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World Journal of Hepatology

Contents

Contents

ABOUT COVER

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EDITORIAL

Dietary factors can protect against liver cancer development

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Abstract

Liver cancer is the third leading cause of cancer mortality worldwide with hepatocellular carcinoma (HCC) representing more than 90% of primary liver cancers. Most HCC patients are also suffering from chronic liver disease (CLD). Evidence is emerging that the composition of diet plays an important role in HCC and CLD development and may also have a chemoprotective role. In contrast to other types of cancer, there are few studies investigating the role of diet in hepatocarcinogenesis. From the available data it is evident that high intakes of red meat and dietary sugar positively correlate with HCC occurrence. On the contrary, high consumption of white meat, fish, vegetables, fruits and cereals are inversely associated with HCC risk. This letter discusses the potential role of dietary interventions in the prevention of hepatocarcinogenesis. The increasing HCC incidence and its high fatality are making HCC prevention an urgent matter. Dietary modifications are found to offer protection against HCC, however, new studies from well-designed and large prospective trials are required to confirm these results.

Key words: Cancer prevention; Diet; Hepatitis virus; Meat; Hepatocellular carcinoma

Core tip: Hepatocellular carcinoma (HCC) is the third leading cause of cancer mortality. Evidence shows that diet relates to HCC risk and may also have a protective role. Several dietary factors such as vegetables, cereals, fruits, white meat and fish have been found to be inversely associated with HCC risk, whereas a positive correlation has been found with red meat and dietary sugar intakes. The increasing HCC incidence makes its prevention an urgent matter and diet intervention represent an attractive potential. Dietary modifications are found to protect against HCC, however, new studies from well-designed and large prospective trials are required to confirm these results.

INTRODUCTION

Hepatocellular carcinoma (HCC) is third leading cause of cancer mortality worldwide and accounts for about 90% of primary liver cancers. The major risk factors for HCC occurrence include chronic infection with hepatitis B virus (HBV) and hepatitis C virus (HCV), excess of alcohol consumption, non-alcoholic fatty liver disease (NAFLD), dietary aflatoxin exposure, obesity, smoking and diabetes mellitus[1]. Exposure to these factors can injure the liver leading to chronic liver disease (CLD) and patients with CLD are at high risk of developing HCC. A substantial proportion of HCC, however, occurs in patients without exposure to these risk factors[2], suggesting the existence of additional factors.

For the past few decades, epidemiological evidence has shown that diet-related factors are closely related with cancer. A healthy diet is known to reduce the development of some types of cancer, while a poor diet increases cancer risk[3]. However, there is no current definition of healthy eating. Observational studies have indicated a protective role of vegetables, fruits and cereals in cancer prevention. In contrast to other types of cancer, there are relatively few studies that investigated the association of diet and HCC risk. Although studies have reached conflicting results, consistent evidence suggests that high intakes of red meat[4,5] and dietary sugar[6] should be avoided in at-risk populations. Higher intakes of white meat or fish[7-10], vegetables[10-16], fruits[10,14,17,18], cereals[6], eggs[10,17,18], milk[18] and yogurt[10] have been reported to decrease HCC development. Dietary patterns can capture interactions between dietary components and other risk factors providing a better understanding of the association between dietary intakes and HCC risk. Here the impact of dietary patterns on the prevention of HCC is being discussed.

RED MEAT

Red meat is an important dietary source of saturated and monounsaturated fatty acids and iron. A number of studies associated meat, especially red and processed meat, with gastrointestinal cancers, including HCC[5,10,13,17-23]. Nanji et al[24] was the first to report in 1985 that high pork intake correlated with liver cancer mortality and since then significant associations between total red meat and an increased risk of CLD and HCC have been found[4,25,26] (Table 1). A large prospective study by Freedman et al[4] with a United States cohort revealed an association between total fat, monounstaturated fat, and saturated fat with both CLD and HCC incident and another smaller study from Greece observed that that high saturated fat intake correlated with liver cirrhosis and HCC[26]. One study of daily beef, pork and poultry intake found a statistically significant positive association between red meat and HCC risk in an age- and sex-adjusted analysis[5]. On the contrary, Polesel et al[27] reported no direct association of HCC risk with saturated fat intake and results from a Greek case-control study showed no

association with any fat type[20]. Notably, Polesel et al[27] did report a positive association between iron intake and HCC. Furthermore, a recent meta-analysis study by Luo et al[28] as well as the multicenter prospective EPIC cohort study, which associates diet with various types of cancer, reported no association between different kinds of meats (red and processed meats or poultry) and increased HCC risk[7,28,29]. The EPIC study, however, found that a 20 g/d substitution of fish with meat results in a 16% decrease in HCC risk.

The link between red meat and liver cancer is biological plausible, since red meat contains high amounts of known carcinogens including heme iron, N-nitroso compounds (NOC) and heterocyclic amines (HCA) that are produced when meat is cooked in high temperatures[30]. Red meat contains high amounts of bioavailable heme iron while reactive oxygen species are being formed when iron undergoes reduction. Interestingly, individuals with hereditary hemochromatosis, an iron overload disease, have substantially increased HCC occurrence[31]. Also excess dietary iron has been shown to contribute to HCC risk in several parts of Africa, and treatment with chelating agents, repeated phlebotomy and low iron diet appear to reduce the HCC incident[32]. Freedman et al[4] 2010 observed that meat processing, its heme iron and NOCs associated with CLD but not with HCC. A case-control study with an Italian cohort revealed a significant positive association between dietary iron intake and HCC risk but did not investigate the role of heme iron[27]. HCA and polycyclic aromatic hydrocarbons, carcinogens are generated during high-temperature cooking, and NOCs compounds have been shown to induce liver tumour development[30] while high doses of HCAs cause liver tumours in primates[30]. The higher fat content of red meat could also explain the harmful effect. Red meat and processed meat contain high levels of cholesterol and saturated fat, and correlate with high risk of obesity and diabetes, which are known as cancer risk factors. In addition, fat intake may play a role in insulin resistance, which relates with liver disease and cancer. Fatty acid deposition in the liver can result in NAFLD therefore increasing the risk of CLD and HCC[33].

Although conflicting results, it can be suggested that red meat intake positively associates with CLD and HCC risk. The observed discrepancies between the studies can be attributed to the limitations of the studies, including differences in the dietary patterns of the various countries studied. However, further large prospective randomized trials investigating the relationship between meat intake and HCC risk are required to reach conclusive results.

WHITE MEAT AND FISH

Evidence from case control and prospective studies from the NIH-AARP Diet and Health study, Italy and Japan reported an inverse association of white meat, including chicken, turkey and fish, with HCC development[4,7,8,10,29] (Table 1). In the EPIC study, the subgroup analyses revealed that lean fish, fatty fish, crustaceans and

Table 1 Main characteristics of studies on dietary factors and hepatocellular carcinoma risk

Conclusions	Study details	Location	Ref.
Inverse association of vegetable and fruit intake with HCC and upper digestive cancers risk	Study design: Case-control Cases: 285 Controls: 6147 Duration: 1983-1990 Intake: Vegetables and fruit	Italy	Negri et al[14], 1991
No association between meat and vegetable intake and HCC risk	Study design: Case-control Cases: 97 Controls: 128 Duration: 1995-1998 Intake: Total meat, fruit and vegetables	Greece	Kuper et al[44], 2000
Association of red meat intake and HCC risk NAT2 gene polymorphisms play a role in the effect of meat in HCC development	Study design: Case-control Cases: 185 Controls: 185 Duration: 1999-2001 Intake: Red and white meat, vegetables and fruits	China	Huang et al[23], 2003
Inverse association of white meat, coffee and vegetables with HCC mortality Association of egg intake with HCC mortality	Study design: Cohort Cases: 401 Controls: 110688 Duration: 1988-1999 Intake: Fish, red meat, processed meat, chicken, vegetables	Japan	Kurozawa et al[17], 2004
Inverse association of white meat, milk, yogurt, eggs, and fruits with HCC risk	Study design: Population based case-control Cases: 185 Controls: 412 Duration: 1999-2002 Intake: Milk, yogurt, white meats, eggs, fruits, vegetables	Italy	Talamini et al[10], 2006
Association of red and processed meat intake with HCC risk	Study design: Case-control Cases: 403 Controls: 567169 Duration: 1995-2006 Type of meat: Processed meat	United States	Cross et al[25], 2007
Association of dietary iron intake and HCC risk Inverse association of linoleic acid (white meat) intake and HCC risk	Study design: Case-control Cases: 185 Controls: 412 Duration: 1999-2002 Intake: Total meat	Italy	Polesel et al[27], 2007
Association of red meat and saturated fat intake with CLD and HCC risk Inverse association of white meat with HCC and CLD risk	Study design: Case-control Cases: 338 Controls: 495006 Duration: 1995-2006 Intake: White and red meat	United States	Freedman et al[4], 2010
Inverse association of fish or n-3 PUFAs intake and HCC risk	Study design: Cohort Cases: 398 Controls: 90296 Duration: 1990-2008 Intake: Fish	Japan	Sawada et al[9], 2012
Inverse association of vegetables intake and HCC risk	Study design: Case control Cases: 267 Controls: 132837 Duration: 1997-2006 Intake: Meat	China	Zhang et al[41], 2013
Inverse association of fish intake and HCC risk HCC risk decreases by 16% for 20 g/d substitution of fish with meat	Study design: Cohort Cases: 157 Controls: 35628 Duration: 1992-2000 Intake: Dietary flavonoids	World-wide	Zamora-Ros et al[46], 2013
Inverse association of fish intake and HCC risk No association of meat and poultry intake and HCC development	Study design: Cohort Cases: 191 Controls: 477206 Duration: 1992-2010 Intake: Total meat, fish, red and white meat	Europe	Fedirko et al[7], 2013
HCC risk decreases by 8% for every 100 g/d increase in vegetable intake	Study design: Meta-analysis Cases: 3912 Controls: 1290045 Duration: 1956-2014 Intake: Vegetables and fruits	World-wide	Yang et al[43], 2014

HCC: Hepatocellular carcinoma; CLD: Chronic liver disease.

molluscs independently associated with low HCC risk[7]. In a large population-based prospective Japanese cohort, Sawada *et al*[9] revealed that the consumption of fish or n-3 polyunsaturated fatty acids (n-3 PUFA) protects against the HCC development even among subjects with HBV and/or HCV infection, and Freedman *et al*[4] reported an inverse association between fish intake and CLD risk.

The finding that both fish and white meat reduce HCC risk is unforeseen. Nutritionally, fish and white meat are a rich source of PUFA and have less cholesterol and saturated fat compared with red meat. Substantial evidence indicates that n-3 PUFA possess anti-inflammatory activity by inhibiting IL-1 and TNF synthesis[34], which can contribute in HCC prevention, considering that chronic inflammation plays a central role in HCC development. PUFA might exert anticancer effects also through their ability to induce apoptosis, to modulate cell cycle and eicosanoid production[35]. In particular, n-3 PUFAs have been shown to inhibit HCC growth *in vitro* through the blockage of β-catenin and cyclooxygenase-2[36]. It is observed that n-3 PUFA supplementation can improve hepatic steatosis in patients with NAFLD in a pilot study[37]. All of this evidence support a possible chemoprotective effect for fish and white meat on HCC development and suggest a molecular mechanism of n-3 PUFA in HCC prevention. However, some fatty acids themselves can also have harmful effects, particularly the saturated fats and trans fatty acids since their increased consumption is strongly linked with the development of non-alcoholic steatohepatitis and its progression to cirrhosis and fibrosis[38]. As there is significant heterogeneity in fat subtypes within most foods, increasing fatty acid consumption should not be encouraged in at-risk populations at least until more studies prove the potential benefits of specific PUFA supplements.

MILK AND EGGS

High intake of milk, yogurt and eggs was found to reduce liver cancer risk in a case control study[10]. Decreased risk of HCC with highest milk intakes was also reported in another case-control study from Italy[18], while a Japanese case-control study revealed a higher risk with greater than average milk consumption[19]. Saturated fat from dairy products was also independently associated with CLD and HCC risk[4]. However, two other studies from China and Japan did not find such associations[17,39]. An inverse correlation between egg and HCC has been observed in two Italian case-control studies[10,18], while a Japanese study reported an increased risk of HCC for high egg consumption in men only[19]. Such discrepancies, however, may be attributed to different dietary habits between the studied populations, such as the use of fat for cooking. Notably eggs are a different diet indicator in Italy and Japan. The inverse association with dairy products and eggs could be explained by their retinol content, since serum retinol levels have been

inversely related to HCC risk in a case-control study from China[40].

VEGETABLE, FRUIT AND CEREALS

The association of vegetable and fruit intakes with HCC incidence has been investigated by a number of observational studies since Negri *et al*[14] revealed in a case-control study in the 1990s that high intake of vegetables and fruit was inversely associated with risk of upper digestive cancers, including HCC. The majority of studies reported inverse associations between high vegetable consumption and liver cancer risk[1,10,11,13-15,17,41] (Table 1). In the large prospective study of a US cohort and two European cohorts it was revealed that adherence to the high vegetable content diets of the dietary recommendations and the Mediterranean diet decreases HCC risk[42,43]. This observation was confirmed by Yang *et al*[43] in a meta-analysis on 19 published studies where it was reported that HCC risk decreases by 8% for every 100 g/d increase in vegetable intake. On the contrary, two case-control studies from Greece reported no association, although they involved a small number of cases[20,44]. The role of fruit consumption in HCC risk is more controversial. Three case-control studies reported a decreased liver cancer risk with higher fruit intake[10,13,18], while other four studies found no such association[19,20,28,41]. Another case-control study in northern Italy found that the population attributable-risk for liver cancer was as high as 40% for low vegetable and fruit consumptions[11].

Vegetables and fruits are major sources of vitamins, minerals, dietary fibres, and other bioactive compounds, including flavonoids. Several *in vitro* studies have shown an anti-tumour effect of flavonoids in some hepatocarcinoma cell lines[45,46] while in animal models, flavonoids have been shown to modulate mechanisms involved in proliferation, invasion, angiogenesis, survival and metastasis[47]. According to the EPIC study and a case-control study from Greece an inverse association exists between the flavonoids subclass, flavones, and HCC occurrence[46,48]. Therefore, flavonoids may explain the favorable effects of vegetables and fruits against liver cancer. In addition, evidence is emerging from cell culture and animal model experiments that phytochemicals and other bioactive components found in vegetables, such as diallyl sulphides, lentinan, apigenin and luteolin, have cancer-inhibitory effects through their anti-oxidative properties, stimulation of the immune system, or inhibition of mutagenesis[41]. An animal study on effects of dietary dry bean on hepatic gene expression in rats, found that the expression of six genes was significantly altered after high bean intakes suggesting that these genes may exert cancer preventive effects in liver[49].

A protective role of dietary fiber has been also suggested in HCC development[6]. High intake of fiber from cereals and cereal derivatives was found to be statistically significantly inverse associated with HCC risk. Consumption of fiber from vegetables or other sources (but not fruits)

was also revealed to have a chemoprotective role in HCC development although a statistical significance was not reached[6]. Diets with a high fiber content, like cereals, could lower HCC occurrence by decreasing subjective appetite and energy intake and hence contributing to the maintenance of normal body weight as well as exerting beneficial effects on postprandial glucose level and blood lipid profile. Further research is needed to understand the mechanisms underlying these associations.

DIETARY SUGAR

Dietary glycemic load (GL) estimates how much the food will raise a person's blood glucose level after eating it and is therefore the extent to which carbohydrate-rich foods increase the concentration of glucose in the blood to represent the total glycemic effect of a diet. GL has been associated with diabetes mellitus and with several types of cancer. The mechanism for the role of high GL in carcinogenesis is thought to be *via* increased insulin concentrations, glucose intolerance and insulin resistance, even in the absence of diabetes mellitus. Foods such as added sugars, syrups, sweets, white bread and soft drinks are the main culprits. One case control study from Italy showed a positive association between GL and HCC overall and interestingly a stronger association was observed in patients with HBV and HCV[50]. On the contrary, the EPIC study reported that GL and total carbohydrate intakes did not correlate with HCC risk[6]. However, when specific carbohydrates were analysed, a positive association was found for total sugar. Increased fructose intake is known to underlie NAFLD and may therefore provide a possible explanation for the positive association seen in HCC[51].

DIET IN VIRAL HEPATITIS-INDUCED HCC

HBV and HCV infections are major risk factors for the development of CLD and HCC. Three studies in the United States and Italy agree that the risk estimates for meat and fish consumption were similar between subjects with or without HBV and/or HCV infection[4,7,10,27]. The association of n-3 PUFA, fibers and flavonoids with HCC risk has also been found to exist independently of the HBV and/or HCV status[6,9,46,48]. However, the positive correlation of GL and HCC was observed to be stronger in patients with HBV and HCV in one case control study from Italy[50]. This evidence indicates that the dietary patterns, possibly except the GL, do not appear to affect HCC outcome in chronically HBV or HCV infected individuals. However, the possibility that dietary intakes can protect against the HCC progression in viral hepatitis infection cannot be excluded.

CONCLUSION

Considering the increasing trend of HCC incidence and its high fatality, prevention of HCC is an urgent matter. At present attempts to prevent HCC mainly include the control of HBV or HCV infection, reduction of alcohol consumption, and reducing the prevalence of obesity and diabetes. It is of great importance to discover novel strategies to prevent HCC and dietary factors represent an attractive potential.

Up to date the results from studies investigating the impact of dietary factors in HCC development are conflicting and often inconclusive. Notably, there are several limitations in the methodology of most studies, including selection bias, errors in diet assessment and insufficient adjustment for potential confounders such as HBV/HCV status, diabetes, alcohol and energy intake. Since both case control and prospective studies include data that comes from questionnaires, which are based on self-reported food intakes it is important to consider the dietary habits. Food consumption categories are different across the studies and this can contribute to the heterogeneity of the results. In addition, the case-control studies assessed diet after HCC diagnosis, at which stage the health of individuals has already been compromised, affecting the accuracy of dietary recall. Indeed, a major problem of the case-control studies on HCC is reverse causation because HCC precedes chronic hepatitis and cirrhosis. Furthermore, in the large prospective EPIC study, the diet was assessed only at baseline without considering any potential dietary changes during the follow-up, the period of exposure to cancer initiation was not taken into account and also that dietary patterns of different European countries may have not been fully accounted. It is therefore possible that dietary errors may have occurred that might underestimate the true associations described in the EPIC study.

Nevertheless from the available data it is evident that dietary factors play an important role in CLD and HCC occurrence and their identification can be used for the development of new public health diet recommendations. In particular, consistent associations indicate that higher consumption of vegetables, white meat, fish, milk and cereals have beneficiary effects in liver cancer development. It should be noted that increased consumption of fatty acids correlates with the progression of cirrhosis and fibrosis. The potential benefits of fat subtypes, deriving from white meat, fish and milk, should not be encouraged until more well designed studies prove their chemoprotective effect. Furthermore, it can be inferred that red meat and dietary sugar consumption intake associates with CLD mortality and HCC risk and hence their intake should be monitored and controlled in at-risk populations to attempt to slow down HCC development. Flavonoids appear to reduce the risk of HCC, but pharmacological doses might be required in order to effectively protect against carcinogenesis. Although current studies on infections with HBV and HCV suggest that the effect of diet is independent of viral hepatitis infection, an association between diet and hepatitis virus related HCC progression is possible.

To prevent disease progression to CLD or HCC it is crucial to investigate the impact of diet and subsequently to lead to the development of clinical trials using new

dietary patterns. Evidence from well designed prospective interventional studies with large sample sizes and long-term follow-up are required to develop diet modifications to lower HCC incidence or to prolong survival in HCC patients. Additional experimental and molecular research is also needed to explore the possible mechanisms involved.

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MINIREVIEWS

Iatrogenic amyloid polyneuropathy after domino liver transplantation

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Abstract

Liver transplantation has been used in treatment of transthyretin amyloidosis, and some patients undergo domino liver transplantation (DLT) with explanted liver being transplanted to another patient with liver failure as the liver is otherwise usually functionally normal. Until end of 2015, there were 1154 DLT performed worldwide. DLT for transthyretin amyloidosis is associated with the risk of developing *de novo* systemic amyloidosis and amyloid neuropathy, and the risk may be greater with some non-Val30Met mutations. *De novo* amyloid neuropathy has been described in up to 23% of transplant recipients. Neuropathy may be preceded by asymptomatic amyloid deposition in various tissues and symptoms of neuropathy started after a median of 7 years following DLT (5.7 ± 3.2 years; range 2 mo to 10 years). Typical initial symptoms include neuropathic pain and sensory loss, while dysautonomia usually starts later. Progression of neuropathy may necessitate liver re-transplantation, and subsequent improvement of neuropathy has been reported in some patients. Explant allograft recipients need close monitoring for signs of systemic amyloidosis, neuropathy and dysautonomia as progressive symptoms may require re-transplantation.

