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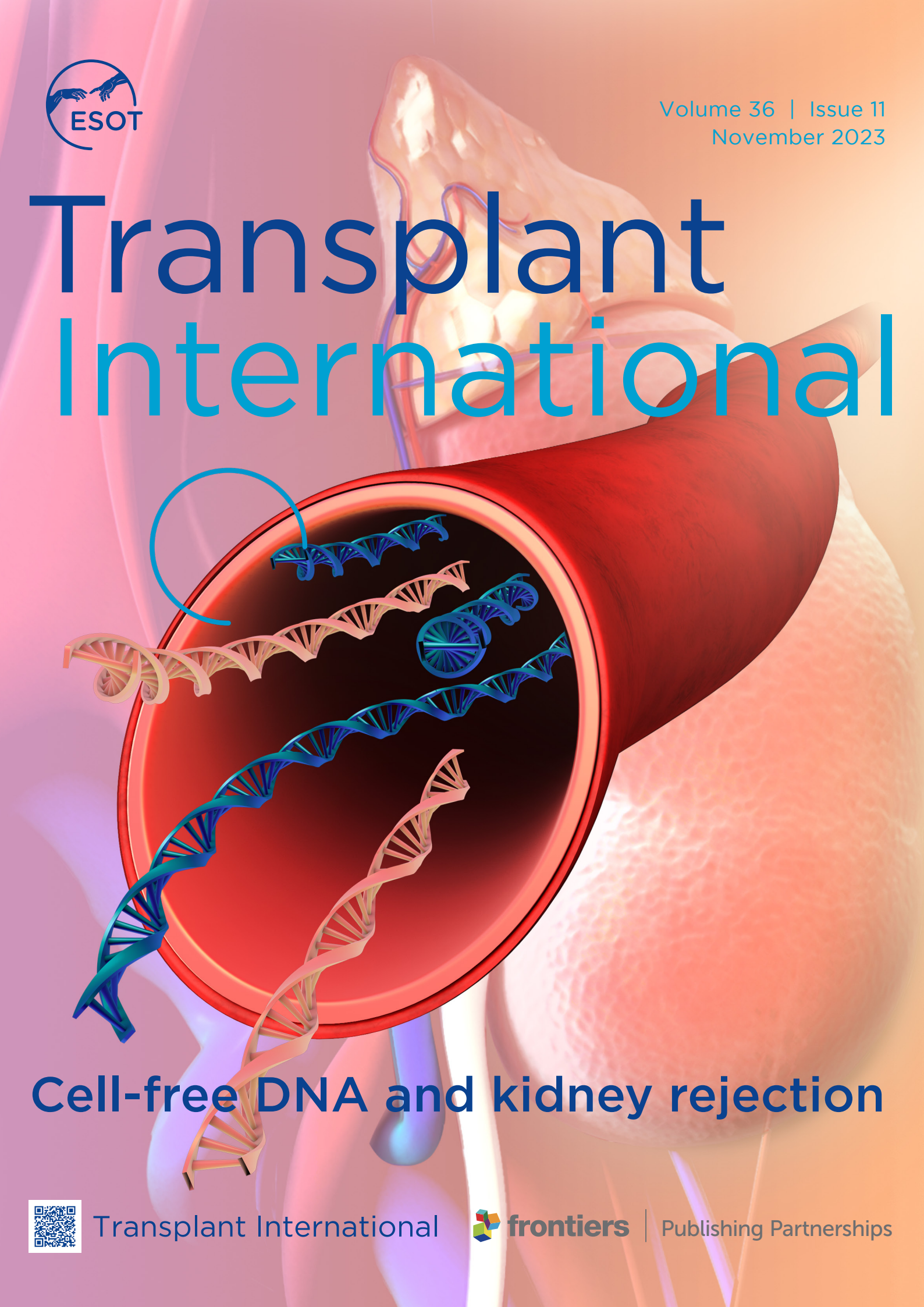

Cell-free DNA and kidney rejection

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DOI: 10.3389/ti.2023.12406
Angelica Pagliazzi, Oriol Bestard and Maarten Naesens
In this forum paper we comment on the results of the prospective studies by Benning at al. and Mantios et al., reporting the diagnostic performance of dd-cfDNA% in discriminating kidney transplant recipients experiencing graft rejection from rejection-free patients, at the time of clinically indicated biopsies. In the paper we discuss the diagnostic value of dd-cfDNA and the aspects of this topic that have yet to be untangled.

Cover article

22 Donor-Derived Cell-Free DNA (dd-cfDNA) in Kidney Transplant Recipients With Indication Biopsy—Results of a Prospective Single-Center Trial

DOI: 10.3389/ti.2023.11899

Louise Benning, Christian Morath, Annette Fink, Markus Rudek, Claudius Speer, Florian Kälble, Christian Nusshag, Jörg Beimler, Constantin Schwab, Rüdiger Waldherr, Martin Zeier, Caner Süsal and Thuong Hien Tran

In our prospective single-center study, dd-cfDNA effectively identified graft injury in kidney transplant recipients with indication biopsy. Our findings suggest that dd-cfDNA may also serve as an indicator for response to therapy and for risk-stratifying patients with borderline changes.

Meeting Report

33 New Approaches to Manage Infections in Transplant Recipients: Report From the 2023 GTI (Infection and Transplantation Group) Annual Meeting

DOI: 10.3389/ti.2023.11859

Alexandra Serris, Julien Coussement, Benoît Pilmis, Victoire De Lastours, Aurélien Dinh, François Parquin, Eric Epailly, Florence Ader, Olivier Lortholary, Emmanuel Morelon, Nassim Kamar, Edouard Forcade, David Lebeaux, Jérôme Dumortier, Filomena Conti, Agnes Lefort, Anne Scemla and Hannah Kaminski

This meeting report of the GTI ("Groupe Transplantation and Infection") summarizes the covered topics: new anti-infective agents and non-antibiotic approaches multidrug-resistant Gram-negative bacteria, staphylococci, fungal infections, as well as new approaches to manage symptomatic urinary tract infections and asymptomatic bacteriuria in kidney transplant recipients.

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41 Organ Repair and Regeneration During *Ex Situ* Dynamic Preservation: The Future is Nano

DOI: 10.3389/ti.2023.11947

Nicholas Gilbo, Joris Blondeel, Jacques Pirenne, Renato Romagnoli, Giovanni Camussi and Diethard Monbaliu

A review of preclinical data suggests that MSC-therapy during organ MP is limited by suboptimal delivery and MSC transient lifespan. Conversely, a cell-free approach utilizing MSC-derived secretome and/or extracellular vesicles appears to capitalize on MSC therapeutic effects more effectively.

Systematic Review and Meta-Analysis

50 Psychological Impact of Living Kidney Donation: A Systematic Review by the EAU—YAU Kidney Transplant Working Group

DOI: 10.3389/ti.2023.11827

Valentine Cazauvieilh, Valérie Moal, Thomas Prudhomme, Alessio Pecoraro, Alberto Piana, Riccardo Campi, Vital Hevia, Angelo Territo and Romain Boissier

This systematic review reported all available evidence on the psychological impact of living donation. Taking into account both quantitative and qualitative studies, we reported that living donation although aglobally positive, was a singular and complex experience for donors.

Original Research

59 Impact of Preformed Donor-Specific Anti-HLA-Cw and Anti-HLA-DP Antibodies on Acute Antibody-Mediated Rejection in Kidney Transplantation

DOI: 10.3389/ti.2023.11416

Timothée Laboux, Rémi Lenain, Jonathan Visentin, Gauthier Flahaut, Paul Chamley, François Provôt, Isabelle Top, Clarisse Kerleau, Myriam Labalette, Gabriel Choukroun, Lionel Couzi, Gilles Blancho, Marc Hazzan and Mehdi Maanaoui

In this multicenter retrospective study, we found a 2 years incidence of acute ABMR of 28% and 12% for preformed DP- and Cw-DSA, respectively. Our results question the need of taking Cw- and DP-DSA into account in the allocation algorithms.

