances (sepsis, pancreatitis and intravascular haemolysis). It is postulated that endothelin may act as final common factor leading to renal damage and subsequent RCN, because both renal hypo-perfusion and endothelial injury stimulate release of endothelin from vascular endothelial cell. However, further detailed studies are required to established the possible role of endothelin in the pathogenesis of RCN.

CLINICAL AND DIAGNOSTIC FEATURES OF RCN

RCN is a rare but catastrophic cause of AKI. Absolute anuria (urine output nil in 24 h) or anuria (urine output < 100 mL/24 h) are the usual presenting symptoms of acute RCN. Because of the systemic nature of illness causing RCN, the lesion is usually bilateral. Prolonged anuria (> 4 wk) in clinical setting of haemorrhage, sepsis, shock or disseminated intravascular coagulation suggests the clinical diagnosis of RCN. The mean duration of absolute anuria from the onset till death or partial recovery of renal function while on dialysis was 24.5 ± 26.2 (range 6-100) d in our study[2,4]. Hematuria (microscopic or gross) can be seen in patients with ACN. The renal biopsy is the gold standard to confirm the diagnosis of RCN. The typical histological feature of RCN is ischemic necrosis of all elements of renal parenchyma of cortical region (Figure 4). RCN was diagnosed on

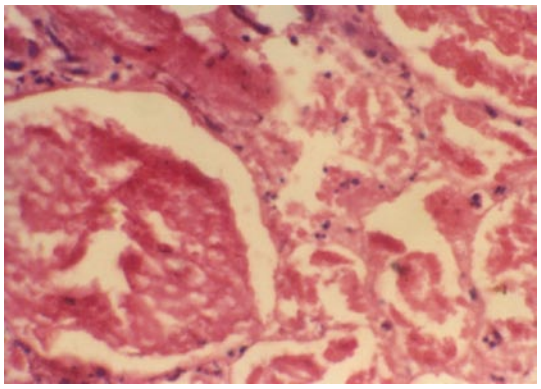

Figure 4 Glomeruli and neighbouring tubules are showing coagulative necrosis. There is complete loss of nuclei in glomeruli and tubules, but connective tissue framework is preserved. Neutrophilic infiltrations are seen around necrosed glomeruli and in interstitium (HE × 250). Typical histologic feature of renal cortical necrosis.

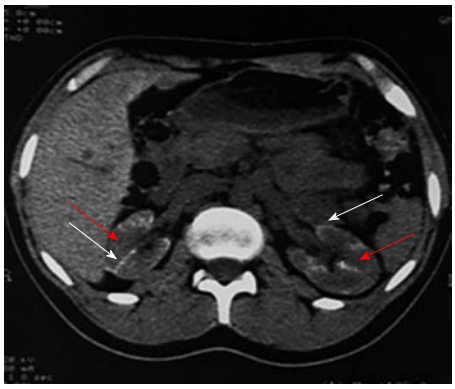

Figure 5 Non contrast computed tomography showing bilateral cortical calcification in a patient with renal cortical necrosis 72 d after acute pancreatitis. Non contrast computed tomography scan of abdomen at the level of renal hila showing linear hyperattenuation along the renal cortical rim (white arrow) with hypoattenuating medulla (red arrow) in bilateral kidney.

kidney biopsy specimen within the first week of the onset of disease. However, contrast enhanced computed tomography (CECT) scan is a suitable non-invasive modality for the early diagnosis of RCN[43]. The presence of hypoattenuated subcapsular rim of renal cortex on CECT scan is atypical radiological abnormalities in patient with RCN (Figure 3). In addition, a non-contrast CT scan (NCCT) is more sensitive in picking up cortical calcification. However, renal cortical calcification develop too late and also not observed in all patients with RCN[20,44,45] (Figure 5). Thus, demonstration of cortical calcification on NCCT scan is not useful in early diagnosis of RCN. Other less invasive method of diagnosis like MRI scan are useful alternative[46]. Recently, contrast enhanced ultrasound scan is found to another non-invasive modality for early diagnosis of RCN[47].

CLINICAL COURSE AND OUTCOMES OF RCN

The clinical course of patients with RCN can be divided into five broad groups: (1) death in uraemia during the acute phase; (2) survival without dialysis; (3) late return to dialysis/transplant; (4) survival only with chronic maintenance dialysis/transplant; and (5) late resumption of sufficient renal function to become dialysis independent. The mortality was 87% during acute phase of illness in our previous study[4]. However, mortality decreased to 19% in 1995-2005 from 72% in 1984-1994[2]. The maternal mortality in obstetric RCN was 72.7% in 1982-1991. Mortality was observed in 1 of 3 patients in 1992-2002 and no mortality in the last decades. Thus, maternal mortality reduced to zero in 2003-2014 from 72.7% in 1982-1991[22]. The causes of death during acute phase of illness are; severe uraemia, sepsis, pulmonary oedema, gastrointestinal haemorrhage and hyperkalemia including multiorgan failure[2]. Thus, the majority of deaths are due to sepsis and uremic complication in those who could not afford dialysis. However, the prognosis and survival of patients with RCN has improved markedly in our recent publication due to availability of renal replacement therapy and overall improved medical care[2,15,22]. Survival without dialysis is possible in patients with patchy cortical necrosis because surviving nephrons carry the function of the remaining kidney. In certain patients, there may be slow rise in creatinine clearance and a gradual gain in renal function over one to two years, so that the glomerular filtration rate may reach a final plateau level of approximately 20-24 mL/min[48,49]. It is assumed that juxtamedullary glomeruli (which comprise 15%-20% of total) escape destruction, even in the complete cortical necrosis and that early functional return is due to recovery of these nephron segment. The deterioration in renal function had been reported several years (1-10 years) after the acute cortical necrosis in a significant number of patients. Factors causing these late functional downturn are not clear but may include pyelonephritis, hypertension and shrinkage of the kidney due to progressive fibrosis and/or calcification[50,51].

The fate and outcome of RCN has changed in developing countries mainly due to decreasing incidence of RCN in patients with acute renal failure. We observed the incidence of RCN decreased to 1.6% in 1995-2005 from 6.7% in 1984-1994[2]. The incidence of RCN in obstetric AKI has decreased to 2.4% in 1992-2002 from 17% in 1982-1991 in our previous publication[15]. The most recent incidence of RCN in obstetric AKI was 1.44% in our study[22]. This changing picture of RCN is mainly due to a decrease in incidence of poost-abortal sepsis. Public awareness, legalization of abortion law, and overall improved health services are other reasons for such improvement in the prognosis and outcome of cortical necrosis at our centre.

CONCLUSION

The renal prognosis of RCN has improved. The partial recovery of renal function had increased to

33.3% in 1995-2005 from 11% in 1984-1994. The mortality of patients with RCN had markedly reduced to 19% in 1995-2005 from higher mortality of 72% in 1984-1994 due to wider availability of dialysis and overall improvement in health care facilities. Because of decreased mortality, higher (47.6%) proportion of patients with cortical necrosis had progressed to ESRD in 1995-2005, compared to lower (16.6%) number of patients in 1984-1994. Thus, both increased number of patient survival and better renal outcome contributed to improved prognosis of RCN in recent years. In addition to improved prognosis of RCN, overall incidence of RCN has decreased to 3% of total cases of ARF. The current incidence of RCN in obstetric AKI is 1.44% at our centre.

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EDITORIAL

Biomarkers in kidney transplantation: From bench to bedside

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Abstract

Immunosuppressive drug level monitoring and serum creatinine are widely used for kidney transplantation (KT) monitoring. Monitoring of drug level is not the direct measurement of the immune response while the rising of creatinine is too late for detection of allograft injury. Kidney biopsy, the gold standard for KT monitoring, is invasive and may lead to complications. Many biomarkers have been discovered for direct monitoring of the immune system in KT and the benefit of some biomarkers has reached clinical level. In order to use biomarkers for KT monitoring, physicians have to understand the biology including kinetics of each marker. This can guide biomarker selection for specific condition. Herein, we summarize the recent findings of donor specific anti-human leukocyte antigen antibody, B lymphocyte stimulator, interferon-gamma induced protein of 10 kDa, and intracellular adenosine triphosphate monitoring, all of which have very strong evidence support for the clinical use in KT.

Key words: Kidney; Transplantation; Biomarkers; Donor specific antibody; B-cell; B lymphocyte stimulator; Interferon induced protein of 10 kDa; Intracellular adenosine triphosphate

Core tip: There are many studies about roles and benefits of biomarkers in nephrology, including transplantation. Only some of them reach the clinical level with strong evidence support. Biomarkers can guide immunosuppressive adjustment, provide prognostic value, and guide early detect of allograft injury, particularly from allograft rejection. We summarized the potential biomarkers for kidney transplantation monitoring, including clinical implication, strength and weakness of each of them.

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INTRODUCTION

Kidney transplantation (KT) has been succeeded for a long-time but the improvement in graft outcome is only limited in the short-term[1,2]. The keys to achieving long-term kidney allograft survival are early detection of treatable cause of allograft dysfunction and the appropriate tailoring of immunosuppression. Over immunosuppression can result in infections and cancers whereas under immunosuppression can cause rejection of the transplanted kidney. Immunosuppressive drug level monitoring is currently the only method broadly used in clinical practice. The gold standard for KT monitoring is biopsy which is invasive. However, kidney biopsy is only useful once rejection has already occurred. The procedure is unable to predict rejection. Recently, there are growing numbers of biomarker studies for KT, ranged from experimental to clinical level. Understanding immunologic and physiologic changes of allograft and directly monitoring through blood and urine testing can guide management and immunosuppressive adjustment.

There are two main objectives for biomarker testing: (1) for diagnosis; and (2) for prognostic and outcome prediction. Some biomarkers can guide diagnosis and management during allograft dysfunction, whereas others can predict outcome and guide long-term management including immunosuppressive adjustment for rejection and drug toxicity prevention. The samples mainly used for monitoring are blood and urine. Blood sample is easily taken and handled but it is not directly excreted from the kidney allograft and can be diluted in the blood stream. Urine sample is directly contacted and excreted from the allograft which more represents kidney environment than blood sample. However, urine sample can be interfered by urine pH, urine protein, and urine volume. Furthermore, urine sample is not easily collected during anuric phase. Herein, we summarized the recent findings in blood and urine biomarkers mainly focusing on methods which can be easily tested in clinical practice of KT.

DONOR SPECIFIC ANTIBODY

Donor specific antibody (DSA) is the anti-human leukocyte antigen (HLA) which is specific to donor HLA. It is the major obstacle in KT. In the study of 1329 KT recipients, the 4-year allograft survival was lower in recipients with positive DSA detected compared with the recipients with negative DSA[3]. There are many methods for DSA detection, ranged from lymphocytotoxic anti-human globulin (LCT-AHG) which has least sensitivity to the most sensitive and specific assay, the solid phase single antigen bead (SAB). DSA is a major cause of antibody mediated rejection (ABMR). Positive DSA by LCT-AHG is the absolute contraindication for KT. However, positive SAB but negative LCT-AHG (SAB positive/LCT-AHG negative) is not the absolute contraindication for KT. DSA is now the most widely used test in KT. DSA can be monitored from pre-transplantation period till many years post-transplantation. The pre-transplant DSA can predict post-transplant outcomes and guide perioperative management[4,5]. In post-transplantation period, DSA is included in one of the criteria for ABMR[6]. The newly presence of DSA (*de novo* DSA) prompts physician for evaluation for ABMR and increasing the level of immunosuppression before allograft function deteriorates (Figure 1)[7]. However, there are certain issues to be concerned in DSA interpretation. Only substantial number of the patients who developed de novo DSA have allograft function deterioration. A cohort study by Wu *et al*[8] showed that 9.5% and 19.0% of *de novo* DSA patients developed early allograft failure and early allograft function deterioration, respectively during a 3-year follow up. Indeed, the graft function of the 70% of *de novo* DSA patients remains stable for years. As such, DSA can be classified into the pathogenic- and non-pathogenic-DSA. The pathogenic DSA is likely to have at least one of these features: (1) DSA to HLA-DQ; (2) mean fluorescence intensity (MFI) > 7000; (3) DSA with C1q activating capacity; and (4) IgG1 or IgG3 subclasses[9]. The presence of DSA together with one of these characteristics prompts physician for allograft biopsy and treatment of ABMR to remove this pathogenic DSA in those who have pathological clues of allograft injury.

B LYMPHOCYTE STIMULATOR

As anti-HLA antibody is the major barrier in KT, plasma cell and B-cell are currently the major targets of treatment. B lymphocyte stimulator (BLyS) is produced mainly by innate immune cells and binds to its receptor on B-cell and plasma cell. There are two cytokines in the BLyS system, B cell-activating factor (BAFF) and a proliferation-inducing ligand (APRIL). BLyS is required for the development of B-cell in earlier stages whereas APRIL is required for plasma cell survival[10]. In a model of murine cardiac allograft, BAFF deficient mice had longer allograft survival when compared to wild-type[11].

In pre-transplantation period, BAFF is correlated with the degree of sensitization. A higher BAFF level was associated with a higher MFI of pre-transplant anti-HLA antibody[12]. Elevated pre-transplant serum BAFF level was also associated with an increased risk of the subsequent ABMR[13]. Patients with high post-transplant soluble BAFF levels had a significantly higher risk of developing de novo DSA[14].

There are some issues to be concerned in interpretation of BLyS in KT. The first is the balancing between BLyS production by innate immune cells and utilization by B-cell. Increments in BLyS levels may be due to

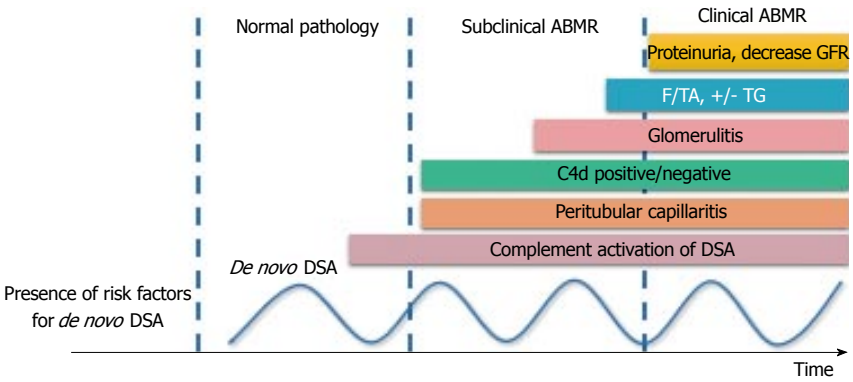

Figure 1 Graft injury and clinical presentation after development of *de novo* donor specific antibody. The pathologic injury of ABMR starts from microvascular inflammation, including peritubular capillaritis, C4d staining in allograft, and glomerulitis, to interstitial fibrosis/tubular atrophy (IF/TA) and transplant glomerulopathy (TG). GFR: Glomerular filtration rate; ABMR: Antibody mediated rejection; DSA: Donor specific antibody.

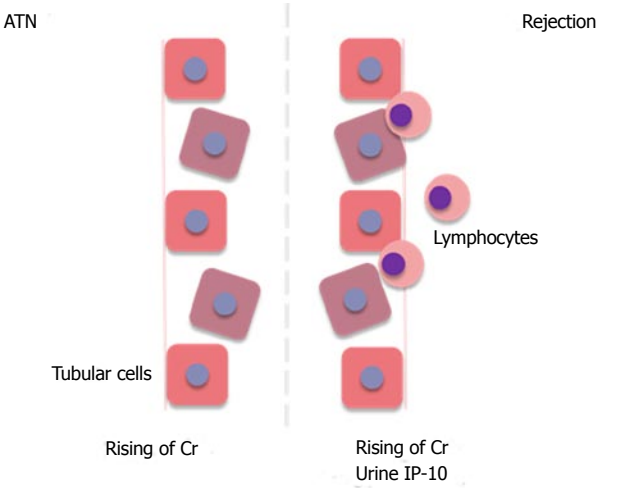

Figure 2 Comparing the mechanism of acute tubular necrosis and rejection. The rejection causes tubular damage similar to ATN but lymphocytes infiltration can leads to elevation of urinary IP-10 in rejection. ATN: Acute tubular necrosis; Cr: Creatinine; IP-10: Interferon-gamma induced protein of 10 kDa.

either increased production and/or reduced B-cell consumption. Recipients who received anti-rejection therapy with rituximab, a potent B-cell inhibitor, had a significant peak of BLyS levels at 3 mo post-treatment which can be explained by lower BLyS consumption from B-cells inhibition[15]. Second, there is a number of evidence in the roles of BLyS in immune regulation. BLyS is not only needed in B-cell or plasma cell activation, but also required by the regulatory B-cell which plays a very important role in immune regulation and transplantation tolerance[16]. Transplantation tolerance is a condition that the recipient immune system accepts allograft as a part of recipient and is the holy grail of transplantation. Recipients with tolerance require less immunosuppression or no immunosuppression needed in some circumstances. Increasing BLyS level in some certain conditions may be favorable as it may be a potential induction of transplantation tolerance.

We recently studied the benefit of BAFF testing in both low risk and high risk newly KT recipients and found that among recipient with positive pre-transplant DSA, the 6-month ABMR rate in recipients with higher perioperative serum BAFF level was significantly higher than those with lower perioperative BAFF level. In recipients with negative DSA, none of the patients with lower BAFF level developed ABMR while 17% of the higher BAFF recipients, despite negative DSA, still experienced ABMR (manuscript in preparation). This finding supports the benefit of adding BAFF in to pre-transplant immunologic risk evaluation together with DSA testing.

INTERFERON-GAMMA INDUCED PROTEIN OF 10 KDA

Induced protein of 10 kDa (IP-10) (also called CXCL-10) is one of the CXCR3 chemokine family. It is produced by tubular cells, mesangial cells and inflammatory cells and can be found in the kidney allograft[17]. In the setting of tubular inflammation, mainly acute rejection, IP-10 is elevated and highly expressed in urine (Figure 2). Recipients with T-cell mediated rejection or ABMR revealed higher urine IP-10 level compared to other pathological findings[18-20]. Many studies found that IP-10 measurement can detect subclinical tubulitis/rejection in surveillance allograft biopsy before allograft dysfunction developed[18,21].