Key words: Transthyretin; Familial amyloid neuropathy; Domino liver transplantation; Systemic amyloidosis; Acquired amyloid neuropathy

Core tip: Domino liver transplantation (DLT) has been used in treatment of transthyretin amyloidosis, with explanted liver being transplanted to another patient with liver failure as the liver is otherwise usually functionally normal. Domino liver explant recipients are at risk of developing *de novo* systemic amyloidosis and amyloid neuropathy has been described in up to 23% of transplant recipients after a median of 7 years following DLT. Typical initial symptoms include neuropathic pain and sensory loss, while dysautonomia usually starts later. Explant allograft recipients need close monitoring for signs of systemic amyloidosis, neuropathy and dysautonomia as progressive symptoms may require re-transplantation.

INTRODUCTION

Transthyretin familial amyloidosis is an autosomal dominant multisystem disorder caused by deposition of insoluble transthyretin (TTR) amyloid deposits in various tissues[1]. Amyloid deposits are found in peripheral nerve, liver, skin, heart and other organs, and peripheral neuropathy is one of the major clinical manifestations. Three main phenotypes of transthyretin familial amyloidosis include familial amyloid polyneuropathy (TTR-FAP), cardiomyopathy and leptomeningeal amyloidosis. Additionally, wild type TTR may be also deposited in senile systemic amyloidosis which more commonly presents with cardiomyopathy, and only rarely as neuropathy. TTR-FAP is a progressive sensorimotor neuropathy with dysautonomia which results in a severe disability. The neuropathy usually starts with distal sensory loss and dysesthesias, followed by autonomic dysfunction, while motor function is usually affected only later[2]. Carpal tunnel syndrome is usually an early feature, but there is a significant variability of symptoms, even among the patients with the same TTR mutation. Overall prevalence of TTR-FAP is estimated at 0.9-1.1 per 10000000 people, with expected survival of 7-12 years after the onset of symptoms[3-5]. To date more than 120 mutations of TTR have been reported to cause TTR-FAP, and high prevalence is found in endemic regions in Portugal, Japan and Sweden (up to 1 in 1000 to 1 in 10000), and the most common genotype with predominant neuropathy is Val30Met TTR mutation[6].

Transthyretin is mainly synthesized in the liver and liver transplantation has been used to ameliorate the progression of systemic amyloidosis by decreasing the synthesis of abnormal TTR[7,8]. There is a shortage of liver grafts available for transplantation, and explanted liver of the patient with TTR amyloidosis is sometimes transplanted to another patient with liver failure as the liver is otherwise usually functionally normal Domino liver transplantation (DLT)[8]. Until end of 2015, there were 1154 DLT s performed worldwide[9]. Best outcomes for liver transplantation have been reported in young Val30Met patients with mild symptoms, and the overall 20-year survival after liver transplantation for TTR-amyloidosis has reached 55%[8,10,11]. In transplant patients (DLT donors) with non-Val30Met genotype, there is a wide spectrum of survival rates which vary a lot depending on the underlying mutations[12]. The overall risk of amyloid production by transplanted domino liver was thought to be low with potential delayed manifestations of amyloidosis in patients with otherwise very short survival if they are not transplanted. Nevertheless, amyloid deposition in the tissue may start soon after domino transplantation and iatrogenic amyloid neuropathy may affect up to 8%-24% of DLT recipients[13,14].

In this manuscript, we review clinical features of acquired amyloid TTR neuropathy after DLT.

LITERATURE

We have identified 16 case reports with detailed description of acquired TTR-FAP in recipients of DLT in the literature search (Table 1)[14-23]. The patients were 73% men with age of 60.7 ± 10.4 years. The most common TTR mutation was Val30Met (*n* = 10; 61.3%), and others included Ser23Asn, Ser77Tyr, Leu58His, Thr49Ala, Gly47Glu, Glu54Gly (*n* = 1 each; 6.7%). Underlying causes of liver failure include hepatitis C infection (*n* = 8), hepatocellular carcinoma (*n* = 6), hepatitis B infection (*n* = 5), primary sclerosing cholangitis, primary biliary cirrhosis and nonalcoholic liver steatosis (*n* = 1 each; some patients had more than 1 cause).

De novo amyloid neuropathy presented at after a median of 7 years following DLT (5.7 ± 3.2 years; range 2 mo to 10 years). Initial symptoms included neuropathic pain (*n* = 14), sensory loss (*n* = 5), erectile dysfunction (*n* = 2), weakness, diarrhea and orthostatic hypotension (*n* = 1, each). Nerve biopsies showed amyloid deposits in 3 reported cases[16,18,23]. Other abnormal tests included positive rectal and abdominal fat (*n* = 2 each), duodenal and endomyocardial biopsies (*n* = 1 each) showing amyloid deposits. Three patients were treated with retransplantation of the liver, with improved outcome in two patients (outcome not reported in the third case).

The age of donors and recipients of domino liver allografts was not associated with an earlier onset of amyloid neuropathy. Non-Val30Met TTR mutations were overall associated with earlier onset of *de novo* TTR-FAP neuropathy (latency 3.95 years *vs* 6.83 years after transplantation; range 2 mo to 10 years).

CONCLUSION

DLT has been advocated to alleviate shortage of liver allografts for patients in liver failure who would not have survived without such procedure. Initial reports estimated a low risk of systemic amyloidosis from synthesis of abnormal transthyretin liver allografts. Nevertheless, subsequent studies demonstrated that recipients of DLT are indeed at risk from systemic amyloidosis and some patients may develop *de novo* amyloid neuropathy after a median of 7 years following transplantation (5.7 ± 3.2 years; range 2 mo to 10 years). Similarly, less favorable survival of patients with non-Val30Met after liver transplantations (domino liver donors) was paralleled by shorter latency of *de novo* amyloid neuropathy in domino liver recipients with allografts with non-Val30Met TTR mutations (3.95 years *vs* 6.83 years)[12]. Nevertheless, there seems to be a marked variability of clinical course, depending on the underlying TTR mutation in domino liver donors (and allografts). Exceptionally short latency of *de novo* amyloid neuropathy in DLT recipients was reported with Ser23Asn and Leu58His TTR mutations

Table 1	*De novo* transthyretin amyloid neuropathy cases after liver transplantation				
Ref.	TTR mutation	Age at transplant	Latency (yr)	Cause of liver failure	Initial Neuropathy symptoms
[14]	Val30Met	61	8	HCV EtOH	Pain
[14]	Val30Met	35	5	HBV	Pain
[14]	Val30Met	26	3.5	HCC	Pain, erectile dysfunction
[14]	Val30Met	28	5	HBV	Pain
[14]	Ser77Tyr	57	2	HCC HBV HCV	Pain
[14]	Leu58Hys	60	0.17	HCV HIV	Pain, weakness
[14]	Thr49Ala	49	2	HCC HCV	Pain, orthostatic hypotension
[15]	Glu54Gly	43	9	HCC	Diarrhea, pain, sensory loss
[16]	Val30Met	60	7	HBV cirr	Pain
[17]	Gly 47Glu	65	10	HCC	Pain
[18]	Val30Met	54	9	HCV	Pain, erectile dysfunction
[19]	Ser23Asn	72	0.5	NASH	Sensory loss, pain
[20]	Val30Met	50	7	PBC	Sensory loss
[21]	Val30Met	35	10	PSC	Pain, sensory loss
[22]	Val30Met	59	12	HBV HCV cirr	Pain, sensory loss
[23]	Val30Met	47	8	HCV cirr	Pain

PBC: Primary biliary cirrhosis; NASH: Nonalcoholic hepatic steatosis; EtOH: Alcoholic liver cirrhosis; HBV: Hepatitis B virus; HCV: Hepatitis C virus; cirr: Cirrhosis of the liver; PSC: Primary sclerosing cholangitis; HCC: Hepatocellular carcinoma.

(< 6 mo)[14,19]. These series did not show an association with donor or recipient age with *de novo* systemic TTR amyloidosis, although other studies suggest that recipient aging may accelerate tissue deposition of amyloid[24].

Clinically, these patients typically present with sensory loss and neuropathic pain, while dysautonomia is often not the initial symptom and follows later (Table 1). Tissue deposition of amyloid in the gastrointestinal tract and skin may precede clinical symptoms of neuropathy by several years[20,25]. and prospective study showed deposition of amyloid in salivary glands in up to 48% of DLT recipients[14]. Additionally, amyloid deposits were also found on sural nerve biopsies in the absence of clinical signs of neuropathy (research study and autopsy)[13,26]. Transthyretin amyloid neuropathy in DLT recipients presents in the context of systemic amyloidosis and *de novo* amyloid produced by transplanted liver is also found in myocardium, gastrointestinal tract, skin and fatty tissues[15,17,19-22]. Autopsy case of a DLT patient with asymptomatic amyloidosis at 8 years after transplantation (died from lymphoma) showed amyloid deposits in the heart, lungs, gastrointestinal tract (upper and lower), pancreas, spleen, testes, epididymis, prostate, skeletal muscle, thoracic sympathetic ganglia and median nerve (carpal tunnel syndrome)[26]. Amyloid deposition in the myocardium after DLT is usually asymptomatic, and so far only two cases of *de novo* cardiomyopathy after DLT have been reported with Val71Ala and Val30Met mutations[27,28]. One of the patients developed cardiac amyloidosis 5 years after re-transplantation for systemic *de novo* amyloidosis after DLT, and it is unclear whether cardiac amyloidosis was related to wild-type or mutant transthyretin[28]. Similarly, DLT donors continue to have deposition of amyloid in different tissues of FAP-TTR patients after transplantation, which is at least partly related to wild-type ("senile") amyloid[29]. Worsening of peripheral neuropathy after transplantation has been reported in 24% of DLT liver allograft donors[5], and ocular deposition of amyloid is not abated after liver transplantation, as mutant TTR continues to be synthesized in the retinal pigment epithelium[30].

While *de novo* amyloid neuropathy is associated with significant morbidity, DLT recipients may also have other potential causes of neuropathy and nerve biopsy may be needed to establish the etiology and exclude alternative etiologies[14]. Nevertheless, nerve biopsy may be false negative with a reported sensitivity of 33%-80%, and this may be attributed to uneven distribution of amyloid along peripheral nerves[31-33]. Biopsies of gastrointestinal tract, myocardium and fat aspirate may also demonstrate systemic deposition of amyloid in DLT recipients[15,17,19-22].

De novo amyloid neuropathy presents in the context of systemic amyloidosis and careful monitoring of clinical manifestations of amyloidosis is needed after DLT (Table 2)[34]. Once iatrogenic amyloid neuropathy and systemic amyloidosis are diagnosed, treatment options are limited and retransplantation has been reported to stabilize or improve neuropathy in some recipients[15,16]. Tafamidis, a tetramer stabilizer, is approved for treatment of FAP-TTR in Europe, Japan, Mexico and Argentina and is used as first-line treatment for early FAP-TTR[35]. In countries where tafamidis is available, liver transplantation is often considered as a second-line option for patients who progressed on tafamidis or did not tolerate the treatment. Liver-retransplantation typically carries worse prognosis and more significant morbidity than the initial transplantation, but the survival after liver retransplantation in DLT recipients seems to be greater than in other subgroups of liver retransplant recipients[36]. Despite a single report of benefits with deflunisal[37], potential benefits of treatment with tetramer stabilizers in DLT recipients remain uncertain. There are also no reports on efficacy of inhibition of expression of *TTR* gene in this setting. At this time, tafamidis remains available in a limited number of countries, and new experimental treatments are still not ready to substitute the role of DLT in treatment of systemic amyloidosis. In conclusion, DLT

Table 2 Evaluation and monitoring of possible *de novo* amyloidosis after domino liver transplantation

Clinical presentation	Signs and symptoms	Testing
Dysautonomia (small fiber neuropathy)	Orthostatic hypotension	Tilt table testing
	Sweating abnormalities (anhidrosis)	QSART with autonomic battery
	Constipation/diarrhea	ECG
	Erectile dysfunction	Neurologic examination
	Neuropathic pain	
	Arrhythmia	
	Neurogenic bladder	
Large fiber polyneuropathy	Sensory loss	EMG/NCS
	Weakness	Nerve (and muscle) biopsy
	Neuropathic pain	Neurologic examination
	Ataxia	
	Areflexia	
Cardiac amyloidosis	Fatigue	ECG
	Arrhythmia	Transthoracic echo
	Syncope	Radionuclide imaging
	Orthostatic hypotension	Cardiac MRI
		Endomyocardial biopsy
		BNP/troponin
Gastrointestinal amyloidosis	Constipation/diarrhea	GI tract biopsy
	Nausea/vomiting	
Ocular amyloidosis	Dry eye	Ophthalmologic evaluation
	Vitreous opacity	
	Glaucoma	
Leptomeningeal amyloidosis	Cerebral infarction/hemorrhage	MRI of brain and spinal cord
	Hydrocephalus	Meningeal biopsy
	Ataxia	
	Spastic paresis	
	Seizures	
	Dementia	
Other system involvement	Coldness	Rectal and abdominal fat biopsy
	Weight loss	Salivary gland biopsy
	Peripheral edema	TSH
	Anemia	Urinalysis/urine protein collection
	Dry mouth	

QSART: Quantitative sudomotor axon reflex test; ECG: Electrocardiograph; EMG/NCS: Electromyography and nerve conduction studies; MRI: Magnetic resonance imaging; BNP: Brain natriuretic peptide; GI: Gastrointestinal; TSH: Thyroid stimulating hormone.

is associated with the risk of developing *de novo* systemic amyloidosis and amyloid neuropathy, and the risk may be greater with some non-Val30Met mutations. Explant allograft recipients need close monitoring for signs of systemic amyloidosis, neuropathy and dysautonomia as progressive symptoms may require re-transplantation.

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Cockcroft-Gault revisited: New de-liver-ance on recommendations for use in cirrhosis

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Abstract

The Cockcroft-Gault (CG) equation has become perhaps the most popular practical approach for estimating renal function among health care professionals. Despite its widespread use, clinicians often overlook not only the limitations of the original serum creatinine (SCr) based equation, but also may not appreciate the validity of the many variations used to compensate for these limitations. For cirrhotic patients in particular, the underlying pathophysiology of the disease contributes to a falsely low SCr, thereby overestimating renal function with use of the CG equation in this population. We reviewed the original CG trial from 1976 along with data surrounding clinician specific alterations to the CG equation that followed through time. These alterations included different formulas for body weight in obese patients and the "rounding up" approach in patients with low SCr. Additionally, we described the pathophysiology and hemodynamic changes that occur in cirrhosis; and reviewed several studies that attempted to estimate renal function in this population. The evidence we reviewed regarding the most accurate manipulation of the original CG equation to estimate creatinine clearance (CrCl) was inconclusive. Unfortunately, the homogeneity of the patient population in the original CG trial limited its external validity. Elimination of body weight in the CG equation actually produced the estimate closest to the measure CrCl. Furthermore, "rounding up" of SCr values often underestimated CrCl. This approach could lead to suboptimal dosing of drug therapies in patients with low SCr. In cirrhotic patients, utilization of SCr based methods overestimated true renal function by about 50% in the literature we reviewed.

Key words: Cockcroft-Gault; Cirrhosis; Renal function; Pharmacokinetics; Creatinine clearance

Core tip: For many health care professionals in the

United States, the Cockcroft-Gault (CG) equation has become perhaps the most popular practical approach for estimating renal function. Despite its widespread use, clinicians often overlook not only the limitations of the original serum creatinine (SCr) based equation, but also may not appreciate the validity of variations used to compensate for these limitations. For cirrhotic patients in particular, the underlying disease pathophysiology contributes to a falsely low SCr, thereby overestimating renal function with use of the CG equation in this population.

INTRODUCTION

In order to optimize efficacy and minimize potential toxicity of pharmacologic agents, appropriate patient-specific dosing of medications remains an inherent responsibility of all healthcare providers. Proper assessment of a patient's renal function is essential when managing medications that are primarily renally excreted[1]. With an estimated 14% of adults in the United States experiencing varying degrees of chronic kidney disease (CKD), optimization of drug therapies poses a frequent challenge to clinicians[2]. Renal impairment may significantly alters the pharmacokinetic (PK) properties of many medications[3]. Therefore, reasonably accurate yet convenient quantification of the degree of renal impairment is an essential tool for clinicians implementing renal dose adjustments[3].

In the majority of clinical settings, calculating the creatinine clearance (CrCl) using the Cockcroft-Gault (CG) equation has become the most popular and practical approach for estimating renal function[4,5]. Many institutions provide dosing recommendations based on calculated CrCl, and even often utilize electronic health record (EHR) software that automatically calculates CrCl based on the CG equation. Unfortunately, some clinicians fail to realize the inherent limitations of a serum creatinine (SCr) based equation and the subsequent variations that stem from many of these limitations[1,4,6].

SCr concentrations can be altered by patient specific factors including age, sex, weight, muscle mass, disease state, diet, and certain drug therapies, thus limiting the generalizability of the CG equation[1,4-6]. For example, patients with hepatic impairment not only experience altered drug metabolism, but also have secondarily reduced creatinine production. If not taken into consideration, these SCr-based formulas can lead to an overestimation of GFR in cirrhotic patients[7].

To help clarify the true applicability of the CG equation, we will discuss the origins of the CG equation and the evidence and reasoning behind specific alterations to the equation used in current practice.

DATA SOURCES AND SELECTION

Data included in this review were identified from a PubMed search of publications starting in 1970 through June of 2016. Searches included the keywords "Cockcroft-Gault", "serum creatinine", "creatinine clearance", "renal function", "cirrhosis" and related search terms. Publications were considered for review if they were designed as meta-analyses, retrospective, or prospective studies that compared different methods of estimating CrCl using the CG equation.

THE ORIGINS OF THE COCKCROFT-GAULT EQUATION

The CG equation (Table 1A; equation I) was derived from 236 patients (96% male), aged 18-92 years old in 1976 at the Queen Mary Veterans' Hospital in Canada. SCr values used in the equation were the mean values calculated from two 24-h SCr levels obtained from blood for each patient at steady state. The CrCl was calculated using 4 different formulas (Table 1A; equations I -IV) that were compared against each other and with each patient's 24-h urine creatinine excretion. The CG equation was found to provide an estimated CrCl that was 80% ± 30% of the actual creatinine clearance calculated from the 24-h urine creatinine excretion test[5].

Limitations acknowledged at the end of this trial included requirements for SCr to be at steady state, the need for normal relationship between muscle mass and total body weight, and factors related to age, sex, and height. In addition to this, the formula was tested in a patient population that was 96% male, which obviously limits the external validity of the results in female cohorts. To compensate for females having different relative amounts of fat and muscle compared to males, a somewhat arbitrary 15% reduction of predicted CrCl was considered appropriate based on previous study estimations[8-10]. Furthermore, it was noted that certain patients had predictably low creatinine excretion for age and body weight. Examples included paraplegics and patients with marked obesity or ascites. To correct for these patients, although no data were presented to support this decision, the authors suggested using ideal body weight (IBW).

Finally, due to the delay in SCr fluctuations and the time needed to establish a new steady state, the authors acknowledged that CrCl can be significantly overestimated in early phases of acute renal failure[5]. This is an extremely important concept for clinicians to grasp. In patients with excellent renal function, the t ½ is on the order of 4 h, and a new steady state could be reached in about 1 d. However, a 75% reduction in GFR would increase the half-life to about 15 h, and the time to steady state would increase to about 2 ½ d[11]. In the

Table 1 Different methods of estimating creatinine-clearance (A): Equations I-IV were evaluated in the original Cockcroft-Gault study, Equation V is a modified Cockcroft-Gault that only incorporates age and serum creatinine into the equation; B: Different body weight equations tested in the Cockcroft-Gault equation to compensate for various body types

A: Formula = CrCl (mL/min)		B: Formula = weight (kg)	
I [1]	$\dfrac{[(140 - age)(weight\ in\ kg)]}{(72 \times SCr)}$	IBW$_{male}$	50 + (2.3 kg × inches > 60)
II	(100/SCr) - 12	IBW$_{female}$	45.5 + (2.3 kg × inches > 60)
III	$\dfrac{98 - 16 \times [(age - 20)/20]}{SCr}$	AdjBW	IBW + [(TBW - IBW) × C^2]
IV	(94.3/SCr) - 1.8	LBW$_{male}$	$\dfrac{9270 \times TBW}{6680 + (216 \times BMI)}$
V	$\dfrac{(140 - age)}{SCr}$	LBW$_{female}$	9270 × TBW
VI	$\dfrac{100}{SCr}$	FFW	Calculated using BIA[15]

[1]CrCl × (1.73 m^2/BSA) to normalize to body surface area (BSA) of 1.73 m^2; [2]0.3 for 30% ABW and 0.4 for 40% ABW. AdjBW: Adjusted body weight; BMI: Body mass index; CrCl: Creatinine clearance; FFW: Fat free weight; IBW: Ideal body weight; LBW: Lean body weight; SCr: Serum creatinine; TBW: Total body weight; BIA: Bioelectrical impedance analysis.