70 Measurement of the Immunosuppressant Possession Ratio by Transplant Clinical Pharmacists Captures a Non-Adherence Associated With Antibody-Mediated Rejection

DOI: 10.3389/ti.2023.11962

Jérémy Chambord, Bertrand Chauveau, Sarah Djabarouti, Jean Vignaud, Benjamin Taton, Karine Moreau, Jonathan Visentin, Pierre Merville, Fabien Xuereb and Lionel Couzi

Calculation of the immunosuppressant possession ratio by the transplant pharmacist makes it possible to assess and quantify non-adherence to immunosuppressants. It is less than 90% in 73% of patients with antibody-mediated rejection and then much more sensitive than clinical suspicion

82 A Decade of Experience With Alemtuzumab Therapy for Severe or Glucocorticoid-Resistant Kidney Transplant Rejection

DOI: 10.3389/ti.2023.11834

Lukas K. van Vugt, Marieke van der Zwan, Marian C. Clahsen-van Groningen, Madelon van Agteren, Daphne M. Hullegie-Peelen, Brenda C. M. De Winter, Marlies E. J. Reinders, Pedro Miranda Afonso and Dennis A. Hesselink

This large, retrospective study shows that alemtuzumab is an effective therapy for severe kidney transplant rejection, but the frequent post-treatment infections and inferior patient survival after therapy should prompt clinicians to weight its risks and benefits before initiation of therapy.

93 Impact of Infection-Related Immunosuppressant Reduction on Kidney Transplant Outcomes: A Retrospective Study Considering the Temporal Dynamics of Immunosuppressive Requirements
DOI: 10.3389/ti.2023.11802
Bo Yang, Qianqian Ye, Changhao Huang and Xiang Ding
This study emphasizes the adverse impact of infection-related immunosuppressant reduction (ISR) on kidney transplant outcomes. It reveals that patients experiencing ISR due to infection between 2-6 months post-transplant face a significantly elevated risk of both graft loss and rejection.

104 The Yield of Routine Post-Operative Doppler Ultrasound to Detect Early Post-Liver Transplantation Vascular Complications
DOI: 10.3389/ti.2023.11611
Iulia Minciuna, Caroline den Hoed, Adriaan J. van der Meer, Milan J. Sonneveld, Dave Sprengers, Robert J. de Knegt, Jeroen de Jonge, Raoel Maan, Wojciech G. Polak and Sarwa Darwish Murad
Routine post-operative ultrasound on POD 0,1 and 7 correctly detects 80.7% of vascular complications after liver transplantation and is a useful tool. However, rDUS generates a high number of false positive results and requires 90 ultrasounds to detect one complication.

Letter to the Editor

113 Women Referred for Liver Transplant Are Less Likely to Be Transplanted Irrespective of Socioeconomic Status
DOI: 10.3389/ti.2023.11667
Emmanouil Giorgakis, Martha M. Estrada, Allison Wells, Mauricio Garcia Saenz de Sicilia, Matthew Deneke, Raj Patel, Gary Barone, Lyle Burdine and Mary K. Rude
On this US-based single center statewide retrospective study it as shown that female-sex patients with end-stage liver disease in need of a transplant are less likely to be referred, enter liver transplant evaluation and be enlisted, irrespective of socioeconomic status.

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TRANSPLANT TRIAL WATCH
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Transplant Trial Watch

John M. O'Callaghan[1,2]* *and Simon R. Knight*[2,3]*

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Keywords: solid organ transplant, liver transplant, deceased donor, randomised controlled trial, reperfusion

To keep the transplantation community informed about recently published level 1 evidence in organ transplantation ESOT and the Centre for Evidence in Transplantation have developed the Transplant Trial Watch. The Transplant Trial Watch is a monthly overview of 10 new randomised controlled trials (RCTs) and systematic reviews. This page of Transplant International offers commentaries on methodological issues and clinical implications on two articles of particular interest from the CET Transplant Trial Watch monthly selection. For all high quality evidence in solid organ transplantation, visit the Transplant Library: www.transplantlibrary.com.

RANDOMISED CONTROLLED TRIAL 1

Results of a Multicenter Cluster-Randomized Controlled Clinical Trial Testing the Effectiveness of a Bioinformatics-Enabled Pharmacist Intervention in Transplant Recipients.

by Taber, D. J., et al. American Journal of Transplantation 2023 [record in progress].

Aims

This study aimed to report the outcomes of the cluster-randomised ISTEP trial, which aimed to examine the effectiveness of a bioinformatics-driven dashboard to guide pharmacist-led medication therapy management intervention in solid organ transplant recipients.

Interventions

Participants were randomised to either standard care combined with the pharmacist-led, bioinformatics dashboard intervention or standard care alone.

Participants

1982 veterans receiving 2196 transplants.

Outcomes

The primary endpoints were the overall rate of veterans affairs (VA) emergency department (ED) visits and VA hospitalisations. Secondary endpoints included patient survival, graft survival and acute rejection episodes.

Follow-Up

24 months.

CET Conclusion

This interesting study from the US randomised 10 VA transplant centres, at a centre level, to use of a computerised alert dashboard designed to identify recipients at risk of non-adherence, drug interactions

Transplant Trial Watch

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and abnormal/missing lab values. The authors found that use of the dashboard significantly reduced the incidence of hospital admissions (by 12.3%) and emergency department visits (by 11.3%), although the incidence of registry-reported acute rejection episodes was increased. There are potential issues with cluster randomisation in this type of study. When the number of centres is small, cluster randomisation can lead to imbalances in the groups in terms of baseline demographics and standard care levels. There is some evidence of this—ED visits and hospitalisations differed significantly in the year preceding the study between the control and intervention groups, and there are demographic and transplant mix differences as well. All of these may affect the risk of the outcomes. It is likely that the intervention was not used optimally by the participating pharmacists, with delays in responding to alerts and a lack of response to many. The key to successful implementation is therefore likely to be in optimising the workflow to ensure that alerts are acted upon in a timely fashion to achieve maximum benefit.

Trial Registration
ClinicalTrials.gov—NCT03860818.

Funding Source
Non-industry funded.

RANDOMISED CONTROLLED TRIAL 2

Prophylactic terlipressin infusion for severe postreperfusion syndrome in patients undergoing deceased donor liver transplantation. The TIPS-DDLT randomized controlled trial.

by Zhang, L., et al. International Journal of Surgery 2023 [record in progress].

Aims
The aim of this study was to assess the effect of prophylactic terlipressin on the incidence of severe postreperfusion syndrome (PRS) in deceased donor liver transplant recipients.

Interventions
Participants were randomised to receive either terlipressin or placebo immediately following portal vein (PV) clamping.

Participants
64 patient scheduled for deceased donor liver transplantation.

Outcomes
The primary endpoint was the occcurence of severe PRS after PV declamping. The secondary endpoints were hemodynamic effects following the start of the trial medication infusion, PV flow velocity after reperfusion, use of renal replacement therapy (RRT), acute kidney injury (AKI), initial poor graft function (IPGF), reoperation, and in-hospital mortality.

Follow-Up
Not reported.

CET Conclusion
This is an interesting randomised controlled trial in deceased donor liver transplantation. The study was small (64 patients), but adequately powered for the primary outcome of severe post-reperfusion syndrome. The study was double-blinded so that patients and clinicians were not aware of the treatment allocation. Following portal vein clamping, the study or control infusion was given at 100 mL over 10 min. The study showed a startling significant reduction in severe post-reperfusion syndrome (9% versus 53%) when using terlipressin. There was a significant difference whether using the Peking definition, van Rijn, Kork or Hilmi definition of post-reperfusion syndrome. The use of terlipressin was also associated with reduced vasopressor requirement, reduced peak ALT, and better early graft function. ICU and hospital stay were unaffected. Of concern, terlipressin was associated with increased pulmonary capillary wedge pressure and duration of mechanical ventilation. Other vasopressors were not administered prior to reperfusion so it is not clear if it is purely prophylactic action that is important, rather than terlipressin compared to other vasopressors.

Jadad Score
5.

Data Analysis
Strict intention-to-treat analysis.

Allocation Concealment
Yes.

Trial Registration
ChiCTR1800019952.

Funding Source
Non-industry funded.

CLINICAL IMPACT SUMMARY

This is a well-written report of an interesting study in deceased donor liver transplantation. The trial was adequately randomised and good steps were taken to blind clinicians to the group allocation through the use of identical infusion bags. Given the trial was double-blinded in this way, one should have faith in the objective outcomes that are recorded; the primary outcome being severe reperfusion syndrome. The study was adequately powered for this outcome, defined by Peking criteria including severe/persistent hypotension during the early reperfusion period, new-onset vasoplegia during the late reperfusion period, or prolonged vasopressor treatment at the end of the surgery. Terlipressin 1 mg or placebo was administered immediately after portal vein clamping.