In the setting of delayed graft function (DGF), early acute rejection, which needs early treatment, has to be differentiated from ischemic acute tubular necrosis (ATN). Concerning the higher risk of bleeding complication during this period, the clinician is reluctant to perform kidney biopsy. Our group studied the usefulness of urine IP-10 monitoring during DGF period. Recipients with early post-operative ABMR had significantly higher urine IP-10 compared with recipients with pure ischemic ATN (manuscript in preparation). However, an adequate amount of urine is needed for IP-10 testing.

ADENOSINE TRIPHOSPHATE MEASUREMENT

Since pharmacokinetic monitoring of immunosuppressive drug dose not directly predict T-cell reactivity, measurement of nucleotide adenosine triphosphate (ATP) from T-cell allows direct assessment of immunosuppression. The Food and Drug Administration has approved the ImmuKnow® assay for measuring intracellular ATP of T-cell for immune system monitoring in KT recipients.

The low ATP patients were associated with infections, whereas the high ATP patients were associated with rejection[22]. A randomized controlled trial from Ravaioli *et al*[23] in liver transplant recipients found that dosing of immunosuppression guided by ATP monitoring provided higher 1-year patient survival compared to convention immunosuppressive drug adjustment. However, some studies revealed no association between ATP level and transplantation outcomes[24-26]. This can be explained by the fact that the ATP level is not associated only with the T-cell reactivity but is also affected by the number of white blood cell (WBC), particularly in patients receiving lymphocyte depleting antibody. The number of WBC has to be considered when interpreting the result of ATP values. ATP measurement should not be used solely without other monitorings.

CONCLUSION

There are many biomarkers for KT monitoring. Each biomarker provides specific purpose for measurement. Knowing the immunologic mechanisms can guide biomarker selection. Together with biomarker monitoring, clinical clues should not be overlooked in taking care of KT recipients.

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REVIEW

Medical and alternative therapies in urinary tract stone disease

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Abstract

Nephrolithiasis is a serious problem for both patients and the health system. Recurrence stands out as a significant problem in urinary system stone disease, the prevalence of which is increasing gradually. If recurrence is not prevented, patients may go through recurrent operations due to nephrolithiasis. While classical therapeutic options are available for all stone types, the number of randomized controlled studies and extensive meta-analyses focusing on their efficiency are inadequate. Various alternative therapeutic options to these medical therapies also stand out in recent years. The etiology of urolithiasis is multifactorial and not always related to nutritional factors. Nutrition therapy seems to be useful, either along with pharmacological therapy or as a monotherapy. General nutrition guidelines are useful in promoting public health and developing nutrition plans that reduce the risk or attenuate the effects of diseases affected by nutrition. Nutrition therapy involves the evaluation of a patient's nutritional state and intake, the diagnosis of nutrition risk factors, and the organization and application of a nutrition program. The main target is the reduction or prevention of calculus formation and growth *via* decreasing lithogenic risk factors and increasing lithogenic inhibitors in urine. This review focuses briefly on classical medical therapy, along with alternative options, related diets, and medical expulsive therapy.

Key words: Urolithiasis; Prevention; Stone medical therapy; Nutrition therapy; Diet; Hypercalciuria; Hyperoxaluria; Hyperuricosuria; Hypocitraturia; Cysteine stones

Core tip: Nephrolithiasis is a serious problem for both patients and the health system. Recurrence stands out as a significant problem in urinary system stone disease, the prevalence of which is increasing gradually. While classical therapeutic options are available for all stone types, the number of randomized controlled studies and extensive meta-analyses focusing on their efficiency are inadequate. Various alternative therapeutic options to these medical therapies also stand out in recent years. This review focuses briefly on classical medical therapy,

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INTRODUCTION

Nephrolithiasis is a widespread medical problem, with an increased incidence in the last 20 years[1-4]. Its prevalence is expected to rise in the upcoming decades, as has been the case for obesity, diabetes, and metabolic syndrome[2,3]. Another problem related to nephrolithiasis is recurrence. In patients who do not receive prophylaxis following the first attack, recurrence rates are reported as 10% in the first year, 35% in the next 5 years, and 50% in 10 years[5].

If recurrence is not prevented, patients may go through recurrent operations due to nephrolithiasis which, even if said operations are only minimally invasive, still results in hospitalization. This leads to higher monetary costs and loss of manpower, whereas preventing stone formation is far more economic.

Numerous reports have revealed that urinary stone disease recurrence rates can be reduced *via* the correction of environmental and metabolic factors, as well as by the use of certain drugs and diet treatments[6-9]. In a meta-analysis of randomized trials focusing on the effects of drugs and diet on stone recurrence, the risk was shown to be reduced by 26%[10].

Further investigation into the possible pathogenic effect of Randall's plaques and the role of renal tubular crystal retention as a precursor in calcium oxalate (CaOx) nephrolithiasis may allow for the development of new drugs for the prevention of plaque formation and crystal adhesion to kidney cells. Nevertheless, all future studies should aim to understand the molecular/genetic level and pathophysiological mechanism of nephrolithiasis for the development of a targeted therapy.

PHARMACOTHERAPY

Hypercalciuria

Thiazide diuretics are the main treatment for idiopathic hypercalciuria (IH)-related calcium stones. There have been at least 10 randomized controlled trials (RCTs) focusing on the efficacy of thiazide in the prevention of idiopathic calcium kidney stones recurrence. Of these, seven have reported a decline in recurrence rates in treated patients[11-17].

Potassium supplements should normally be applied alongside thiazide treatment in order to prevent hypokalemia and hypocitraturia secondary to thiazide, as well as any possible subsequent side effects[18]. A reduction in bone mineral intensity and an increase in osteoclasis accompanies IH, with some authors claiming that reducing urinary calcium excretion can improve bone histology[19]. Bisphosphonates are effective in the inhibition of bone resorption, and the lower urinary calcium secretion and higher bone mineral intensity provided *via* bisphosphonate treatment was reported in a few studies[20]. Heller *et al*[21] have reported that alendronate prevented the excretion of urinary calcium, decreased 24-h urine calcium, and adjusted calcium equilibrium in 9 calcium stone patients within a short time period. Since no RCTs have yet been conducted to evaluate the efficacy of bisphosphonates on recurrence, bisphosphonate treatment is not currently recommended.

Hyperoxaluria

Two pharmacological agents that can reduce urinary oxalate are magnesium and pyridoxine.

The effect of magnesium in CaOx stone and non-hypomagnesuria patients is explained by the complexes formed among magnesium and oxalate that can lead to a reduction in CaOx supersaturation and inhibit the development of CaOx crystals. Increased magnesium intake additionally leads to an increase in urinary citrate and pH levels. On the other hand, dietary magnesium can reduce intestinal oxalate absorption in a manner similar to that of dietary calcium[22].

Calcium supplements, such as calcium carbonate and calcium citrate, are another potential therapeutic to magnesium supplements, as the latter are relatively less studied in hyperoxaluria treatment and form complexes with oxalate anions by a similar mechanism.

The logic behind vitamin B_6 use, on the other hand, is that its deficiency may cause oxalate leakage in urine[13]. RCTs on the use of pyridoxine in the prevention of recurrent stone disease are lacking in the literature, yet studies without controls in CaOx stone patients suggest that vitamin B_6 decreases urinary oxalate and stone recurrence[14,15].

The discovery of the relationship of oxalate with bacterial flora in the gastrointestinal system has paved the way for research related to the function of probiotics in the management of repeated CaOx stones. Studies revealed that lacking *Oxalobacter formigenes* colonies, which use oxalate as their solitary source of nutrition, could lead to an increase in the incidence of CaOx stones. In multivariate analyses, stone recurrence risk is reduced by 70% in *O. formigenes* colonized individuals[23]. Batislam *et al*[24] investigated *O. formigenes* levels for the first time in stool samples of cases with hyperoxaluria and recurrent nephrolithiasis by real-time PCR, and eventually found reduced levels of *O. formigenes* in cases with hyperoxaluria and recurrent nephrolithiasis.

An increase in the intestinal colonization of oxalate-producing bacteria decreases oxalate absorption, which in turn causes a reduction in urinary oxalate. Use of *O. formigenes* enteric capsules in primary hyperoxaluric

patients decreases urinary oxalate levels substantially, which shows that oral intake is effective on the enteric metabolism of endogenously-produced oxalate[25]. Prospective trials are required to corroborate the efficacy of probiotics on reducing urinary oxalate levels, as well as the side effects. Although current uncontrolled studies show that use of lactic acid bacteria[26] decreases urinary oxalate levels, these are not prospective double-blind placebo-controlled studies[27].

The function of oxalate-reducing bacteria, such as *O. formigenes* in CaOx stone formation, is currently under investigation. A pilot study has shown that *O. formigenes* reduces blood oxalate levels and urinary oxalate excretion in many IH-related calcium stones cases[26], yet these results could not be reproduced completely in a recent multi-centered study. Furthermore, the results acquired from expending lactic acid bacteria as a probiotic for the reduction of urinary oxalate elimination are inconsistent[28]. Treatments involving increased anion transport activity or the upregulation of intestinal luminal oxalate secretion through the use of oxalate binders are among other potential treatment approaches[29].

As calcium binds diet oxalate in the intestinal lumen, calcium supplements may decrease oxalate absorption[30]. Consumption of 2-4 g cholestyramine with every meal is much more beneficial in binding oxalate, yet brings along such inconveniences as an unpleasant taste in the mouth and vitamin K insufficiency[31]. Although the phosphate binding agent sevelamer hydrochloride is thought to decrease oxalate absorption, the conclusions are discrepant[32,33].

Hyperuricosuria

Dietetic purine limitation should be the initial medicinal approach[34], with alternative approaches required for incompatible patients and actual non-responders.

The main strategy in the treatment of uric acid cases is alkalization of the urine, which is more important than uricosuria reduction[35,36]. Typical starting doses to preserve the urine pH between 6.5-7.46 are 40-60 mEq for potassium citrate (KCit) in divided doses, or 1300 mg 2 × 1/d for sodium bicarbonate[37].

Allopurinol addition should be considered for patients with persistent acidic urine that does not alkalize easily. Hyperuricosuric CaOx nephrolithiasis is traditionally a xanthine oxidase inhibitor that is treated with allopurinol, which decreases endogenous uric acid formation and urinary uric acid excretion. The typical daily dose of allopurinol is 100-300 mg[37].

Recent interesting research on uric acid metabolism suggests that novel therapeutic methods may be developed in the future for hyperuricosuric nephrolithiasis and uric acid nephrolithiasis. More specifically, recent studies have shown that novel xanthine oxidase inhibitor (febuxostat) and the recombinant form of uricase enzyme (rasburicase) had superior serum uric acid-reducing effects compared to allopurinol, and are more successful in reducing the periodicity of gout recurrences[38,39]. These drugs should be considered for potential therapeutic agents of stone disease, although they are not yet tested.

Hypocitraturia

While both potassium citrate (KCit)[40] and potassium-magnesium citrate[19] were demonstrated to remarkably reduce hypocitraturia and recurrent urolithiasis formation in randomized stone case studies, sodium-potassium citrate did not exert any beneficial effect[20]. Although KCit is available commercially in tablet, liquid, and powder forms, investigations are ongoing in terms of developing a drug form of potassium-magnesium citrate[37].

The standard initial dose of KCit is 40-60 mEq daily, in divided doses, until the desired citraturia level is achieved[37]. These patients should be closely followed due to the potential for hyperkalemia, which is a theoretical risk due to the use of potassium-containing preparations and the increased glomerular filtration rate of the patients. In addition, some patients speak of gastrointestinal complaints while using KCit. Due to the high monetary cost and low patient compatibility of KCit therapy, its substitution with a dietary treatment was investigated. Lemon juice is naturally rich in citrate, and while it was concluded that drinking lemonade for 21 d increased urinary citrate levels in an uncontrolled metabolic study[41], 2 other recently carried out controlled-metabolic studies have raised doubts concerning the effect of lemonade in reducing recurrence.

Koff *et al*[42] conducted a randomized comparative study where they compared lemonade and KCit therapy in a group of patients with recurrent stones. While no changes were detected in basic urinary citrate or pH levels following lemonade therapy, a notable increase in urine citrate was reported in cases who received KCit. In accordance with these findings, while the use of lemonade is unsuccessful in increasing urine citrate and pH levels, it provides a prominent advantage over KCit due to the increased urine volume.

Cystine stones

Dilution and alkalization of urine, as well as thiol-binding drug and claw (chelation) combination therapy, constitutes the main guidelines of the cystine stone treatment approach. Combinatory use of these drugs may be more effective compared to individual use, due to the relatively higher pKa (8.5) of cysteine[43].

Patients should wake up at least once every night to drink water, as well as drink additional water to prevent concentration of urine at night. Patients can take 10-20 mEq 3 × 1/d KCit to increase urinary pH if it is < 7. Cystine excretion can be mildly decreased with < 100 mmol/d per sodium and 0.8 g/kg per day/protein restriction diets.

If stone recurrence occurs in spite of appropriate fluid intake and base urinary pH, cystine-linking drugs should be added to the treatment. D-penicillamine and tiopronin are the most widely-used thiol-linking

pills. D-penicillamine and tiopronin therapy has been indicated to be beneficial in the reduction of urolithiasis formation in cases where there was no benefit from hydration and alkaline urine.

The frequently-used thiol group anti-hypertensive captopril is another theoretical pharmacological agent in cystinuria treatment. Nevertheless, it is reported that it was not adequately effective in the solubility of cystine in urine, and that it also gave speculative results in a number of small scale studies regarding its ability to decrease urine cystine levels[43].

Struvite stones

Early diagnosis and eradication is essential for struvite stones, due to their fast growth potential and significant morbidity[44].

Long-term, low dose culture-specific antibiotic treatment is significant in the prevention of post-operative new stone growth and progression. Furthermore, minimizing urease concentration may even provide post-operative eradication of small fragments. Treatment with antibiotics only is not a standard approach[45].

Even in the presence of hydroxyurea, acetohydroxamic acid (AHA) is the most frequently used medical agent. In three randomized double-blind studies where AHA was used, stone growth and formation was decreased[46-48]. AHA and antibiotic suppression regimes can typically be recommended in patients that may not be surgery candidates due to serious side effect profiles, and in which potential significant side effects of AHA can be considered as acceptable risks.

MEDICAL EXPULSIVE THERAPY

Medical expulsive therapy (MET) exerts its effects *via* relaxation of the ureter and augmentation of the hydrostatic physical force proximal to the calculus[30].

In a patient that admits with lumbar pain due to ureteric calculi, the most substantial elements predicting the unpremeditated transition of the calculi are the dimension and localization of the stone. Meta-analysis has given the unpremeditated transition ratio of ureteric calculus as 68% and 47% for < 5 mm and 5-10 mm dimensions, respectively[49]. The most widely-used drugs for premedication of ureteric stones are α-1 adrenergic receptor antagonists and calcium channel blockers (CCB). α-1D receptors are the most widely-localized α-adrenergic receptors in the ureter, and are most densely localized in the distal ureter[50]. α-1 adrenergic receptor antagonists reduce the frequency and strength of the urethral contractions[51]. The CCB nifedipine was demonstrated to soften urethral smooth muscles *in vitro*, and to exert its impact mainly in the distal urethra[52].

There are 2 meta-analyses examining the effects of CCB and α-1 adrenergic receptor antagonists. Hollingsworth *et al*[31] published a meta-analysis where described cases who received α-blockers or CCBs displayed 65% higher spontaneous calculi transition, compared to the unmedicated group. Guidelines show that cases with a urethral calculus of < 10 mm and well-controlled symptoms can be followed for a while with application of MET as an initial therapy, and recommends α-1 blockers alongside the drugs recommended for MET[49].

In spite of the useful effects of α-1 blockers that have been shown in many studies, there are also studies that report negative effects. Hermanns and Pedro did not find any superiority of α-blockers over placebo in stone expulsion time[53].

Use of corticosteroids in order to decrease edema and inflammation, and thereby ease calculi transition, are under testing. Dellabella *et al*[54], in a small scale study, compared the calculi transition ratio in cases that received tamsulosin with or without the addition of deflazacort. No change was observed in the calculi transition ratio, yet the corticosteroid + tamsulosin group passed their calculus 2 d earlier on average. Larger scale future studies are needed in order for corticosteroids to gain widespread use.

NSAIDs do not provide any benefit in calculi transition time or calculi transition in renal pain[55].

The development of and increasing experience in endoscopic approaches, such as r/f URS, have led to the questioning of whether MET application to urethral stone disease patients admitted with acute renal colic is a loss of time and money. However, recent studies comparing MET and early endoscopic stone removal report less direct and indirect costs with MET, while no difference was detected in hospitalization numbers[56,57].

NUTRITION THERAPY

The etiology of urolithiasis is multifactorial and not always related to nutritional factors. However, nutrition therapy still seems to be useful, either in combination with pharmacological therapy or as a monotherapy. Nutrition therapy involves evaluation of a patient's nutritional state and intake, diagnosis of nutrition risk factors, and the organization and application of a nutrition program[58]. The main target of nutrition therapy is the reduction or prevention of calculus formation and growth *via* decreasing lithogenic risk factors and increasing lithogenic inhibitors in urine.

There are two approaches for nutrition therapy. The first is the empirical approach that is applied to all patients. This approach is a general mixture of various nutrition strategies that target multiple risk factors and that can be applied to patients with no known specific urinary risk factors. The second is the planned/specific approach, and is an alteration aiming to decrease or eliminate specific risk factors of patients. In two studies that included calcium stone patients, the empirical diet side showed a greater decrease in stone recurrence compared to general nutrition, yet these were not compared to direct planned approaches[59,60]. On the other hand, in a study where the empirical and planned nutrition therapy approaches were compared, stone

recurrence rate was reported to decrease with planned therapy[9].