Figure 1 Impact of various body weights used in estimating creatinine clearance from Winter *et al*[1]. In patients with a BMI $\geq$ 25 kg/m^2, using AdjBW0.4 was the most accurate weight to estimate CrCl when compared to a 24-h urine CrCl. Under Wt: BMI < 18.5 kg/m^2; Normal Wt: BMI 18.5-24.9 kg/m^2; Overweight: BMI 25-29.9 kg/m^2; Obese: BMI 30-39.9 kg/m^2; Morbidly obese: BMI $\geq$ 40 kg/m^2; CrCl: Creatinine clearance; ActBW: Actual body weight; IBW: Ideal body weight; AdjBW: Adjusted body weight; LBW: Lean body weight; BMI: Body mass index.

case of oliguric or anuric renal failure, it may take several days to reach a new steady state for the SCr[11,12].

Despite the above acknowledged limitations of the CG equation, it has become the most popular renal function prediction method used for renal dosing by clinicians[1,13,14]. Attempts to validate CrCl calculated using the CG equation have produced mixed results[6,13]. In their 2010 Guidance for Industry, the Food and Drug Administration (FDA) advocated for the use of the CG equation in drug development because it has been widely used in PK studies[14]. For instance, where CrCl may be inaccurate (muscle wasting, malnutrition, amputation, *etc*.), alternative methods of calculating CrCl are suggested but not required[14].

As clinicians, it is important to understand that attempts to modify the equation to compensate for some of these patient-specific factors often lead to variable results. In the sections that follow, we discuss the rationale and results that these various adjustments yield in predicting actual CrCl.

WEIGHING YOUR OPTIONS

Once again, recall that the CG equation was derived based on the assumption that SCr represents muscle mass as a definite percentage of the patient's body weight, and that both of these values decline in a linear manner as patients age[13]. In obese patients, these assumptions may not be true, as body fat becomes the major contributor to body mass[13]. Given that over 50% of the United States population > 20 years old are overweight or obese, reviewing available literature comparing accuracy of different weights used in the CG equation may help clinicians optimize dose selection[1,15].

The CG equation was derived from a population of

normal weight individuals (mean = 72 kg) using actual body weight; and therefore, its use in obese patients may lead to significant estimation errors[5,16]. Despite 40 years of clinical experience and numerous studies evaluating different weight calculations in obese patients (Table 1B), no uniform consensus appears to exist for estimating CrCl using the CG equation in this patient population[1,15-17].

Winter *et al*[1] studied the impact of various body weights used when calculating CrCl in obese and non-obese patients. They estimated CrCl using the CG equation with actual body weight (actBW) for body mass index (BMI) < 18.5 kg/m^2; IBW and actBW for BMI 18.5-24.9 kg/m^2; and actBW, IBW, adjusted body weight (adjBW0.3), adjBW0.4, and lean body weight (LBW) for all patients with BMI > 25 kg/m^2. The calculated CrCl was compared to a CrCl derived from a measured 24-h urine collection for all 952 patients in the study. ActBW was shown to underestimate CrCl by 0.221 mL/min in underweight patients (BMI < 18.5 kg/m^2); in normal weight patients (BMI: 18.5-24.9 kg/m^2), IBW was shown to be more accurate than actBW (IBW underestimated CrCl by 1.3 mL/min *vs* actBW overestimated by 4.7 mL/min); and in patients with a BMI > 25 kg/m^2, adjBW0.4 was shown to be the most accurate method of predicting CrCl (BMI 25-29.9 kg/m^2 -2.4 mL/min; BMI 30-39.9 kg/m^2 -6.2 mL/min; BMI > 40 kg/m^2 -5.9 mL/min) (Figure 1).

In a similar study, Demirovic *et al*[15] prospectively evaluated the impact different body-size descriptors would have on the accuracy of the CG equation when compared to a timed 24-h urine collection. They estimated the CrCl in only obese patients with a BMI $\geq$ 40 kg/m^2 and used ActBW, IBW, AdjBW0.3, AdjBW0.4, fat free weight (FFW), and LBW in the CG equation. Bioelectric impedance analysis (BIA) was used to estimate the FFW in patients. The calculated CrCl was compared to

Method[1]	Mean estimated CrCl ± SD	Mean bias (mL/min)	± 30% of measured CrCl	± 50% of measured CrCl
Measured CrCl	109.5 ± 44.4			
ActBW	217 ± 113	-107	13%	30%
IBW	85 ± 29	+24	48%	89%
AdjBW$_{0.3}$	129 ± 55	-20	54%	76%
AdjBW$_{0.4}$	142 ± 63	-33	52%	67%
FFW	103 ± 48	+7	61%	83%
LBW	102 ± 43	+8	56%	87%
MDRD4	96.3 ± 29.4	+13.3	51.90%	87%
Salazar-Corcoran	155.2 ± 65.1	-45.7	46.20%	55.60%

Table 2 Estimating creatinine clearance in morbidly obese patients by Demirovic *et al*[15] showed that using fat free weight and lean body weight provided the closest estimate to the control 24-h urine creatinine clearance

[1]All weight variables used in CG equation only. MDRD4[18]: $186 \times SCr^{-1.154} \times age^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if black})$; Salazar-Corcoran[19]: Male: $(137 - age) \times [(0.285 \times Wt) + (12.1 \times height\ meters)^2], 51 \times SCr$; Female: $(146 - age) \times [(0.287 \times Wt) + (9.74 \times height\ meters)^2], 60 \times SCr$; ActBW: Actual body weight; AdjBW$_{0.3}$: 30% adjusted body weight; AdjBW$_{0.4}$: 40% adjusted body weight; CG: Cockcroft-Gault; CrCl: Creatinine clearance; FFW: Fat free weight; IBW: Ideal body weight; LBW: Lean body weight; MDRD: Modification of diet in renal disease study equation; SD: Standard deviation; Wt: Weight.

a CrCl derived from a measured 24-h urine collection for all 54 patients in the study (Table 2). On average, the CG equation using a patient's actBW overestimated the CrCl by 107.4 mL/min; using IBW underestimated CrCl by 24.3 mL/min; using AdjBW$_{0.3}$ and AdjBW$_{0.4}$ both overestimated the CrCl by 19.8 and 32.3 mL/min, respectively; FFW and LBW were found to be the most accurate estimate of the measured CrCl, as the FFW underestimated CrCl by 6.8 mL/min and LBW underestimated by 8.1 mL/min (Table 2)[15,18,19].

A meta-analysis by Wilhelm *et al*[17] analyzed a total of 1197 patients from 13 different trials and compared CrCl calculated with CG using ActBW, IBW, AdjBW$_{0.3}$, AdjBW$_{0.4}$, and no body weight (NBW) with a measured 24-h urine collection. For NBW, the authors assumed the patient weight to be 72 kg, as this was the average weight from the original CG trial[5]. NBW slightly modified the CG equation as it only incorporates age and SCr (Table 1A; Equation V). When using actBW, the mean difference in the CG estimated CrCl was an overestimation of 15.91 mL/min; using IBW underestimated CrCl by 5.15 mL/min; using adjBW$_{0.3}$ slightly underestimated the CrCl by 4.55 mL/min whereas the adjBW$_{0.4}$ considerably underestimated the CrCl by 19.94 mL/min. The most accurate method of estimating CrCl was using the modified CG equation without a variable for body weight (NBW), which underestimated CrCl by 0.43 mL/min.

The studies presented above reiterate the challenges faced by many clinicians when estimating CrCl using the CG equation. The CG equation was not originally studied in obese patients, and therefore, has limited applicability in this population. The study by Winter *et al*[1] showed that in patients with a BMI > 25 kg/m², use of adjBW$_{0.4}$ was the most accurate method of estimating CrCl when

using the CG methods. Unfortunately, Demirovic *et al*[15] did not come to the same conclusion with the results found by Winter *et al*[1] and Demirovic *et al*[15]. They found that FFW and LBW provided the most accurate estimate of CrCl when compared to a measured 24-h urine collection. These findings support what was originally assumed by CG. That is, that SCr can best be used as a surrogate marker for renal function when an accurate assessment of a patient's muscle mass is used to calculate CrCl[5]. Unfortunately, calculating FFW and LBW on a daily basis is not practical in most clinical settings. Finally, Wilhelm's study illustrated that in a large, heterogeneous sample, removing the weight variable from the CG equation actually produced the estimate closest to the measured CrCl[17]. Although body weight remains controversial, utilizing the NBW equation assumes SCr predictable declines with age. Like the original CG equation, the NBW equation may be of limited use in patients with low SCr or falsely low SCr due to muscle mass or underlying disease. Certainly, the evidence presented by the authors of this review reiterate the potential limitations of the CG equation, and why this equation cannot be used as the sole means of estimating renal function in all patients.

SERUM CREATININE - HOW LOW CAN YOU GO?

It is well known that, due to a decrease in muscle mass beyond about age 40, SCr and CrCl decline as a patient ages. The CG equation assumes this decline is linear[5,20]. In patients with a SCr ≤ 0.6 mg/dL, CrCl estimation using the CG equation often overestimate CrCl, and may consequently lead to supratherapeutic dosing of renally excreted drugs[21]. To compensate for this, clinicians often arbitrarily round a SCr ≤ 0.6 mg/dL to a closer-to-normal value (0.8-1 mg/dL)[21]. Although "rounding up" of SCr is a widely used technique by many clinicians, it has not been robustly validated[21].

Dooley *et al*[21] performed a study comparing measured GFR using diethyl triamine penta-acetic acid (DTPA) to an estimated CrCl calculated using the CG equation in patients with low SCr levels (< 0.6 mg/dL), and determined the impact of rounding SCr to 0.6 mg/dL. This retrospective study analyzed 26 patients with an average age of 57 years old. When compared to the measured GFR, the CG equation, using actual SCr overestimated CrCl by 12.9%, whereas the rounded SCr of 0.6 mg/dL underestimated CrCl by 7% (Table 3). Although rounding of SCr to 0.6 mg/dL was more accurate when calculating CrCl in this study, it was noted by the authors that clinicians typically round to either 0.8 or 1 mg/dL which would increase the underestimation when calculating CrCl. Furthermore, in patients with a measured CrCl that was > 100 mL/min, the rounding of SCr to 0.6 mg/dL underestimated CrCl by 18.9% *vs* 0.1% using the actual SCr.

Smythe *et al*[22] performed a prospective study in elderly patients, but chose to round SCr to 1.0 mg/dL

Table 3 Results from Dooley *et al*[21] illustrated that rounding of serum creatinine to 0.6 mg/dL underestimated creatinine clearance by 7%; of note, the majority of clinicians round low serum creatinine values to 0.8 or 1.0 mg/dL

		Mean ± SD (mL/min)	Range (mL/min)	Mean % error	*P* value
DTPA	All	111 ± 46	45-256		
	≤ 100 mL/min	77 ± 14	45-96		
	> 100 mL/min	140 ± 45	103-256		
CG (no rounding)	All	117 ± 38	55-207	12.9	0.352
	≤ 100 mL/min	98 ± 28	55-152	29.2	0.024
	> 100 mL/min	135 ± 38	86-207	-0.1	0.631
CG (rounding SCr to 0.6 mg/dL)	All	97 ± 30	46-172	-7.0	0.029
	≤ 100 mL/min	82 ± 23	46-127	7.9	0.543
	> 100 mL/min	110 ± 29	72-172	-18.9	0.003

CG: Cockcroft-Gault; DTPA: Diethyl triamine penta-acetic acid; SCr: Serum creatinine.

Table 4 Results from Smythe *et al*[22] showed that rounding of serum creatinine to 1.0 in elderly patients was less accurate than using the patients actual serum creatinine

Method	Bias = CrCl~meas~ - CrCl~calc~ (CI)	Precision
CG using IBW without gender adjustment		
Actual SCr	2.3 (-10.3-14.8)	22.5
Rounded SCr	28.8 (19.1-38.4)	17.4
CG using ActBW without gender adjustment		
Actual SCr	-13.6 [-26.8-(-0.43)]	23.6
Rounded SCr	16.3 (4.5-28.1)	21.2
CG using ActBW with gender adjustment		
Actual SCr	-5.2 (-17.2-7.1)	22.1
Rounded SCr	22.6 (11.5-33.7)	19.9

ActBW: Actual body weight; CG: Cockcroft-Gault; CrCl: Creatinine clearance; IBW: Ideal body weight; SCr: Serum creatinine.

when calculating CrCl using the CG. This study included 23 patients (age 69.2 ± 8.1 years old) and compared the calculated CrCl using various body weights with or without rounding of SCr to 1 mg/dL with a 24-h measured CrCl. The results of this study showed that of all three examples of calculating CrCl, using the actual SCr values produced the most accurate estimate of CrCl (Table 4).

The inverse relationship between SCr and CrCl has lead clinicians to further deviate from the studied CG equation in order to broaden the applicability of the equation[22]. Rounding of low SCr values (≤ 0.6 mg/dL) when calculating CrCl is often used by clinicians to prevent overestimation of renal function and over-dosing of renally excreted drugs[21]. The fact that "rounding up" of SCr has not been validated by strong evidence, clinicians who routinely round low SCr in patients may underestimate CrCl and consequently overcompensate for a perceived problem[21,22]. The studies presented in this section confirm the limitations of the CG equation in elderly patients and the use of SCr as a surrogate marker of GFR. Unfortunately, the limitations of SCr based equations extend to additional populations where SCr is falsely low due to underlying disease.

CIRRHOTICS - THE EXCEPTION TO THE RULE

Among the many complications that arise in patients with liver cirrhosis, renal dysfunction has become a well-established predictor associated with poor prognosis and increased mortality[7,23]. The overall survival of patients with cirrhosis who develop hepatorenal syndrome (HRS) is approximately 50% at 1 mo and 20% at 6 mo[24]. Given the frequency at which cirrhotics demonstrate "cryptic" renal impairment and so often go on to develop HRS, it is critical that clinicians appropriately dose all drugs in cirrhotic patients, particularly those that are nephrotoxic[25]. Unfortunately, due to the underlying disease pathophysiology producing a falsely low SCr, SCr based calculations of CrCl are of limited use in cirrhotics[7].

Underlying CKD in cirrhotics results from alterations in hemodynamics, renal autoregulatory mechanisms, and cardiac function (Figure 2)[26,27]. Hemodynamically, because of increased portal vein pressure, compensatory vasodilators such as nitrous oxide (NO) decrease peripheral vascular resistance and dilate the splanchnic circulation[26,27]. Progressive vasodilation in the presence of portal hypertension results in a decrease in effective arterial blood volume and activation of sodium retention mechanisms such as the renin-angiotensin-aldosterone system (RAAS)[26,27]. Unfortunately, these compensatory mechanisms lead to renal vasoconstriction and reduced GFR[26,27].

In addition to the above hemodynamic changes, cirrhotics have falsely low-to-normal levels of SCr, thus further complicating a clinician's assessment of renal function. Creatine is originally produced in the liver before it is transferred to the skeletal muscles to be stored for energy. In the muscles, it is then phosphorylated, converted to creatinine, and then transferred back into the bloodstream[28]. As cirrhosis progresses, creatine production declines and becomes inconsistent[28]. Furthermore, due to malnutrition and low androgen levels, muscle wasting in cirrhotics limits the storage capacity and phosphorylation of creatine, thereby further decreasing the serum concentration of creatinine[28-30]. Finally,
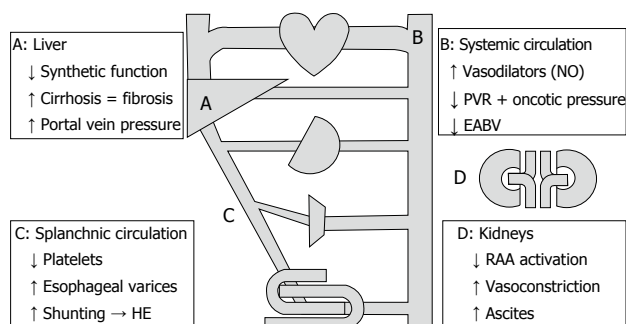
Figure 2 Systemic effects of cirrhosis. Increased portal vein pressure results in vasodilation decreasing peripheral vascular resistance (PVR) and effective arterial blood volume (EABV). To compensate for this, increased renin-angiotensin-aldosterone (RAA) activation leads to sodium and fluid retention along with renal vasoconstriction and reduced glomerular filtration rate. Adopted with permission from Ho *et al*[27]. HE: Hepatic encephalopathy.

detecting early acute kidney injury (AKI) in cirrhotics using SCr is already tenuous, and may require far greater than 24 h given the pharmacokinetic properties of creatinine in patients with reduced GFRs[11].

MacAulay *et al*[31] compared estimates of GFR using three SCr based formulas (CG, MDRD and SCr$_{rec}$) with standard radionuclide measurements (DTPA) of GFR in patients with advanced liver disease. Of the 57 patients in their trial, the mean GFR *via* DTPA was 83 mL/min per 1.73 m^2 (range 28-173 mL/min per 1.73 m^2). On average, estimation using the MDRD was most accurate (mean difference % = +4.0; CI = -5.73-20.39), followed by the CG equation (mean difference % = +18.56; CI = 8.48-22.83), and the SCr$_{rec}$ (mean difference % = +28.68; CI = 14.3-30.28). The authors concluded that using the CG and SCr$_{rec}$ (Table 1A equation VI) equations to estimate GFR in this population can lead to a significant overestimation of GFR.

A similar study by Rognant *et al*[32] compared GFR estimates using the CG and MDRD equations to a measured GFR using inulin. Estimating CrCl using the CG was normalized to 1.73 m^2 body surface area (Table 1A equation I). The 143 patients in this study all had decompensated alcoholic cirrhosis. The mean measured GFR using inulin was 76.9 ± 28 mL/min per 1.73 m^2, and 30.4% of patients had a GFR ≥ 90 mL/min per 1.73 m^2 (group 1), 39.2% had a GFR between 60-89.9 mL/min per 1.73 m^2 (group 2), 26.3% had a GFR < 60 mL/min per 1.73 m^2 (group 3) with 4.1% of these patients having a GFR ≤ 30 mL/min per 1.73 m^2. Mean GFR estimates using the CG and MDRD equations were 98.7 ± 32 mL/min and 99.4 ± 34 mL/min per 1.73 m^2. The mean estimates using the CG and MDRD equations both overestimated the GFR mean by 21.8 mL/min (28.3%) and 22.5 mL/min (29.3%), respectively. For patients in group 1, the mean absolute bias for the CG was 20 ± 25 mL/min and 18 ± 23 mL/min per 1.73 m^2. In group 2, the mean absolute bias for the CG was 25 ± 18 mL/min and 27 ± 19 mL/min per 1.73 m^2 using the MDRD. For those in group 3, the mean absolute bias using the CG was 21 ± 19 mL/min and 19 ± 25 mL/min per 1.73 m^2.

The authors of the study concluded that although the differences between the CG and MDRD estimations were not statistically significant, their findings suggest both equations significantly overestimated renal function in cirrhotics, particularly in those with lower GFRs.

A third study assessing renal function in cirrhotics by Caregaro *et al*[33] was designed to evaluate the sensitivity of SCr and CrCl in detecting renal insufficiency and the magnitude of overestimation of GFR by CrCl. Estimation of CrCl was made using the CG equation and a 24-h urine collection. Estimates of CrCl were compared to measured GFR using inulin (Inulin Clearance = InCl). Patients in this study were divided into 2 groups based on measured GFR; group 1 (*n* = 29) had a GFR > 80 mL/min per 1.73 m^2 and group 2 (*n* = 27) had a GFR ≤ 80 mL/min per 1.73 m^2. For the patients in groups 1 and 2, the mean measured GFR (InCl) was 113.5 ± 27.9 mL/min per 1.73 m^2 and 56.8 ± 19.8 mL/min per 1.73 m^2, respectively. Estimating CrCl using the CG and 24-h urine collection provided an adequate assessment of measured GFR (InCl) in group 1 (CG = 106.3 ± 34.0 mL/min; 24-h = 121.5 ± 28.8 mL/min) but significantly overestimated measured GFR in group 2 (CG = 75.9 ± 40.1 mL/min; 24-h = 78.7 ± 39.2 mL/min) (Figure 3). Only 18.5% of patients in group 2 had a SCr level above normal limits and 81.5% of patients with a GFR ≤ 60 mL/min per 1.73 m^2 had normal SCr levels. Overall, the sensitivity of SCr, CrCl estimated using the CG equation and 24-h urine collection in detecting renal insufficiency was 18.5%, 51% and 74%, respectively.

The authors concluded renal failure in cirrhotic patients is greatly underestimated because of the low sensitivity and accuracy of SCr levels in this population (Figure 3). Based on the data presented in this study, utilization of SCr based methods overestimated true renal function by about 50% in cirrhotic patients with a GFR ≤ 80 mL/min per 1.73 m^2.