The trial identified a very significant reduction in the rate of severe reperfusion syndrome with the prophylactic use of terlipressin (9% versus 53%), accompanied by a significant reduction in vasopressor requirement, poor early graft function, and post-operative peak ALT. There was no difference in acute kidney injury or in-hospital mortality.

Of concern, terlipressin was associated with increased pulmonary capillary wedge pressure at 5 min after reperfusion, but this had settled by 2 h later. Mechanical ventilation was longer

following terlipressin, but only by 1 h on average. These 2 issues do raise the concern for intensive monitoring for potential cardiorespiratory complications following terlipressin administration. The other fundamental concern is whether this study has identified a benefit of prophylactic pretreatment with vasopressor, or if the effect is specific to terlipressin compared to other vasopressors.

The findings of this study are in concordance with prior work done in live donor liver transplantation going back over 10 years.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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NEWS AND VIEWS
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Vitrification and Nanowarming. Is this the Future of Kidney Transplantation

Sarah A. Hosgood and Michael L. Nicholson*

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Keywords: kidney, cryopreservation, nanowarming, preservation, normothermic machine perfusion, vitrification

Transplanting an organ within a set timeframe to facilitate allocation and matching is a challenge. The commonest method of kidney preservation is to simply flush the organ with cold preservation solution at the time of retrieval to remove the blood and cool it to below 4°C. The kidney is then stored in ice until transplantation. This technique of static cold storage reduces the metabolic demand and requirement for oxygen to limit the rate of deterioration. The storage time is kept below 24 h to reduce cold ischaemic injury and risk of graft loss [1].

The recent paper by Han et al. [2] and colleagues at the University of Minnesota addresses the issue of time by successfully demonstrating an innovative technique of vitrification and nanoparticle rewarming which allowed rodent kidneys to be preserved for up to 100 days prior to successful transplantation [2]. This is a significant step forward towards the concept of organ banking. The ability to bank organs would allow a more elective approach to transplantation, with better matching and allocation and tailored induction protocols for the recipients.

Cryopreservation is the storage of cells or organs at very low temperatures. It was first attempted in the 1800s but with limited success. One of the major problems is the formation of ice crystals within the cell due to instant freezing and thawing [3, 4]. Ice crystals disrupt the cellular membranes causing deformities in the cell structure [3, 4]. Increases in solute concentration also occur as ice crystals form intracellularly during cooling [3, 4]. To reduce the formation of ice crystals two protective actions are needed [5–7]: slow increments in the speed of freezing and rewarming and the use of cryopreservation agents such as dimethyl sulfoxide (DMSO), glycerol or polyethylene glycol [5–7]. Cryopreservation agents increase the porosity of the cellular membrane and interact strongly with water through hydrogen bonding to reduce the freezing point and the formation of ice crystals [8]. The formation of solid water with an irregular, amorphous structure is known as vitrification. This is achieved with the use of a cryopreservation solution and the appropriate cooling rate. During vitrification cells or organs are cooled from 37°C to −150°C in a stable, ice-free, glass-like state [8]. To reduce toxicity, vitrification mixtures are added in a stepwise manner. This allows the successful storage of cells in a solid phase at supercool temperatures to halt biochemical processes without the formation of ice [8].

Han et al. demonstrated that rodent kidneys can be vitrified and rewarmed to sustain kidney function for 30 days after transplantation [2]. This is a significant step forward from the work of Fahy et al. in 1984 who reported a single rabbit kidney transplant after vitrification for 8 min [9]. They found that vitrification and rewarming were hampered by inadequate tissue cooling due to reduced quantities of cryopreservation solution to avoid toxicity and by the formation of ice crystals upon rewarming [9].

Han et al. overcame these issues with the administration of iron oxide nanoparticles throughout the organ vasculature with a newly formulated cryopreservation solution called VMP [2]. The iron oxide nanoparticles are silica and polyethylene glycol (PEG) coated to increase the stability of the cryopreservation solution and provide biocompatibility and organ washout. The organ was vitrified by first perfusing or loading the kidney gradually with VMP solution. Iron oxide nanoparticles were perfused at the final step of loading. The kidney was then placed in a controlled rate freezer and cooled at a rate faster than the cryopreservation solution's

News & Views

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critical cooling rate to enter a stable glassy state. The vitrified organ was then stored at −150°C. Rewarming occurred by placing the kidney in a radio frequency coil that induced alternating magnetic fields from an electric current flowing through the coil. The magnetic fields generated an oscillatory response in the nanoparticles that generated heat throughout the system. The radio frequencies penetrate tissues without causing damage. Histologically the kidneys had no evidence of ice formation. Several kidneys were tested during a period of *ex vivo* normothermic machine perfusion with an oxygenated acellular solution. Vascular resistance was comparative to fresh control kidneys, and they demonstrated metabolic function by consuming oxygen and glucose.

In the final series of experiments the nanowarmed rodent kidneys were transplanted and the animals recovered for 30 days post-transplant. There was some initial graft dysfunction but after day 14 post-transplant renal function recovered to that of healthy controls. At day 30, renal function was similar to fresh control kidneys. Histology showed some focal tubular necrosis and hyaline change but intact basement membranes and vasculature. This study is a significant breakthrough in the application of cryopreservation using biochemical and engineering principles to overcome the toxicity associated with cryopreservation solutions and rewarming in a unified manner using nanoparticle technology to prevent crystallisation. Nonetheless, the question remains as to whether this technology can be applied in clinical kidney transplantation.

The rising incidence of chronic renal failure has increased the burden on kidney transplantation. The use of donation after circulatory death (DCD) and expanded criteria donors has increased rates of transplantation but the gap between supply and demand is growing. There is also a high rate of kidney discard due to insufficient organ quality [10]. Although cryopreservation extends the time that kidneys can be preserved several questions remain, the most crucial being can this be applied to human kidneys? The perfusion of cryopreservation solution into the rat kidney has solved the problem of damaging ice crystal formation during freezing but it is questionable whether this can be scaled up to a human kidney. Can 200 g of tissue have its water replaced by cryopreservation solution quickly enough to protect the tissue deep in the kidney, especially the medulla where blood flow is low? Furthermore, the use of iron oxide nanoparticles to allow magnetic rewarming is a novel and clever idea but again, can this be scaled up to a human kidney? Would the central deep tissues of the kidney be warmed efficiently and is there any potential toxicity of iron oxide? Moreover, if the technique was successfully applied to kidneys from marginal donors, would they be of sufficient quality for transplantation? With cryopreservation techniques a post-thaw reduction in viability is inevitable.

Organ perfusion technologies are a fast-developing area of research [11]. Recent focus has been on developing normothermic or subnormothermic machine perfusion (NMP) techniques [12, 13]. These preserve a level of cellular metabolism and restore function to avoid or limit cold ischaemic injury. In the liver, NMP has been used to extend the preservation interval to days rather than hours [14]. Experimental evidence with human organs suggests that prolonged perfusion may also be possible in the kidney [15]. Prolonged NMP may extend the preservation interval to allow better matching and allocation and also provide an opportunity to treat the kidney to repair damaged cells. The administration of regenerative or gene therapies is gaining interest in this area [16, 17]. One other advantage of NMP is the ability to assess the quality of the kidney to determine suitability for transplantation. Although, the exact assessment criteria have not yet been defined, basic functional perfusion parameters such as flow, appearance and urine production can provide a measure of kidney quality [18, 19].

The concept of organ banking using cryopreservation and nanoparticle rewarming would certainly ease constraints on allocation and allow better matching. However, it is likely that upon rewarming an assessment of viability would be needed. Rather than a competing technology, NMP could be complementary and used in conjunction with cryopreservation to assess the quality before transplantation.

Vitrification and nanoparticle rewarming is an exciting new approach that offers many advantages in transplantation and the work by Han et al. provides proof of principle that it can be achieved. The next step of this research would be to study it in human kidneys.

AUTHOR CONTRIBUTIONS

SH wrote the manuscript. MN co-wrote and reviewed the manuscript.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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NEWS AND VIEWS
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Prolonging Preservation or Assessment of Organ Quality—What is Key?