Evaluation of the patient's normal diet and the supplements they use is useful in detecting the effects of an excess, lack, or imbalance of the consumed food or non-food ingredients. A targeted evaluation should be performed in order to detect suitable nutritional factors from the list of foods consumed in the last 24 h provided by the patient in one-to-one conversation or the multi-day nutrition chart kept by the patient.

Hypercalciuria

If sodium intake is identified as a nutritional risk factor, high sodium foods, along with the other foods consumed alongside them, should be examined in preparation for nutritional therapy.

A scale has been developed for predicting the renal acid load capacity (RALC) of foods[61]. This scale calculates the anion/cation ratios of foods, and is accepted as a suitable model in calculating the effects of diet on renal net acid excretion. Foods that carry an acid load proportional to the sulfur amount in their amino acid structures are all meat-based foods (red or white), cheeses (all types), eggs (mostly egg yolks), and grains.

Milk, yoghurt, and fats naturally appear in the RALC scale. Alkaline content foods (those with negative numbers in the RALC scale) are almost all of the fruits and vegetables. A few fruits and vegetables, namely Cornelian cherries (*Cornus mas*) and lentils (*Lens culinaris*), have low acid loads. However, their restriction is not necessary, as their acid loads are far lower than other foods known to have high acid contents. Furthermore, increased fruit and vegetable consumption is usually recommended.

Fiber may reduce gastrointestinal absorption of calcium[62]. If high fiber food consumption is not at the desired levels (25-30 g/d for adult individuals), and calcium and bone statuses look normal, it may be appropriate to recommend higher dietary fiber intake or combination with fiber-reinforced supplements. Caffeine and alcohol may contribute to urinary calcium excretion and thus restriction of these may be useful[63,64].

Some reports show the efficiency of omega-3 fatty acids in reducing urinary calcium excretion[65-67], and supplementation is available *via* current commercial formulations in certain amounts.

Hyperoxaluria

Restriction of food-based oxalate is controversial. The majority of oxalate-containing foods are healthy, and, furthermore, contain special nutrients that frequently have general health benefits and contain fiber, potassium, magnesium, and antioxidants. Restriction or elimination of such foods from the diet will thus do greater harm than good. Reduction of dietary oxalate intake also requires a simultaneous reduction in dietary calcium, as it is essential to maintain the appropriate low calcium/oxalate ratio in the urine, and thus some authors question the low oxalate strategy for this reason.

Specific gastrointestinal microbiota profiles containing separate combinations of bacteria species have been recently identified, and these were observed to be regulated with diet habits[68]. For instance, individuals who consume diets with high fiber content have a different microbiota profile than those that do not[69]. In another study, individuals consuming diets with high meat content were shown to have different bacterial enterotypes than those that consume diets rich in carbohydrates[70]. As related research proceeds, it is possible that some diet patterns (such as oxalate-decreasing bacteria adjusting to suitable concentrations) will be shown to provide anti-lithogenic effects by leading to alterations in enteral microbes.

Hyperuricosuria

If a nutrition evaluation reveals a high content of purine-rich foods, a lack of foods with high purine concentration, and a reduction in the consumption foods with low purine, concentration should be recommended.

Another potential concern is blaming red meat as the only main culprit in uric acid synthesis. Recently, consumption of fish and chicken has also been shown to increase the concentration of serum and urine uric acid to at least the same extent as red meat[71]. As recommending that the patient decrease their red meat consumption will result in a higher consumption of chicken and fish instead, reducing the intake of all these foods is necessary in order to obtain suitable results. Reduction can be organized by decreasing portion sizes and the frequency of consumption during the week.

The quantity of alcohol and fructose consumptions should be evaluated in hyperuricosuria patients, and ways to reduce their intake should be discussed if they are believed to increase stone formation.

Hypocitraturia

When a diet with a high acid load that shows a hypocitraturic effect with its renal citrate reabsorption-improving effect is detected, small amounts of cheese, meat, and other meat products should be recommended in order to lower the acid load[61,72]. For instance, patients usually don't want to eliminate meat and other meat products from their diet, or are unable to apply such a change. Instead, special recommendations to restrict such foods to only small portions in one meal per day will have the same effect. If calorie load is not an issue for the patient, simply balancing the current high acid foods with low acid or alkaline foods (more fruits and vegetables) may be recommended.

Increased dietary intake of citric acid is useful and can increase urinary citrate excretion[40,73-75]. This can be partially achieved *via* the consumption of lemons (which contain concentrated citric acid) and lemonade. Recommendations on increasing consumption of citrus fruits will also provide benefit in terms of increased fiber, potassium, antioxidants, and prebiotics.

Recently, consumption of low sugar and calorie drinks sweetened with citrate and other organic acids has been recommended, as they have the capacity to increase urinary citrate[76]. On the grounds of presenting these drinks as therapeutics targeting the urinary citrate levels in a certain group of patients, liquid volumes provided by these drinks will also contribute to augmentation of overall liquid consumption.

If citrated fruit juices contain mainly citric acid, any bicarbonate obtained is neutralized by hydrogen ions. In that event, the net alkaline response will not take place and the eventual citraturic response will be at a minimum level. In contrast, if potassium accompanies citrate, the net alkaline response will take place, and urinary pH and citrate will rise. Ideal replacement therapy should be low in calories and oxalate, and rich in KCit. Yilmaz *et al*[77] have evaluated tomato, orange, lemon, and mandarin juices in terms of nutritional content. Interestingly, fresh tomato juice contains two times the amount of citrate compared to lemon juice or orange juice; however, the potassium concentration in fresh tomato juice is equal to orange juice and its oxalate content is 40% lower. In the light of these data, although fresh tomato juice seems to be suitable for preventing stone formation, its application is more difficult compared to lemon juice and orange juice. Ripe tomato juice, on the other hand, is rich in sodium.

Haleblian *et al*[78] evaluated 12 different commercial drinks that contained citrate, in an attempt to find natural treatment modalities that are more effective in preventing stone formation. Grapefruit juice was reported to have the highest citrate content, followed by lemon juice, orange juice, lemonade, and Cornelian cherry (*Cornus mas*) juice, respectively.

If frequent diarrhea is thought to contribute to hypocitraturia *via* increased renal citrate reabsorption as a result of excessive bicarbonate loss in the stool, nutrition strategies can be applied that target diarrhea treatment[79]. Probiotic supplements are recommended in the current literature for correction of diarrhea, and many probiotic formulations are commercially available for such use[80,81].

Low liquid consumption

All liquid types induce urinary output, and this is probably the most useful method that can spontaneously reduce the risk of stone formation on its own[8]. Low sugar- and low-calorie drinks are preferred.

Some patients may benefit more from a simple increase in liquid intake than far more specific recommendations. The liquid intake schedule is designed for these situations. The day may be separated into 3 equal parts (of 5 h blocks, for instance, depending on the lifestyle of the patient) and the individual may consume approximately 4 L of (120 oz) liquid by drinking 1200 mL in each part.

Hyperphosphaturia

Phosphate is widely present in all plant and animal-based foods, and so a reduction of dietary phosphate is not practically possible in calcium phosphate stone patients. Control of urinary citrate, calcium, pH, and volume is instead far more important in these patients.

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REVIEW

Pre-treatment considerations in childhood hypertension due to chronic kidney disease

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Abstract

Hypertension (HTN) develops very early in childhood chronic kidney disease (CKD). It is linked with rapid progression of kidney disease, increased morbidity and mortality hence the imperative to start anti-hypertensive medication when blood pressure (BP) is persistently > 90th percentile for age, gender, and height in non-dialyzing hypertensive children with CKD. HTN pathomechanism in CKD is multifactorial and complexly interwoven. The patient with CKD-associated HTN needs to be carefully evaluated for co-morbidities that frequently alter the course of the disease as successful treatment of HTN in CKD goes beyond life style modification and anti-hypertensive therapy alone. Chronic anaemia, volume overload, endothelial dysfunction, arterial media calcification, and metabolic derangements like secondary hyperparathyroidism, hyperphosphataemia, and calcitriol deficiency are a few co-morbidities that may cause or worsen HTN in CKD. It is important to know if the HTN is caused or made worse by the toxic effects of medications like erythropoietin, cyclosporine, tacrolimus, corticosteroids and non-steroidal anti-inflammatory drugs. Poor treatment response may be due to any of these co-morbidities and medications. A satisfactory hypertensive CKD outcome, therefore, depends very much on identifying and managing these co-morbid conditions and HTN promoting medications promptly and appropriately. This review attempts to point attention to factors that may affect successful treatment of the hypertensive CKD child and how to attain the desired therapeutic BP target.

Key words: Anaemia; Childhood; Chronic kidney disease; Hypertension; Hyperparathyroidism; Renin-angiotensin; Vascular calcification

Core tip: Hypertension (HTN) is often difficult to control in chronic kidney disease (CKD). Failure to achieve the desired therapeutic BP target in the hypertensive CKD child could be due to comorbidities and toxic effects of HTN promoting medications. So, before starting or altering anti-hypertensive medications, it is important that patients are evaluated for the roles that HTN promoting medications and co-morbidities like chronic

anaemia, hyperphosphataemia, progressive tunica media calcifications, and serum parathyroid hormone levels that are well above the acceptable limits for CKD stage could be playing in the entire process. Ways of solving this important clinical problem are the focus of this article.

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INTRODUCTION

In the non chronic kidney disease (CKD) paediatric population, hypertension (HTN) is a significant cause of morbidities[1,2] that are further escalated when it co-exists with CKD[3]. HTN develops very early in childhood CKD[3,4]. It is linked with rapid progression of kidney disease hence the Kidney Disease: Improving Global Outcomes recommendation that non-dialyzing hypertensive CKD children should commence antihypertensives when blood pressure (BP) is consistently > 90th percentile and not wait until it is ⩾ 95th percentile for age, gender, and height[5]. Therapeutic BP target in such children, particularly those with proteinuria, should be < 50th percentile for age, gender and height except hypotension is a limitation[5].

Pathophysiology of HTN in CKD is multifactorial and complex. In as much as this is so, the management should not be expected to be simple. An individual with CKD-associated HTN (CKD/HTN) needs to be carefully evaluated for co-morbidities that frequently alter the course of the disease as successful treatment of hypertensive CKD goes beyond life style modification and anti-hypertensive therapy alone. Chronic anaemia, volume overload, endothelial dysfunction, and metabolic derangements like hyperparathyroidism, hyperphosphataemia, 1, 25 $(OH)_2$ vitamin D_3 (calcitriol) deficiency, and tunica media vascular calcification (VC) are some of the co-morbidities that may cause or worsen HTN in CKD. A satisfactory hypertensive CKD outcome, therefore, depends very much on identifying and managing these co-morbid conditions promptly and appropriately. Before initiating a life style modifying plan or any form of antihypertensive treatment, it is important to know if the index patient has: Hyperphosphataemia, secondary hyperparathyroidism (SHPT), endothelial dysfunction, VC, anaemia, volume overload, and an estimated glomerular filtration rate (eGFR) that is < 15 mL/min per 1.73 m^2. Questions should be asked. Will the patient require dialysis? If so, is the patient on calcium-containing phosphate binder? Can the patient be dialyzed with a dialysis fluid that contains the standard concentration of calcium ions (1.75 mmol/L)? The doctor needs to know if the patient is regularly dialyzed or has received a kidney transplant. It is important to know if the patient is on HTN promoting medications like erythropoietin, cyclosporine, tacrolimus, corticosteroids and non-steroidal anti-inflammatory drugs (NSAID). Successful answers to these questions should guide the physician to further steps in tackling the HTN and achieving the therapeutic BP target for the patient.

This review attempts to point attention to factors that may affect successful treatment of the hypertensive CKD child and how to attain the desired therapeutic BP target.

EPIDEMIOLOGY OF HTN IN CKD

High CKD and co-morbidities, including HTN, prevalence have been reported in many studies. Severe CKDs are most commonly associated with the worst co-morbidities. The frequencies of co-morbidities, including HTN, rise with increasing severity of CKD stage[3,4]. Figure 1, generated from data from reference[3], shows the prevalence pattern of HTN by CKD stage in a population of children. Data on CKD incidence and prevalence from different countries vary widely, depending on whether they are hospital-based or obtained from national renal registries. A hospital-based study from Nigeria showed that the overall CKD incidence in children increased from 6.0 in year 2000 to 20.0 per million children population (pmcp) per year in 2009 while the prevalence increased from 8 to 101 pmcp; the incidence and prevalence of severe CKD (eGFR < 30 mL/min per 1.73 m^2) were, however, 3 pmcp/year and 22 pmcp, respectively[3]. Also from Nigeria, another hospital-based study puts the median annual incidence of severe CKD (creatinine clearance, CrCL: < 30 mL/min per 1.73 m^2) at 3.0/million age-related population (MARP) per year with a prevalence of 15 patients per MARP[6]. From a hospital-based study in Jordan, the estimated annual incidence and prevalence of severe CKD were reported to be 10.7/MARP per year and 51/MARP, respectively[7]. An Italian national survey reported a median annual incidence and prevalence of 7.7/MARP per year and 21/MARP, respectively for severe CKD[8]. However, in a French study severe CKD incidence was estimated at 7.5/MARP per year in children younger than 16 years while the prevalence was between 29.4 and 54/MARP[9]. Clearly from the above, the burden of CKD is very high and expectedly, the burden of co-morbidities is also high. The prevalence of HTN in childhood CKD is frequently high; it is reported to range between 20.0% and 80.0%[3,10-13]. This contrasts sharply with the 3.2%-3.6% HTN prevalence in the normal paediatric and adolescents' population[14-16]. Commonly, children with CKD are associated with high nocturnal[17] and masked HTN prevalence[12,13].

Target-organ abnormalities are common features of HTN in children and adolescents. Curiously, CKD children with mild HTN have been reported to have

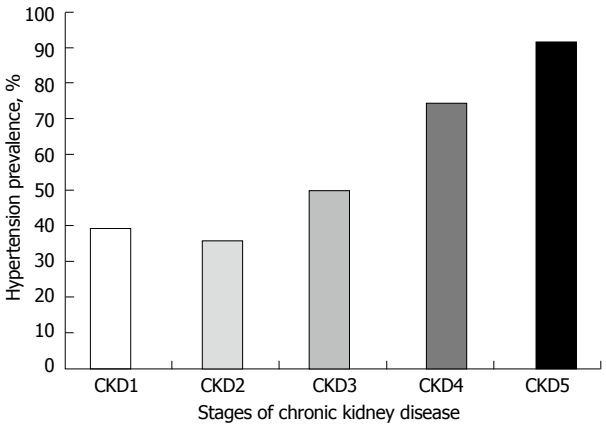

Figure 1 Prevalence of hypertension by chronic kidney disease stage in children. Data for this Figure were obtained from reference[3]. CKD: Chronic kidney disease.

target-organ damage[18-20]. Left ventricular hypertrophy (LVH) is common target-organ damage in HTN[2]. About 34%-38% of paediatric patients with mild and untreated HTN have LVH[21-23]. When associated with proteinuria, HTN has been found to escalate CKD progression and mortality in children and adults[24-26]. In a report, mortality was escalated from 55.5% in non-hypertensive CKD children with heart failure to 84.0% in hypertensive CKD patients with heart failure[3].

It is often difficult to control HTN in CKD. Irrespective of anti-hypertensive medications used, HTN cannot be controlled in more than 50% of children with end-stage renal disease (ESRD)[27-29]. Following treatment with combination antihypertensive medications, only 56% of hypertensive CKD children were able to achieve a BP target of < 50th percentile for age, gender and height[3]. But why is it so difficult to achieve good BP control in hypertensive CKDs? This might be due to failure to critically appraise some of the CKD co-morbidities highlighted above, before starting antihypertensive medications. In a cohort of ESRD children, poor BP control was associated with very young age, post dialysis fluid overload, and hyperphosphataemia. In that report, only 23.5% of treated patients were able to achieve a KDOQI BP target of < 90th percentile[29].

PATHOPHYSIOLOGY OF HTN IN CKD

BP regulation is a complex coordination of physiological functions namely cardiac output, fluid volumes, and peripheral resistance among organ systems in the human body. These organ systems encompass the central nervous system, cardiovascular system, kidneys, and adrenal glands[30]. CKD/HTN develops through a number of complexly interwoven pathomechanisms (Figure 2). Fluid overload and renin-angiotensin-aldosterone-system (RAAS) activation are long recognized important HTN pathophysiological pathways. More recently, increased parathyroid and sympathetic activity and endothelial dysfunction have been reported as contributing to CKD/HTN[31]. HTN may possibly be due to angiotensin Ⅱ (ANG Ⅱ)-related vascular constriction and aldosterone-related sodium retention due to renin hyper secretion by under perfused renal scars/cysts and/or severe renal tissue damage from microangiopathy or tubulointerstitial inflammation[32,33]. Furthermore, high circulating levels of ANG Ⅱ contribute to HTN and end organ injury by promoting mesangial cell proliferation, endothelial cell damage, cardiac enlargement, inflammation, and fibrosis[34]. A further mechanism for CKD/HTN which may be in line with Brenner hypothesis is that reduced nephron number following progressive kidney damage may result in reduced salt and water excretion which may predispose to HTN. The Brenner[35] hypothesis which has since been confirmed in other studies[36,37] is that low sodium excretion with attendant HTN may result from congenital nephron number deficit in the low birth weight infant[35]. While sodium retention and volume overload are established aetiological factors in CKD/HTN, sympathetic hyperactivity remains an important volume-independent cause of HTN whose pathomechanism is unclear[38,39]. Renal afferent signals, dopaminergic abnormalities and leptin accumulation in CKD may be contributory[38,39]. Renal sympathetic nerves in renal tubular epithelial cells and blood vessels are stimulated by ANG Ⅱ to cause an increase in the local release of norepinephrine which then causes renovascular constriction leading to decreased renal blood flow and GFR and HTN[40]. This excessive sympathetic activity is blocked by an ANG Ⅱ receptor blocker (ARB)[41]. Hyperparathyroidism is a common disorder in CKD that interferes with cardiovascular structural geometry and functions. Chronic hyperparathyroidism increases vascular smooth muscle cells' (VSMC) sensitivity to calcium and norepinephrine by promoting calcium ions accumulation within the VSMCs[42,43]. The consequence of this is vasoconstriction and HTN. This action may be countered with calcium channel blockers (CCB)[42,43]. Furthermore, chronic hyperparathyroidism promotes VSMC transformation to osteoblasts and vascular wall mineralization or calcification leading to vascular stiffening, increased peripheral resistance to blood flow with consequent HTN.