CONCLUSION

The importance of accurate and appropriate dosing of all medications remains a critical component of healthcare to maximize efficacy while limiting toxicity. For renally excreted medications, assessment and interpretation of renal function often dictates dosage selection. Due to the impractical nature of a 24-h urine collection, SCr has become a widely accepted surrogate marker of renal function used in several equations including the CG equation. With now over 40 years since its development, the CG equation remains one of the most widely used methods of assessing renal function. Unfortunately, because of its seemingly ubiquitous use and acceptance, many have forgotten its limitations and provider-specific variations used to compensate for these limitations.

Many of the limitations of the CG equation largely stem from the original study supporting its accuracy. The homogenous sample population limits the external validity and creates opportunity for the implementation of empiric correction factors that may or may not be
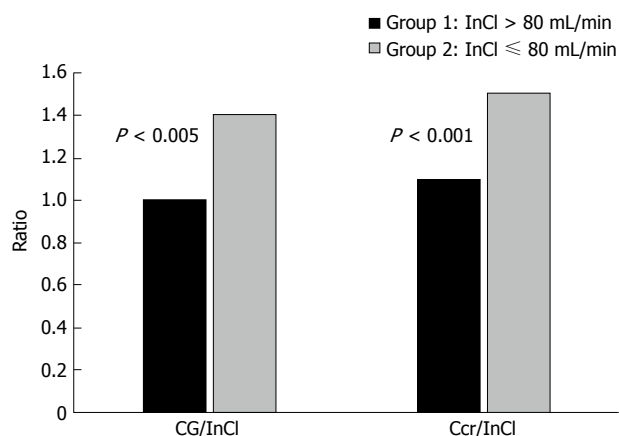
Figure 3 In cirrhotics, as renal function declines, conventional methods of estimating renal function are no longer accurate. Both the Cockcroft-Gault (CG) equation and a 24-h urine creatinine clearance (Ccr) significantly overestimated true renal function as measured by inulin clearance (InCl) in cirrhotics with a baseline InCl ≤ 80 mL/min. The CG equation and Ccr were better estimates in those with a baseline InCl > 80 mL/min[33].

supported by data[5]. As outlined in this review, selection of appropriate weight and rounding of low SCr levels are two examples of techniques used to broaden the applicability of the CG equation. These techniques vary among clinicians, largely because of a lack of evidence unanimously supporting one method over another. Unfortunately, additional limitations in the CG equation extend beyond body composition and habitus to include the subtle manifestations of the underlying disease process in a patient.

In cirrhotic patients, in addition to declining liver function, secondary physiological hemodynamic changes lead to a resultant reduction in GFR. Meanwhile, reductions in creatinine production and reduced muscle mass result in low SCr levels. Because of this, a sort of "cryptic renal failure" picture ensues, whereby SCr-based formulae will overestimate actual GFR by an average of about 50%[28-30]. Based on the evidence presented in this review and in the authors experience, multiplying the SCr by 1.5 in patients with decompensated cirrhosis provides a better CrCl estimate using the CG equation.

Utilization of the CG equation plays a significant role in the dosing decisions of many clinicians. In order to appropriately utilize this equation, clinicians have an inherent responsibility to understand its origins and limitations. True clinicians comprehensively assess each patient and consider SCr and CrCl as two variables that carry equal weight with several other parameters. Regardless of one's approach to dosing medications, sole reliance on CrCl will undoubtedly lead to the ultimate realization that there is indeed fault in the CG.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Advanced non-alcoholic steatohepatitis cirrhosis: A high-risk population for pre-liver transplant portal vein thrombosis

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Abstract

AIM

To examine if liver transplant recipients with high-risk non-alcoholic steatohepatitis (NASH) are at increased risk for pre-transplant portal venous thrombosis.

METHODS

Data on all liver transplants in the United States from February 2002 through September 2014 were analyzed. Recipients were sorted into three distinct groups: High-risk (age > 60, body mass index > 30 kg/m^2, hypertension and diabetes), low-risk and non-NASH cirrhosis. Multivariable logistic regression models were constructed.

RESULTS

Thirty-five thousand and seventy-two candidates underwent liver transplantation and of those organ recipients, 465 were transplanted for high-risk and 2775 for low-risk NASH. Two thousand six hundred and twenty-six (7.5%) recipients had pre-transplant portal vein thrombosis; 66 (14.2%) of the high-risk NASH group had portal vein thrombosis *vs* 328 (11.8%) of the low-risk NASH group. In general, all NASH recipients were less likely to be male or African American and more likely to be obese. In adjusted multivariable regression analyses, high-risk recipients had the greatest risk of

pre-transplant portal vein thrombosis with OR = 2.11 (95%CI: 1.60-2.76, *P* < 0.001) when referenced to the non-NASH group.

CONCLUSION
Liver transplant candidates with high-risk NASH are at the greatest risk for portal vein thrombosis development prior to transplantation. These candidates may benefit from interventions to decrease their likelihood of clot formation and resultant downstream hepatic decompensating events. Prospective study is needed.

Key words: Liver transplantation; Non-alcoholic fatty liver disease; Portal hypertension; Hepatology; Coagulopathy

Core tip: Non-alcoholic steatohepatitis (NASH) is increasing in prevalence and is expected to be the leading indication for liver transplantation in the foreseeable future. There is a growing body of evidence supporting the clinical importance of a thrombophilic state in patients with NASH. In NASH patients, the most severe hypercoagulable environment is found in patients with NASH cirrhosis. High-risk NASH patients (concomitant age > 60 years, obesity, diabetes and hypertension) have inferior post transplantation outcomes, however, how this group's risk of clotting compares to other etiologies of liver disease is unknown. In a retrospective nationwide United States based cohort, we provide further evidence of coagulation derangement in NASH and identify a new high-risk subtype in the high-risk NASH population. Whether or not this high-risk group may benefit from preventative anticoagulation remains unknown.

INTRODUCTION
Non-alcoholic fatty liver disease (NAFLD) is a spectrum of hepatic disorders ranging from simple steatosis with or without mild inflammation to non-alcoholic steatohepatitis (NASH) which is diagnosed by the presence of inflammation, cellular injury with hepatocyte ballooning and accumulation of Mallory-Denk bodies and in the most advanced cases, fibrosis[1]. NAFLD is increasing in prevalence in Western society[2] with rates approaching 20%-30%[3] and more importantly, NASH is projected to become the number one indication for liver transplantation in the foreseeable future[4], especially in light of the new all oral direct acting antiviral treatment regimens for

hepatitis C virus (HCV), and it is the most rapidly growing indication for simultaneous liver-kidney transplantation[5]. High-risk NASH (HR-NASH) is a subtype of NASH defined by the presence of the following: Age > 60 years, body mass index (BMI) > 30 kg/m^2, hypertension and diabetes[6,7]. In general, liver transplant recipients with NASH have similar liver graft and overall one-, three- and five-year survival rates when compared to other etiologies[4-6,8,9]. Outcomes for HR-NASH recipients are less promising with single center experiences showing significantly lower one-[7] and five-year survival rates[6]. While the exact explanation for this remains relatively unexplored, post-transplant cardiovascular events[8], some of which are attributable to macrovascular arterial thrombosis[7,9], and chronic renal dysfunction[10] are more common in NASH patients and these could contribute to lesser outcomes.

Venous thromboembolism (VTE) including portal vein thrombosis (PVT) is a common affliction in patients with cirrhosis[11]. Incidence rates of PVT are reported to be as high as 16%[12] and 30-d mortality is increased in patients with pulmonary embolism (PE) or deep vein thrombosis (DVT)[11]. In a matched retrospective case-control study of 414 patients, Di Minno *et al*[13] found that NAFLD was associated with VTE (PE or DVT) with an OR of 1.8 on adjusted multivariable analysis controlling for additional VTE and NAFLD risk factors. While the presence of PVT may mirror the degree of liver disease burden, it is nonetheless associated with adverse outcomes including increased pre- and post-liver transplant mortality and impaired quality of life, as well as technical challenges during the transplant procedure[14,15]. We have previously shown that liver transplant recipients with NASH are predisposed to pre-transplant PVT[16]. Patients with NASH and metabolic syndrome, which encompasses many of the features of the HR-NASH definition, are known to have increased degrees of fibrosis[17], and presumably increased thrombotic risk. To date, there is a lack of data investigating the relationship between pre-transplant PVT and HR-NASH. We aim to explore this potential association and hypothesize that liver transplant recipients with HR-NASH are at increased risk for PVT when compared directly to other NASH patients and all other etiologies of liver disease.

MATERIALS AND METHODS
Study design and recipient characteristics
Data on all transplants in the United States during the model for end-stage liver disease (MELD era) through September 2014 were reviewed from the Organ Procurement and Transplantation Network (OPTN) with permission from the United Network for Organ Sharing (UNOS). Status 1a, multi-organ, living donor, re-transplants, pediatric recipients, donation after cardiac death, recipients with pre-transplantation transjugular intrahepatic portosystemic shunts and malignancy (hepatocellular carcinoma, hepatoblastoma, cholangiocarcinoma) were excluded. Recipients with cryptogenic

cirrhosis were also excluded due to the potential for misclassification of NASH. The conclusions of the model were not significantly changed with the exclusion of the cryptogenic recipients. Recipients were sorted into two distinct groups: Those with NASH and those without NASH (all other etiologies except cryptogenic cirrhosis, which was excluded due to the potential for misclassification of NASH). The NASH group was then subdivided into HR and low-risk (LR) subgroups. HR-NASH was based on the standard definition used in previous large-scale single center experiences and was defined as the presence of all of the following: Age > 60 years, BMI > 30 kg/m^2, and pre-transplantation hypertension and diabetes[6,7]. Recipient characteristics (age at listing and at transplantation, ethnicity, gender, BMI, diabetes), severity of liver disease based on native laboratory MELD score at allocation, laboratory values [international normalized ratio (INR), bilirubin, creatinine, albumin], and clinically relevant manifestations of portal hypertension (ascites and hepatic encephalopathy) were reviewed in each of the three groups to compare baseline covariates.

Outcomes analysis

Separate analyses were performed comparing recipients with NASH to non-NASH controls and comparing HR-NASH to LR-NASH. In the UNOS data set, PVT is categorized as "Present", "Not present", or "Unknown" and the data are based upon direct surgical evaluation of the veins at the time of hepatectomy. The degree of clot burden is not specified in the dataset nor is the chronicity. Based on previously validated methodology and due to the potential for misclassification bias[16], 831 recipients with "Unknown" PVT status were excluded. In general, univariate comparisons of the excluded cases to the included cohort with known PVT status did not reveal any baseline differences with the exception that the included cohort had a greater percentage of patients with cholestatic liver disease (10.2% *vs* 5.5%, *P* < 0.001). It was felt by the study team that this was not a significant clinical factor in the analysis. The dataset also does not contain information on treatment of PVT or testing for thrombophilia.

Statistical analysis

Recipients were statistically evaluated in multiple factors including demographics, medical comorbidities, waiting list and transplantation characteristics. Univariate comparisons were performed using the Student-*t* test, Wilcoxon sign rank test, χ^2 test, or Fisher exact test as appropriate. Multivariable models were constructed using logistic regression and analysis of maximum likelihood estimates to test the primary hypothesis that patients with HR-NASH are at increased risk for the development of PVT and to assess statistical associations and risk factors for the development of PVT. Individual covariates were included in the multivariable model if they were statistically significant to *P* < 0.20 in univariate analysis, have been shown in the literature to be important or

were deemed to be clinically important by the study team[18,19]. In separate models, individual components of the HR-NASH definition (hypertension, age, BMI and diabetes) were entered into the model as individual variables to ensure one of these did not dominate. Final variables included in the regression model included HR-NASH, LR-NASH, individual laboratory values at transplant (creatinine, bilirubin, INR, albumin, sodium), HCV, cholestatic liver disease, male gender, African American race, Hispanic race, encephalopathy (which was dichotomized into those with severe encephalopathy with score > 2), ascites (similarly dichotomized), pre-transplant dialysis treatment and autoimmune liver disease. No data imputation was performed. All statistical tests for significance were two sided and a significance level *P* less than or equal to 0.05 was considered statistically significant. All data set manipulation and statistical analyses were performed using SAS (version 9.4, Cary, NC). No transplants involving prisoners were included in this analysis. Institutional review board approval was not required for this study as the UNOS/OPTN dataset is de-identified.

RESULTS

Thirty-five thousand and seventy-two candidates underwent liver transplantation and of those organ recipients, 3240 (9.2%) were transplanted for NASH of which 465 met criteria for HR-NASH (1.3%) and 2775 for LR-NASH (7.9%). Two thousand six hundred and twenty-six (7.5%) recipients had pre-transplant PVT, of which 394 (12.2%) were in the NASH group (Figure 1). The prevalence of PVT was not significantly different between HR-NASH and LR-NASH (*n* = 66, 14.2% *vs n* = 328, 11.8%, *P* = 0.145). In general, NASH recipients were older, more likely to be female, less likely to be African American or Hispanic, had higher BMI values and were more likely to have diabetes, hypertension and renal dysfunction (Table 1). Severity of liver disease, while statistically significantly different, was not deemed to be clinically significantly different (*e.g.,* MELD at listing of 20.0, 95%CI: 19.8-20.3 for NASH *vs* 19.6, 95%CI: 19.5-19.7 for non-NASH). The leading indication for transplantation in the non-NASH group was chronic HCV (46.6%) while alcoholic liver disease was the second leading indication (19.0%).

When comparing HR-NASH recipients to LR-NASH recipients, several differences were noted (Table 2). As expected by definition, HR-NASH recipients were older both at listing (64.0 years, 95%CI: 63.8-64.3 *vs* 56.7 years, 95%CI: 56.2-56.9, *P* < 0.001) and at transplantation (64.5 years, 95%CI: 64.2-64.7 *vs* 57.0 years, 95%CI: 56.8-57.4, *P* < 0.001). BMI values were greater for HR-NASH (35.1 kg/m^2, 95%CI: 34.7-35.5 *vs* 31.8 kg/m^2, 95%CI: 31.5-32.0, *P* < 0.001) as was renal dysfunction (mean creatinine 1.98 g/dL, 95%CI: 1.85-2.11 *vs* 1.78 g/dL, 95%CI: 1.73-1.85, *P* = 0.003).

Severity of liver disease based on MELD scores and portal hypertensive manifestations of ascites and

Table 1 Baseline characteristics comparing non-alcoholic steatohepatitis recipients to all other etiologies of liver disease *n* (%)

	NASH (*n* = 3240)	Other etiologies (*n* = 31832)	*P* value
Recipient characteristics			
Age at listing, mean years (95%CI)	57.6 (57.3-57.9)	52.2 (52.1-52.3)	< 0.001
Age at transplant, mean years (95%CI)	58.1 (57.8-58.4)	52.7 (52.6-52.8)	< 0.001
Male gender	1747 (52.9)	22099 (67.3)	< 0.001
African American race	65 (2.0)	3.559 (10.9)	< 0.001
Hispanic race	348 (10.5)	4009 (12.2)	0.005
BMI at transplant, kg/m^2, mean (95%CI)	32.3 (32.0-32.5)	27.8 (27.7-27.9)	< 0.001
Hypertension requiring medical treatment	160 (33.2)	2155 (19.2)	< 0.001
Diabetes	1698 (51.4)	6211 (18.9)	< 0.001
Portal vein thrombosis	394 (12.2)	2232 (7.1)	< 0.001
Etiology of liver disease			
Alcohol alone		6236 (19.0)	
Autoimmune disease		1.202 (3.7)	
Cholestatic disease		3638 (11.1)	
Hepatitis B		986 (3.0)	
Hepatitis C		15298 (46.6)	
Other		4472 (14.0)	
Severity of liver disease			
MELD score at listing, mean (95%CI)	20.0 (19.8-20.3)	19.6 (19.5-19.7)	0.014
MELD score at transplantation, mean (95%CI)	23.5 (23.2-23.8)	22.8 (22.7-22.9)	< 0.001
Laboratory values			
Serum bilirubin, mg/dL, mean (95%CI)	7.4 (7.1-7.8)	9.1 (9.0-9.2)	< 0.001
INR, mean (95%CI)	1.92 (1.90-1.95)	1.93 (1.92-1.94)	NS
Serum albumin, g/dL, mean (95%CI)	3.0 (3.0-3.1)	3.0 (2.9-3.0)	0.002
Creatinine, g/dL, mean (95%CI)	1.81 (1.76-1.86)	1.65 (1.63-1.67)	< 0.001
On dialysis at transplantation	488 (10.6)	4135 (12.2)	< 0.001
Portal hypertension manifestations			
Moderate-severe ascites at transplant	1210 (36.7)	10782 (32.9)	< 0.001
Moderate-severe hepatic encephalopathy at transplant	375 (11.7)	3708 (11.3)	NS

In general, NASH recipients were older, more likely to be female, less likely to be African American or Hispanic, had higher BMI values and were more likely to have diabetes, hypertension and renal dysfunction. BMI: Body mass index; NASH: Non-alcoholic steatohepatitis; NS: Not significant; INR: International normalized ratio; MELD: Model for end-stage liver disease.
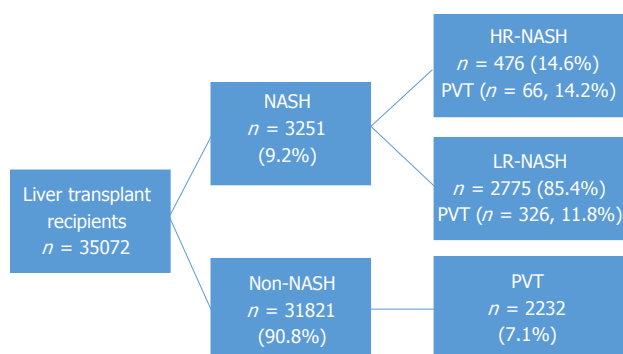
Figure 1 Study enrollment. HR: High-risk; LR: Low-risk; NASH: Non-alcoholic steatohepatitis; PVT: Portal vein thrombosis.

encephalopathy were similar between the two groups. Interestingly, non-NASH recipients with HR features were not at increased odds of PVT (*P* = 0.11), albeit only 58 patients met criteria for this subgroup.

In adjusted multivariable analysis (Table 3), recipients with HR-NASH had the greatest risk of pre-transplant PVT with OR = 2.11 (95%CI: 1.60-2.76, *P* < 0.001) when referenced to the non-NASH cohort and 30% increased odds when compared to LR-NASH recipients (OR = 1.71, 95%CI: 1.49-1.96, *P* < 0.001). Other significant associations with pre-transplant PVT included male gender (OR = 1.18, 95%CI: 1.07-1.29, *P* < 0.001), Hispanic race (OR = 1.24, 95%CI: 1.10-1.39, *P* < 0.001) moderate-to-severe ascites (OR = 1.14, 95%CI: 1.04-1.25, *P* = 0.007) and autoimmune liver disease (OR = 1.43, 95%CI: 1.14-1.79, *P* = 0.002). African Americans were less likely to have pre-transplant PVT with OR = 0.75 (95%CI: 0.64-0.89, *P* < 0.001), similar to our previous findings[16].

While MELD was significantly different on univariate analysis, the individual factors (bilirubin, INR, creatinine) involved in the MELD regression equation were not clinically important statistically significant predictors in adjusted multivariable analysis nor was pre-transplantation dialysis.

DISCUSSION

Based on a large national liver transplant database and building on our previous work in transplant recipients with NASH[16], we have shown an independent cross-sectional association documenting an increased risk of pre-transplant PVT in recipients undergoing transplantation for HR-NASH. This association was significant despite adjustment for multiple established risk factors for pre-transplant PVT. Given the technical difficulties associated with pre-transplant PVT, careful recipient selection is paramount in preventing post-transplant vascular complications.