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Keywords: organ transplantation, vitrification, organ utilization, organ preservation, organ assessment

In their recent article [1], Han et al demonstrate successful vitrification and nanowarming of rat kidneys for up to 100 days, with subsequent transplantation in a rat transplant model. This is a milestone and represents unequivocally the longest out of body preservation time for a solid organ. The authors achieved such a remarkable result by a complex procedure, involving several important steps. First, loading of specific cryoprotective agents is performed by a short initial organ perfusion with ice blockers and iron oxide nanoparticles. Secondly, super rapid cooling is achieved with a cooling rate of 24°C per minute by a controlled freezer. Third, a deep temperature storage follows at −150°C, keeping the organ in a glassy state for up to 100 days. Fourth, super rapid and uniform rewarming is realized with a temperature increase of 78°C per min by a radiofrequency alternating electromagnetic field in a coil. Fifth, unloading of the cryoprotective agents is needed through another organ perfusion step. Finally, a final perfusion period is suggested with an assessment of organ quality under normothermic conditions (40 min, 37°C). The presented results suggest that such cryobanking is potentially more effective than earlier published work on super cooling at −6°C, which prolonged *ex situ* rat liver viability only up to 7 days [2], and human liver viability only up to 27 h [3].

As the authors state, the study is limited by the small size of the experimental groups in a rodent transplant model, and by a very short follow up of rat recipients, i.e., 1 month. It is therefore unclear if these experiments can be reproduced in large animal models or human organs, and whether a long-term high quality of transplanted cryopreserved organs can be assured. Undoubtedly though, successful cryobanking of human organs would completely change the field of organ transplantation in terms of scheduled procedures.

The disadvantage of this concept is on the other hand, that a static procedure, i.e., storage at −150°C, is unlikely to allow organ assessment. After cryobanking, the authors suggest therefore a short period of normothermic perfusion to check for organ quality. This is yet the most debatable point in the field, as reliable biomarkers are not available for kidneys and also not for livers, lungs, and hearts. Therefore, and in contrast to what the authors state, improved organ utilization will not necessarily increase by prolongation of preservation alone, but rather by improved assessment of organs before transplantation [4, 5].

There remains currently an inherent and unsolved difficulty in interpreting liver or kidney function during any kind of *ex situ* preservation, leading to the report of several so-called biomarkers, measured, for example, in circulating machine perfusates [6] or in produced bile and urine [7]. These include machine perfusate transaminanses, LDH, cytokines, danger proteins, lactate clearance, bile flow, bile pH, bile glucose, NGAL, creatinine, INR, factor V, or methacetin metabolism [6]. While most of these parameters are used during normothermic machine perfusion (NMP) of livers or kidneys, their potential to distinguish between good or bad organs remains very limited. This is based on the fact, that the above mentioned clinical markers are rather down-stream consequences of

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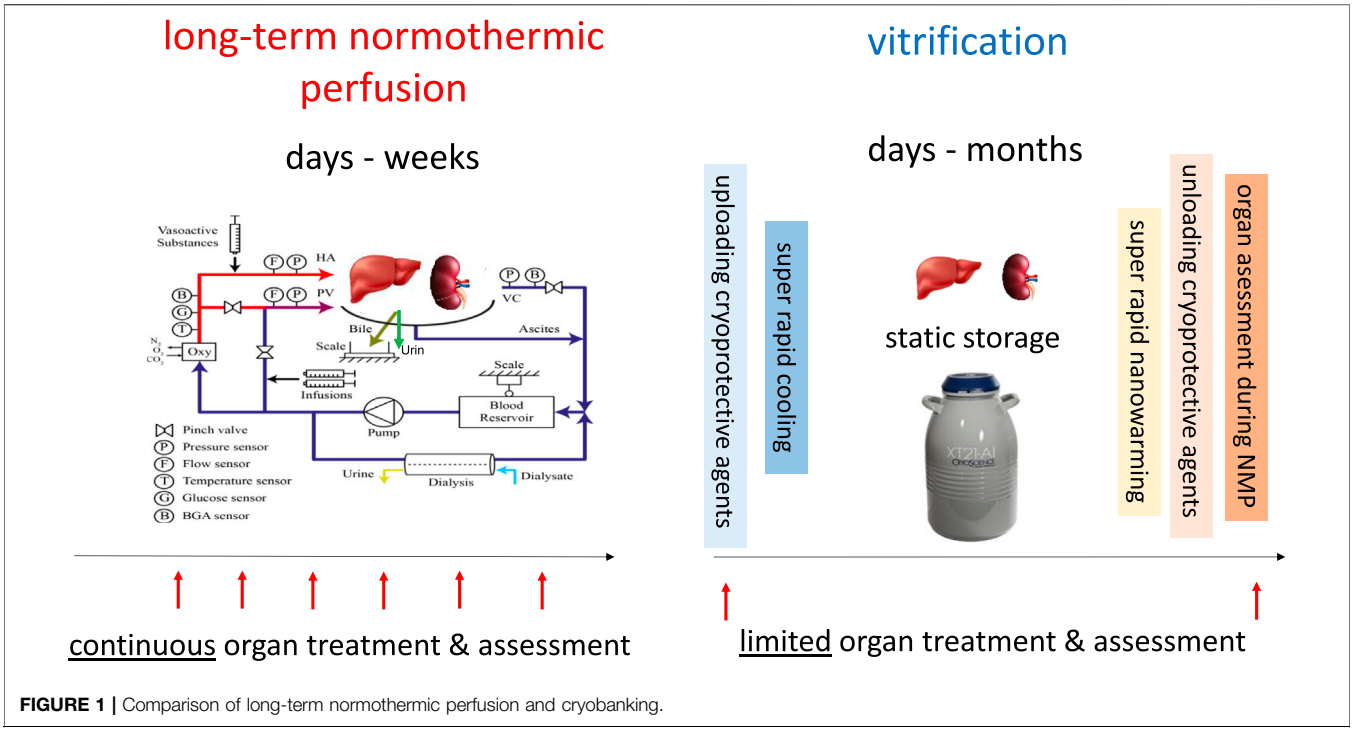

FIGURE 1 | Comparison of long-term normothermic perfusion and cryobanking.

impaired organ function, but not cause related. In contrast, on the subcellular level, clear evidence points to mitochondria as the source of ischemia reperfusion injury in all solid organs [8]. Mitochondrial complex I and II injury, transition pore opening, release of mitochondrial DNA and danger signals, are therefore more upfront signals of organ cellular injury [8], and are also representative for an impaired organ function. Measurement of mitochondrial injury during *ex situ* machine perfusion has therefore gained attendance but needs further research [9, 10]. Besides, it is also unclear, which time period is needed for reliable organ assessment.

Another limitation is that the authors used healthy kidneys for the cryo-approach, i.e., kidneys without significant cold or warm ischemia. The realistic scenario in the transplant world is however the use of injured organs, which need additional transport to recipient centers in most cases. Successful cryobanking will require possibly already before vitrification organ pretreatment by machine perfusion, such as, for example, by initial hypothermic oxygenated perfusion, to minimize mitochondrial oxidative stress [11, 12], and to upload organ energy resources before cryobanking [13, 14].

Notably, the described procedure of cryobanking is the opposite to the alternative idea to keep organs in a functional status rather than to minimize metabolism. In fact, normothermic long-term kidney- or liver perfusions have been performed, but currently only for periods up to 2 days in kidneys [15] and for 7–10 days in livers [16, 17]. The shortcoming of normothermic perfusion systems is therefore an extensive effort and the need for sophisticated devices, due to *ex situ* simulation of the physiologic environment of human organs. The advantage of long-term normothermic perfusion is however the continued accessibility to the perfused organ with the option to treat and monitor outcome parameters, although it remains unsolved which parameters should be best tested (**Figure 1**).