Children on maintenance dialysis are reported to have significant incidence of HTN that is as high as 53%-65% and 45%-58% in haemodialysis and peritoneal dialysis patients, respectively[44]. Haemodialysis substantially contributes to HTN by increasing both plasma renin activity and catecholamines[45].

Nitric oxide (NO) is a major vasodilator factor that vascular endothelia secrete, and lack of it causes severe HTN[46]. Endothelium-dependent vasodilatation is impaired in uraemia due to deficient NO synthesis[47]. A circulating endothelium-derived NO synthase inhibitor, presumably asymmetric dimthylarginine, which

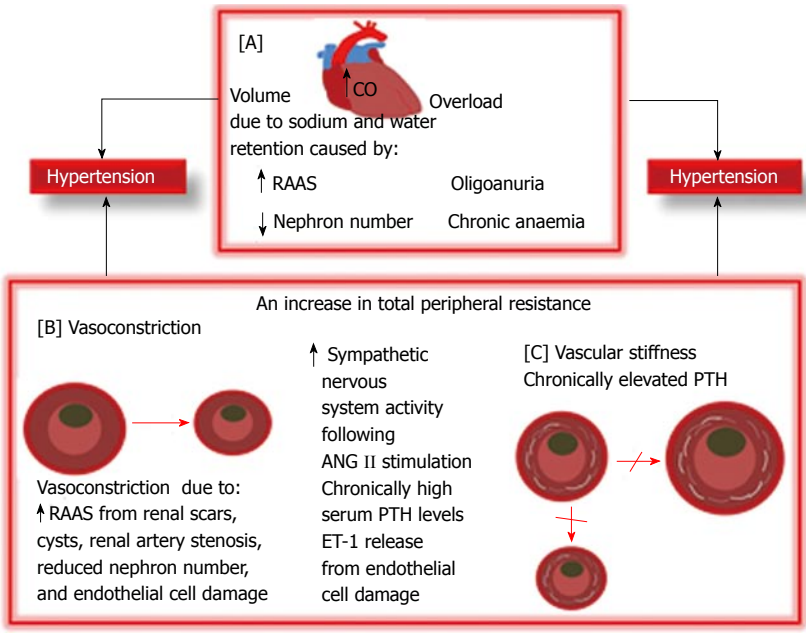

Figure 2 Pathophysiologic mechanisms of hypertension in chronic kidney disease. A: Volume overload is associated with increase in cardiac output (CO) which ultimately leads to hypertension; B: Increase in total peripheral resistance (TPR) due to systemic vasoconstriction leads to hypertension; C: Arterial tunica media calcification causing vascular stiffening and failure of vasodilatation and vasoconstriction are illustrated. Chronic hyperparathyroidism promotes vascular wall mineralization or calcification leading to vascular stiffening and increase in TPR with consequent hypertension. Blood pressure = CO × TPR. RAAS: Renin-angiotensin-aldosterone system; ANG II: Angiotensin II; ET-1: Endothelin 1; PTH: Parathyroid hormone.

accumulates in uraemia is possibly responsible. Endothelin-1 (ET-1), the most potent vasoconstrictor known, is secreted by the vascular endothelium. Plasma ET-1 concentrations rise directly with BP increase in ESRD, suggesting a role for ET-1 in the causation of CKD/HTN[48]. By preventing the breakdown of vasodilatory kinins, angiotensin converting enzyme inhibitors (ACEi) are able to reduce ET-1 expression and suppress ET-1 induced HTN[49,50].

For a number of reasons, CKD patients receive medications like erythropoietin, NSAID, cyclosporine, tacrolimus, and corticosteroids that could predispose to or make HTN worse. These agents cause HTN through a variety of mechanisms that involve interference with arachidonic acid metabolism, ET-1 and NO syntheses. The ultimate result of this interference is HTN through increased TPR due to vasoconstriction with reduced renal perfusion and GFR, increased sodium and water reabsorption as a consequence of RAAS activation. In a review by Krapf *et al*[51], post erythropoietin therapy HTN occurs through increased syntheses of vasoconstrictors like ET-1 and thromboxane (TXB2) but decreased productions of vasodilators like prostacyclin (PGI2) and NO. Reduced production of NO is secondary to decreased expression of endothelium-derived nitric oxide synthase (NOS), an enzyme that catalysis the production of NO. NSAID associated HTN occurs through cyclooxygenase inhibition by preventing arachidonic acid conversion to vasodilator prostanoids like prostaglandin E2 (PGE2) and PGI2[52]. This action leads to increased TPR and volume overload and HTN through increased production of ET-1, increased Na^+ and Cl^- ions reabsorption in the loop of Henle (thick ascending segment) and anti-diuretic hormone-mediated increased water reabsorption. Through another metabolic pathway, NSAID may cause HTN by promoting the release of cytochrome P450-mediated vasoconstricting metabolites of arachidonic acid such as epoxyeicosatrienoic and hydroxyeicosatetraenoic acid[52]. Calcineurin inhibitors, namely cyclosporine and tacrolimus cause HTN through reduced productions of PGI2 and NO but increased productions of ET-1 and TXB2[53] with consequent vasoconstriction, reduced renal blood flow and GFR, sodium and water retention. The mechanism by which corticosteroids causes HTN is not yet clear; however, one of the mechanisms known currently is inhibition of the release of arachidonic acid from phospholipids thereby preventing prostaglandins formation leading to decreased production of vasodilator prostanoids[54]. *In-vitro*, cortisol has been demonstrated to potentiate vascular smooth muscles cells pressor responsiveness to epinephrine and norepinephrine by inhibiting catechol-o-methyl transferase, an enzyme that degrades catecholamines neurotransmitters such as dopamine, epinephrine, and norepinephrine[54]. While, for obvious reasons, these drugs cannot be stopped the dosages can be lowered, in order to achieve good BP control, to levels that will not compromise the primary indications for their prescription.

HTN in the transplanted CKD patient is often caused by volume overload, corticosteroids, and calcineurin inhibitors. ACEi and ARB are avoided in the first few weeks post-transplant to avoid renal insufficiency in the setting of diminished effective arterial blood

volume[55]. To prevent calcineurin inhibitor-induced graft dysfunction, therefore, HTN is treated with CCB in the immediate post-operative period[55]. CCBs like nifedipine and amilodipine have been used with satisfactory outcomes in paediatric transplant patients[56].

WHAT FACTORS MAY PREVENT ATTAINMENT OFTHERAPEUTIC BP TARGET IN CKD-ASSOCIATED HTN?

It is inconceivable that in the setting of chronic anaemia, progressive arteriosclerosis, uncontrolled SHPT, and HTN promoting medications, the therapeutic BP target can be attained in CKD/HTN. Successful HTN treatment outcome demands that these factors be carefully evaluated and managed accordingly.

Volume overload due to chronic anaemia

Anaemia is a frequent comorbidity in childhood CKD[3,4,57]. Failure to attain the target therapeutic BP goal in CKD/HTN may be due to untreated or poorly treated anaemia. Anemia-associated tissue hypoxia causes peripheral vasodilatation. Reduced BP caused by vasodilatation stimulates increased sympathetic activity with attendant tachycardia and increased stroke volume. This is accompanied by increased cardiac output and vasoconstriction. The latter causes reduced renal blood flow, increased RAAS activity and anti-diuretic hormone production leading to salt and water retention[58]. The long term effect of this is HTN or worsening of existing HTN. All hypertensive CKD patients should be carefully assessed for anaemia and volume overload and managed accordingly. Anaemia in childhood CKD is defined as haemoglobin (Hb) concentration < 11.0, < 11.5, and < 12.0 g/dL in children aged 0.5-5, 5-12, and 12-15 years, respectively[57]. It is suggested that when correcting anaemia, the target Hb concentration in all paediatric CKD patients receiving erythrocytes stimulating agent therapy should be maintained within 11.0 to 12.0 g/dL range[57]. Excess volume can be removed with a low ceiling diuretic, like a thiazide, when eGFR is $\geqslant$ 60 mL/min per 1.73 m^2 or with frusemide, a high ceiling diuretic, when eGFR is < 60 mL/min per 1.73 m^2. eGFR $\leqslant$ 15 mL/min per 1.73 m^2 will rarely respond to diuretics. Fluid removal will have to be by dialytic ultrafiltration. It is important to note that when treating anaemia with erythropoietin, HTN may occur following weeks of therapy; this is partly due to increase in the red blood cell mass, increased blood viscosity and resistance to blood flow. Other mechanisms include vascular wall remodeling with resultant rise in vascular resistance[59]. It is also possible that due to direct action of erythropoietin on voltage-independent Ca^{2+} channels in the VMSCs, the sensitivity of the latter to the vasodilatory action of NO may be diminished[60]. Erythropoietin has been reported to exacerbate HTN in both non-dialyzing and dialyzing CKD children[61,62]. This complication can be ameliorated by reducing the dose of erythropoietin.

Tunica media VC or arteriosclerosis

It is a well-known fact that tunica media VC, a form of CKD-mineral and bone disorder, is associated vascular wall rigidity with attendant progressive vascular pulse wave deceleration and abnormal vascular wall geometry. Increasing vascular rigidity ultimately leads to cardiac damage from long standing cardiomyocytes ischaemia, from high oxygen consumption, and diminished coronary blood flow[63]. Dialysis history, consumption of high doses of active vitamin D, deficiencies of inhibitors of calcification, hypercalcaemia and hyperphosphataemia are risk factors for VC in CKD[64]. Hyperphosphataemia is an important and possibly a principal promoter of VC because it has been clearly linked with increased VC and mortality[65,66]. Fibroblast growth factor-23 (FGF23) together with its anti-ageing cofactor, Klotho have been recognized as major regulators of phosphate homeostasis, in addition to inhibiting production and release of parathyroid hormone (PTH) and suppressing renal production of 1, 25 $(OH)_2$ vitamin D. In an experiment by Sitara *et al*[67], FGF23 null mice and Klotho null mice developed similar phenotypes, characterized by very high serum concentrations of phosphate and 1, 25-dihydroxyvitamin D3 with disordered bone mineralization including multiple soft-tissue calcifications. A 13-year-old child with a Klotho gene mutation suffered severe vascular and soft-tissue calcifications, despite markedly elevated serum FGF23. Thus, deficiency of Klotho in this patient prevented FGF23 from exerting its phosphate-lowering effects and its protection against soft tissue calcification[68]. This shows that without Klotho, FGF23 cannot correctly exert its normal physiological functions. Klotho prevents soft tissue calcification by three main mechanisms namely, phosphaturia, kidney function preservation and directly inhibiting phosphate uptake and dedifferentiation by the VSMCs[69]. In CKD, serum levels of FGF23 increase in proportion to the decrease of GFR[70]. This increase can be considered as an appropriate compensatory mechanism in the defense against phosphate retention, in concert with PTH, although it also leads to an inhibition of renal calcitriol synthesis, in contrast to PTH which promotes renal calcitriol synthesis[71]. Of note, chronic dialysis patients and uremic animals have been shown to exhibit a relative resistance to the inhibitory action of FGF23 on parathyroid gland function[72-74]. This is probably due to down regulation of Klotho and FGF23 receptor expression in CKD. Increased systolic BP, resulting in elevated cardiac afterload and LVH and decreased diastolic BP and impaired coronary perfusion are initial major consequences of arterial stiffening[75]. Cardiovascular calcification (CVC) is not only a progressive disorder; it is also severer among CKD patients, with poorer cardiovascular outcome, compared with other populations[76]. VC must, therefore, be recognized very early in CKD and aborted as progression will worsen both the kidney disease and HTN thereby making BP

therapeutic goal unattainable with dire consequences for the patient. High pulse pressure suggests arterial stiffening/rigidity and therefore, should be an indication for anyone or combination of the following investigations: flow mediated dilation for endothelial dysfunction, carotid intimal medial thickness (cIMT), pulse wave velocity (PWV) and echocardiography for valvular calcification; plain X-rays of the hands including the wrists can also detect VC in the radial and digital arteries[76,77]. Similarly, lateral lumbar spine (lateral abdominal X-ray) and pelvic radiographs can detect VC in the abdominal aorta and femoral and iliac arteries[64]. However, in detecting and quantifying CVC, including the coronary arteries, the electron-beam computed tomography (EBCT) and multislice CT (MSCT) are the most sensitive radiologic techniques available[78-82]. cIMT, PWV, EBCT and MSCT are established indicators of structural and functional anomalies of blood vessels, including calcification in children and adults[78-82]. cIMT in paediatric CKD patients was adversely affected by high plasma phosphate[78-80]. In 85 dialyzing children, the cIMT increased by 0.15 mm for each mmol/L rise in the serum phosphate concentration[78].

Can VC be treated or reversed?

Currently, there is no definitive treatment for VC reversal but the process leading to it can be halted through preventive measures. The most important preventive measure is to ensure that serum phosphorous level is kept within the normal age-specific range. The approaches to reducing high plasma phosphate level should include reducing dietary phosphate intake[83], and gastrointestinal absorption with phosphate binders[84], and giving more dialysis to increase clearance in those with 5D-CKD[85,86]. Stages 3-5 CKD patients can have their serum phosphorous kept within acceptable limits of 0.81-1.45 mmol/L (2.5-4.5 mg/dL); high values should, however, be brought down to the normal limits in CKD-5D. On the other hand, serum calcium should be kept within the normal limits of 2.1-2.6 mmol/L (8.8-10.5 mg/dL) in individuals with 3-5D CKD[85,86]. However, a dialysate fluid having low calcium concentration of 1.25-1.50 mmol/L (2.5-3.0 mEq/L) is advised for use in order to avoid hypercalcaemia, adynamic bone disease and rapid VC progression that may occur with the standard dialysate fluid, containing 1.75 mmol/L of calcium, when used in CKD-5D[76]. It is recommended that serum concentrations of calcium, phosphorus, PTH, and alkaline phosphatase should be determined starting from CKD-2 in paediatric patients[76]. Furthermore, it is suggested that serum calcium and phosphorous be measured in CKD 3, CKD 4, and CKD 5/5D at 6-12, 3-6, and 1-3 mo intervals, respectively[76]. In hyperphosphataemic individuals with 3-5D CKD, calcium-based phosphate binders are best avoided when there is evidence for arterial calcification and/or adynamic bone disease and/or persistently low serum concentrations of PTH. Calcium-based phosphate binders and/or calcitriol or vitamin D analog are similarly contraindicated when such patients have hypercalcaemia that is persistent or recurrent[76]. Increased dialytic phosphate removal is suggested for CKD stage 5D if hyperphosphataemia is persistent. Effective alternatives to calcium-based phosphate binders include non calcium-based phosphate binders like sevelamer, and lanthanum salts. Although Sevelamer hydrochloride possesses the additional benefit of reducing total cholesterol and low density lipoprotein cholesterol concentrations in the plasma, patients may need to be on calcium supplement when there is overt hypocalcaemia[76]. Sevelamer hydrochloride has been reported in some studies to attenuate arterial calcification progression in stages 3-5 and 5D CKD patients when compared to similar patients treated with calcium-based phosphate binders[87-91]. Zhang *et al*[92] have shown in their systematic review of literature on adult patients that lanthanum carbonate efficaciously reduces serum phosphorus and intact PTH levels without raising the serum calcium concentration. The author is currently not aware of any published study on lanthanum carbonate use in children.

The use of pyrophosphate, bisphosphonate and thiosulfate in the prevention of VC is largely experimental. With current level of information available from various experimental studies, they show a lot of future promise for the prevention of VC in humans when they become clinically available. Schibler *et al*[93] were able to demonstrate that high dose pyrophosphate could inhibit tunica media calcification in rats that were intoxicated with vitamin D. High dose pyrophosphate was used to prevent its rapid hydrolysis to orthophosphate. However, to obviate the need for high dose pyrophosphate, bisphosphonate a non hydrolysable analogue of the former was developed. Medial calcification has been effectively inhibited with bisphosphonate in uraemic rats[94]. Pasch *et al*[95] demonstrated that tunica media calcification developed within four weeks in a Wister rat model of uraemic renal failure caused by adenine diet-induced severe interstitial nephritis. Using thiosulfate at doses and frequencies that were similar to that used in patients with calcific uraemic arteriolopathy, Pasch et al were able to completely prevent VC in their animal model. However, the drawbacks with the thiosulfate study of Pasch *et al*[95] are that: (1) the mode of action is unknown; (2)thiosulfate prevents but does not reverse VC; (3) its safety limits in man are unknown; and (4) there is the possibility of reduced bone mineralization.

VC is a common complication of high doses of vitamin D receptor agonists (VDRAs) especially when associated with hypercalcaemia[96-99]. However, using lower doses of VDRAs that are currently in use in clinical practice, Lau *et al*[100], were able to demonstrate that active vitamin D (calcitriol, 30 ng/kg) and its analog (100 ng/kg paricalcitol) prevented arterial medial VC in CKD mice given high phosphate diet (1.5%). Independently of serum calcium and PTH both VDRAs reduced the degree of VC *via*: (1) elevated serum Klotho, increased phosphaturia as well as normalized serum phosphate and FGF23 levels; and (2) up regulation of VSMC osteopontin but reduced

circulating osteopontin that is associated with VC reduction. Using much lower (physiological) dosages, Mathew *et al*[97] had earlier noted that both calcitriol and paricalcitol prevent VC. The clinical benefit of both studies with regard to VC needs to be determined by further studies.