Table 2 Baseline characteristics comparing high-risk non-alcoholic steatohepatitis to low-risk non-alcoholic steatohepatitis recipients

	High-risk NASH (*n* = 465)	Low-risk NASH (*n* = 2775)	*P* value
Recipient characteristics			
Age at listing, mean years (95%CI)	64.0 (63.8-64.3)	56.7 (56.2-56.9)	< 0.001
Age at transplant, mean years (95%CI)	64.5 (64.2-64.7)	57.0 (56.8-57.4)	< 0.001
Male gender	247 (52.4)	1500 (53.0)	NS
African American race	7 (0.2)	58 (1.6)	NS
Hispanic race	43 (9.1)	305 (10.8)	NS
BMI at transplant, kg/m², mean (95%CI)	35.1 (34.7-35.5)	31.8 (31.5-32.0)	< 0.001
Portal vein thrombosis	66 (14.2)	326 (11.8)	NS
Severity of liver disease			
MELD score at listing, mean (95%CI)	19.5 (18.7-20.3)	20.1 (19.8-20.4)	NS
MELD score at transplantation, mean (95%CI)	22.8 (21.9-23.6)	23.7 (23.3-24.0)	NS
Laboratory values			
Serum bilirubin, mg/dL, mean (95%CI)	6.2 (5.4-7.0)	7.7 (7.3-8.0)	0.002
INR, mean (95%CI)	1.81 (1.75-1.86)	1.94 (1.91-1.97)	0.002
Serum albumin, g/dL, mean (95%CI)	3.1 (3.0-3.2)	3.0 (2.9-3.1)	0.006
Creatinine, g/dL, mean (95%CI)	1.98 (1.85-2.11)	1.78 (1.73-1.83)	0.003
On dialysis at transplantation	73 (15.5)	415 (14.7)	NS
Portal hypertension manifestations			
Moderate-severe ascites at transplant	178 (37.8)	1032 (36.5)	NS
Moderate-severe hepatic encephalopathy at transplant	51 (10.8)	324 (11.5)	NS

HR-NASH recipients were older, had higher BMI values and were more likely to have renal dysfunction. BMI: Body mass index; HR: High-risk; NASH: Non-alcoholic steatohepatitis; NS: Not significant; INR: International normalized ratio.

Table 3 Adjusted multivariable analysis for predictors of portal vein thrombosis at the time of liver transplantation

	Odds ratio	95%CI	*P* value
African American race	0.75	0.64-0.89	< 0.001
AIH	1.43	1.14-1.79	0.002
Hispanic race	1.24	1.10-1.39	< 0.001
HR-NASH	2.11	1.60-2.76	< 0.001
LR-NASH	1.71	1.49-1.96	< 0.001
Male gender	1.18	1.07-1.29	< 0.001
Moderate-severe ascites	1.14	1.04-1.25	0.007

Recipients with HR-NASH had the greatest risk of pre-transplant PVT when referenced both to the non-NASH cohort and to LR-NASH recipients. Variables that were not significant in the model: Albumin levels, bilirubin levels, cholestatic liver disease, creatinine levels, hepatitis C, international normalized ratio (INR); moderate-severe encephalopathy, pre-transplant dialysis treatment, sodium levels. AIH: Autoimmune hepatitis; HR: High-risk; LR: Low-risk; NASH: Non-alcoholic steatohepatitis.

Patients with NASH and in particular NASH cirrhosis, have *in vivo* abnormalities in primary, secondary and tertiary hemostasis[20,21]. The role of platelet dysfunction *via* increased activation, adherence and aggregation is the best described abnormality in primary hemostasis[22-24], however multiple investigators have shown elevations in vonWillebrand factor as a surrogate for endothelial dysfunction as well[21,25]. Secondary hemostasis is impaired in NASH due to elevations in fibrinogen, factor Ⅷ, Ⅸ, Ⅺ, Ⅻ, increased clotting activity of factor Ⅶ, and low levels of antithrombin Ⅲ[20,21,25-27]. Protein C levels may be increased or decreased in patients with NASH[20]. Tertiary hemostasis is disrupted due to elevations in plasminogen activator inhibitor-1 and low levels in both thrombin activatable fibrinolysis inhibitor and tissue plasminogen activator[20,21,27]. The aggregate of these impaired mechanisms of coagulation leads to the hypercoagulable milieu responsible in part for the development of PVT.

Whether or not the presence of pre-transplant PVT plays a role in the decreased survival of HR-NASH[6,7] remains unknown as our study did not investigate patient centered outcomes such as graft and overall patient survival. We did not attempt to do this due to the limitations of the UNOS/OPTN dataset, which does not contain information on the use of anticoagulants and data on post-transplant vascular complications is hindered by a large degree of missing data. The lack of this information introduces significant heterogeneity into the dataset and concrete post-transplant outcomes based conclusions for patients with PVT are problematic. However, what is clear is that the high-risk subgroup of NASH is the most at risk for PVT both in comparison to other NASH recipients not meeting the HR definition and also to all other etiologies of liver disease. We have previously shown that the independent factors of diabetes and obesity do not predispose to PVT on an individual basis[16]. It is only in combination with advanced age > 60 years and hypertension that these factors interact in a way to produce clinically meaningful thrombotic disease, perhaps due to increased physiologic endothelial dysfunction with advancing age[28].

In general, treatment outcomes for patients with PVT are impaired by a lack of large-scale, randomized, placebo-controlled trials that are generalizable. Villa *et al*[29] demonstrated in an un-blinded, single center randomized controlled trial that daily prophylactic dosing of low molecular weight heparin (40 mg daily) for twelve months prevented the development of PVT in patients with compensated cirrhosis at 48 wk, an effect that persisted through the 5-year follow-up period when compared to standard of care[29]. This study also demonstrated significantly less hepatic decompensation in the low

molecular weight heparin arm and a survival benefit in the absence of a single bleeding event[29]. Building on this, Cui *et al*[30] recently published a controlled trial evaluating the efficacy and safety of anticoagulation therapy with different doses of enoxaparin (1 mg/kg twice a day *vs* 1.5 mg/kg daily) for PVT in patients with cirrhosis secondary to chronic hepatitis B in 65 patents, the majority of which had partial thrombosis. Importantly, 79% of patients achieved partial or complete response with anticoagulation based on follow-up imaging, however, non-variceal bleeding was significantly greater in the daily group (23.5% *vs* 6.4%) and the authors concluded that dosing at 1 mg/kg of enoxaparin subcutaneously twice a day was the preferred anticoagulation regimen. While the inclusion criteria were stringent limiting generalizability and the imaging guided definition of PVT open for criticism in these studies, the findings are nonetheless intriguing. A recent meta-analysis by Qi *et al*[31] of 16 studies (the authors did not include either of the aforementioned studies) and 960 patients found a pooled OR of 4.16 (95%CI: 1.88-9.20, *P* < 0.001) for complete portal vein recanalization with anticoagulation. Interestingly, the pooled rate of bleeding was only 3.3% (95%CI: 1.1%-6.7%). A recently published animal model found that 3 mo of dabigatran significantly reduced fibrin deposition, inflammation, hepatocellular injury, steatosis and weight gain[32] through mitigation of thrombin generation, the end-result of the coagulation abnormalities in NASH, suggesting a potential novel therapeutic approach. Newer data regarding the potential use of prothrombin complex concentrates in combination with antithrombin with or without concurrent fibrinogen administration to restore the delicate homeostasis of coagulation and normal thrombin and fibrinogen is emerging[33,34]. However, this combination of therapy has not been broadly studied in patients with chronic liver disease and concrete recommendations about the utility of this treatment cannot be made at this juncture.

In general, prospective, randomized, placebo-controlled studies are sorely needed in all patients with cirrhosis, however, targeting those most at risk including patients with HR-NASH, may provide the most substantial bene-fits including the potential to reduce disease burden from cerebrovascular accidents that this population is at risk for. If the reduction in inflammation and fibrosis with the direct thrombin inhibitors is validated in human subjects, these agents may provide an antifibrotic therapy which could alter the prognosis of liver disease.

Our study has several limitations. Despite containing a large number of transplant recipients in the MELD era, it is a retrospective study. Furthermore, missing data and correct diagnostic coding are potentially problematic with all large datasets. We attempted to control for missing data by excluding the small percentage of patients with unknown PVT status who were demographically similar to our included cohort to ensure bias towards or away from the null was not introduced. Although transplant centers are gaining increased experience transplanting recipients with PVT, it is possible that small volume centers may not have the same surgical technique or experience and pre-transplant PVT may in fact preclude transplantation in a subset of patients that would go on to be excluded from this study. The PVT variable in the dataset also has inherent heterogeneity as there is no differentiating between partial and complete thrombus or the chronicity of the clot. Our analysis also could not account for thrombophilia disorders and therapy in the pre-transplant phase. Additionally, our study excluded HCC patients in the event that PVT was associated with HCC as tumor thrombus, which may limit generalizability.

In conclusion, as the *in vivo* evidence of a throm-bophilic state in patients with NASH continues to grow, epidemiologic evidence continues to lag behind. Building on our previous work, we have shown that liver trans-plant candidates with HR-NASH are at the highest risk for PVT development when compared to other NASH patients and also to all other etiologies of liver disease. Prospective study enrolling HR-NASH patients in anticoagulation trials seems warranted in order to determine a direct benefit in improving patient centered outcomes including the potential for overall and post-transplantation graft survival.

ACKNOWLEDGMENTS

COMMENTS

Background
Non-alcoholic steatohepatitis (NASH) is increasing in prevalence and will soon be the leading indication for liver transplantation in western nations. Patients with NASH are at increased risk for thrombosis. Patients with high-risk NASH have inferior liver transplantation outcomes. Whether or not this high-risk group has an increased risk of portal vein thrombosis (PVT) remains unknown.

Research frontiers
The field of coagulation disorders in chronic liver disease continues to grow. Much of the research focuses on PVT and/or venothromboembolic disease. Identifying high-risk groups for possible preventative intervention through clinical trials remains a goal of the liver and hematology fields alike.

Innovations and breakthroughs
In the present study, the authors investigated the association between high-risk NASH and PVT in liver transplant recipients with cirrhosis. This is the first report of PVT risk in patients with high-risk NASH.

Applications
The present report furthers understanding regarding the thrombophilic state of NASH and highlights a potential high-risk group who may benefit from further prospective study.

Peer-review
This is an excellent very large retrospective review that clearly shows that high risk NASH patients are more thrombophilic than low risk NASH patients and much more thrombophilic than non-NASH cirrhotic patients.

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ORIGINAL ARTICLE

Retrospective Study

Biliary complications following liver transplantation: Single-center experience over three decades and recent risk factors

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Data sharing statement: No additional data are available.

Abstract

AIM
To identify independent risk factors for biliary complications in a center with three decades of experience in liver transplantation.

METHODS
A total of 1607 consecutive liver transplantations were analyzed in a retrospective study. Detailed subset analysis was performed in 417 patients, which have been transplanted since the introduction of Model of End-Stage Liver Disease (MELD)-based liver allocation. Risk factors for the onset of anastomotic biliary complications were identified with multivariable binary logistic

regression analyses. The identified risk factors in regression analyses were compiled into a prognostic model. The applicability was evaluated with receiver operating characteristic curve analyses. Furthermore, Kaplan-Meier analyses with the log rank test were applied where appropriate.

RESULTS

Biliary complications were observed in 227 cases (14.1%). Four hundred and seventeen (26%) transplantations were performed after the introduction of MELD-based donor organ allocation. Since then, 21% ($n = 89$) of the patients suffered from biliary complications, which are further categorized into anastomotic bile leaks [46% ($n = 41$)], anastomotic strictures [25% ($n = 22$)], cholangitis [8% ($n = 7$)] and non-anastomotic strictures [3% ($n = 3$)]. The remaining 18% ($n = 16$) were not further classified. After adjustment for all univariably significant variables, the recipient MELD-score at transplantation ($P = 0.006$; OR = 1.035; 95%CI: 1.010-1.060), the development of hepatic artery thrombosis post-operatively ($P = 0.019$; OR = 3.543; 95%CI: 1.233-10.178), as well as the donor creatinine prior to explantation ($P = 0.010$; OR = 1.003; 95%CI: 1.001-1.006) were revealed as independent risk factors for biliary complications. The compilation of these identified risk factors into a prognostic model was shown to have good prognostic abilities in the investigated cohort with an area under the receiver operating curve of 0.702.

CONCLUSION

The parallel occurrence of high recipient MELD and impaired donor kidney function should be avoided. Risk is especially increased when post-transplant hepatic artery thrombosis occurs.

Key words: Biliary complications; Liver transplantation; Prognostic model; Risk factors; Multivariable analyses

Core tip: This retrospective study investigates the occurrence of biliary complications in a total of 1607 consecutive liver transplant patients throughout three decades. Since introduction of Model of End-Stage Liver Disease (MELD)-based liver allocation, the recipient's MELD-score at transplantation, the development of hepatic artery thrombosis post-operatively, as well as the donor creatinine prior to explantation were identified as independent risk factors, thus a combination of high recipient MELD-score and impaired donor kidney function should be avoided. Risk is especially increased when post-transplant hepatic artery thrombosis occurs. A prognostic model for the prediction of anastomotic biliary complications was developed and successfully internally validated.

INTRODUCTION

Since its introduction as standard procedure in 1983, liver transplantation is nowadays widely accepted as the only live-saving treatment for end stage liver diseases. Nevertheless, several serious complications still endanger successful short- and long-term outcome. Biliary complications appear to be one of the most common issues during follow-up[1]. Diverse studies reveal their notable association with mortality and an overall incidence of 10%-40% is described[2]. Moreover, the socio-economic implications due to prolonged morbidity are of increasing relevance and represent a serious burden to health care systems[3].

As the bile duct is supplied only arterially without the benefit of portal vein nourishment, there is a pre-determined breaking point to cause repercussions[4]. Furthermore, donor parameters, surgical aspects as well as the recipients' condition prior to transplant seem to affect the outcome[2]. In general, anastomotic lesions are distinguished from non-anastomotic stenosis or leakage[5]. The most common manifestations of biliary complications are strictures of the bile duct[5]. Anastomotic lesions are usually due to mechanical and surgical issues which alter the bile duct's arterial support and occur mainly within the first 90 d after transplantation, whereas non-anastomotic lesions show a predominantly manifestation period of about six to nine month after transplant[5]. Under the term of non-anastomotic lesions systemic complications such as post-thrombotic, inflammatory and immunological processes as well as the presence of cytotoxic hydrophobic bile salts are summarized, which all lead to damage of the biliary epithelium[6].

In recent studies, inadequate surgical technique, arterial complications such as hepatic artery thrombosis, as well as donor age and macrovesicular graft steatosis could be identified as relevant risk factors for the occurrence of biliary complications in risk-adjusted multivariate analyses[5,7,8].

This study has two aims. Firstly, a historical overview over three decades of biliary complications after liver transplantation should show the development of their incidence. Secondly, main focus was to identify independent risk factors in the most recent years, which contribute to the development of early biliary complications, occurring during the direct hospital stay after liver transplantation.

MATERIALS AND METHODS

In this single-center, retrospective, observational study, the influence of pre-, inter- and post-transplant aspects as relevant risk factors for the occurrence of early biliary

complications, which occur during the direct hospital stay after liver transplantation, were investigated.

Follow-up period and exclusion criteria

During follow-up patients were routinely seen in the transplant outpatient clinic at least once per year and follow-up visits consisted of physical examination, blood chemistry, as well as standardized abdominal ultrasound. Mean follow-up was 9.4 years (SD: 7.5 years). Included were all consecutive adult liver transplantations (pediatric was defined as younger than 18 years of age). Excluded from analysis were combined transplantations, split liver transplantations, and patients with re-transplantation during initial hospital stay. Since donation after cardiac death is not allowed in Germany by current law, there are no such transplantations included in this study.

Definition of eras in 30 years of liver transplantation

In this study, the three decades of liver transplantation were divided into four eras. Era 1 reaches from 01.01.1983-31.12.1991, Era 2 from 01.01.1992-31.12.1999. Era 3 (Child-Pugh) includes the years 2000-2006 until the Model of End-Stage Liver Disease (MELD) allocation (Era 4) started in 2006.

Study endpoints

Onset of biliary complications during the post-transplant hospital stay is defined as primary study endpoint. In the MELD-era, a more detailed analysis was performed. The further respective study endpoints are complications occurring at the bile duct anastomosis, defined as anastomotic biliary leak or stricture.

Regular operative procedure and clinical diagnosis scheme of biliary complications

For the detection of early biliary complications after liver transplantation, daily ultrasound/Doppler investigations, daily laboratory works, and daily clinical rounds were applied. It is standard operating procedure to implant abdominal drainages during the transplant procedure, which are regularly pulled after the secretion is less than 100 mL post-transplant. The biliary anastomosis was usually performed as end- to end-anastomosis between donor common bile duct and recipient common hepatic duct using a 6/0 prolene suture in continuous manner. University of Wisconsin preservation solution was routinely used until recently, the application of HTK solution increased since its introduction in the early 1990s and is nowadays the mostly applied preservation solution. A more detailed analysis of preservation solutions and their application at the study center is given elsewhere[10]. Due to certain indications for liver transplantation, such as primary biliary diseases, a hepaticojejunostomy in Roux-Y-technique is implemented[11]. The implantation of T-tube was omitted as standard procedure at our center around 2004 and has not been applied since.

Treatment schemes for biliary complications

There are various therapy options for biliary com-

plications. Early biliary complications, such as biliary leaks can be managed *via* endoscopic retrograde cholangiopancreatography with stent implantation, whereas late complications, such as biliary stenosis or a diffuse leakage often require a percutaneous transhepatic biliary drainage, surgical revision with a Y-Roux hepaticojejunostomy or at last resort a re-transplantation.

Ethics statement

The study was reviewed and approved by the institutional review board of Hannover Medical School (application number 1683-2013).

Statistical analysis

Risk factors for the onset of study endpoints after liver transplantation were identified with univariable and multivariable binary logistic regression analyses. The alpha-level for inclusion into multivariate modeling was set at 0.05. All variables which were significant in univariable binary logistic regression analysis were considered for the multivariable binary regression model. Variables which were included in the multivariable regression model were compiled as the prognostic score for the prediction of anastomotic biliary complications. The clinical usefulness of this score was assessed with receiver operating characteristic curve analysis. Areas under the receiver operating curve (AUROCs) larger than 0.700 indicate a clinically useful prognostic model[9]. For internal validation of the developed score, randomized backwards bootstrapping was applied. Kaplan-Meier analysis with the Log-Rank test was applied were appropriate. For all statistical tests a *P*-value < 0.05 was defined as significant. The SPSS statistics software version 21.0 (IBM, Somers, NY, United States) was used to perform statistical analysis.

RESULTS

Descriptive statistics of the investigated study population of 1607 consecutive liver transplants is summarized in Tables 1-3. During 30 years of follow-up, 561 (35.1%) patients deceased. The documented causes of death are summarized in Table 4.

Biliary complications during 30 years of liver transplantation

During 30 years of liver transplantation at a single center, biliary complications were observed in 227 cases (14.1%). The development of biliary complication incidence since 1983 is shown in Figure 1. Patient as well as graft survival were significantly associated to the occurrence of biliary complications during 30 years of follow-up, as shown in Kaplan Meier analysis (Figure 2).

Detailed analysis of biliary complications in the MELD era

Of the 1607 included transplantations, 417 (26%) were performed after introduction of MELD-based donor

Table 1 Clinical characteristics of recipient data

Variable	Mean (SD)	Median (range)	*n* (% of cohort)
MELD	21 (11.5)	18 (6-40)	
BMI	24.2 (4.5)	24.7 (15.1-40)	
Days on the waiting list	277 (41.5)	143 (0-4299)	
Male: Female		915 (57%) to 692 (43%)	
Age	46.1 (12)	47.5 (18-73.6)	
ICU stay in days	23 (33)	9 (1-276)	
Pre-transplant PVT			143 (9)
Creatinine (μmol/L)	121 (92)	86 (38-707)	
Bilirubine (μmol/L)	177 (209)	72 (7-930)	
Indication HCC			244 (15)
Indication PSC			153 (9.5)
Indication ALF			137 (8.5)
Indication HCV cirrh.			101 (6.3)
Indication alc. cirrh.			104 (6.4)
Indication biliary dis.			326 (20.3)

MELD: Model of End-Stage Liver Disease; BMI: Body mass index; ICU: Intensive care unit; PVT: Portal vein thromboses; HCC: Hepatocellular carcinoma; PSC: Primary sclerosing cholangitis; ALF: Acute liver failure; HCV: Hepatitis C virus.

Table 2 Clinical characteristics of transplant specific data

Variable	Mean (SD)	Median (range)	*n* (% of cohort)
Gender mismatch			929 (58)
Cold ischemic time (min)	6.9 (220)	593 (152-1696)	
Era 1 of transplantation			303 (19)
Era 2 of transplantation			434 (27)
Era 3 of transplantation			453 (28)
Era 4 of transplantation			417 (26)
> 1 arterial anastom.			71 (4.5)
Aortal anastomosis			164 (10.8)
Portal vein interpos. Graft			14 (0.8)
Hepaticojejunostomy			353 (22.4)
Post-transplant HAT			59 (3.7)

HAT: Hepatic artery thrombosis.

organ allocation in December 2006. During this MELD-era, 21% of patients (*n* = 89) suffered from early biliary complications during the initial post-transplant hospital stay. The distribution of complication type in the MELD-era is shown in Figure 3. In 46% (*n* = 41) of the patients an anastomotic bile leak occurred, whereas 25% (*n* = 22) showed an anastomotic stricture. Cholangitis occurred in 8% (*n* = 7), non-anastomotic strictures in 3% (*n* = 3) of the cases. The remaining 18% (*n* = 16) were not further classified.