In conclusion it is unclear, whether long-term perfusion strategies or advanced cryobanking will have the highest impact on organ availability in the future, but a combination of both could be the best option. Both methods should therefore be further elaborated. True organ treatment and repair will likewise only be feasible at a functional state, i.e., with dynamic preservation procedures.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

JE and PD both drafted the article and revised it critically.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Donor-Derived Cell-Free DNA: Attractive Biomarker Seeks a Context of Use

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Keywords: donor-derived cell-free DNA, post-transplant monitoring, context of use, predictive values, clinical utility

A Forum discussing:

Donor-Derived Cell-Free DNA (ddcfDNA) in Kidney Transplant Recipients With Indication Biopsy—Results of a Prospective Single-Center Trial
by Benning L, Morath C, Fink A, Rudek M, Speer C, Kälble F, Nusshag C, Beimler J, Schwab C, Waldherr R, Zeier M, Süsal C and Tran TH (2023). Transpl Int. 36:11899. doi: 10.3389/ti.2023.11899 and

Assessment of Donor Derived Cell Free DNA (dd-cfDNA) at Surveillance and at Clinical Suspicion of Acute Rejection in Renal Transplantation
by Mantios E, Filiopoulos V, Constantoulakis P, Liapis G, Vittoraki A, Casas S, Marinaki S, Boletis JN (2023). Transpl Int. 36:11507. doi: 10.3389/ti.2023.11507

dd-cfDNA, A PROMISING BIOMARKER

The search for biomarkers for clinical use in kidney transplant monitoring sometimes seems a tantalizing torment.

The first hint about the potential application of donor-derived cell-free DNA (dd-cfDNA) in post-transplant monitoring dates back 25 years [1]. Specifically released by donor tissue cells (graft cells or donor hematopoietic cells residing within the graft) mainly at the time of cell death, dd-cfDNA is tightly linked to the graft status and, therefore, a promising non-invasive biomarker (**Figure 1**).

The advancement of more comprehensive and scalable DNA sequencing technologies, coupled with the easy accessibility and short half-life of cfDNA, has paved the way for the development of commercially available assays for measuring dd-cfDNA in the plasma of transplanted recipients. In the past 10 years, after demonstration of analytical robustness, these assays have been validated in clinical practice for kidney transplant recipients, and consistently demonstrated a significant correlation between plasma dd-cfDNA levels and graft damage [2–5].

These promising findings have led to the clinical adoption of dd-cfDNA assays for monitoring the occurrence of graft rejection and injury in kidney transplant recipients in the Unites States. This was boosted by Medicare reimbursement in 2017, and positive coverage decisions from several commercial payers. In Europe, however, the adoption of dd-cfDNA assays in clinical practice

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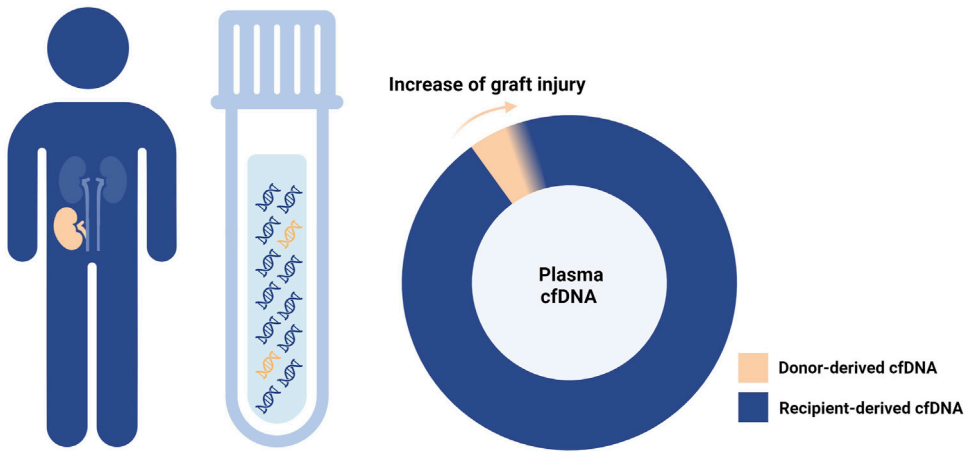

FIGURE 1 | Donor-derived cell-free DNA and graft injury. As the graft damage advances, cell-free DNA (cfDNA) is increasingly released from the graft tissue into the recipient plasma.

lags behind, due to cost concerns of the test and a lack of data demonstrating their clinical utility and context of use.

DIAGNOSTIC VALUE OF dd-cfDNA IN EUROPEAN CONTEXT

In two prospective single-center studies in this journal, Benning et al. and Mantios et al. report on the diagnostic performance of dd-cfDNA% in discriminating kidney transplant recipients experiencing graft rejection from rejection-free patients, at the time of clinically indicated biopsies [6, 7]. The optimal discriminative threshold for dd-cfDNA% was consistent across the two studies, underlining the analytic robustness of the assay. The overall dd-cfDNA% performance in discriminating rejection from no rejection was greater for full-blown rejection diagnoses (Antibody-mediated rejection (AMR) vs. no rejection Area under the ROC curve (AUC) 0.90; T cell-mediated rejection (TCMR) vs. no TCMR AUC 0.73) than for borderline changes (borderline vs. no rejection AUC 0.66), in accordance with previous studies [6].

A major added value of the study by Benning et al [6] is the reporting on negative (NPV) and positive predictive values (PPV), essential parameters to identify the best clinical context of use of a test [8]. The authors conclude that dd-cfDNA% might help in clinical decision making, warranting, or excluding the need of a kidney transplant biopsy in recipients at higher-risk of graft rejection, i.e., when the clinician decides to perform an indication biopsy based on other blood and/or urine biomarkers.

CAN BIOPSIES BE SAFELY AVOIDED WITH dd-cfDNA TESTING?

However, a NPV of 77% with the best cut-off (0.57%) is far from excluding all rejection cases. As outlined by the authors, the lower sensitivity of dd-cfDNA for borderline changes, could be a major downside of the test at time of graft dysfunction. Borderline changes in indication biopsies are considered as clinically meaningful [9] and are also treated similarly like TCMR by the majority of centers. How reassured can one be by testing negative for severe rejection with dd-cfDNA%, and how safely can a biopsy be omitted, when clinically meaningful borderline changes and sometimes even TCMR are not detected with the test and proposed threshold?

Instead of proposing single thresholds, more work is needed to identify the thresholds below which rejection (including borderline changes) can be safely excluded, and to calculate how many biopsies could be avoided with such test. This would allow for establishing the true clinical benefit of dd-cfDNA% testing at the time of clinical suspicion of injury/rejection and help calculate cost-effectiveness in such a context.

NON-SPECIFICITY OF dd-cfDNA, AND DETECTION OF SUBCLINICAL INJURY

In addition, these two studies [6, 7] highlight other aspects that remain to be untangled on this topic.

First, while typically higher in severe active rejection, dd-cfDNA% shows considerable variability within specific rejection categories, correlation with both active and chronic lesions, and possibly increased levels in case of rejection-free graft injuries (such as calcineurin inhibitor toxicity or acute tubular injury). These observations suggest that although dd-cfDNA may be used as an intuitive biomarker of graft injury, what exactly is being measured at the biological level has not yet been elucidated. Coupling plasma dd-cfDNA and biopsy gene expression data, a weak association between dd-cfDNA and injury as well as atrophy-fibrosis gene sets was noted. This supports the idea that dd-cfDNA correlates with unspecific parenchymal injury and not primarily with alloimmune mediated inflammation [10].

Such non-specificity of dd-cfDNA for graft rejection is not necessarily a disadvantage *per se*. By being more comprehensive, such non-invasive biomarker could indicate invasive confirmation of a potentially treatable condition, in addition to rejection. Nonetheless, larger prospective studies, including heterogeneous real-life kidney transplant populations and integrating multiple layers of information (detailed demographic, clinical, serological, virological, and histological data, activity and chronicity indices, blood, and biopsy omics data) are needed to untangle dd-cfDNA biology in renal allograft recipients and eventually extend the applicability of dd-cfDNA testing in post-transplant monitoring.

Second, beyond its value at time of clinical suspicion by avoiding some biopsies, timely detection of subclinical and/or incipient immunological activation is an even greater unmet need in post-transplant monitoring. Besides protocol biopsies, which cannot be performed as a serial testing approach, there are not many other options available for frequent surveillance of kidney transplant status and identification of subclinical rejection or graft injury [11, 12]. Whether dd-cfDNA% has sufficient diagnostic performance in such specific context of use remains to be studied. Important here will be the false positive rate (and herewith related PPV). When PPV is too low (few true positive cases in the test positive group), this could lead to anxiety and performance of more non-informative biopsies, instead of less.

CONCLUSION

In conclusion, the graft-specificity and apparent intuitive use of dd-cfDNA% resulted in an acceleration of its clinical implementation in the United States. Despite a large body of research been done and slowly advancing insights into its added value, many questions and confusion remains, hindering more global implementation of dd-cfDNA% as biomarker in kidney transplantation. We must remain critical and focus on intrinsic biology and the best context of use. Especially the latter will be very important for reimbursement discussions with payers in, e.g., European countries.