SHPT

As discussed above, hyperparathyroidism causes HTN through vasoconstriction and vascular medial wall calcification[42,43]. PTH level should be determined early in the course of managing CKD/HTN as this may be elevated beyond the expected level for the CKD stage in the patient. Appropriate management of the inappropriately elevated PTH for CKD stage may impact significantly on HTN outcome. In children with CKD, 25 (OH) vitamin D (calcidiol) is a common deficiency; it is one of the factors that may be responsible for SHPT in CKD. The serum level of calcidiol (normal: 8-50 ng/mL) should be determined at baseline in every CKD patient. The ways by which active vitamin D sterols suppress PTH levels include: Increased intestinal calcium absorption, and PTH gene transcription suppression. Given either in daily or intermittent doses, calcitriol and alfacalcidol effectively suppress PTH and improve growth in childhood CKD[101,102]. Hypercalcaemia is, however, a serious side effect especially when ingested with phosphate binders containing calcium. The newer vitamin D analogues namely 22-oxacalcitriol, 19-nor-1, 25-dihydroxy vitamin D2 (paricalcitol) and 1α-hydroxyvitamin D2 (doxercalciferol) are associated with minimal intestinal calcium and phosphorus absorption. PTH levels are effectively reduced by doxercalciferol and paricalcitol; both have the ability to reduce serum calcium levels better than calcitriol in CKD children and adults[103,104]. Where SHPT is due to hyperphosphataemia, appropriate use of phosphate binders may just be sufficient. Cinacalcet is a type II calcimimetic that allosterically modulates the calcium sensing receptor, CaSR thus making it more sensitive to circulating calcium ions with resultant reduction in PTH release[105]. Studies have shown that calcimimetics effectively act on the parathyroid gland of CKD-5 patients to promote reasonable decreases in circulating serum phosphorus and calcium ions[106,107]. Calcimimetics have on the other hand been associated with unwanted increases in serum phosphorus, through unknown pathways, in CKD-3/4. They should, therefore, be avoided in such patients[108,109]. Calcimimetics have been found useful in the few paediatric CKD-5 patients studied so far[110,111]. Six CKD 5D children aged between 11 mo and 14 years who had uncontrolled SHPT and treated with cinacalcet (doses: 0.4-1.4 mg/kg) showed satisfactory and sustained correction of the hyperparathyroidism[112]. Whatever medication that is chosen for the hyperparathyroidism, it is suggested that the target serum PTH in CKD 3, CKD 4, and CKD 5/5D should, respectively be in the 35-70, 70-110, and 200-300 pg/mL range to avoid adynamic bone disease from too low serum PTH[113]. In CKD, the serum PTH should be maintained within 2-9 times the upper limits of the normal laboratory range[76]. It is important that serum PTH and alkaline phosphatase are determined at baseline, every 6-12, and 3-6 mo, respectively in patients with progressive CKD 3, CKD 4, and CKD 5/5D[76].

CONCLUSION

The pathomechanism of HTN in CKD is multifactorial and complexly interwoven. Successful treatment of HTN in CKD, therefore, goes beyond life style modification and anti-hypertensive therapy alone. The patient with CKD/HTN needs to be carefully evaluated for co-morbidities that frequently alter the course of the disease. It is also important to know if the HTN is caused or made worse by the toxic effects of medications like erythropoietin, cyclosporine, tacrolimus, corticosteroids and NSAID. A satisfactory therapeutic outcome in the hypertensive CKD, therefore, depends very much on identifying and managing these co-morbid conditions promptly and appropriately.

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MINIREVIEWS

Hepatorenal syndrome: Update on diagnosis and treatment

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Abstract

Acute kidney injury (AKI) is a common complication in patients with end-stage liver disease and advanced cirrhosis regardless of the underlying cause. Hepatorenal syndrome (HRS), a functional form of kidney failure, is one of the many possible causes of AKI. HRS is potentially reversible but involves highly complex pathogenetic mechanisms and equally complex clinical and therapeutic management. Once HRS has developed, it has a very poor prognosis. This review focuses on the diagnostic approach to HRS and discusses the therapeutic protocols currently adopted in clinical practice.

Key words: Hepatorenal syndrome; Cirrhosis; Acute kidney injury; Diagnosis; Treatment; Terlipressin; Liver support system

Core tip: Hepatorenal syndrome is a functional and potentially reversible form of kidney failure. The pathophysiological bases of this disease are complex and not fully understood. The aim of this review is to focus the current diagnostic approach and the updated therapeutic protocols adopted in clinical practice.

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PATHOGENESIS

Hepatorenal syndrome (HRS) can be considered the final stage of a pathophysiological condition charac-

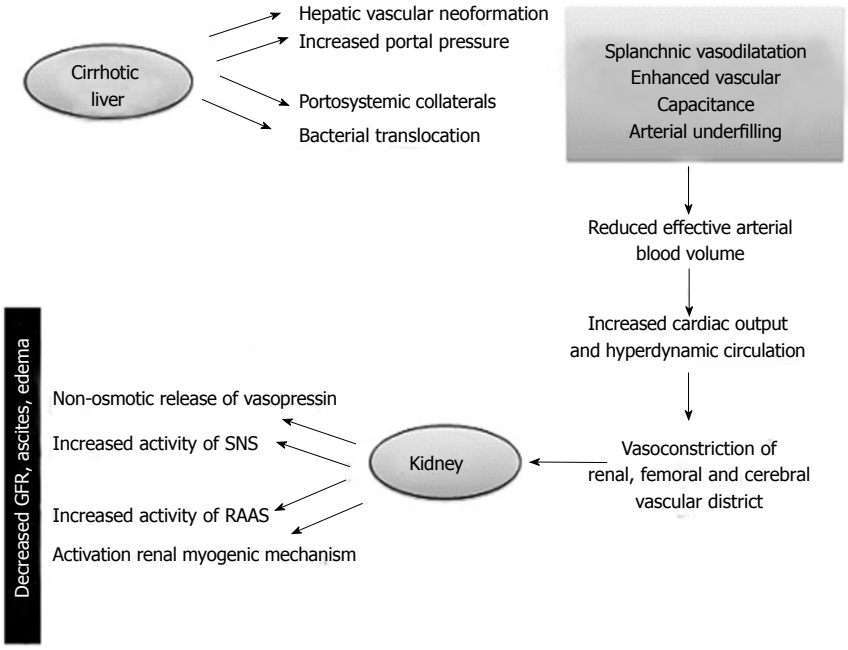

Figure 1 Hepatorenal syndrome: Pathogenesis. In cirrhotic patients portal hypertension can lead to markedly dilated splanchnic arterial vessels. The bacterial translocation of intestinal germs, the gradual decrease in systemic vascular resistances, the hepatic vascular neoformation are potential risk factors. The fall in mean arterial pressure is compensated by increase in cardiac output and by activation of renin-angiotensin-aldosterone system (RAAS) and sympathetic nervous system (SNS) to improve systemic vascular resistence. The response mechanisms to the decreased effective circulating volume caused by Enhanced vascular capacitance (so-called "arterial underfilling") include the non-osmotic release of vasopressin accounting for renal tubular sodium resorption and water retention leading to the onset of ascites, edema and hypervolemic hyponatremia. These compensatory mechanisms ultimately have repercussions on kidney function causing reduced glomerular filtration rate (GFR) and further water retention thereby worsening the water overload.

terized by decreased renal blood flow resulting from deteriorating liver function in patients with cirrhosis and ascites[1-5].

Hemodynamic changes associated with endothelial shear stress occur before the onset of ascites and are sustained by an increase in pro-angiogenic factors like the vascular endothelial growth factor and platelet-derived growth factor and vasodilators (carbon monoxide, endocannabinoids and nitric oxide) able to promote the formation of hepatic, splanchnic and portosystemic collateral vessels[6-11] (Figure 1).

The ensuing hemodynamic instability may give rise to many clinical events that further interfere with the compensatory mechanisms. These include the onset of spontaneous bacterial peritonitis, gastrointestinal bleeding and post-paracentesis circulatory dysfunction[12].

The renal impairment is worsened by a progressive cardiac dysfunction known as cirrhotic cardiomyopathy. The latter is characterized by diastolic impairment with septal ventricular hypertrophy, blunted ventricular response to stress, systolic and diastolic dysfunction, and electrophysiological abnormalities (prolongation of QT interval)[7]. Systolic dysfunction is due to impairment of both β-adrenergic receptor and increasing in endogenous cannabinoids and cardiosuppressants such as nitric oxide and inflammatory cytokines and myocyte apoptosis. Furthermore it is possible that several intracellular signaling pathways are involved.

On the other hand the activation of renin-angiotensin system and salt retention play a role in diastolic disfunction. Recent studies have stated myocardial dysfunction in cirrhosis as a contributing, or even a precipitant factor, of HRS[13,14] .

EPIDEMIOLOGY

According to Fede *et al*[15], approximately 20% of cirrhotic patients with diuretic-resistant ascites potentially develop HRS, while a prospective study by Ginès *et al*[4] on 229 patients with cirrhosis found an 18% incidence of HRS at one year, rising to 39% at five years after initial diagnosis.

HRS may also arise in patients with acute liver failure as shown in Akriviadis *et al*[16]: They considered 101 patients with alcoholic hepatitis of whom 28 developed HRS after a four-week follow-up. Planas *et al*[17], in a study enrolling 263 cirrhotic patients with a follow-up of 41 ± 3 mo after the onset of ascites, found prevalence rates of 2.6% and 5% for HRS types Ⅰ and Ⅱ respectively, with a cumulative probability of 11.4% at five years. The prevalence of HRS increases with liver disease progression, Wong *et al*[18] reporting a rate of 48% in patients on the waiting list for liver transplant.

Despite discrepancies in literature data, the prevalence of HRS has dropped in recent years, probably as a result of a better understanding of its pathophysiology and improved clinical management[19]. Nonetheless the long-term survival of HRS patients remains poor and the only effective treatment for this condition is liver transplantation.

Table 1 Diagnostic criteria for hepatorenal syndrome

Cirrhosis with ascites
Serum Creatinine > 1.5 mg/dL
Absence of shock
No improvement of serum creatinine (decrease to a level of 1.5 mg/dL or less) after at least 2 d of diuretic withdraw and volume expansion with albumin (The recommended dose of albumin is 1 g/kg of body weight per day up to a maximum of 100 g/d)
No current o recent exposure to nephrotoxic drugs
Absence of parenchymal disease as indicated by proteinuria > 500 mg/d, microscopic hematuria (50 red blood cells per high power field) and abnormal renal ultrasonography

HRS: Hepatorenal syndrome.

Table 2 Characteristics of type Ⅰ and type Ⅱ hepatorenal syndrome

HRS I	Doubling of serum creatinine in < 2 wk	A precipitating event is present in the most of case	No history of diuretic resistant ascites	10% survival in 90 d without treatment
HRS II	Renal impairment gradually progressive	No precipitating events	Always ascites diuretic resistance	Median survival 6 mo

HRS: Hepatorenal syndrome.

DIAGNOSIS

The diagnostic criteria for HRS were initially defined by the International Ascites Club (IAC) in 1994[20-22]. Since then, advances in our understanding of HRS pathogenesis and the introduction of new therapies led to repeated revisions of the criteria. The latest version of 2007 excludes the use of creatinine clearance (due to its poor correlation with kidney function in patients with cirrhosis), and has eliminated minor criteria (sodium excretion fraction, urinary output) deemed less sensitive and specific. Concomitant bacterial infection does not rule out a diagnosis of HRS but it is crucial to identify the absence of septic shock[1] (Table 1).

Two forms of HRS, types Ⅰ and Ⅱ, have been described. They differ in severity and rate of progression and can be considered two separate clinico-pathological entities[23] (Table 2).

Type Ⅰ HRS is characterized by acute onset and rapidly progressing kidney failure with a doubling of serum creatinine to > 2.5 mg/dL (corresponding to a 50% reduction in the creatinine clearance rate) in less than 2 wk, usually associated with multiorgan damage. The prognosis is poor with only 10% of patients surviving longer than 90 d[4].

This type of HRS can develop spontaneously but more often tends to follow a precipitating event, mostly spontaneous bacterial peritonitis or other infections like pneumonia, urinary tract infections or cellulitis[24]. Other potential risk factors include viral, alcoholic, toxic or ischemic hepatitis (*e.g.,* TIPS), gastrointestinal bleeding and surgical procedures (Table 3).

Type Ⅱ HRS represents the final kidney response to hemodynamic impairments in cirrhosis. This type presents as a less severe and more gradual decline in renal function associated with refractory ascites. The increase in creatinine is gradual with mean values of 1.5-2.0 mg/dL. Type Ⅱ HRS predisposes patients to the development of type Ⅰ HRS after a precipitating event. The average survival rate is six to eight months after onset.

The differential diagnosis between the two types of HRS is based on the rate of progression and extent of renal impairment, whereas the pathophysiological differences have not yet been fully clarified.

A spontaneous recovery is rare in both cases unless there is a significant improvement in liver function.

The differential diagnosis between HRS, other causes of kidney disease and septic shock remain extremely difficult. Despite the widespread circulation of the IAC criteria, a serum creatinine cut-off of 1.5 mg/dL appears limited as it does not take into account its physiological fluctuations. In addition, creatinine values ≤ 1.5 mg/dL may overestimate the true reduction in GFR[25].

The AKI network (AKIN) has proposed a new definition of AKI for the diagnosis of HRS designed to implement the traditional IAC criteria for prompt recognition of kidney damage. AKI is defined as the abrupt loss of kidney function resulting in a 0.3 mg/dL increase in serum creatinine in 48 h or a 50% increase over the basal value. The aim is to apply the AKI criteria to decompensated cirrhotic patients for an early identification of kidney failure and thereby implementing prompt aggressive treatment[26].

Two recent prospective studies assessed the applicability of the AKI criteria in patients with cirrhosis. The study by Fagundes *et al*[27] on 375 patients and another by Piano *et al*[28] on 233 cirrhotic patients both divided the populations into two groups based on kidney function. The first group comprised patients with a serum creatinine increase ≥ 0.3 mg/dL but below the threshold of 1.5 mg/dL, whereas the second enrolled patients with creatinine > 1.5 mg/dL. In both cases renal decline and mortality rates were significantly higher in the group with serum creatinine > 1.5 mg/dL, with a lower probability of kidney disease regression. These results suggest that AKI with serum creatinine values < 1.5 mg/dL is a relatively benign and potentially reversible condition, whereas the progression of renal deterioration to a significant decrease in GFR (values > 1.5 mg/dL) carries a poor prognosis[27,28].

Nonetheless, a recent editorial by Arroyo *et al*[29] pointed to a lack of evidence demonstrating the real advantage of the IAC guidelines with respect to AKI criteria. The stratification of cirrhotic patients according to single organ damage (kidney, liver or brain) appears to simplify the complex changes occurring in patients with decompensated liver failure.

Mindikoglu *et al*[2] proposed a new classification

Table 3 Risk factors for the onset of hepatorenal syndrome

Spontaneous bacterial peritonitis
Large volume paracentesis (> 5 L) with inadequate albumin substitution
NSAID and other nephrotoxic drugs, iv contrast
Bleeding from esophageal varices
Post TIPS syndrome
Diuretic treatment

Spontaneous bacterial peritonitis are leading trigger of HRS. One-third of patients with SBP develop HRS in the absence of septic shock. Diuretic treatment has been suggested as a potential trigger of HRS, but there are no clear supportive data for this. HRS: Hepatorenal syndrome; NSAID: Non-steroidal anti-inflammatory drug; TIPS: Transjugular intrahepatic portosystemic shunt.

associating GFR measurement and renal blood flow to stratify renal dysfunction, introducing the new concept of "pre-HRS", *i.e.,* patients with reduced renal blood flow but still normal or slightly reduced GFR. However, further studies are required to establish the clinical utility of this concept[30].

In all patients with acute renal failure and even more in patients with cirrhosis, serum creatinine may not reflect the reduction of kidney function with a significant difference between male and female. Because of that it was proposed using cystain C as alternative marker of renal function.

Seo *et al*[31] and Sharawey *et al*[32] showed that serum cystatin C level is a good marker for predicting HRS and survival in patients with cirrhotic ascites.

In the last 2 years the IAC organised a consensus development meeting in order to analyse the new definition of AKI in patients with cirrhosis and HRS: All the experts agreed on the removal of a fixed cut-off value of serum creatinine from the diagnostic criteria of HRS and they didn't suggest to evaluate Cystain C determination[33] (Table 1).

As there are currently no specific tests to identify HRS, diagnosis rests on the exclusion of other causes of kidney failure. It is important to establish the etiology of kidney injury in order to institute the appropriate treatment.

The onset of AKI in patients with cirrhosis enters into the differential diagnosis with other forms of kidney injury: Pre-renal (45%), organic, including acute tubular necrosis and glomerulonephritis (32%), and less frequently obstructive nephropathy (< 1%)[34,35] (Table 4).

The parameters traditionally used to distinguish AKI from chronic kidney disease (CKD) (urinary sodium concentration, serum and urine osmolarity) are not applicable in patients with cirrhosis and ascites. Likewise, serum urea values are usually reduced in cirrhotic patients due to the impaired hepatic synthesis.

Belcher *et al*[36] proposed the use of urinary biomarkers of AKI to improve the diagnostic process: urinary levels of neutrophil gelatinase-associated lipocalin (NGAL), interleukin 18 (IL-18), kidney injury molecule-1 and liver fatty acid-binding protein are elevated in liver disease patients with kidney injury due to acute tubular necrosis.

Table 4 Differential diagnosis of renal failure in cirrhosis

Pre-renal	History of fluid loss, gastrointestinal bleeding, treatment with diuretics or non-steroidal anti-inflammatory drugs
Organic	Medical history, laboratory tests (cryoglobulinemia, complementemia, *etc.*)
Obstructive	Ultrasound imaging
Chronic kidney disease	Anemia, proteinuria, secondary hyperparathyroidism, ultrasound evidence of renal cortical thinning

Two recent trials studied patients admitted to hospital for cirrhosis-induced complications. They both demonstrated that raised urinary levels of NGAL may serve to distinguish functional kidney damage from acute tubular necrosis or necrosis arising in HRS[37,38].

Barreto *et al*[39] confirmed that urinary NGAL predicts clinical outcome, namely persistent kidney injury and mortality at three months in hospitalized patients with cirrhosis and bacterial infections. Although further clinical trials are required, NGAL appears to predict short-term mortality in cirrhotic patients.