Since the biliary anastomosis can be influenced the most by the operating surgeon, risk factors were evaluated for anastomotic biliary complications, which included biliary strictures and anastomotic leakage. In 63 patients (15.1%) anastomotic complications were observed. Table 5 shows the results of univariable and multivariable binary regression analysis for identification of significant, independent risk factors for the development of anastomotic biliary complications during the initial post-transplant hospital stay. After adjustment for all univariably significant variables, the recipient MELD-score at transplantation (*P* = 0.006;

Table 3 Clinical characteristics of donor data

Variable	Mean (SD)	Median (range)	*n* (% of cohort)
Age	42 (16.9)	43 (15-88)	
Male: Female		969 (60%) to 624 (40%)	
BMI	24.8 (16.6)	24 (20-44)	
Pre-transplant ICU stay	8.7 (19.7)	6 (1-383)	
Body temperature ℃	36.4 (1.0)	36 (33-39)	
Bilirubine (μmol/L)	12.5 (11.7)	9.9 (0.9-154)	
Creatinine (μmol/L)	96 (79)	80 (12-885)	
CRP (mg/L)	127 (108)	114 (0.1-818)	
ALT (u/L)	48 (190)	22 (0-1136)	
AST (u/L)	59 (2.67)	29 (0-1074)	
GGT (u/L)	51.2 (78.6)	24 (0-912)	
Urea (mg/dL)	7.2 (8.4)	5.2 (0.2-103)	
CMV positivity			805 (50)

BMI: Body mass index; ICU: Intensive care unit; ALT: Alanine aminotransferase; AST: Aspartate transaminase; GGT: Gamma glutamyltransferase; CMV: Cytomegalovirus; CRP: C-reactive protein.

Table 4 Causes of death in transplanted patients over 30 years

Cause of death	No. of patients (% of cohort)
Sepsis	109 (6.8)
Tumor recurrence	103 (6.4)
De novo malignancy	33 (2.1)
Pneumonia	31 (1.9)
Liver graft: Biliary complications	20 (1.3)
Cardiovascular event	18 (1.1)
Liver graft: Chronic rejection	15 (0.9)
Cerebral ischemia	12 (0.8)
Cerebral bleeding	11 (0.7)
Liver graft: HCV reinfection	11 (0.7)
Cerebral edema	9 (0.6%)
Liver graft: HBV reinfection	9 (0.6)
Gastrointestinal bleeding	7 (0.4)
Gastrointestinal perforation	6 (0.4)
Liver graft: Venous thrombosis	6 (0.4)
Lung: Acute respiratory distress syndrome	4 (0.3)
Polytrauma	4 (0.3)
Cerebral infection	3 (0.3)
Liver graft: HCV *de novo* infection	3 (0.3)
Liver graft: Initial non function	3 (0.3)
Pulmonary embolism	6 (0.4)
Gastrointestinal ischemia	2 (0.1)
Liver graft: Arterial thrombosis	2 (0.1)
Suicide	2 (0.1)
Liver graft: Portal vein thrombosis	1 (0.1)
Non-compliance to immunosuppression	1 (0.1)
Recurrent alcoholism	1 (0.1)
Unknown	129 (8.1)
Total	561 (35.1)

HCV: Hepatitis C virus; HBV: Hepatitis B virus.

OR = 1.035; 95%CI: 1.010-1.060), the development of HAT post-operatively (*P* = 0.019; OR = 3.543; 95%CI: 1.233-10.178), as well as the donor creatinine prior to explantation (*P* = 0.010; OR = 1.003; 95%CI: 1.001-1.006) were revealed as independent risk factors.

Retrospective prediction of anastomotic biliary complications in the MELD-era

Compiling all variables which were included in multi-
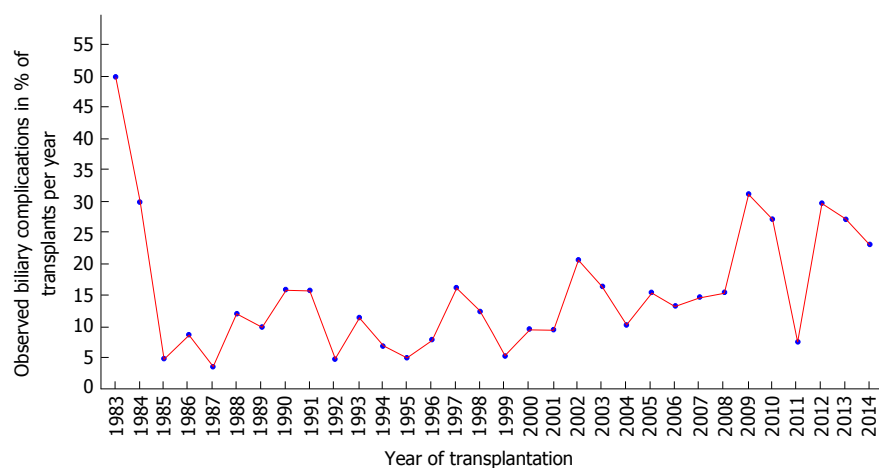
Figure 1 Observed biliary complications as a percent of transplant per year over 30 years.
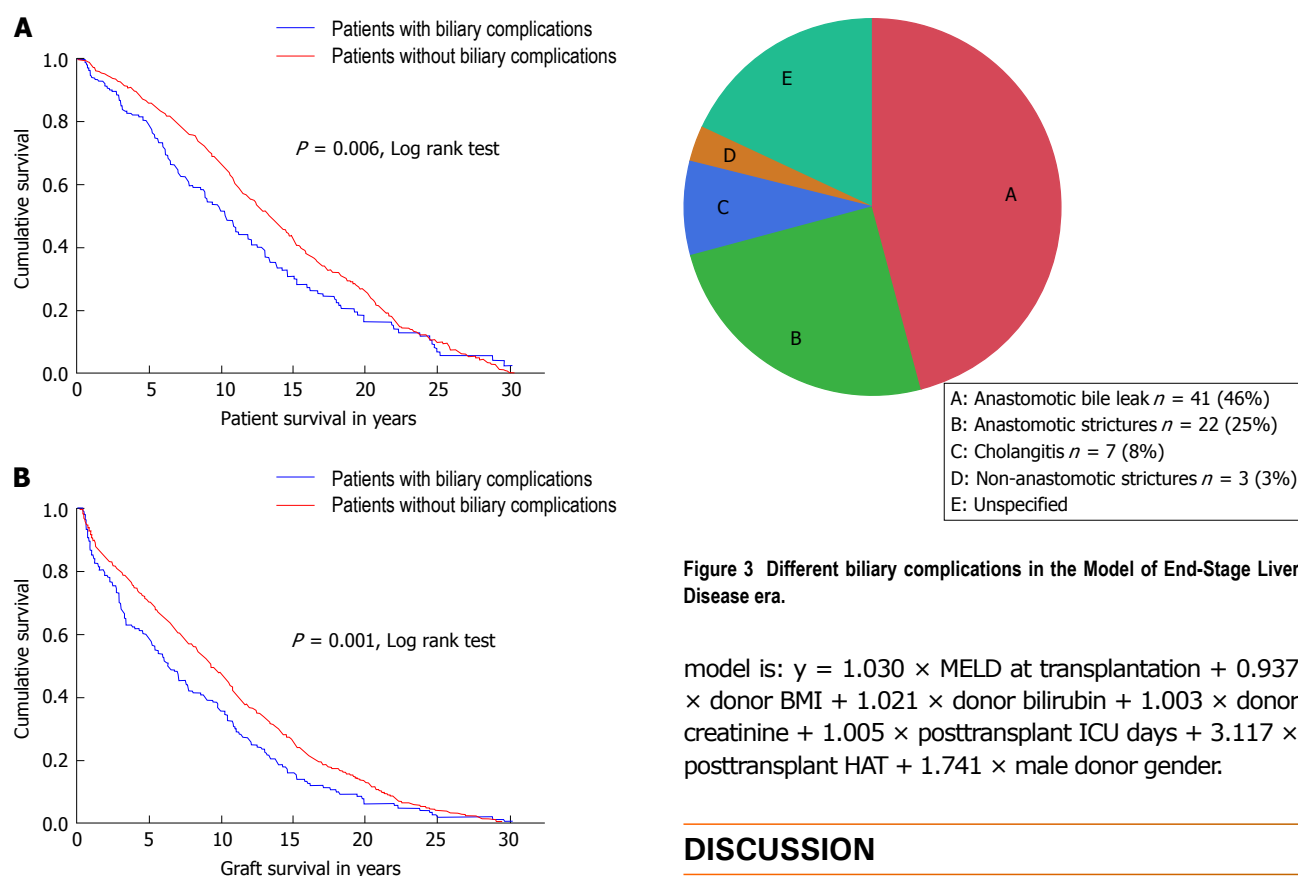
Figure 2 Kaplan Meier curve showing the cumulative survival of (A) patients with and without biliary complications (B) graft survival of both groups.

A: Anastomotic bile leak *n* = 41 (46%)
B: Anastomotic strictures *n* = 22 (25%)
C: Cholangitis *n* = 7 (8%)
D: Non-anastomotic strictures *n* = 3 (3%)
E: Unspecified

Figure 3 Different biliary complications in the Model of End-Stage Liver Disease era.

variable analysis [recipient MELD-score, post-operative HAT, donor creatinine, donor body mass index (BMI), recipient intensive care unit (ICU) stay in days, donor gender, donor bilirubin] in a regression equation, this model provides good prognostic abilities in the investigated cohort with an AUROC of 0.702 (Figure 4). The model was internally validated applying a backwards randomized bootstrap analysis in 100 cases [mean AUROC: 0.720 (SD: 0.040)]. The proposed prognostic model is: y = 1.030 × MELD at transplantation + 0.937 × donor BMI + 1.021 × donor bilirubin + 1.003 × donor creatinine + 1.005 × posttransplant ICU days + 3.117 × posttransplant HAT + 1.741 × male donor gender.

DISCUSSION

Biliary complications are a common post-operative issue after liver transplantation. Liver transplantation has been established in the 1980s as the only life-saving standard treatment for many conditions leading to end-stage liver disease. Therefore, the number of performed liver transplantations has been increasing ever since. Biliary complications endanger early as well as long-term success of liver transplantation and are thus constantly in focus of research to improve care for transplant recipients. However, evidence from large single-center databases on the long-term follow-up of liver transplant recipients including risk-adjusted identification of probable risk factors for the development of biliary complications is still scarce. In the current study,

Table 5 Univariable and multivariable analyses for identification of risk factors for anastomotic biliary complications since 2006

Variable		Univariable analysis		Multivariable analysis	
		P-value	OR (95%CI)	*P*-value	OR (95%CI)
Recipient data	MELD	0.029	1.036 (1.003-1.050)	0.006	1.035 (1.010-1.060)
	BMI	0.438			
	Days on the waiting list	0.594			
	Gender	0.494			
	Age	0.752			
	ICU stay in days	0.025	1.018 (1.001-1.012)	0.093	
	Pre-transplant PVT	0.056			
	Creatinine	0.042	1.003 (1.001-1.005)		
	Bilirubine	0.034	1.001 (1.001-1.002)		
	Indication HCC	0.328			
	Indication PSC	0.415			
	Indication ALF	0.620			
	Indication HCV cirrh.	0.685			
	Indication alc. cirrh.	0.769			
	Indication biliary dis.	0.115			
Transplant-specific data	Gender mismatch	0.620			
	Cold ischemic time	0.417			
	Era of transplantation	0.124			
	Preservation solution	0.746			
	> 1 arterial anastom.	0.396			
	Aortal anastomosis	0.331			
	Portal vein interpos. Graft	0.251			
	Hepaticojejunostomy	0.425			
	Post-transplant HAT	0.048	2.999 (1.010-8.056)	0.019	3.543 (1.283-10.178)
	Operative duration	0.624			
Donor-specific data	Age	0.738			
	Gender male	0.003	1.835 (1.050-3.303)	0.066	
	BMI	0.014	0.923 (0.861-0.990)	0.056	
	Pre-transplant ICU stay	0.115			
	Body temperature	0.921			
	Bilirubine	0.022	1.027 (1.005-1.050)	0.073	
	Creatinine	0.020	1.003 (1.001-1.005)	0.010	1.003 (1.001-1.006)
	CRP	0.406			
	ALT	0.765			
	AST	0.613			
	GGT	0.278			
	Urea	0.581			
	CMV positivity	0.100			

MELD: Model of End-Stage Liver Disease; BMI: Body mass index; ICU: Intensive care unit; ALT: Alanine aminotransferase; AST: Aspartate transaminase; GGT: Gamma glutamyltransferase; CMV: Cytomegalovirus; PVT: Portal vein thromboses; HCC: Hepatocellular carcinoma; PSC: Primary sclerosing cholangitis; ALF: Acute liver failure; HCV: Hepatitis C virus; CRP: C-reactive protein.

a large European center reports its results regarding biliary complications overlooking over three decades of transplant experience. Moreover, in the recent era with the introduction of MELD-based organ allocation in late 2006 as defined starting point, relevant independent risk factors were investigated.

It could be shown that the onset of post-transplant biliary complications endangers patient as well as graft survival even in the long run (Figure 2; *P* = 0.006; *P* = 0.001, resp.). This also has serious implications for healthcare economy. A recent analysis of a large dataset with more than 12800 liver transplantations could show that biliary complications in recipients receiving a graft after brain death donation were responsible for an increment of cost of nearly 55000$ in the first post-transplant year[3]. These findings could be confirmed in the following post-transplant years as well as in donation after cardiac death transplantations.

As shown in Figure 1, the number of patients suffering from biliary complications after liver transplantation decreased very early in the observed series to a minimum of closely over 5% in 1985. This early drop might well be a result of the early surgical learning curve. It can also be assumed that some biliary complications might not be detected in these years due to technically less developed diagnostic capabilities, such as computed tomography or ultrasound. Furthermore, early mortality was comparatively high and recipients might have died before developing detectable/treatable biliary complications.

In the years between 1985 and 2006, the incidence of biliary complications was ranging from 5% to 25% of performed transplantations per year, which is comparable to other reported data[3,5,6]. These years were characterized by introduction of the Child-Pugh score-based center allocation scheme in the year 2000, which represents a paradigm shift and is therefore regarded in
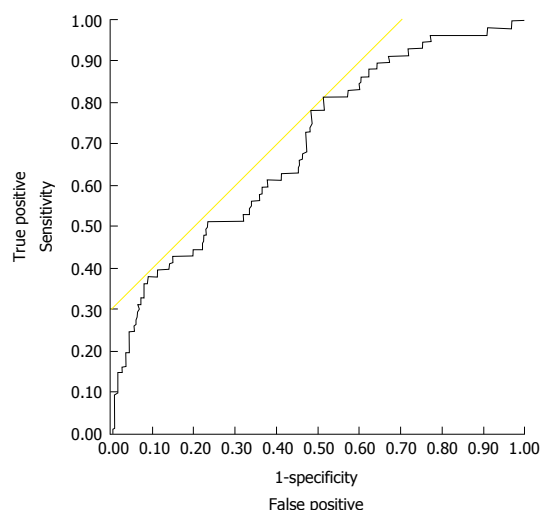
Figure 4 Receiver operating characteristic-curve of the developed prognostic model for the prediction of anastomotic biliary complications in the Model of End-Stage Liver Disease-era (*n* = 417 liver transplants). The area under the receiver operating-curve is 0.702 indicating a useful and clinical applicable prognostic model.

the analysis as a new era. After introduction of MELD-based organ allocation in late 2006, the rate of biliary complications starts to fluctuate in a wider range from 10% to as high as 30%. The notion that donor organ quality might have decreased as well as the general condition of the transplant recipients since the start of this recent era has led to a controversial discussion in the German transplant community about the usefulness of this current allocation policy.

As early as 2009, Weismüller *et al*[12] reported of decreased short-term survival since the introduction of MELD-based liver allocation. An association with longer surgery duration and higher recipient morbidity could be revealed as possible underlying causes. As another example, it was reported recently that indications with high chances for successful long-term survival after liver transplantation such as primary sclerosing cholangitis did not show any improved outcome since MELD-introduction in Germany and that there is relevant outcome stagnation for this entity[13].

Therefore, a more detailed analysis was applied on the MELD-era data, in which a categorization of the biliary complication into different subtypes was possible due to consequent and clear documentation, as well as electronic patient files. Since the biliary anastomosis seems to be the most susceptible part for surgical improvement measures, the anastomotic complications are in a special focus of this investigation. Multivariable, risk-adjusted analysis revealed that the MELD-score at transplantation, the donor creatinine at time of graft donation and the development of HAT after transplantation were statistically significant, independent risk factors for the onset of anastomotic biliary complications. The association of post-transplant HAT and the development of biliary complications has been observed several times and can be explained by an anatomical circumstance. The biliary tract tissue is

especially vulnerable to impaired arterial vascular supply. Whereas the liver parenchyma is nourished *via* a dual vascular supply *via* portal vein and hepatic artery, the bile ducts are supplied only arterially[5]. Therefore, the biliary epithelium is more susceptible to decreased perfusion than hepatocytes, which is the case in ischemic injury and severe hypotension, both occurring in the donor organ during transplantation and after HAT.

The finding that the recipient MELD score has influence on the onset of post-transplant biliary complications seems not to be surprising. The MELD-score was shown to accurately depict the recipients state of morbidity prior to transplantation[14,15], thus identifying patients with a risk profile to have impaired healing capabilities at the bile duct anastomosis. This is further confirmed by the finding that impaired donor kidney function as depicted *via* increased donor creatinine levels contributes to the development of anastomotic bile duct lesions, since this further intensifies the unfavorable metabolic situation at the anastomosis, which is at risk for ischemic injury.

After a relevant drop of the incidence of biliary complications in 2011, the number of observed complications increased again in 2012. The data does not clearly provide insights into the root-causes of this observed development, thus, only assumptions can be discussed here. There was no notable change in allocation policies at that time, MELD-based allocation was introduced in late 2006 in Germany as mentioned above. Furthermore, the clinical setting did not change significantly, just a slight increase in the application of Histidine-Tryptophane-Ketoglutarat (HTK) preservation solution could be detected in 2012. HTK-solution was suspected to be associated to biliary complications previously and a trend towards this association was shown in our center in previously published research[10,16].

The proposed prognostic model, which is basing on the results of regression analyses, was shown to have good predictive capabilities with an AUROC > 0.700. Furthermore, it could be internally validated successfully with 100 randomized backwards bootstraps. These promising results regarding this model warrant its validation in an external dataset, which is definitely necessary before a broad application in clinical transplantation seems useful. This validation is preferably performed in a prospective, multi-centric cohort or a large transplant registry, which contains all relevant data as described above.

This study is limited by its single center design and its retrospective character. Furthermore, the long observation period of three decades naturally includes changes in diagnostics and management of biliary complications. This circumstance was addressed with the categorization of the data into four eras, which was taken into account during statistical analysis.

Taken together, the results of the current study lead to the assumption that high recipient MELD scores in combination with impaired donor kidney function as depicted in donor creatinine levels at the time of transplantation should be avoided to protect the recipient from

the onset of early biliary complications. This is especially the case, when HAT occurs in the post-transplant setting, which further endangers the recipient to develop serious anastomotic bile duct issues.

COMMENTS

Background

Biliary complications account for a great part of early issues after liver transplantation leading to re-intervention, serious morbidity and even mortality. Moreover, they are responsible for a high amount of healthcare costs after transplantation.

Research frontiers

In recent years, studies were outlined and published to identify relevant risk factors for the development of biliary complications. However, long-term follow-up data and large series overlooking decades of liver transplant experience are scarce. There is especially no prognostic model available so far, which helps to identify patients who are threatened by this serious complication.

Applications

The results of this study should reduce the incidence of anastomotic biliary complications after liver transplantation. The proposed prognostic model for the prediction of anastomotic biliary complications can be a tool for the transplant clinician to identify patients at high risk for the development of biliary complications, which then can be included into a stricter diagnostic observation scheme, *e.g.*, with repeating abdominal ultrasound examinations and blood works.

Terminology

Biliary complications do regularly occur after liver transplantation. They can be classified into anastomotic (bile leak *vs* strictures) and non-anastomotic lesions. In many countries, liver grafts are currently allocated on the basis of the Model of End-Stage Liver Disease (MELD)-score, which is a score including the recipient's bilirubine levels, creatinine levels as well as the coagulation state *via* the international normalized ratio-value. The MELD-score is able to reliably predict short-term death on the liver transplant waiting lists in many transplantation systems.

Peer-review

An interesting well-written study.