Moving from a promising biomarker to a widely used standard biomarker goes through larger prospective studies in real-life patient populations, and even randomized trials with clinically meaningful endpoints, such as the number of biopsies that can be avoided with non-invasive monitoring. This requires tireless efforts to integrate current monitoring practice with the results of dd-cfDNA measurements in a wide range of clinical scenarios.

Unlike Tantalus, who is eternally close to food and water without ever reaching them, we are rapidly closing the knowledge gap around dd-cfDNA testing for kidney transplantation. Well-conducted studies evaluating clinical utility and context of use are needed to implement dd-cfDNA testing in routine clinical care in Europe.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

AP and MN wrote the original draft. AP, MN, and OB reviewed and edited the draft. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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ORIGINAL RESEARCH
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Donor-Derived Cell-Free DNA (dd-cfDNA) in Kidney Transplant Recipients With Indication Biopsy—Results of a Prospective Single-Center Trial

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Donor-derived cell-free DNA (dd-cfDNA) identifies allograft injury and discriminates active rejection from no rejection. In this prospective study, 106 kidney transplant recipients with 108 clinically indicated biopsies were enrolled at Heidelberg University Hospital between November 2020 and December 2022 to validate the clinical value of dd-cfDNA in a cohort of German patients. dd-cfDNA was quantified at biopsy and correlated to histopathology. Additionally, dd-cfDNA was determined on days 7, 30, and 90 post-biopsy and analyzed for potential use to monitor response to anti-rejection treatment. dd-cfDNA levels were with a median (IQR) % of 2.00 (0.48–3.20) highest in patients with ABMR, followed by 0.92 (0.19–11.25) in patients with TCMR, 0.44 (0.20–1.10) in patients with borderline changes and 0.20 (0.11–0.53) in patients with no signs of rejection. The AUC for dd-cfDNA to discriminate any type of rejection including borderline changes from no rejection was at 0.72 (95% CI 0.62–0.83). In patients receiving anti-rejection treatment, dd-cfDNA levels significantly decreased during the 7, 30, and 90 days follow-up compared to levels at the time of biopsy ($p = 0.006$, $p = 0.002$, and $p < 0.001$, respectively). In conclusion, dd-cfDNA significantly discriminates active rejection from no rejection. Decreasing dd-cfDNA following anti-rejection treatment may indicate response to therapy.

Clinical Trial Registration: https://drks.de/search/de/trial/DRKS00023604, identifier DRKS00023604.

Keywords: donor-derived cell-free DNA, dd-cfDNA, kidney transplantation, rejection, response to therapy

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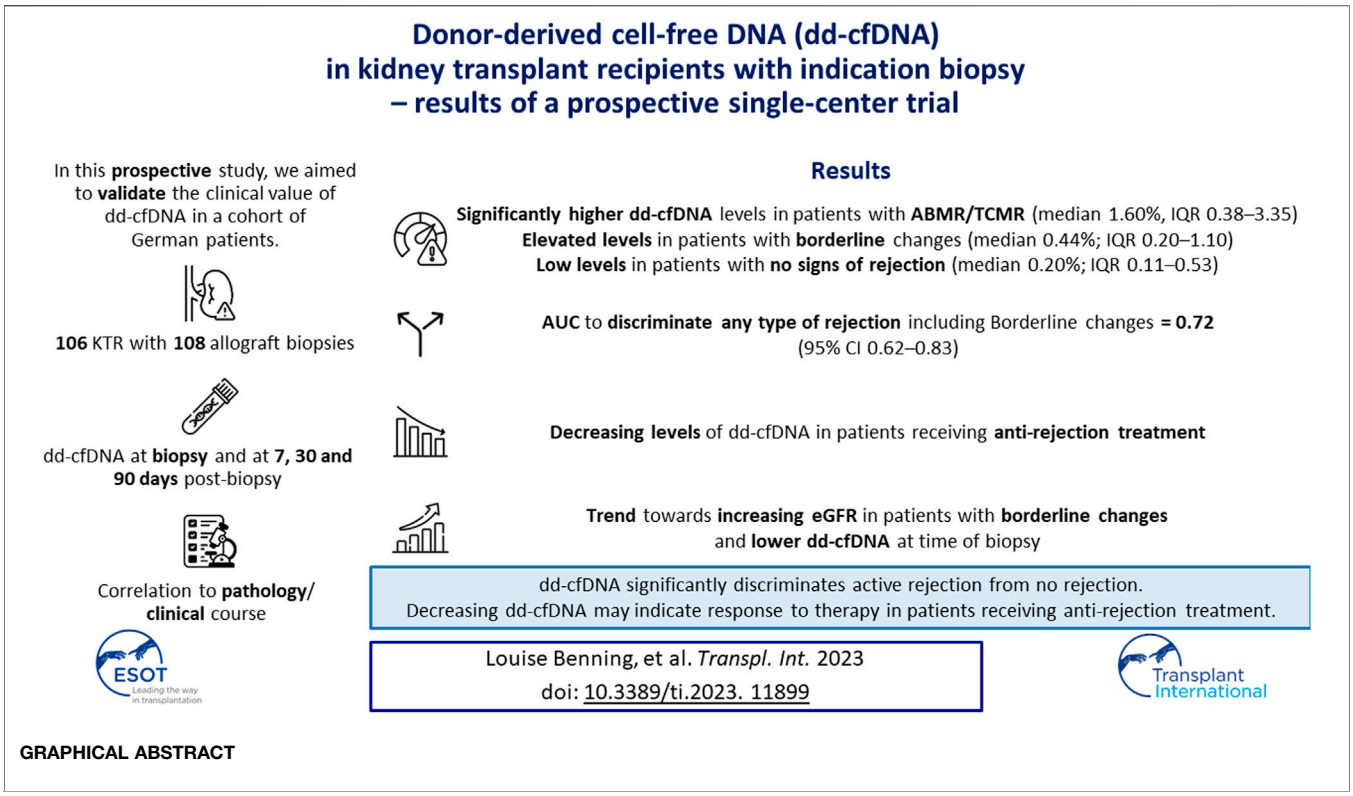
INTRODUCTION

Despite improvements in short-term outcomes after kidney transplantation, mainly driven by improvements in 1 year allograft survival, late allograft failure remains an issue [1, 2]. In a study of 252,910 patients who received kidney transplants in the United States between 1989 and 2009, Lamb et al found that the zero to 1 year rate for graft loss dropped dramatically from 19.8 to 6.7 during this period while rates beyond the first year only showed marginal improvements [3]. Analyzing 108,787 patients



GRAPHICAL ABSTRACT

from the Collaborative Transplant Study transplanted between 1986 and 2015 and accounting for the evolution of donor and recipient characteristics, Coemans et al found that short-term improvement in more recent years since 2000 was less pronounced, while long-term improvement remained largely unchanged in Europe [4]. Both studies emphasize the pressing need for innovation aimed at improving long-term graft survival.

Meier-Kriesche et al. noted that the limited improvements in long-term allograft survival, despite reduced rejection rates, could be due to acute rejection episodes without complete functional recovery [1], which was supported by results from other clinical trials [5]. Currently, biopsy remains the gold standard for the diagnosis of kidney graft rejection and for the differential diagnosis of kidney graft damage. However, its accessibility is sometimes limited, the right time for biopsy is difficult to determine, and the procedure itself may pose risks, e.g., in obese patients or those requiring anticoagulation. Therefore, there is a need for minimally invasive biomarkers capable of identifying high-risk patients requiring biopsy in the outpatient setting.

In recent years, several advances have been made in follow-up after kidney transplantation, including big data-driven models such as the iBOX to predict allograft survival or new biomarkers such as donor-derived cell-free DNA (dd-cfDNA) to detect early graft damage [6, 7]. Elevated dd-cfDNA levels reflect allograft damage, and studies have shown that dd-cfDNA can effectively distinguish active rejection from no rejection [7–9]. The biomarker was validated in a large US multicenter study of 1,092 kidney transplant recipients over a 3 years period, with an increase in dd-cfDNA to 0.5% or more indicating clinically apparent and subclinical rejection [10]. However, European data on the use of dd-cfDNA is still scarce. In this prospective study, our objective was to analyze dd-cfDNA within a group of German kidney transplant recipients who underwent clinically indicated biopsies, presenting diverse histopathological findings. Our primary aim was to evaluate the sensitivity and specificity of dd-cfDNA in detecting rejection among these patients, and the secondary aim was to explore whether dd-cfDNA levels exhibited changes following anti-rejection therapy, potentially serving as an indicator of treatment response.