Renal biopsy is not used for diagnostic purposes but can be entertained when a decline in renal function is associated with active urinary sediment or clinical status not corresponding to IAC criteria or unresponsive to therapy.

TREATMENT

Despite improvements in the clinical management of HRS patients in the past twenty years, currently available treatments enhance patients' short-term survival but offer little benefit in the longer term.

The current therapeutic armamentarium includes drugs with specific vasoconstrictive effects on the splanchnic circulation in addition to renal and liver replacement therapies which can be artificial or natural (liver transplantation). Liver transplant remains the only truly effective treatment but is limited by the high mortality rate in HRS patients and the shortage of available organs.

A recent literature review by Fabrizi *et al*[40] noted that pre-transplant kidney function is the most important predictor for patient survival after liver transplant. Pharmacological treatment and medical care serve as a "bridge" to transplant to improve the patient's prognosis.

Prevention and general patient management

The cirrhotic patient with ascites must be closely monitored to prevent and treat precipitating factors[41-45] (Table 5).

If multiorgan damage is present, some patients, especially those with type I HRS, may require a high level of care, and admission to an intensive care facility. In addition, a patient-tailored diet and physical rehabi-

Table 5 Prevention of hepatorenal syndrome and general patient management strategies

Avoid drugs that reduce renal perfusion or nephrotoxic substances
Minimize exposure to organ-iodated contrast agents
Intravenous albumin is recommended for volemic filling after large volume paracentesis (8 g of albumin for each liter of ascites removed)
Diuretic therapy should be suspended
Pentoxifylline as drug's anti-TNFa activity
Antibiotic prophylaxis to prevent infections reducing intestinal bacterial translocation (norfloxacin 400 mg/d)
Intravenous albumin administered in association with ceftriaxone in SPB
Adrenal insufficiency should be identified and treated
Drug dosages must be adjusted according to renal function

ligation program should be planned and each patient assessed for eligibility for liver transplantation to avoid aggressive treatment.

The aim of treatment must be to stabilize patients until liver transplantation and optimize their clinical condition for a successful transplant[6].

Medical management

Medical management is targeted at the pathogenetic mechanisms underlying HRS. The ideal treatment is designed to improve liver function by exerting splanchnic vasoconstriction and renal vasodilation to reduce portal hypertension and raise systemic arterial pressure[34]. The specific drug approach is based on the use of vasoconstrictor agents (terlipressin, norepinephrine, midodrine) to correct circulatory changes.

As reported in a review by Davenport *et al*[41], intravenous administration of terlipressin and albumin is currently the treatment of choice for patients with type Ⅰ and type Ⅱ HRS, resulting in an overall reduction in short-term mortality rates.

The vasopressin synthetic analogue terlipressin is a V1 agonist of the receptors expressed on vascular smooth muscle cells in the splanchnic circulation. It is enzymatically transformed from the inactive to biologically active form (lysine-vasopressin) with a longer half-life than other vasopressin analogues, *e.g.,* ornipressin. Terlipressin's long half-life accounts for its initial administration as an intravenous bolus, now replaced by continuous infusion[46,47]. The vasoconstrictive effect of terlipressin corrects the circulatory dysfunction typical of end-stage liver disease, indirectly rebalancing intrarenal vasoconstriction and lowering levels of renin, noradrenaline and ultimately serum creatinine. As a result, the kidney regains control of its self-regulatory system. In addition, terlipressin has a major impact on the portal circulation reducing portal venous flow and porto-systemic pressure with a concomitant increase in hepatic arterial blood flow and an improvement in hepatocellular oxygenation.

Terlipressin can be administered as an intravenous bolus starting from a dose of 0.5 mg every 4-6 h or as a continuous infusion (2 mg/d). The dosage can be doubled after three days of treatment if there is no improvement in serum creatinine (*i.e.,* a reduction of at least 25%)[12]. The total daily dose should not exceed 2 mg Ⅳ bolus every 4-6 h or 12 mg/d in continuous infusion[40]. Continuous infusion is associated with a better clinical response and fewer side-effects[48].

Terlipressin should be associated with albumin (at a dose of 1 g/kg per day on the first day, without exceeding 100 g/d, followed by 20-40 g/d). Albumin serves to expand the circulating plasma volume by raising the oncotic pressure. In addition, it has metabolic, immune and vasoconstrictor effects by binding to endotoxins, nitric oxide, bilirubin and fatty acids[49,50]. The terlipressin-albumin association improves renal function by 40%-60%[48], increasing the number of patients eligible for liver transplant thereby enhancing their outcome[51-53]. When serum creatinine values reach < 1.5 mg/dL, treatment is deemed complete[48]. The average recovery time is seven days up to a maximum of two weeks after which terlipressin should be suspended if there is no improvement in kidney function[54]. Even when there is a complete response, HRS recurrence is common (50% of cases) and treatment should be resumed.

Terlipressin has an acceptable side-effects profile. Side effects include abdominal pain with cramps and diarrhea until intestinal ischemia; cardiac tachiarrhythmias and chest pain can be observed, in generale ECG monitoring is recommended. Vasoconstriction induced by terlipressin may cause also cyanosis, livedo reticularis, necrosis of the skin and extremities[53]. Terlipressin could also associated with hyponatremia but without impairment of patients' survival[55].

If patient shows side effects the dosage should be reduced or administration discontinued. Continuous infusion is safer and less burdened by side effects[52].

The incidence of ischemic events ranges from 5% to 30% even though many studies exclude patients at risk of cardiovascular ischemia. Fabrizi *et al*[56]'s literature meta-analysis of 243 patients compared the effects of terlipressin *vs* placebo on kidney function and survival in HRS patients. Their data confirm the regression of HRS in a significant number of treated patients but no effect on survival rates.

The association albumin and terlipressin showed an improvement of survival rates for positive effects of albumin on cardiac function, on the reduction of nitric oxid and on improving the responsiveness of arterial wall to vasoconstrictors. Other studies in patients treated with terlipressin and differents colloids didn't showed the same positive response[52,53].

Terlipressin is not available in the United States and Canada so therapeutic protocols with other vasoconstrictor agents need to be considered in those countries.

The alpha-adrenergic receptor agonist norepinephrine has proved effective in the treatment of HRS. Continuous norepinephrine infusion (at a dose of 0.5-3 mg/h) must be associated with albumin administered as an Ⅳ bolus at least twice daily (1 g/kg up to a maximum of 100 g/d). The aim is to raise mean

arterial pressure by 10 mmHg and urinary output > 200 mL every four hours. The maximum period of treatment must not exceed 2 wk[57,58].

A pilot study by Ghosh *et al*[59] compared terlipressin *vs* noradrenaline in 46 patients with type Ⅱ HRS. Neither treatment proved superior to the other and the outcome was broadly the same in terms of HRS regression. Noradrenaline can be deemed as effective as terlipressin but its lower costs makes it an interesting option for the treatment of HRS.

Another alpha-adrenergic agent, midodrine, can be considered a good alternative to terlipressin and is the drug most commonly used in the United States. Midodrine is a prodrug metabolized by the liver into its active metabolite (desglymidodrine) and then excreted in the urine. When administered in association with octreotide (a somatostatin analogue and splanchnic vasodilator) it has a positive effect on renal function in HRS patients with 50% likelihood of disease reversal[49,60-62].

Midodrine can be administered orally (initial dose 7.5 mg every 8 h up to a maximum of 12.5 mg three times daily) or octreotide can be given by continuous infusion (50 mcg/h) or subcutaneously (from 100 to 200 mcg 12.5 mg three times daily). Albumin must be associated at the usual dose[6]. Midodrine dosage has a major effect on its effectiveness: Patients treated at the maximum dose have shown a complete response to therapy, whereas octreotide administered alone has no effect on kidney function[62].

Transjugular intrahepatic portosystemic shunt

The creation of a portosystemic shunt to treat refractory ascites can improve renal function in cirrhotic patients as it increases venous return of splanchnic blood to the right heart thereby raising the effective arterial blood volume and reducing hepatic sinusoidal pressure. Although literature reports on the use of transjugular intrahepatic portosystemic shunt (TIPS) in HRS patients are scant, Brensing *et al*[63] analyzed the trend of creatinine clearance in patients treated with TIPS, finding a twofold increase in clearance values from 9 to 27 mL/min two weeks after the procedure. Despite its side-effects (namely the high incidence of hepatic encephalopathy), TIPS can be used in the short term to gain potential benefits in patients awaiting liver transplant[64,65].

Renal replacement therapy

The indications for renal replacement therapy (RRT) in patients with HRS are the same as those for AKI patients without cirrhosis. HRS patients, particularly those with type Ⅰ, may need to undergo dialysis because of metabolic acidosis or hyperkalemia due to water or sodium retention or less frequently uremic intoxication.

RRT is among the so-called bridging therapies designed to support patients awaiting liver transplant, but there is no evidence that dialysis improves the long-term survival of patients not eligible for transplantation[66].

By definition, patients with cirrhosis are at higher risk of bleeding and hemodynamic complications (hypotension, arrythmias) hampering the decision to initiate and manage dialysis treatment. Cirrhotic patients on RRT have a 2%-8% higher mortality rate than other patients[67].

Continuous renal replacement therapy (CRRT) is usually preferred to intermittent dialysis due to its greater hemodynamic stability ensuring fewer fluctuations in intracranial pressure. However, prospective studies show that the choice of RRT has no significant effect on survival rates in patients awaiting liver transplantation[68-71]. Anticoagulation of the extracorporeal circuit is needed to maintain the filter patency without increasing the risk of hemorrhage. Regional citrate anticoagulation emerged as possible alternative but no specific protocols are currently recommended for patients with liver diseases[72].

Peritoneal dialysis is an option to resolve ascites and correct other complications of cirrhosis without exposing patients to the complications of hemodialysis[73,74].

The precise timing and dose of RRT have yet to be established but some studies demonstrate that the early initiation and maintenance of a constantly negative fluid balance have a positive effect on survival rates[75].

Extracorporeal artificial liver support therapy

More complex therapies known as liver support measures may be required to replace the liver's detoxifying system. RRT removes water-soluble toxins whereas most of the molecules accumulated in the course of liver failure are linked to albumin and hence are not removed by conventional hemodialysis.

Liver support systems are designed to enhance and optimize these results, increasing the removal of water-soluble toxins and those linked to albumin.

To date these treatments have served as bridging therapies for patients awaiting liver transplantation.

Molecular adsorbent recirculating system

Molecular adsorbent recirculating system (MARS) combines the conventional CRRT monitor or a standard hemodialysis machine with an albumin dialysate circuit. The system is based on the removal of albumin-bound toxins (bile acids and nitric oxide) and water-soluble cytokines (IL-6 and TNF-α) to stabilize liver function and improve organ damage.

The MARS system consists of an albumin-impermeable membrane separating the patient's blood from the albumin dialysate solution. The free albumin in the dialysate attracts and binds the liver toxins in the patient's blood. The albumin dialysate, in its turn, is regenerated by a low flux dialysis filter and two adsorber cartridges, one filled with activated charcoal, the other with an anion exchanger resin. The regenerated albumin solution is then ready for new uptake of toxins from the blood, entering the circuit through a high permeability filter to undergo standard dialysis to remove water-

soluble toxins.

Some studies have reported better survival rates in patients treated with MARS compared to conventional CRRT, but overall survival remains very poor (37% at 7 d and 25% at 30 d). The main factor affecting survival is the patient's clinical status before treatment[75,76].

In 2000, a trial by Mitzner *et al*[75] assessed survival rates in 13 patients with type I HRS. The eight patients treated with MARS had significantly better survival rates at 30 d than patients receiving standard medical therapy. By contrast, the randomized RELIEF study failed to show any significant differences in terms of survival in 189 patients treated with MARS *vs* standard medical therapy even though some benefit was noted in the management of encephalopathy in patients with type I HRS who underwent MARS[77].

After a one-year follow-up, Donati *et al*[78] reported that among 64 patients treated with MARS, the best survival rates were found in the 11 patients who subsequently underwent liver transplant. The same authors observed an improvement in both systolic and diastolic blood pressure in 5 patients with type 2 HRS treated with MARS and standard medical therapy.

Fractionated plasma separation and absorption (Prometheus)

The Prometheus system consists of a primary circuit (plasma filter and dialyzer) and a secondary circuit (adsorbent filters to remove bilirubin) for the combined removal of toxin albumin-bound and water-soluble molecules using a fractionated plasma separation and adsorption (FPSA) system. Unlike MARS, the plasma is separated from the blood through a high cut-off point polysulfone membrane (250 kDa, albumin permeable) and purified from the albumin-bound toxins by direct adsorption on resin-containing cartridges. The purified plasma is then returned to the blood circuit through a high efficiency dialyzer to remove water-soluble toxins.

The HELIOS study on 179 patients with liver failure treated with standard medical therapy *vs* extracorporeal treatment showed no significant advantage in terms of overall survival except in the subgroup of patients with type I HRS treated with FPSA who had a significant survival benefit[79].

Liver transplantation

Liver transplantation remains the treatment of choice in HRS patients despite its mortality rate which is particularly high in patients with type I HRS whose survival is so poor that many die while awaiting transplant.

Recovery of renal function is not universal: Marik *et al*[80], in a study on 28 patients, noted a complete recovery of kidney function in only 58% of transplanted patients, a partial recovery in 15% and no recovery in 25% (observation time 110 d). Renal sodium excretion, serum creatinine and neurohormonal levels may normalize within a month whereas renovascular resistance may take more than a year to return to normal after transplantation[81,82].

Organ allocation is mainly based on the MELD score, a system devised to stratify disease severity on the basis of laboratory parameters (serum creatinine, bilirubin and INR) to assign organs according to the so-called sickest first policy[83].

Considering all liver transplant recipients, those with HRS are more exposed to post-transplant complications, at greater risk of developing CKD and have a shorter overall survival[84,85]. Those patients who fail to recover renal function and need to continue hemodialysis have an even worse survival rate (70% mortality at one year)[86].

RRT prior to liver transplant is an important predictive factor. Patients undergoing hemodialysis for more than eight weeks have a markedly reduced probability of renal recovery and a combined liver-kidney transplant is recommended in these cases[87,88] .

Vasopressor treatment of HRS before liver transplant does not seem to affect subsequent patient outcome[89]. Nonetheless, Angeli *et al*[83] reported that liver transplantation may be delayed in patients treated with vasopressors following a response to treatment and hence an improvement in clinical and hemodynamic status. This paradoxical situation must be avoided and the clinical criteria adopted to establish the priority of patients on the waiting list for transplant (first and foremost the MELD score) must always refer to the patient's initial condition and not to the status reached after treatment.

There are no specific recommendations as to post-transplant immunosuppressive therapy, but it may be advisable to delay the start of cyclosporine or tacrolimus to 48-72 h after transplantation to enhance renal recovery as suggested by Guevara and Arroyo[90].

CONCLUSION

HRS is a life-threatening complication arising in patients with liver cirrhosis and triggered by a series of complex hemodynamic and neurohormonal changes linked to the liver disease. The condition carries a very poor prognosis and high morbidity and mortality rates.

Recent years have seen a reduction in HRS prevalence and an improvement in patient outcome probably reflecting a better understanding of HRS pathophysiology and advances in therapeutic strategies.

Treatment consists of medical management (mainly based on vasopressor administration), surgery (TIPS) or instrumental therapies (*e.g.,* renal replacement and liver support systems). Although the therapeutic armamentarium at our disposal will control the syndrome and obtain temporary remission, there is no guarantee of disease resolution.

The only effective treatment offering patients the hope of complete recovery is liver transplantation or combined kidney-liver transplant in selected cases. The decision to embark on transplantation must be carefully assessed in HRS patients considering all the potential

factors likely to influence transplant surgery and its outcome.

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When to initiate renal replacement therapy: The trend of dialysis initiation

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Abstract

The timing of renal replacement therapy for patients with end-stage renal disease has been subject to considerable variation. The United States Renal Data System shows an ascending trend of early dialysis initiation until 2010, at which point it decreased slightly for the following 2 years. In the 1990s, nephrologists believed that early initiation of dialysis could improve patient survival. Based on the Canadian-United States Peritoneal Dialysis study, the National Kidney Foundation Dialysis Outcomes Quality Initiative recommended that dialysis should be initiated early. Since 2001, several observational studies and 1 randomized controlled trial have found no beneficial effect when patients were placed on dialysis early. In contrast, they found that an increase in mortality was associated with early dialysis initiation. The most recent dialysis initiation guidelines recommend that dialysis should be initiated at an estimated glomerular filtration rate (eGFR) of greater than or equal to 6 mL/min per 1.73 m^2. Nevertheless, the decision to start dialysis is mainly based on a predefined eGFR value, and no convincing evidence has demonstrated that patients would benefit from early dialysis initiation as indicated by the eGFR. Even today, the optimal dialysis initiation time remains unknown. The decision of when to start dialysis should be based on careful clinical evaluation.

Key words: End-stage renal disease; Renal replacement therapy; Dialysis; Estimated glomerular filtration rate; Creatinine clearance; Survival

Core tip: In the United States, the number of patients who were placed on dialysis early increased dramatically from 1996 to 2010 and then decreased slightly. To investigate the proper timing of renal replacement therapy (RRT), we reviewed the literature and found that the results from different studies were conflicting, so that the optimal time of dialysis initiation remained unknown. Early initiation of RRT may contribute to the

current high incidence of RRT. If properly delayed RRT initiation is demonstrated to be safe for patients, this strategy may reduce the high incidence of RRT.

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INTRODUCTION

Over the past 2 decades, the numbers of patients with uremia and patients who have received renal replacement therapy (RRT) have increased worldwide. At the same time, the percentage of patients who initiated dialysis early increased dramatically from 1996 to 2010[1], which might have contributed to the high incidence of end-stage renal disease. RRT initiation can be intentionally delayed with careful monitoring. Delaying the initiation of RRT might be a strategy to reduce the incidence of RRT, if it is safe for patients. To investigate the effects of RRT timing on patient outcome, we conducted a literature review; we report its results in this paper.