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CASE REPORT

Primary mucosa-associated lymphoid tissue lymphoma of the liver: A report of two cases and review of the literature

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Abstract

Mucosa-associated lymphoid tissue (MALT) lymphoma of the liver is a very rare condition and thus the diagnosis may be challenging. The clinical presentation is usually variable, ranging from minimal clinical symptoms to severe end stage liver disease. In this paper, we describe the clinicopathologic findings in two cases of primary hepatic MALT lymphoma. One case is an 80-year-old female with no underlying chronic liver disease and the second case is a 30-year-old female with autoimmune hepatitis complicated by MALT lymphoma. In both specimens, there was diffuse infiltration of atypical B-lymphocytes that were positive for CD20 and CD79a, but negative for CD5, CD43 and CD10. There were occasional lymphoepithelial lesions involving the hepatocytes or bile ducts. Polymerase chain reaction analysis showed monoclonal immunoglobulin heavy chain gene rearrangement in both cases. The first case was treated with surgery but developed pulmonary recurrence a year after complete resection but went into remission following treatment with rituximab. A second recurrence occurred in the right parotid gland 7 years later, which was treated with idelalisib. The second case was effectively treated with rituximab. To our knowledge, the second case is the first reported case linked to autoimmune hepatitis.

Key words: Extranodal; Mucosa-associated lymphoid tissue; Lymphoepithelial; Lymphoma; Polymerase chain reaction

Core tip: The diagnosis and management of mucosa-associated lymphoid tissue lymphoma of the liver can be a clinical dilemma. Recognition of the clinic-pathologic

pattern and its associations with underlying autoimmune disease can prevent misdiagnosis. This case report not only represents the first reported association with autoimmune hepatitis and the development of multiple recurrences of the lymphoma in the literature, but also it applies new successful treatment regimens as an alternative to current clinical practice.

INTRODUCTION

Mucosa-associated lymphoid tissue (MALT) lymphoma, is a distinct subgroup of non-Hodgkin's lymphoma (NHL) that accounts for 7%-8% of all B cell lymphomas[1]. MALT lymphomas are considered low-grade and they can occur in a variety of organs, including the stomach, orbit, conjunctiva, salivary gland, skin, thyroid, lung, stomach, intestine, dura and rarely liver. MALT lymphomas usually arise in areas that are devoid of lymphoid tissue, but are preceded by chronic inflammation, either infectious or autoimmune, which result in the accumulation of ex-tranodal lymphoid proliferation[1]. Prolonged lymphoid proliferation can eventually result in the development of a malignant clone. The stomach is the most common site of MALT lymphoma and its association with *H. pylori* is well documented[2-4]. An increased occurrence of MALT lymphomas, especially in the salivary glands has been reported in patients with Sjögren's syndrome[5-7]. Patients with Hashimoto's thyroiditis have a 67- to 80-fold increased risk of developing primary thyroid lymphoma[8-10] and B-cell type NHL is the most common type and features of MALT lymphoma can be seen in over one-third of cases[10]. Case studies on MALT lymphoma of the liver have been rarely reported and very little is known about this disease entity. Here we present two cases with primary MALT lymphoma of the liver, one with no underlying chronic liver disorder and the other is associated with autoimmune hepatitis.

CASE REPORT

Case 1

An 80-year-old Caucasian female, presents with a history of nausea, loss of appetite and a 20-pound weight loss. History was negative for any underlying infectious or autoimmune process. Physical examination was notable for weight loss. Abnormal laboratory results obtained was as follows: WBC 13.5 K/UL, aspartate aminotransferase (AST) 137 U/L and alanine aminotransferase (ALT) 166 U/L, alkaline phosphatase 53 U/L, albumin 2.5 g/dL, bilirubin total 1.1 mg/dL, Bilirubin direct 0.2 mg/dL. Abdominal computed tomography (CT) scan identified

a bi-lobed mass in the left hepatic lobe (Figure 1A). No dilatation of the intra- or extra-hepatic bile ducts was seen. The spleen, pancreas, gallbladder kidneys and adrenal glands were unremarkable. Histological sections of the mass revealed a nodular infiltrate of atypical lymphocytes with small irregular nuclei and abundant clear cytoplasm surrounding occasional reactive germinal centers (Figure 1B and C). Focal fibrosis and plasmacytosis was identified at the periphery of the nodules. There were occasional lymphoepithelial lesions (Figure 1D). Immunohistochemical staining showed that the neoplastic cells were positive for CD20 (Figure 1E), CD79a and BCL-2 and negative for CD10, CD5 (Figure 1F), CD23, BCL-6, CD43, CD3, CD21, CD138 and IgD. Ki-67 was positive in approximately 30% of the cells. Polymerase chain reaction (PCR) analysis by capillary electrophoresis was clonal for immunoglobulin heavy chain (IgH) rearrangement. Bone marrow biopsy showed normocellular marrow with trilineage hematopoiesis and no evidence of lymphoma. These results supported the diagnosis of MALT lymphoma of the liver. On further follow-up, a year later, the patient developed pulmonary nodules which were proven to be MALT lymphoma on biopsy. She went into remission following treatment with rituximab for one year. Seven years later she presented with a right neck mass which was positive for MALT lymphoma of the parotid gland. The lung and parotid MALT lymphoma showed the same IgH rearrangement by PCR, which indicated that they were the same clones.

Case 2

A 30-year-old lady presented to the clinic for mana-gement of a previously diagnosed autoimmune hepatitis. At 10 years of age, she was diagnosed with Hashimoto's thyroiditis and further work up revealed autoimmune hepatitis on a liver biopsy which was managed on azathioprine. In the previous year prior to presentation, she had a flare up of the autoimmune hepatitis when her liver enzymes were found to be in the 500's range and remained abnormal despite having been on me-dication. At the time of presentation, the patient was asymptomatic. The laboratory results obtained were as follows: WBC 1.5 K/UL, platelet 70 K/UL, HB 11.1 g/dL, AST 209 mg/dL, ALT 232U/L, alkaline phosphatase 239 U/L, bilirubin total 1.4 mg/dL, bilirubin direct 0.5 mg/dL. Biochemical investigation for chronic viral hepatitis was negative. Although previously positive, her current report for anti-smooth muscle antibodies was negative. Positron emission tomography/CT abdomen showed an enlarged liver (20 cm) with heterogeneity and diffuse FDG activity (Figure 2A), which was highly suspicious for malignancy. The spleen was slightly enlarged with mild portacaval and left paraaortic adenopathy. Liver biopsy identified atypical lymphoid infiltrate with focal interface activity, fibrosis and lymphoepithelial lesions on histopathological examination (Figure 2B). Immunohistochemistry analysis showed predominantly CD20 positive B-lymphocytes in the infiltrate (Figure 2C) that were negative for CD5, CD10, or CD43. Ki-67 proliferative index was low (30%)
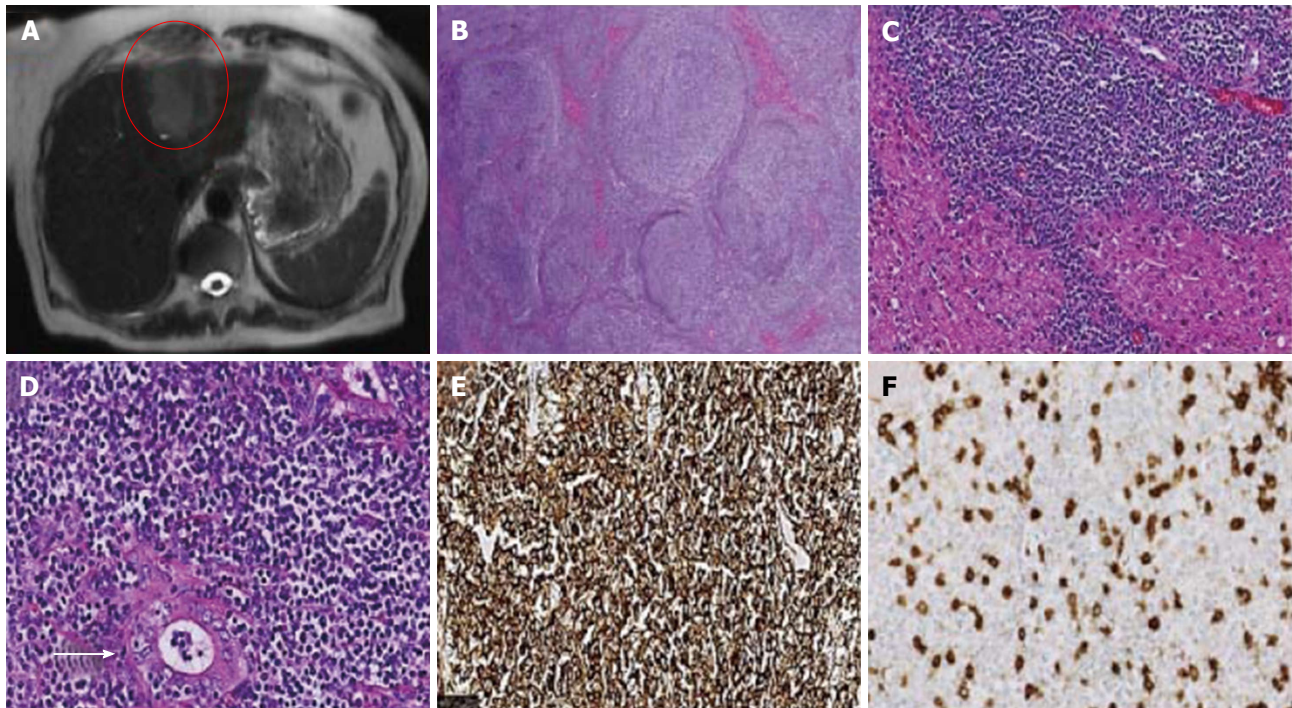
Figure 1 Abdominal computed tomography scan and histology of the mass. A: Computed tomography of the abdomen reveals a large mass, highlighted in red, in the left lobe; B: Effacement of normal liver by nodules of atypical lymphocytes [hematoxylin-eosin (H and E), 2.5 ×]; C: Hepatocytes are admixed with neoplastic lymphocytes (H and E, 20 ×); D: Lymphoma cells infiltrate into the bile duct forming lymphoepithelial lesions (arrows) (H and E, 40 ×); E, F: By immunohistochemistry, the neoplastic cells are positive for CD20 (E) and negative for CD5 (F) (40 ×, each).
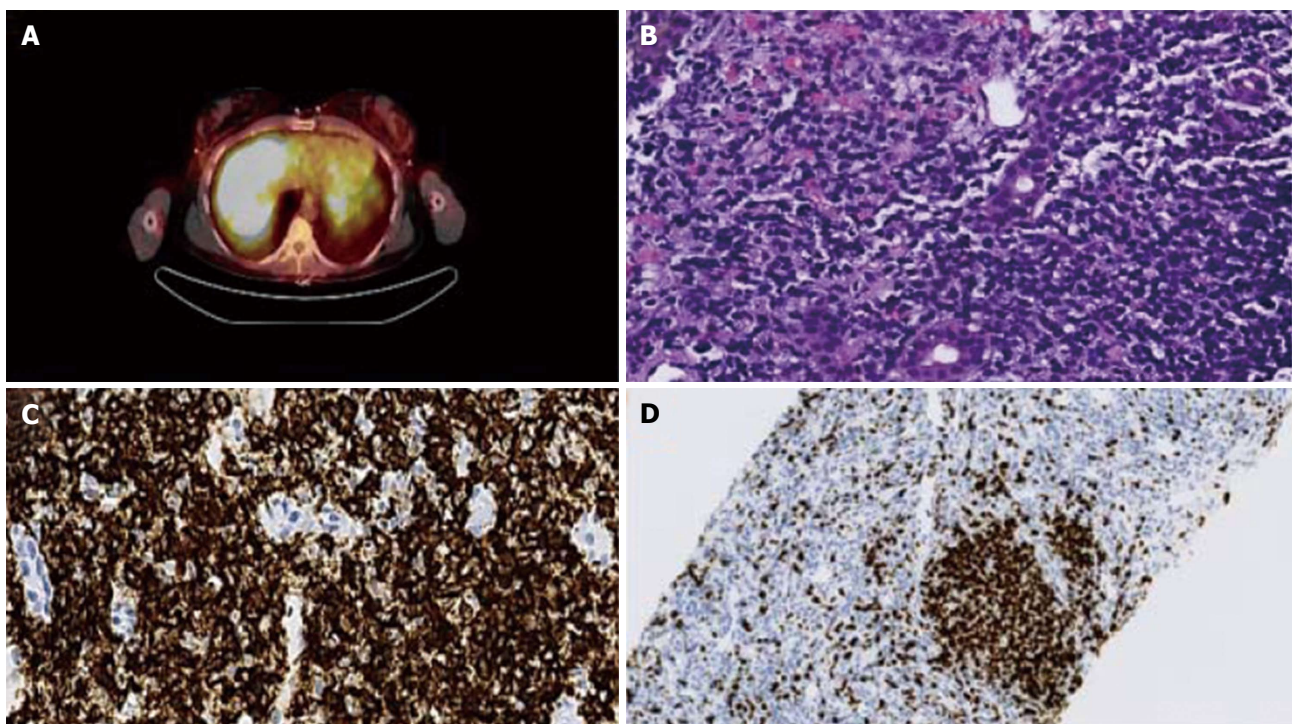
Figure 2 Positron emission tomography-computed tomography and histology of the liver mass. A: Liver shows diffuse fluorodeoxyglucose activity throughout the parenchyma with the maximum standardized uptake value of 8.6; B: The atypical lymphocytes grow diffusely forming mass with occasional lymphoepithelial lesions (40 ×); C, D: By immunohistochemistry, the neoplastic lymphocytes are positive for CD20 (C, 40 ×) and have low MIB-1 proliferative index (D, 40 ×).

except in the reactive germinal center (Figure 2D). PCR analysis of the liver biopsy was positive for the *IgH* gene rearrangement, indicating monoclonal B cell proliferation.

Her bone marrow biopsy showed trilineage maturation with no evidence of lymphoma. Treatment with rituximab was commenced and the patient is still in remission three

years later.

DISCUSSION

The etiology of MALT lymphoma of the liver is still unclear. In most extranodal MALT lymphomas, chronic inflammation due to either an infectious process or autoimmune process has been implicated. Several disease conditions have been associated with the development of hepatic MALT lymphoma which makes initial diagnosis very difficult. Nagata et al[11] reviewed 51 cases of MALT lymphoma of the liver. They reported that 25% was not associated with any disease condition. However, hepatic MALT lymphoma was associated with carcinomas (21%), viral and drug related-hepatitis (20%), biliary cirrhosis (10%), liver cirrhosis (10%), ascariasis (4%), gastric MALT lymphoma (4%), rheumatoid arthritis (2%), multiple biliary unilocular cysts (2%) and no information was reported in 2% of the cases. Majority of the cases presented as a solitary mass and were effectively treated with surgical resection without any adjuvant therapy. Our first case had no underlying history of hepatitis, infection, cancer or autoimmune condition. The only concerning clinical sign was drastic weight loss. The abnormal liver enzymes and liver mass were incidental findings. Clinically an initial diagnosis of hepatocellular carcinoma was made and the patient underwent surgery with complete resection of the lesion, which prevented recurrence in the liver but the lymphoma recurred in the lungs. The most frequent location of recurrence of hepatic MALT lymphoma following treatment appears to be the lungs[11,12] and this occurs at a mean average of 65 mo. Of the 3 reported cases in the literature, 2 patients were treated with resection and the remaining one, with radiation. Hepatic recurrence is rare after complete resection and only one case has been reported[11]. Our patient developed pulmonary recurrence only after one year, but after treatment with rituximab the patient remained in remission for 7 years. However, the lymphoma recurred in the right parotid gland which was treated with 7 mo of idelalisib. The patient is currently in remission a year later. MALT lymphoma generally has an indolent course but recurrences can occur over many years and it tends to involve other common extranodal sites[13]. In our experience, we report for the first time multiple recurrences of MALT lymphoma following complete resection of hepatic MALT lymphoma.

The mean age of patients with MALT lymphoma of the liver is about 60 years of age[11,14], however our second case was 30 years old with an underlying confirmed diagnosis of autoimmune hepatitis which is a disorder frequently seen in young women. Wöhrer *et al*[15] analyzed 158 patients with MALT lymphoma and 39% had an autoimmune disease. The patients were predominantly women and significantly younger at lymphoma diagnosis. The most commonly reported autoimmune disorder associated with liver MALT lymphoma is biliary cirrhosis[14,16,17] and the usual presentation is as a solitary mass. To our knowledge, this is the first reported case associated with autoimmune

hepatitis. Autoimmune hepatitis is a chronic progressive liver disease, characterized by hepatocellular inflammation and liver damage and a tendency to progress to liver cirrhosis. Most patients also have other autoimmune diseases including type 1 diabetes, thyroiditis, vitiligo and Sjögren's syndrome. In support of this, in addition to autoimmune hepatitis, our patient had hashimoto thyroiditis. Similar to MALT lymphoma in other organs, chronic immune activation in autoimmune hepatitis may contribute to lymphomagenesis in the liver. Perhaps the prolonged history and severity of the disease in case 2 explains the diffuse liver involvement with MALT lymphoma. It is important to note that reactive lymphoid hyperplasia, also known as pseudolymphoma, is associated with autoimmune hepatitis[18]. Interestingly pseudolymphoma of the liver can cause a focal liver mass with atypical lymphoid proliferation on histology, which predominantly stain positive for CD20 and reactive germinal center formation[19]. The benign entity can be difficult to differentiate from hepatic MALT lymphoma without further molecular investigation and majority of these lesions are resected due to suspicion for a malignancy. Sato *et al*[20] reported transformation of a pseudolymphoma of the liver, in a background of biliary cirrhosis, into a diffuse B cell NHL. Accordingly, patients with pseudolymphoma will require close follow-up to prevent a misdiagnosis of MALT lymphoma. Indeed, further studies are needed to determine if there is an association between pseudolymphoma and subsequent transformation into low grade lymphomas such as MALT lymphoma.

Rituximab has shown promise as an effective treatment in extra-gastric MALT lymphoma[21,22] but very few cases involving treatment with rituximab have been reported in hepatic MALT lymphoma. Stable remission have been described in patients treated with rituximab following surgical resection[23] or in combination with chemotherapy[24] or radiofrequency ablation[25]. Both of our patients were treated with rituximab and our second case had no previous surgical treatment or chemotherapy. Although high remission rates are achieved by chemotherapy[26], treatment with rituximab can minimize toxicity while maintaining efficacy. Idelalisib, a selective inhibitor of the delta isoform of phosphatidylinositol 3-kinase[27], which plays an important role in B-cell development, proliferation, migration, adhesion and survival[28] has shown efficacy by inducing apoptosis in malignant B-cells in patients with relapsed follicular lymphoma or refractory chronic lymphocytic lymphoma[27,29]. To our knowledge, our study is the first reported case to use idelalisib in the treatment of recurrent MALT lymphoma of the liver and should be considered as a possible effective therapy following treatment failure with rituximab.

Primary hepatic MALT lymphoma is very rare. From our experience, we recommend that young patients with autoimmune hepatitis with sudden elevation of liver enzymes, should raise the suspicion of MALT lymphoma of the liver. Since the liver was diffusely involved, rituximab as the sole agent can be used as an effective treatment of choice. Patients with hepatic MALT lymphoma should

be closely followed up for recurrence especially in common extranodal sites. Rituximab or Idelalisib can be a suitable treatment in patients with relapse. In solitary cases of MALT lymphoma of the liver, complete resection of the lesion with adjuvant rituximab therapy should be considered to prevent recurrence.

COMMENTS

Case characteristics
Mucosa-associated lymphoid tissue (MALT) lymphoma of the liver is a very rare condition, which can be misdiagnosed.

Clinical diagnosis
Hepatic MALT lymphoma is associated with various inflammatory and autoimmune diseases, and one of the authors' patients had autoimmune hepatitis. The disease may also occur in the absence of any underlying disorder.

Differential diagnosis
Hepatocellular carcinoma (HCC), hepatic reactive lymphoid hyperplasia, cholangiocarcinoma, metastatic carcinoma to the liver.

Laboratory diagnosis
The liver enzymes were elevated in both cases of MALT lymphoma of the liver.

Imaging diagnosis
Lesions of hepatic MALT lymphoma may resemble HCC, cholangiocarcinoma or hepatic metastasis on computed tomography or position emission tomography scan, often leading to misdiagnosis.

Pathological diagnosis
MALT lymphoma of the liver often presents as infiltration of the liver with atypical lymphocytes, forming lymphoepithelial lesions on histologic sections and monoclonal immunoglobulin heavy chain gene rearrangement on PCR analysis.

Treatment
Complete surgical excision of lesion or rituximab in localized disease and the use of rituximab or idelalisib in recurrent or advanced disease.

Related reports
Although MALT lymphoma of the liver is considered a low grade lymphoma, patients should be closely followed up for recurrence especially in common extranodal sites. Recurrences or advanced disease can be treated with biological targeted therapy to achieve remission while avoiding the toxic effects of chemotherapy.

Term explanation
MALT lymphoma of the liver is associated with an inflammatory or autoimmune condition such as, primary biliary cirrhosis, hashimoto disease or like in our patient, autoimmune hepatitis. In some cases, there may be no underlying associations.