MATERIALS AND METHODS

Study Design

From November 2020 to December 2022, we enrolled 106 kidney transplant recipients from the Department of Nephrology at Heidelberg University Hospital with 108 clinically indicated biopsies into this prospective single-center study. The study was approved by the ethics committee of the University of Heidelberg and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all study participants. The study is registered in the German Clinical Trials Register (DRKS00023604).

Serum creatinine and the estimated glomerular filtration rate (eGFR), proteinuria, donor-specific HLA antibodies (DSA) and non-HLA antibodies, as well as dd-cfDNA were measured the day of biopsy (before biopsy, T0), as well as 7 (T_1), 30 (T_2), and 90 (T_3) days post-biopsy. Clinical follow-up was until day 180 post-biopsy (T_4, **Figure 1A**).

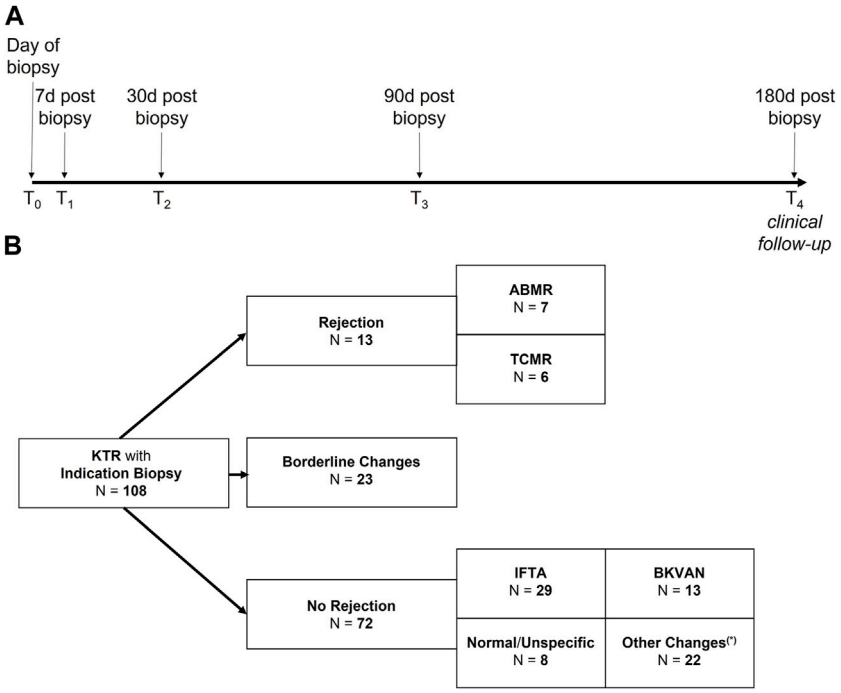


FIGURE 1 | Study design to evaluate the diagnostic potential of donor-derived cell-free DNA (dd-cfDNA) in kidney transplant recipients with indication biopsy. **(A)** Donor-derived cell-free DNA, donor-specific HLA antibodies (DSA) and non-HLA antibodies were determined on the day of biopsy as well as 7, 30 and 90 days post-biopsy. Clinical follow-up was at 180 days post-biopsy. **(B)** Of the 108 allograft biopsies, 36 (33%) were classified as different types of rejection, whereof 7 biopsies were graded as ABMR, 6 as TCMR, and 23 as borderline changes. The 72 biopsies with no signs of rejection were graded either as interstitial fibrosis and tubular atrophy (IFTA, *N* = 29), polyomavirus nephropathy (BKVAN, *N* = 13), normal/unspecific (*N* = 8), or with other changes (*N* = 22). Other changes (*) included acute tubular injury (ATI, *N* = 8), recurrent disease (*N* = 4), infection (*N* = 1), CNI toxicity (*N* = 1), or IFTA with signs of CNI toxicity (*N* = 8). ABMR, antibody-mediated rejection; BKVAN, BK virus-associated nephropathy; CNI, calcineurin-inhibitor; IFTA, interstitial fibrosis and tubular atrophy; KTR, kidney transplant recipients; N, number; T, time point; TCMR, T cell-mediated rejection.

Indication Biopsy and Clinical Management According to Histopathological Reporting

Indications for biopsy included acute graft dysfunction (*N* = 12), creeping creatinine (*N* = 64) development or worsening of proteinuria (*N* = 16), detection of donor-specific HLA-antibodies (DSA) with concomitant proteinuria or graft dysfunction (*N* = 4), or detection of BK viremia with worsening kidney function (*N* = 12). The biopsy was examined by two board-examined pathologists and reported using the BANFF 2018 reference guide [11]. Following histopathological reporting, clinical management involved corticosteroid pulse therapy in 27/36 (75%) patients with signs of active rejection, including 19/23 (83%) patients with borderline changes and excluding patients with concomitant infection (*N* = 4). In addition, 4/6 (67%) patients with T cell-mediated rejection (TCMR) received anti-thymocyte globulin (ATG) and 3/7 (43%) patients with antibody-mediated rejection (ABMR) immunoadsorption. In all 13 patients with BK virus-associated nephropathy (BKVAN, SV40+), immunosuppression was altered from a calcineurin-inhibitor (CNI)-mycophenolic acid (MPA) to a CNI-mTOR regimen. In 12 patients with suspected CNI-toxicity (ah ≥ 1), CNI medication was adapted to lower trough levels (*N* = 6) or changed to Belatacept (*N* = 6).

Quantification of Donor-Derived Cell-Free DNA

Venous blood samples were collected into 10 mL Streck cell-free DNA BCT tubes (Streck, Omaha, NE) and examined within 7 days. Plasma was separated by centrifugation at 1,600 × g for 20 min, followed by a second centrifugation at 16,000 × g for 10 min, and either plasma was stored at −80°C or cfDNA was extracted immediately using the Circulating Nucleic Acid kit (Qiagen, Redwood City, CA). cfDNA was then amplified using the AlloSeq cfDNA assay (CareDX, Brisbane, CA), a multiplex PCR including PCR primers for 202 single nucleotide polymorphisms (SNPs). Variations in the SNPs loci are used to determine the proportion of donor-derived (dd)-cfDNA in relation to the total cfDNA present in the plasma sample. The PCR products were subsequently sequenced on a MiniSeq (Illumina, Inc.). Sequence data was analyzed using the CareDx AlloSeq cfDNA software. All steps were performed according to the manufacturers' instructions and as described previously [12, 13].

Determination of Donor-Specific HLA Antibodies (DSA) and Non-HLA Antibodies

All patients were screened for DSA and non-HLA antibodies at time of biopsy, as well as 7, 30, and 90 days post-biopsy if serum

was available for analysis. Luminex technology was employed to determine HLA antibodies using the LABScreen Single Antigen kit of One Lambda/Thermo Fisher Scientific (West Hills, CA). MFI cutoff of >500 or >1,000 was used to identify DSA against mismatched donor HLA. Testing for non-HLA antibodies included antibodies targeting the major histocompatibility complex class I-related chain A (MICA), angiotensin II type 1 receptor (AT1R) and endothelin receptor subtype A (ETA). MICA antibodies were detected with the LABScreen Mixed kit of One Lambda/Thermo Fisher Scientific (West Hills, CA), whereas AT1R and ETA antibodies were determined with AT1R-IgG-Antibody-ELISA and ETAR-IgG-Antibody-ELISA, respectively (both kits were obtained from CellTrend, Luckenwalde, Germany). Anti-MICA antibodies were found to be associated with ABMR and *de-novo* anti-MICA development was linked to reduced graft survival [14]. AT1R and ETA antibodies were also reported to correlate with a higher prevalence of ABMR and a decline in graft function [15, 16]. Soluble CD30 (sCD30) was assessed using the Human sCD30 Instant ELISA kit of Invitrogen eBioScience/Thermo Fischer Scientific (Bender MedSystems GmbH, Vienna, Austria). Early posttransplant measurements of sCD30 were shown to be predictive of subsequent graft loss, however, the evidence regarding the use of sCD30 as a biomarker in late posttransplant period is limited and its clinical utility remains uncertain [17–19].