RRT TRENDS

The early dialysis initiation trend

Twenty years ago, dialysis was not initiated until patients had life-threatening or severe symptoms of uremia. Emergency dialysis was needed for resistant hyperkalemia, with the emergence of metabolic acidosis. In 1995, Hakim *et al*[2] identified the indicators for RRT initiation: Patients with pericarditis, fluid overload, pulmonary edema, hypertension, advanced uremic encephalopathy, clinically significant coagulopathy, persistent and severe nausea and vomiting and who were poorly responsive to drug therapy should be placed on RRT immediately. If conservative non-dialysis management was ineffective for the following manifestations, the initiation of RRT was also suggested: (1) a general but fairly severe decline in quality of life (QOL), including vomiting, resistance and severe pruritus; and (2) decreased attentiveness, memory, cognitive abilities and depression that could affect the interpersonal relationships of the patient.

Hakim's standard was mainly based on clinical symptoms, and the timing of RRT initiation was apparently late. The point at which dialysis is initiated should be neither too late nor too early. If dialysis is started too late, patients risk the complication of uremia, which leads to low QOL and a higher risk of mortality. Dialysis is not a physiological process. As such, It (1) places the patient under the dangers of complications related to the RRT process; (2) accelerates the reduction of endogenous renal function[3], especially for elderly or frail patients with high rates of comorbidities; and (3) provokes or aggravates depression and other psychosocial problems. All of the above conditions correlate with an increased risk of mortality[4,5].

In the 1990s, the initiation of early dialysis was determined by the estimated glomerular filtration rate (eGFR), in accord with a Modification of diet in renal disease (MDRD) equation; the criterion was an eGFR greater than or equal to 10 mL/min per 1.73 m^2. Many researchers in the academy thought that early dialysis initiation would improve patient QOL and patient survival by reducing the complication of dystrophy. Furthermore, it was also believed that a decreased glomerular filtration rate GFR at dialysis initiation was associated with an increased probability of hospitalization and death[6-9]. They held the idea that early dialysis initiation was indispensable for preventing and reversing the deteriorated nutritional status associated with progressive uremia. The National Dialysis Cooperative Study[9] introduced the Kt/V_{urea} metric as a predictor of morbidity and mortality. Then, in 1996, the Canadian-US Peritoneal Dialysis (CANUSA) study[10] recommended a potential renal survival benefit of a weekly Kt/V_{urea} of greater than 2.0 [peritoneal creatinine clearance (CC) of > 70 L per 1.73 m^2]. This threshold is equivalent to a CC of 9-14 mL/min per 1.73 m^2. Based on the CANUSA study, the National Kidney Foundation Dialysis Outcomes Quality Initiative hemodialysis Adequacy Guideline (1997)[11] recommended that dialysis be initiated when the GFR decreased to 10.5 mL/min per 1.73 m^2 unless the normalized protein nitrogen appearance was more than 0.8 g/kg and the patient had a stable weight and a good appetite. Since then, the majority of national and international guidelines have promoted early dialysis for patients with deteriorating nutritional status and with symptoms or co-morbidities[12]. The Canadian Society of Nephrology (CSN) (1999)[13] suggested that dialysis be initiated when eGFR less than 12 mL/min per 1.73 m^2 in the presence of uremia symptoms or malnutrition. In the meantime, the indicator for dialysis initiation changed from the Kt/V_{urea} to the eGFR. The European Renal Best Practice (ERBP) (2002)[14] advocated for closer supervision of high-risk patients (those with eGFR < 15 mL/min per 1.73 m^2 plus symptoms and signs, the inability to control hydration status or blood pressure, and progressive nutritional status deterioration). High-risk patients, such as diabetics, may benefit from an earlier start. In 2006, the Kidney Dialysis Outcomes Quality Initiative (KDOQI)[15] updated these guidelines and suggested that RRT be considered when eGFR of < 15.0 mL/min per 1.73 m^2. Particular clinical considerations and certain characteristic complications may prompt the initiation of therapy before the onset of end-stage renal disease (ESRD). When the eGFR is greater than 15.0 mL per minute, RRT may also be warranted for patients with coexisting conditions such as diabetes or with symptoms of uremia.

All of the studies and guidelines mentioned above support early dialysis, and they have all been promoted

Table 1 Study and recommendations that support early dialysis initiation

Study/ recommendations	Year	Time/eGFR (mL/min per 1.73 m^2)	Journal
CANUSA study	1996	9 to 14	*J Am Soc Nephrol*
NECOSAD study	2001	No beneficial effect of earlier dialysis initiation	*Lancet*
NKF-DOQI	1997	10.5	*Am J Kidney Dis*
CSN	1999	< 12	*J Am Soc Nephrol*
KDOQI	2006	< 15.0	*Am J Kidney Dis*

eGFR: Estimated glomerular filtration rate.

as conventional wisdom (CW)[2,9,12]. The CW can be summarized as follows: (1) low levels of dialytic and endogenous renal clearance are associated with improved morbidity and mortality; (2) nutrition can be improved with the early initiation of dialysis; (3) dialysis should be initiated earlier in diabetics than in nondiabetics; and (4) dialysis initiated at eGFRs below 6 mL/min per 1.73 m^2 is potentially dangerous.

The trend toward early initiation of dialysis can also be seen internationally. According to the United States Renal Data System (USRDS)[1], with the eGFR calculated using the chronic kidney disease epidemiology calculation (CKD-EPI equation) (CKD-EPI eGFR, mL/min per 1.73 m^2), the percentage of ESRD patients who started RRT at higher eGFR levels increased steadily from 1996 until 2010. In 1996, 9.48% of patients initiated RRT with an eGFR of 10-14.9 mL/min per 1.73 m^2, and only 3.01% had an eGFR > 15 mL/min per 1.73 m^2. In 2010, these percentages had more than doubled (to 27.85% and 14.71%, respectively). This phenomenon was more prominent in the elderly dialysis population. The percentage of incident ESRD patients who started dialysis at an eGFR < 5 mL/min per 1.73 m^2 decreased from 34.4% in 1996 to 12.6% in 2010[1]. In Beijing, the percentage of patients who initiated hemodialysis with an eGFR > 10 mL/min per 1.73 m^2 rose gradually from 13.2% to 20.7% between 2007 and 2010[16]. In Europe[17], dialysis initiation when eGFR > 10.5 mL/min per 1.73 m^2 had risen from 16.4% to 23.6% between 1999 and 2003. The United Kingdom Renal Registry data[18] showed that, between 1997 and 2010, the mean eGFR at dialysis initiation increased from 6.2 to 8.7 mL/min per 1.73 m^2. In data from the Canadian Organ Replacement Registry[19], the percentage of patients who started peritoneal dialysis at an eGFR > 10.5 mL/min per 1.73 m^2 rose from 29% (95%CI: 26%-32%) to 44% (95%CI: 41%-47%) between 2001 and 2009. The average eGFR at dialysis initiation increased from 9.3 ± 4.6 to 10.7 ± 6.1 mL/min per 1.73 m^2 (Figure 1 and Table 1).

Studies and recommendations that support late dialysis initiation

Recently, certain registry and observational studies that included a total of > 900000 analyzable patients all demonstrated that late dialysis initiation was associated with improved survival[14,20].

The Netherlands Cooperative Study on the Adequacy of Dialysis (NECOSAD)[21] showed that, though not significant (adjusted HR = 1.66; 95%CI: 0.95-2.89), the early group (which was initiated according to the first KDOQI guidelines) gained an estimated survival benefit of 2.5 mo *vs* late starters after 3 years of dialysis. In the NECOSAD study, the eGFR was calculated from timed urine collections (as the mean of urea and CC). However, there was a delay of at least 4.1 mo before dialysis initiation in the late-start group[21]. After taking the lead time bias (discussed below) into account, there was no beneficial effect of earlier dialysis initiation. In 2002, Traynor *et al*[22] also found that there was no significant survival benefit from earlier initiation of dialysis and that patients who started dialysis with a lower estimated CC survived longer. More recent observational data[17,23-34] found a comorbidity-adjusted survival disadvantage for early dialysis initiation, as 12 studies found an increase in mortality associated with early dialysis initiation. Beddhu *et al*[23] found that for each 5 mL/min increase of the MDRD eGFR, the associated risk of death was 27% higher (HR = 1.27; $P < 0.001$). However, this phenomenon was not observed for the CC value. In a Chinese study in Taiwan[29], the median eGFR level at dialysis initiation was 4.7 mL/min per 1.73 m^2 from July 2001 to December 2004 in > 23000 incident patients. Based on the eGFR level at dialysis initiation, patients were divided into quintiles, and the best survival was observed at < 3.29 mL/min per 1.73 m^2. In another report, the best survival was achieved in patients with eGFRs of between 0 and 5 mL/min per 1.73 m^2[27] among American subjects. This study included 81176 uremic subjects, aged 20-64 with no substantial comorbidities other than hypertension, from the USRDS dataset[27]. In 2012, Yamagata *et al*[31] analyzed 20854 patients who had started RRT in 1989 and 1990 and found that the timing of RRT initiation had no impact on the long-term prognosis after adjustments were made for co-morbid conditions. In 2014, Crews *et al*[33] found that, compared with patients who started at a lower eGFR, patients with early dialysis initiation at an eGFR ⩾ 10 mL/min per 1.73 m^2 showed greater mortality and more frequent hospitalization, even after adjusting for comorbid conditions. In 2014, a study of 310932 patients who had started dialysis between 2006 and 2008[32] demonstrated that no harm or benefit was associated with early dialysis initiation. A meta-analysis of cohort studies and trials by Susantitaphong *et al*[34] found that a 1 mL/min per 1.73 m^2 increase in the GFR at dialysis initiation was associated with 3%-4% higher all-cause mortality after adjustment for comorbid conditions.

Possible explanations for the conflicting results

Previous studies provide reproducible evidence that dialysis initiation with higher eGFR is associated with increased mortality. However, these studies also have

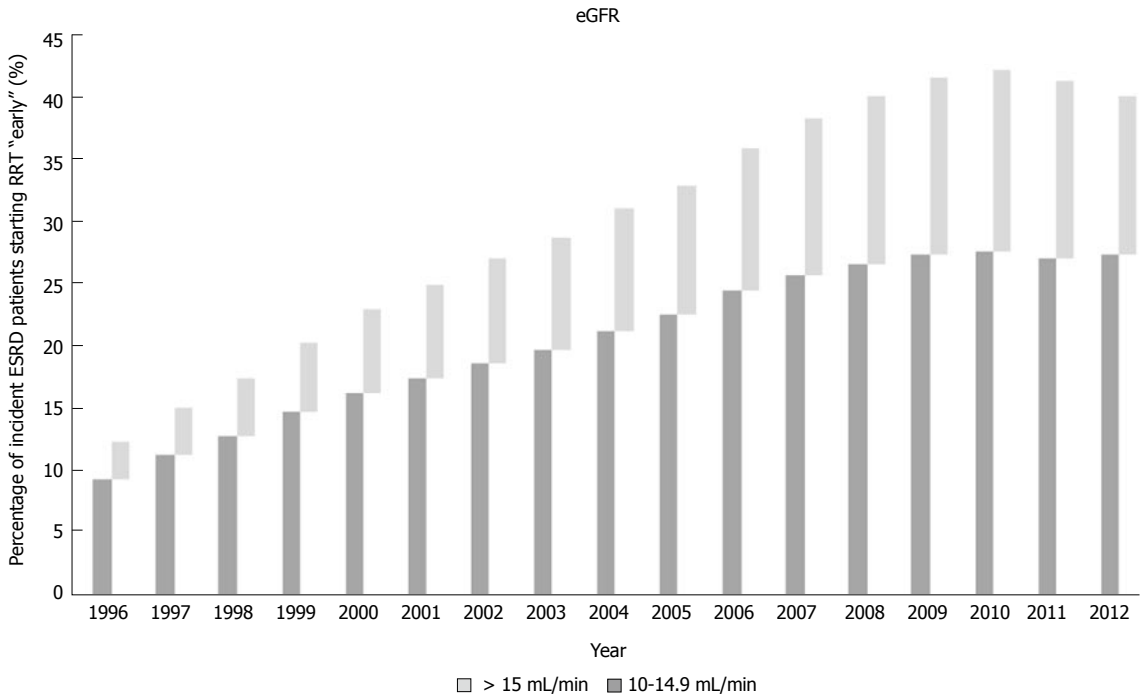

Figure 1 Percentage of patients initiating renal replacement therapy "early" in the United States during the years 1996-2012. Data from the United states Renal Data system. eGFR calculated using the CKD-EPI equation [CKD-EPI eGFR (mL/min per 1.73 m^2)]. ESRD: End-stage renal disease; eGFR: Estimated glomerular filtration rate.

shortcomings. The limitations of the prior studies are discussed below.

Inaccurate eGFR values

The decision to initiate RRT has relied heavily on the eGFR[12]. The ERBP[23] concluded that creatinine-based measures of the eGFR in pre-dialysis patients were fundamentally flawed and were thus invalid. In studies using GFR measures that were based on 24-h urine urea and/or creatinine clearance, the adverse effect of early initiation was not found. The MDRD equation accounts for the average loss of muscle over time with age (sarcopenia) but does not account for unusual body habitus or diet. In other words, the MDRD equation may be erroneous for patients with ESRD. Craig *et al*[35] concluded that, when compared with the reference standard radionuclide GFR (rGFR), the MDRD equations performed poorly in patients with advanced renal failure, while the Cockcroft-Gault (CG) equation showed a smaller bias and was more accurate. In this study, an intravenous injection of 51Cr-EDTA (3 MBq) was used for the measurement of rGFR, and plasma samples were taken approximately 120 and 240 min later. The study recommended using the CG equation when the rGFR method is unavailable. It must be kept in mind that the differences between the GFR methods may greatly influence the decision for RRT initiation[36].

There are possible explanations for falsely over-estimated eGFR. First, patients with low muscle mass due to inactivity or malnutrition have a lower creatinine generation rate, which would overestimate the true residual renal function (RKF). Second, fluid overload would dilute patient serum creatinine levels. All such patients would have higher co-morbidity rates and lower serum creatinine levels. The serum creatinine-based eGFR might overestimate the true GFR in the above patients and thus risk including such patients in the "earlier" start groups[14] when they should in fact start late.

Elderly or frail patients were more likely to start dialysis early

Patients with symptoms or co-morbidities were more likely to be started on dialysis early. The multivariate adjustment for co-morbidities indeed decreased the benefit of initiating dialysis with a low eGFR, but the effects did not disappear. The most common reason to initiate dialysis early was a nutritional decline. Compared with nondiabetic dialysis patients, the association of an early start with higher mortality was much stronger among patients with diabetes[26]. It was confirmed that patients with low comorbidity burdens showed reduced survival compared to higher starting eGFR values[17,22-27,29].

Lead time bias

Unfortunately, the studies that support early dialysis initiation fail to take the effect of the lead time bias into consideration. The lead time bias is related to the initiation time of treatment within the duration of the disease. The prolonged survival may be due merely to earlier diagnosis and treatment. Alternatively, it may be expected that earlier disease detection would be correlated with longer survival. After eliminating the

effect of the lead time bias, the NECOSAD study[21] demonstrated that there was no beneficial effect of earlier dialysis initiation.

High-risk patients may die before dialysis initiation in the late group

Some studies only included patients who actually started dialysis. Those who died before dialysis had been initiated (possibly because of uremia) were excluded. In other words, the late-start subjects may not suffer from severe disease who died before the initiation of dialysis. Only the fittest patients who survived long enough were included in the late-start groups.

Treatment time on RRT

Mortality might be a result of "insufficient" dialysis. Long treatment times (> 4 h per session) and more frequent dialysis sessions reduced the risk of low albumin, which is correlated with decreased mortality[37,38].

Cardiovascular comorbidities and infection

Early-dialysis patients were more likely to die in the hospital. Compared with patients who underwent conservative management, patients who underwent dialysis expected longer days in the hospital and were more likely to die in the hospital, especially when debilitated, frail, or elderly[39]. In the first year of hemodialysis, deaths were mainly due to cardiovascular disease and infection[1]. The all-cause mortality, cardiovascular disease mortality, and mortality due to other causes peaked in month 2 and then decreased thereafter[13]. Dialyzed patients had twice the rate of sudden death, which was connected with ultrafiltration volumes, decreased blood pressure, lower Kt/V_{urea}, and low potassium concentrations[40]. Cardiovascular disadvantages can also appear when accompanied with life-threatening diseases such as pulmonary edema. The risk of infection was associated with the modality and access type. In a study of 55 inpatients who underwent tunneled hemodialysis catheter (TDC) removals, 36.4% had proven bacteremia, 41.8% had a fever and 20% had clinical signs of sepsis with hemodynamic instability or respiratory failure[41]. The risk of TDC is thus apparent. Once a patient has started dialysis, the risks of all forms of infection are much higher, and the patient is more likely to have septicemia, which is especially prevalent among elderly patients.

The IDEAL study

The randomized controlled trial of early *vs* late initiation of dialysis (IDEAL) study[42] showed no difference in mortality between the early and late groups. The early group was expected to start dialysis when the CC (calculated with the CG equation) was 10-14 mL/min per 1.73 m^2, and the late group was expected to start dialysis at 5-7 mL/min per 1.73 m^2. It was allowed to start dialysis based on clinical indications, disregardfulness CC in either group. The average CC values were 12.0 and 9.8 mL/min per 1.73 m^2 at the time of dialysis initiation in the early and late groups, respectively. Compared with the early group, the late group showed a 6-mo delay in initiation. However, 76% of the patients who were allocated to the late group actually commenced dialysis with a higher CC, and the mean difference in the estimated GFR between the late and early groups was only 2.2 mL/min. The gap between the 2 groups was too small to generate a difference in the mortality rates. However, for some patients, who started RRT after their eGFR values dropped below 5-7 mL/min per 1.73 m^2, no harm was detected. In other words, initiating dialysis late might be safe for some patients with fluid overload or other accompanying complications if they are carefully monitored.