Experiences and lessons
MALT lymphoma of the liver should be considered when imaging studies show a hepatic lesion with an underlying autoimmune condition or when a solitary liver mass is identified in a patient with no clear risk factors for HCC and cholangiocarcinoma.

Peer-review
Well written and excellent case report on two patients with MALT lymphoma of the liver.

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CASE REPORT

Miliary tuberculosis infection during hepatitis C treatment with sofosbuvir and ledipasvir plus ribavirin

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Abstract

Chronic hepatitis C virus (HCV) infection is one of the main causes of chronic liver disease worldwide. In the last 5 years, treatment for HCV infection has experienced a marked development. In 2014, the use of ledipasvir/sofosbuvir with or without concomitant weight-based ribavirin was approved with a very significant increase in the sustained virological response. However, new side effects have been associated. We report the first case of an HCV infected patient treated for 12 wk with the combination of sofosbuvir/ledipasvir plus ribavirin who developed a miliary tuberculosis (TB) infection while on therapy. The patient was a 65-year-old woman, who referred malaise, asthenia, hyporexia, 7 kg weight loss, productive cough, evening fever and night sweats, right after finishing the treatment. The chest computed tomography-scan revealed a superior mediastinal widening secondary to numerous lymphadenopathies with extensive necrosis and bilateral diffuse lung miliary pattern with little subsequent bilateral pleural effusion, highly suggestive of lymph node tuberculosis with lung miliary spread. A bronchoscopy was performed and bronchial suction showed more than 50 acid-alcohol resistant bacillus per line. A *Mycobacterium tuberculosis* DNA was detected in blood by polymerase chain reaction, which confirmed the diagnosis of miliary tuberculosis. Some cases of TB infection have been identified with α-interferon-based therapy and with the triple therapy of pegylated interferon, ribavirin and boceprevir or telaprevir. However, significant infection has not been reported with sofosbuvir/ledipasvir plus ribavirin.

We believe that the case is relevant to increase awareness of opportunistic infections and particularly TB infection. Although the international guidelines offer no recommendation regarding TB screening, we wonder whether it would be advisable to screen for opportunistic infections prior to the introduction of HCV therapy.

Key words: Tuberculosis; Ledipasvir; Ribavirin; Sofosbuvir; Hepatitis C

Core tip: Cases of tuberculosis (TB) infection have been identified with α-interferon-based therapy and the triple therapy with pegylated-interferon, ribavirin and boceprevir or telaprevir. This is the first case of a TB infection during treatment with sofosbuvir/ledipasvir plus ribavirin. It is relevant to increase awareness of TB due to its variety of symptoms, which can be confused with those associated to the hepatitis C virus or the antiviral treatment. Considering the impaired immune system of cirrhotic patients and that these drugs arrived slightly more than one year ago it is important to be conscious of the potential events that can be related with the treatment.

INTRODUCTION

Affecting more than 160 million people and at an increasingly higher rate, hepatitis C virus (HCV) infection is one of the main causes of chronic liver disease worldwide. In the last 5 years, treatment for HCV infection has experienced a marked development, especially in the case of treatment for genotype 1. Previously, treatment was based on pegylated interferon and ribavirin for 48 wk, which achieved a sustained virological response (SVR) rate of 45% to 50%. In 2011, the first generation of protease inhibitors-telaprevir and boceprevir-reached the market. The triple therapy with peg-interferon, ribavirin and a protease inhibitor was then adopted as the standard of care, followed in 2013 by the approval of the second generation of protease inhibitor, simeprevir, and the polymerase inhibitor, sofosbuvir. In 2014, the use of ledipasvir/sofosbuvir was approved, as well as the use of ombitasvir/paritaprevir/ritonavir plus dasabuvir for the treatment of HCV genotype 1 infections with or without concomitant weight-based ribavirin. These changes have brought a very significant increase in the SVR, with rates rising above 90%. However, new side effects, such as decompensation of cirrhosis, liver toxicity and some

infections, have been associated[1].

We report the first case of an HCV-infected patient treated for 12 wk with the combination of sofosbuvir/ledipasvir plus ribavirin who developed a miliary tuberculosis infection while on therapy.

CASE REPORT

The patient was a 65-year-old woman from Equatorial Guinea, who had been residing in Spain since 2000 (16 years), without any recent trip reported. Her medical history included a blood transfusion event in 1996, diabetes mellitus type 2 diagnosed in 1997 under treatment with insulin, no current nor previous smoking habits and no underlying pulmonary disease or symptoms. In 2000 the patient was admitted to the hospital with abdominal pain. On physical examination hepatomegaly was detected. The blood test revealed hypertransaminasemia and the serology test permitted the diagnosis of chronic HCV infection. HCV genotype was 1a and viral load was 720000 IU/mL. An abdominal ultrasonography revealed a discrete homogeneous hepatomegaly with no focal liver lesions and a transient elastography resulted in 7.9 kPa. A liver biopsy was performed showing chronic HCV infection with Batts and Ludwig[2] stage 1, grade 1. In January, 2008, the patient was treated with the standard at that time, based on the combination of peg-interferon-α 2a (180 μg/wk) with ribavirin (1200 mg/d) during 12 mo. Unfortunately, treatment had to be stopped three mo after starting due to the appearance of side effects: Gluteus abscess, leucopenia and anemia. The patient was followed up in outpatients' clinic without further therapy for hepatitis C until 2015 when she was evaluated for starting treatment with the new direct antiviral agents (DAAs). She was asymptomatic and there were no signs of ascites, edema, bleeding or encephalopathy nor lymphadenopathies on physical examination. Body weight was 54 kg. Hemoglobin level was 12.9 g/dL (reference range -RR-: 11.5-15.5), leucocyte count 5.56 cells/μL (RR: 3.9-11), absolute neutrophil count 1.14 cells/μL (RR: 2.5-7.5), absolute lymphocyte count 2.46 cells/μL (RR: 1.5-4.5) and platelet count 144.000 cells/μL (RR: 160.000-400.000). Albumin was 4 g/dL (RR: 3.5-5.2), total bilirubin 0.43 mg/dL (RR: 0.10-1.00), INR was 1.09 (RR: 0.85-1.35), alanine aminotransferase (ALT) 126 U/L (RR: 1-31), aspartate aminotransferase 120 U/L (RR: 1-31), gamma glutamyl transpeptidase 52 U/L (RR: 1-38) and alkaline phosphatase 90 U/L (RR: 30-120). Alpha-fetoprotein was 9.3 ng/mL (RR: 0.0-7.0) and glycosylated-hemoglobine was 5.2% (RR: 4.0-6.1). Human immunodeficiency virus (HIV) serology was negative. Viral load was 1300000 IU/mL and HCV genotype was 1a and the *IL28B* gene was TC. An abdominal ultrasound exam revealed signs of hypertrophy of the left and caudate liver segments with a focal benign lesion (hemangioma) of 9 mm in segment IV with no changes when compared to previous tests, normal portal vein caliber with hepatopetal flow and a splenomegaly of 13 cm. Transient elastography scored a
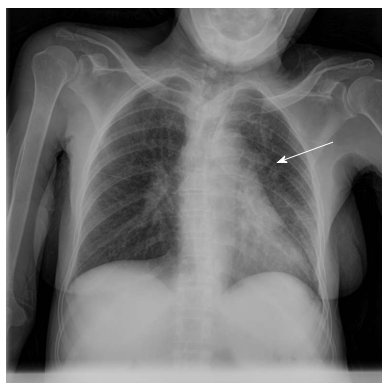
Figure 1 Chest X-ray: Left hilar widening (arrow).

result of 11.1 kPa. Treatment with ledipasvir/sofosbuvir plus ribavirin during 12 wk was initiated in June, 2015. The check-up at the clinic one month later showed no symptoms or pathological signs on physical examination. The treatment was completed but in September, 2015, the patient complained about malaise, asthenia, hyporexia, 7 kg weight loss and dry cough. She referred that these symptoms had appeared one month before she was attended at the emergency unit. However, due to the clinical and hemodynamic stability, the patient was discharged with further outpatient control. In the next 2 wk, in early October, a more productive cough with white sputum, evening fever and night sweats were added to the previous symptoms and upon examination at the emergency unit again, the patient's temperature was 39 ℃, blood pressure was 131/94 mmHg, heart rate was 130 bpm with 98% oxygen saturation. She was conscious with no signs of neurologic impairment. Cardiac and pulmonary auscultation were normal, the abdomen was soft with a 3 cm hepatomegaly and a 2 cm splenomegaly without signs of ascites or abdominal pain, limbs showed no signs of edema and no cervical, axillary or inguinal adenopathies were found. The blood test highlighted a sodium level of 129 mmol/L (RR: 135-145), ALT 38 U/L, CRP 94 mg/L (RR: < 5), hemoglobin level 9.6 g/dL, leucocyte count 5.98 cells/μL, absolute neutrophil count 4.84 cells/μL, absolute lymphocyte count 0.78 cells/μL, platelet count 114000 cells/μL and INR 1.26 with the rest of parameters standing within the normal range. The arterial gasometry showed pH 7.51 (RR: 7.35-7.45), pO2 126 mmHg (RR: 83-108), pCO2 29 mmHg (RR: 35-45), lactate 1.6 mmol/L (RR: 0.6-1.17) and bicarbonate 25 mEq/L (RR: 20-29). The urine test, abdominal X-ray and cranial computed tomography (CT) scan that were carried out revealed no abnormalities. The chest X-ray showed a left hilar widening (Figure 1) and the patient was admitted to the hospital for further studies. The blood culture was negative, as well as the malaria and leishmania tests. The sputum direct vision showed mixed microbiota with predominance of gram-negative bacillus with no acid-alcohol resistant bacillus (AARB) seen. Nevertheless, *Mycobacterium tuberculosis* showed up in the culture of the sputum after 10 d. Chest and abdomen CT scan revealed a superior mediastinal

widening secondary to numerous lymphadenopathies with extensive necrosis, causing a displacement of the esophagus and trachea, and contiguous to these lymphadenopathies, a left hilar mass displaying an air bubble communicated with the left bronchial three (Figure 2), as well as a bilateral diffuse lung miliary pattern with little subsequent bilateral pleural effusion (Figure 3). These findings were highly suggestive of lymph node tuberculosis with lung miliary spread, being less likely to be attributed to a malignant left hilar mass. A flexible bronchoscopy was performed showing a bossing in the upper part of the trachea and mucosa thickening in both main bronchi with partial stenosis of the left upper lobule bronchi. The retrotracheal mass was biopsied, displaying acute inflammation in the pathological study with negative Zielh Neelsen test. However, in the bronchial suction there were more than 50 AARB per line. A *Mycobacterium tuberculosis* DNA was detected in blood by RCP which confirmed the diagnosis of miliary tuberculosis. The first line treatment for tuberculosis with rifampin, isoniazid, pyrazinamide and ethambutol was initiated, presenting remission of the symptoms and a good tolerance with no signs of liver toxicity (Figure 4).

DISCUSSION

Tuberculosis, caused by *Mycobacterium tuberculosis*, is a worldwide health problem, remaining as the leading cause of death from infectious diseases. It is estimated that 30% of the global population hosts TB in its latent form, which can be reactivated with the presence of several factors, such as aging, smoking, alcohol use, diabetes, chronic renal failure, cancer, a weakened immune system, glucocorticoids or tumor necrosis factor-α inhibitors use. Furthermore, miliary tuberculosis form, has only been described in immunocompromised hosts, especially in patients with underlying T-cell deficiencies such as HIV infection. The classic presenting symptoms of pulmonary TB include persistent fever, weight loss, drenching night sweats, persistent cough (often with sputum production), and hemoptysis; whilst extra-pulmonary TB can affect any organ with a wide variety of symptoms, and therefore requires a high index of clinical suspicion. Without treatment, TB has a mortality rate of 50% within 5 years. Various cell types and cytokines are crucial: T cells (CD4^{+}, CD8^{+}, and natural killer) and macrophages participate in protection against TB, and interferon-α and tumor necrosis factor-α (TNF-α) are essential cytokines for the control of acute TB infection[3].

Two conditions might have contributed to the TB infection. On one hand, cirrhosis by itself is associated with lymphocyte and macrophage dysfunction and decreased production of interferon-α and TNF-α and it may be linked to a higher TB risk. A Taiwan study, showed that active TB incidence rates were significantly higher among cirrhotic patients compared with non-cirrhotic patients, particularly those with alcoholism and HCV infection[4].

On the other hand, TB infection has been reported
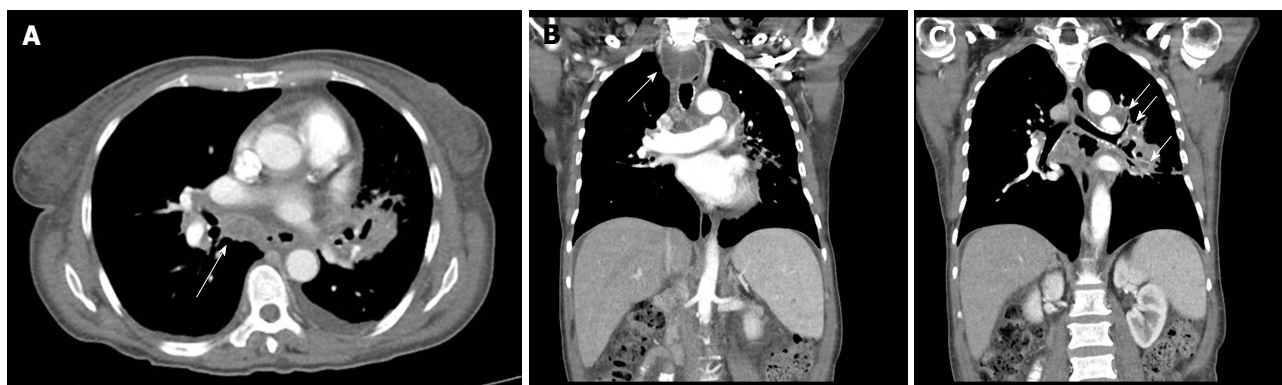
Figure 2 Chest computed tomography scan (scale W500:L50): Soft tissue window. Axial (A) and coronal (B, C) views: Superior mediastinal widening secondary to numerous lymphadenopathies (arrows) and a left hilar mass.

Figure 3 Chest computed tomography scan (scale W500:L50): Pulmonary windows. Axial view: Bilateral lung miliary pattern (arrows) with little bilateral pleural effusion.
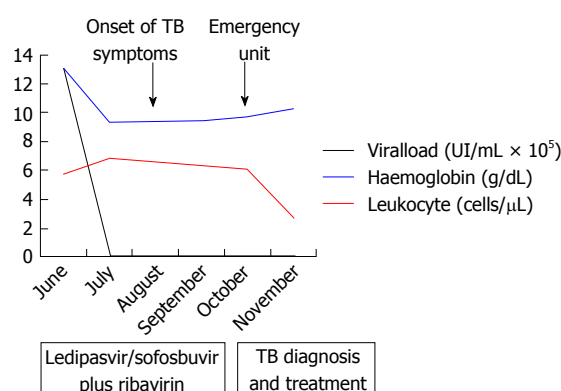
Figure 4 Course of haemoglobin, leucocytes and viral load during and after antiviral treatment. Attendance to the emergency unit, tuberculosis diagnosis and start of therapy are shown according to time. TB: Tuberculosis.

anecdotically in patients with HCV infection undergoing α-interferon-based therapy, usually as a reactivation of latent cases. As an example, 18 cases of TB were observed in patients under HCV treatment in Brazil[5]. Many studies have already shown that α-interferon inhibits type 1 immune response, which is characterized by IL-2, interferon-α and TNF-α production, cytokines that restrain *Mycobacterium tuberculosis*. On the contrary, ribavirin is a guanosine analogue that has demonstrated to have an immunomodulatory effect, shifting a type 2 response to a type 1 in plaque-forming cells *in vivo*. When human T-lymphocytes are activated, ribavirin enhances a type 1 cytokine response producing increased levels of IL-2, interferon-α and TNF-α while suppressing the type 2 response with IL-4, IL-5 and IL-10[6]. Therefore, ribavirin stimulates the host adaptive immune response responsible for protection against TB infection and it may act as a protective factor; however, no studies have been specifically performed to prove it. Some other cases of TB infection have also been reported with the triple therapy of pegylated interferon, ribavirin and boceprevir or telaprevir, the first generation of protease inhibitors[1]. Ledipasvir and sofosbuvir are both DAAs. Sofosbuvir works as an inhibitor of the HCV NS5B RNA-dependent RNA polymerase. Ledipasvir is

an NS5A inhibitor and its exact mechanism of action is unknown, but one suggested mechanism is its inhibition of hyperphosphorylation of NS5A, which seems to be required for viral production. Ledipasvir/Sofosbuvir phase Ⅲ studies for HCV infection treatment showed that the most common adverse events reported by patients were fatigue, headache and nausea[7]. Addition of ribavirin to ledipasvir/sofosbuvir for HCV therapy for patients without cirrhosis or with compensated cirrhosis was associated with a greater incidence of common adverse effects, concomitant medication use and laboratory abnormalities such as anemia, increased levels of total bilirubin and lymphopenia; but rates of severe side effects and interruptions of treatment resulted similar, and no increased infection episodes were detected despite the reported decrease in lymphocytes[8]. In order to reduce the side effects of these drugs, advanced technologies are used during their development such as computer-aided leading drug optimization[9].

Indeed, significant infection has not been reported with the new era of free-interferon regiment treatment. This case is, to the best of our knowledge, the first case report of a miliary TB infection during HCV infection treatment with sofosbuvir/ledipasvir plus ribavirin. Although the association with the DAAs has not been proven and

it may be a coincidence that the two infections have occurred in a close time frame, several data should make clinicians wonder: The patient had diabetes and chronic liver disease as a risk factors for TB, nevertheless both were well controlled; she was asymptomatic and with no signs on physical examination neither before starting the treatment nor after one month of the beginning, which goes against the hypothesis that she may have reactivated TB prior to therapy and it has just presented late; moreover, the mechanisms underlying these drugs effects are currently unknown and further immunological studies should be performed in order to find out how the innate and adaptive immune responses are altered by the different treatment regimens[10].

We believe that the case we have reported is relevant to increase awareness of opportunistic infections, particularly TB infection due to its variety of symptoms which can be confused with those associated to the HCV infection or the antiviral treatment and the high mortality rate of TB infection without treatment. Considering the impaired immune system of cirrhotic patients and the fact that these DAAs arrived on the market slightly more than one year ago and no long-term side effects have been described, we consider that it is important to be conscious of the potential events that can be related with the HCV treatment. In addition, although the international guidelines for the management of HCV infection[11,12] offer no recommendation regarding TB screening, we wonder whether it would be advisable to screen for opportunistic infections, *via* tuberculin skin test and/or interferon gamma releasing assays, prior to the introduction of HCV therapy.

COMMENTS

Case characteristics
A 65-year-old woman with history of diabetes mellitus type 2, chronic hepatitis C virus (HCV) infection and no current nor previous smoking habits and no underlying pulmonary disease or symptoms.

Clinical diagnosis
The patient presented with malaise, asthenia, hyporexia, 7 kg weight loss, productive cough with white sputum, evening fever and night sweats right after finishing the HCV treatment with sofosbuvir/ledipasvir plus ribavirin for 12 wk.

Differential diagnosis
Respiratory infection, side effects of HCV treatment.

Laboratory diagnosis
The blood test highlighted a sodium level of 129 mmol/L (RR: 135-145), ALT 38 U/L, CRP 94 mg/L (RR: < 5), hemoglobin level 9.6 g/dL, leucocyte count 5.98 cells/μL, absolute neutrophil count 4.84 cells/μL, absolute lymphocyte count 0.78 cells/μL, platelet count 114000 cells/μL and INR 1.26 with the rest of parameters standing within the normal range. The arterial gasometry showed pH 7.51 (RR: 7.35-7.45), pO2 126 mmHg (RR: 83-108), pCO2 29 mmHg (RR: 35-45), lactate 1.6 mmol/L (RR: 0.6-1.17) and bicarbonate 25 mEq/L (RR: 20-29).

Imaging diagnosis
The chest X-ray showed a left hilar widening. The chest computed tomography scan revealed a superior mediastinal widening secondary to numerous lymphadenopathies with extensive necrosis, causing a displacement of the esophagus and trachea, and contiguous to these lymphadenopathies, a left hilar mass displaying an air bubble communicated with the left bronchial three, as well as a bilateral diffuse lung miliary pattern with little subsequent bilateral pleural effusion.

Treatment
The first line treatment for tuberculosis with rifampin, isoniazid, pyrazinamide and ethambutol.

Related reports
Cases of tuberculosis infection have been identified with α-interferon-based therapy and the triple therapy with pegylated-interferon, ribavirin and boceprevir or telaprevir.

Experiences and lessons
This is the first case of a TB infection during treatment with sofosbuvir/ledipasvir plus ribavirin.

Peer-review
The manuscript is reasonably well written and is thought to have useful information for readers.

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