Recommendations that support late dialysis

Notably, most patients are symptomatic and need to be dialyzed in a GFR range of 6-9 mL/min per 1.73 m^2. Many guidelines, including the ERBP 2002[14], the Australia 2005[43] and the United Kingdom 2009[44], recommend that RRT should be initiated before the GFR reaches 6 mL/min per 1.73 m^2. The ERBP 2002[14] recommends that dialysis preparation should be initiated at a GFR of 8 mL/min per 1.73 m^2 and that dialysis must be initiated at a GFR of 6 mL/min per 1.73 m^2. Caring for Australians with Renal Impairment (2005)[43] recommends that dialysis should be initiated when the GFR is less than 10 mL/min per 1.73 m^2 if symptoms of uremia or complications such as malnutrition are present or when the GFR is less than 6 mL/min per 1.73 m^2 in the absence of symptoms or complications. The United Kingdom Renal Association 2009[44] recommends RRT initiation when the eGFR is less than 6 mL/min per 1.73 m^2, even if the patient is asymptomatic. The 2012 Kidney Disease Improving Global Outcomes[45] suggests that dialysis should be initiated when the eGFR is approximately 5-9 mL/min per 1.73 m^2. The CSN 2014 clinical practice guidelines[46] suggest that chronic dialysis should be initiated when the eGFR drops to 6 mL/min per 1.73 m^2, even if there are no clinical indications. However, the existing guidelines do not specify a dialysis initiation point (with respect to eGFR or serum creatinine level). In the USRDS[1], the percentage of incident ESRD patients who began RRT at higher eGFR levels decreased slightly in 2011 and again in 2012. The percentage of patients who began RRT at an eGFR $\geqslant$ 10 mL/min per 1.73 m^2 decreased from 42.6% in 2010 to 40.5% in 2012, and the percentage of patients who initiated RRT at an eGFR < 5 mL/min per 1.73 m^2 rose from 12.6% in 2010 to 13.7% in 2012 (Table 2).

CONCLUSION

There is still considerable doubt with respect to the optimal timing of dialysis initiation in uremic populations. The timing of dialysis is often affected by multiple factors, including age, diabetes mellitus, individual desire, socioeconomic status, personal beliefs, and

Table 2 Study and recommendations that support late dialysis initiation

Ref./ recommendations	Year	Time/eGFR (mL/min per 1.73 m^2)	Journal
Beddhu *et al*[23]	2003	5-mL/min increase of the associated risk of death was 27% higher	*J Am Soc Nephrol*
Chinese Taiwan study	2010	< 3.29	*Nephrol Dial Transplant*
Rosansky *et al*[27]	2011	Between 0 to 5	*Arch Intern Med*
Yamagata *et al*[31]	2012	No difference	*Ther Apher Dial*
Crews *et al*[33]	2014	< 10	*J Am Soc Nephrol*
Susantitaphong *et al*[34]	2014	1 mL increase 3%-4% higher all-cause mortality	*Am J Kidney Dis*
Scialla *et al*[32]	2014	No difference	*Kidney Int*
ERBP	2002	8, and to be sure at 6	*Nephrol Dial Transplant*
Australia	2005	Evidenced symptoms or complications: < 10, no symptoms or complications < 6	
United Kingdom	2009	< 6	
K/DIGO	2012	5-9	
CSN	2014	< 6	*CMAJ*

eGFR: Estimated glomerular filtration rate.

the patient's cultural and educational background. Initiating dialysis early based solely on a single objective measurement (specific level of GFR) can be harmful. Most patients begin dialysis because of renal failure-related symptoms. Importantly, dialysis therapy is not innocuous, and it does not replace all the functions of the kidney. Compared with patients who received dialysis, the native Kt/V_{urea} of an able-bodied man is more than 15-fold higher. Some scholars believe that the biggest advantage of dialysis is the alleviation of fluid overload. Thus far, we lack validated and objective measures of the uremic state that could be used to guide the timing of dialysis initiation. Currently, the established guidelines for the timing of dialysis are based on the conclusions of many observational studies. Data from randomized controlled trials that establish optimal timing for RRT are lacking. The time at which dialysis is initiated must be individualized, and further studies are required to explore a comprehensive, systematic dialysis index that is associated with the GFR, with symptoms, and with assumed indications for dialysis initiation.

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CASE REPORT

Lupus-associated thrombotic thrombocytopenic purpura-like microangiopathy

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Author contributions: Blum D performed the chart review of the case being described, searched, reviewed and critically appraised the literature for cases of thrombotic microangiopathy in patients with lupus; Blake G was involved in making the presumptive diagnosis of the TTP-like syndrome in the patient with lupus described in this case report, and in initiating the management of this rare complication of lupus; Blake G also reviewed and amended this case report manuscript prior to its submission.

Institutional review board statement: Not applicable; this report describes the clinical course of a patient under the care of the general medicine team at the Montreal General Hospital.

Informed consent statement: Explicit written informed consent was obtained from the patient described in this case prior to submitting this case report. A copy of the consent form signed by this patient can be provided upon request.

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Abstract

Recently reported cases of lupus complicated by a thrombotic thrombocytopenic purpura (TTP)-like syndrome suggest a survival benefit to early treatment with plasma exchange. The following is a report of the eighth such case in the last ten years. A 44-year-old lady known for lupus presented with the nephrotic syndrome and a renal biopsy was consistent with class 4G lupus nephritis. She was given high-dose steroids and cytotoxic therapy, but her induction therapy was complicated by the classic pentad of TTP. She was subsequently treated with another course of high-dose steroids, a different cytotoxic agent, and plasma exchange, with clinical resolution shortly thereafter. Similar to seven recently reported cases of microangiopathy in lupus, this lady's TTP-like syndrome improved dramatically after initiation of plasma exchange, despite not having a severely deficient ADAMTS13. This has implications on both current clinical practice and on the pathogenesis of TTP-like syndromes in lupus.

Key words: Microangiopathic hemolytic anemia; Microangiopathy; Thrombotic thrombocytopenic purpura; Atypical hemolytic-uremic syndrome; Hemolytic uremic syndrome; Systemic lupus erythematosus associated thrombotic thrombocytopenic purpura-like microangiopathic hemolytic anemia; Lupus nephritis; Lupus; Plasma exchange

Core tip: In patients with lupus who develop thrombotic microangiopathy, early initiation of plasma exchange appears to carry a survival benefit - even in those patients whose ADAMTS13 activity levels are not severely deficient. This improved survival has become apparent in the last ten years during which time seven cases of thrombotic microangiopathy complicating lupus and treated with plasma exchange have been reported. The present article describes the eighth such case, reviews the previously described cases and outcomes of microangiopathy in lupus, and hypothesizes as to why plasma exchange appears to be beneficial in this subset of patients with atypical haemolytic uremic syndrome.

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INTRODUCTION

Microangiopathic hemolytic anemia (MAHA) is caused by a number of conditions, several of which are fatal. One such condition is thrombotic thrombocytopenic purpura (TTP), which has been reported to be less responsive to therapy in patients with lupus as compared to the general population[1]. Recent research has revealed a subset of patients with lupus who have a TTP-like syndrome distinct from TTP[2]. We report a case of a patient with lupus and a TTP-like syndrome who received early plasma exchange and survived. A review of this under-recognized clinical entity follows.

CASE REPORT

A 44-year-old lady with a five-year history of systemic lupus erythematosus (SLE) on hydroxychloroquine presented to the Montreal General Hospital with edema, nausea, and vomiting in January 2014. On exam she was noted to have anasarca and hypertension. She was found to have a creatinine of 335 μmol/L and a urea of 54 mmol/L (normal 2-10 mmol/L). Her serum albumin was low and her urine tested positive for protein. Her urine sediment revealed white cell casts, red cells, and oval fat bodies. She was diagnosed with new-onset nephrotic syndrome. Further workup was consistent with a lupus flare with an anti-dsDNA > 800 (negative < 20), and excluded alternative causes such as ANCA-related vasculitides, hepatitis C, and human immunodeficiency virus. She had a renal biopsy which was consistent with lupus nephritis class Ⅳ-G with a predominance of active lesions and 75% cellular crescents; there was no thrombotic microangiopathy.

She was treated with a 3-d pulse of higher dose steroids followed by daily steroid doses equivalent to 1 mg/kg of prednisone. She was started on mycophenolate. Her in-hospital course was marked by persistent vomiting requiring prolonged admission.

Ten days after presentation she was noted to have a falling platelet number and falling hemoglobin. Twelve days after presentation, her hemoglobin fell to a nadir of 39 g/L (from 83 g/L on presentation) (normal 120-150 g/L), requiring transfusion of 2 units of pRBC, and her platelets fell to a nadir of 70 × 10^9/L (from 330 × 10^9/L on presentation) (normal 150-450 × 10^9/L). Her white count was normal. She had no evidence of bleeding, fibrinogen of 5.2 g/L (normal > 1.5 g/L), and normal PT, aPTT. She did not have anti-cardiolipin antibodies. Her reticulocytes were 48. Her Coombs test was weakly positive for IgG. Her LDH was slightly elevated at 246 U/L (normal < 220 U/L), her total bilirubin was 12.5 mmol/L (normal < 20 μmol/L), her haptoglobin was decreased at 0.66 g/L, and several blood smears consistently showed a 10%-15% fragmentation index (normal < 0.5%) in addition to rouleaux formation. There was no bloody diarrhea. Her creatinine was higher than it was at presentation, peaking at 417 mmol/L on the same day as the hemoglobin nadir. Shortly thereafter she became acutely hypoxemic from volume overload refractory to diuretics, and required ultrafiltration. She then became drowsy, and neurological exam was significant for increased reflexes bilaterally.

TTP could not be excluded; therefore she was treated with five days of plasma exchange with fresh frozen plasma (FFP), followed by plasma exchange every second day. She was given a second course of pulse steroids, cyclophosphamide was started, and mycophenolate was stopped. She was also given a dose of intravenous iron sucrose and was started on erythropoietin given the inappropriately normal reticulocyte count.

Following a course of eight plasma exchanges, her platelet count recovered completely to > 200 × 10^9/L, her fragmentation index decreased to below 1%, and her hemoglobin stabilized around 75 g/L. Her cell counts stabilized off of plasma exchange. She remains on prednisone and cyclophosphamide. She now responds to diuresis, and her markers of active inflammation have all reduced. Her anti-dsDNA was 50 in February 2014. Ultimately her ADAMTS13 activity, which was measured prior to initiation of plasma exchange, returned at 49%. This level is only slightly below the lower limit of 56% and therefore both excludes the diagnosis of TTP and is consistent with the diagnosis of an SLE-associated TTP-like MAHA.

DISCUSSION

MAHAs are characterized by intravascular hemolysis. The usual laboratory findings are normocytic anemia, thrombocytopenia, elevated LDH, reduced haptoglobin, and elevated unconjugated bilirubin. The peripheral

Table 1 Differential diagnosis of microangiopathic hemolytic anemia

Disseminated intravascular coagulation
Thrombotic thrombocytopenic purpura
Hemolytic uremic syndrome
Atypical hemolytic-uremic syndrome
[1]TTP-like MAHA, an aHUS presenting as part of a connective tissue disease
[1]Anti-phospholipid syndrome
Hemolysis with elevated liver enzymes and low platelets of pregnancy
[1]Malignant hypertension
Medications
Malignancy
Mechanical cardiac valves or other foreign bodies in the circulatory system

[1]Indicates conditions at which patients with lupus are at higher risk compared to the general population. TTP: Thrombotic thrombocytopenic purpura; aHUS: Atypical hemolytic-uremic syndrome.

blood smear classically shows schistocytes, or fragmented red cells, which are required to make the diagnosis. The differential diagnosis of MAHA is limited to only a few conditions (Table 1). Patients with lupus are at particular risk for acquiring the anti-phospholipid syndrome, malignant hypertension, and SLE-associated TTP-like MAHA. As its name suggests, SLE-associated TTP-like MAHA is a condition manifested by otherwise unexplained MAHA in a patient meeting the American College of Rheumatology (ACR) criteria for systemic lupus; this condition may be associated with the other classic findings of TTP including acute renal failure, fever, and neurological deterioration, but is not associated with a severe reduction in ADAMTS13 activity, nor is it associated with diarrhea. TTP itself is defined by a MAHA where the activity level of ADAMTS13 is severely deficient. TTP-like MAHAs that occur simultaneously with connective tissue diseases are rare but clinically relevant illnesses that are becoming increasingly recognized as a distinct subgroup of atypical hemolytic uremic syndrome.

Over the last 60 years there have been 127 reported cases of MAHA resembling TTP occurring in patients with SLE. A 2003 review of the English literature from 1968 to 2002 had identified 56 such cases and had suggested that TTP in patients with SLE was associated with a higher mortality than idiopathic TTP, even with optimal treatment[1]. However, the vast majority of the patients in that review were treated prior to the advent of plasma exchange as the optimal therapy for TTP; instead these patients were generally treated with multiple modalities including plasmapheresis without exchange.

In the last ten years, there have been seven additional reported cases of purported TTP occurring in patients meeting the ACR criteria for SLE. Six of these patients were treated with plasma exchange in a timely manner, with or without steroids or cytotoxic therapy, and survived[3,4]. One patient did not receive plasma exchange promptly upon diagnosis and died in hospital[5].

A retrospective study from Japan in 2009 that reviewed a university hospital database for cases of thrombotic microangiopathy occurring in patients with connective tissue disease identified an additional sixty-four patients with thrombotic microangiopathy and SLE. Forty-five of these sixty-four patients received plasma exchange, with or without steroid therapy. Eighteen of the sixty-four died, although the reported data did not clearly address if there was a higher risk of death in patients who were not treated with plasma exchange[2].

Including the case reported here, there are now at least eight cases over the last ten years that suggest that early initiation of plasma exchange, with or without additional therapy, has the potential to be curative for TTP-like MAHA in patients with SLE.

Interestingly, the aforementioned Japanese study found that more than three quarters of the cases of thrombotic microangiopathy occurring in SLE were associated with normal or near normal ADAMTS13 activity. This suggests a different pathogenetic mechanism than that which occurs in idiopathic TTP[2,6], which is classically associated with a severely reduced ADAMTS13 activity. There is no consensus on the mechanism of TTP-like MAHA in lupus patients, but there are several hypotheses that are being investigated. These hypotheses implicate abnormal endothelial activation, elevated d-Dimers, ADAMTS13-resistant von Willebrand Factor, and defects in regulation of the complement system as culprits in causing the illness[6]. We did not measure a d-Dimer in our patient. She did have low C3 and C4 levels, 0.56 and 0.11 respectively, but these are expected given her active lupus. Investigations for mutations in genes encoding complement regulators were not sent. The other hypotheses mentioned could not be tested or confirmed easily in the clinical setting.

Returning to the 2003 review of 56 cases of TTP-like MAHA, an important observation is that plasma exchange appears to be associated with better outcomes than plasmapheresis without FFP infusion in lupus patients with a TTP-like syndrome. This implies that there may be a property of FFP that contributes to the reversal of the underlying pathogenetic process.

COMMENTS

Case characteristics

This 44-year-old lady known for lupus presented with the nephrotic syndrome, was found to have lupus nephritis, and her course of induction therapy was complicated by microangiopathic hemolytic anemia (MAHA), fever, rising creatinine with volume overload, and altered mental status.

Clinical diagnosis

The constellation of finings was suggestive of thrombotic thrombocytopenic purpura (TTP) complicating lupus nephritis.

Differential diagnosis

Atypical hemolytic-uremic syndrome (including a TTP-like syndrome occurring

in the context of a connective tissue disease), disseminated intravascular coagulation, antiphospholipid syndrome.

Laboratory diagnosis

In the context of intravascular hemolysis with schistocytes, rising creatinine, normal coagulation parameters, the absence of antiphospholipids, and an ADAMTS13 level that was not severely deficient, the most likely diagnosis is a TTP-like syndrome occurring in the context of lupus.

Imaging diagnosis

Chest radiography revealed pulmonary edema.

Pathological diagnosis

Histologic examination of the renal biopsy done on presentation revealed class 4G lupus nephritis without evidence of thrombotic microangiopathy; there was no repeat biopsy when she developed the constellation of features described above ten days after her induction therapy. Review of blood films taken after she developed the TTP-like clinical syndrome revealed elevated schistocytes.

Treatment

This lady was initially treated with a pulse of intravenous solumedrol and mycophenolate mofetil (MMF) for induction therapy for class 4 lupus nephritis; when she developed the constellation of features described above ten days later, she was given a second course of pulse IV solumedrol, cyclophosphamide instead of MMF, and plasma exchange.

Related reports

There have been over 50 reported cases of TTP-like syndromes occurring in patients with lupus in the literature, but only 7 such cases have been reported in the last ten years during which time plasma exchange has been the standard of care. Taking the most recent 7 cases as a case series, 6 were treated early with plasma exchange and survived while 1 did not receive plasma exchange early and died. Additionally, retrospective data from Japan has identified a subset of patients with microangiopathy complicating lupus who have near-normal ADAMTS13 levels. The implication is that in these patients, plasma exchange may have a survival benefit even in the absence of a severely deficient ADAMTS13, as suggested by the outcomes of the most recent case series.

Term explanation

MAHA: Characterized by elevated LDH, total bilirubin, decreased haptoglobin, and fragmented cells and schistocytes on blood film; TTP: A thrombotic microangiopathy manifested by fever, acute kidney injury, altered mental status, and intravascular hemolysis. Characterized by a severely deficient ADAMTS13 activity level; Atypical hemolytic-uremic syndrome (HUS): A thrombotic microangiopathy variably associated with acute kidney injury and intravascular hemolysis but with ADAMTS13 activity levels above the severely deficient range. It is often, but not always, associated with gene defects involving inhibitors of the alternative complement cascade; Lupus related TTP-like MAHA: A thrombotic microangiopathy syndrome similar to TTP but with near-normal ADAMTS13 activity; it is a subset of atypical HUS that occurs in patients with lupus and its etiology is unknown.

Experiences and lessons

TTP-like syndromes may complicate the course of active lupus and appear to respond favorably to treatments involving early plasma exchange despite being characterized by near-normal ADAMTS13 activity levels.

Peer-review

This is a very interesting clinical case of a rare complication of patients with LES.

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