Statistics

Data are presented as number (*N*) and percent (%), median and interquartile range (IQR) or mean and Standard Deviation (SD). Categorical data were compared using the Fisher's exact test. To compare non-parametric continuous variables between two independent groups, the Mann-Whitney *U* test was used. When dealing with more than two independent groups, the Kruskal-Wallis test was employed, followed by Dunn's post-test for multiple comparisons. Wilcoxon matched-pairs signed rank test was used when comparing non-parametric paired variables. A multiple linear regression analysis was performed to differentiate possible confounders of elevated dd-cfDNA levels. The area under the ROC curves (AUC) was used to evaluate the performance of dd-cfDNA and eGFR in discriminating acute rejection from no rejection. Rejection status was based on histopathological diagnosis of rejection using the BANFF 2018 reference guide [11]. The Youden index was calculated to give the optimal cut point for dd-cfDNA to discriminate active rejection. In addition, specificity, sensitivity, positive predictive value (PPV) and negative predictive value (NPV) for different dd-cfDNA cutoffs to discriminate acute rejection were calculated using a contingency table. Thresholds of dd-cfDNA levels ≥1%, ≥0.74% and ≥0.5% were applied according to results of the Circulating Donor-Derived Cell-Free DNA in Blood for Diagnosing Active Rejection in Kidney Transplant Recipients (DART) trial [7], early experiences using dd-cfDNA to detect rejection in US American kidney transplant recipients [8], and a recent trial by Stites et al. to identify TCMR1A and borderline patients with elevated risk of graft injury [9], respectively. Spearman's rho was calculated to assess the correlation between dd-cfDNA levels and histopathological lesion scores or the presence of DSA/non-HLA antibodies. Statistical significance was assumed at a p-value < 0.05. The statistical analysis was performed using GraphPad Prism version 9.5.1 (GraphPad Software, San Diego CA, United States). For analysis purposes, serum creatinine and estimated glomerular filtration rate (eGFR) for patients returning to dialysis were arbitrarily set at 10 mg/dL and 5 mL/min, respectively.

RESULTS

Baseline Characteristics

From November 2020 to December 2022, 106 kidney transplant recipients with a total of 108 graft biopsies were enrolled. dd-cfDNA was quantified at day of indication biopsy (T_0), and a median (IQR) of 7 (6–9, T_1), 38 (28–48, T_2), and 88 (84–100, T_3) days post-biopsy. The analytical sample included 370 dd-cfDNA measurements. Clinical follow-up was at a median (IQR) of 185 (172–191) days post-biopsy (T_4). Patients with a biopsy of <7 days post- transplantation were excluded from analysis.

Of the 108 allograft biopsies, 36 (33%) were classified as different types of rejection, whereof 7 biopsies were graded as ABMR, 6 as TCMR, and 23 as borderline changes (**Figure 1B**). Subcategories of ABMR and TCMR with respective dd-cfDNA levels are given in **Supplementary Table S1**. The 72 biopsies with no signs of rejection were either graded as interstitial fibrosis and tubular atrophy (IFTA, N = 29), polyomavirus nephropathy (BKVAN, N = 13), normal/unspecific (N = 8), or with other changes (N = 22, **Figure 1B**). **Figure 2** displays dd-cfDNA levels, the presence of DSA at an MFI cutoff >500 or >1,000, the presence of any non-HLA antibodies determined, and corresponding histopathological lesions for each biopsy.

Patient characteristics stratified for active rejection vs. no rejection are shown in **Table 1**. Since no protocol but only indication biopsies in the presence of allograft dysfunction had been performed, patients with borderline changes were included into the active rejection group. No statistically significant differences in sex or age were seen between patients with rejection and those without (p > 0.99 and p = 0.1, respectively). Patients with active rejection had significantly higher levels of proteinuria (p = 0.002), and were more likely to be DSA+, albeit without reaching statistical significance (p = 0.07 for DSA with MFI >500, p = 0.31 for DSA with MFI >1,000, **Table 1**).

Donor-Derived Cell-Free DNA at Time of Biopsy

Patients with histopathological signs of active rejection had significantly higher levels of dd-cfDNA at time of biopsy than patients without signs for rejection, whereas estimated glomerular filtration rate (eGFR) did not differ significantly between the two groups (p < 0.001 and p > 0.99, respectively; **Table 1**). The diagnosis of active rejection remained independently associated with higher dd-cfDNA levels when stratified for age, gender, BMI, time since transplantation, eGFR, and the presence of donor-specific HLA or non-HLA antibodies (β: −1.071; 95% CI: −1.811, −0.331; p = 0.005; **Supplementary Table S2**).

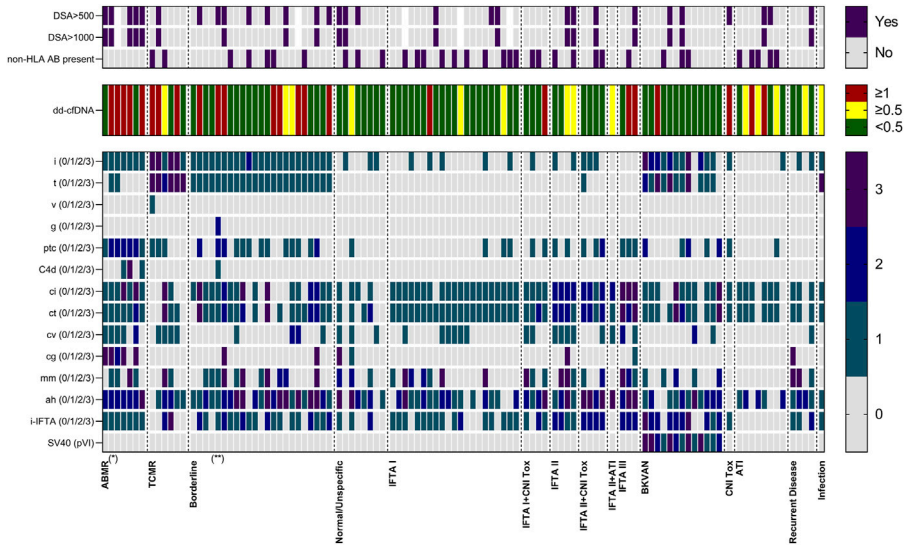


FIGURE 2 | Heat map of histopathological lesion scores according to the BANFF classification and the polyomavirus-associated interstitial nephritis score as well as donor-derived cell-free DNA levels and the presence of donor-specific and non-HLA antibodies for 108 kidney allograft biopsies. The 108 allograft biopsies are grouped according to histopathological diagnosis. Color-coding indicates BANFF lesion scores and dd-cfDNA levels. The presence of donor-specific antibodies with a mean fluorescence intensity of >500 and >1,000, as well as the presence of any non-HLA antibodies is indicated in purple. ABMR, antibody-mediated rejection; ah, hyaline arteriolar thickening; ATI, acute tubular injury; BKVAN, BK virus-associated nephropathy; cg, glomerular basement membrane double contours; ci, interstitial fibrosis; CNI, calcineurin-inhibitor toxicity; ct, tubular atrophy; cv, vascular fibrous intimal thickening; dd-cfDNA, donor-derived cell-free DNA; DSA, donor-specific antibodies; g, glomerulitis; i, interstitial inflammation; IFTA, interstitial fibrosis and tubular atrophy; mm, mesangial matrix thickening; non-HLA AB, non-HLA antibodies (angiotensin II type 1 receptor/endothelin receptor subtype A/major histocompatibility complex class I-related chain A); ptc, peritubular capillaritis; PVI, polyomavirus-associated interstitial nephritis score; t, tubulitis; v, intimal arteritis; TCMR, T cell-mediated rejection; i-IFTA, inflammation in the area of IFTA. (*) Two biopsies showed mixed rejection with concomitant borderline lesions and were categorized as ABMR due to low numbers of mixed rejections. (**) Based on clinical judgement, this biopsy was categorized as borderline changes, despite the presence of glomerulitis, peritubular capillaritis, C4d deposition, and low-level DSA (MFI 505). Of note, the biopsy was conducted 14 days after a living kidney donation, DSA were not detected subsequently and eGFR as well as dd-cfDNA improved upon sole corticosteroid treatment.

TABLE 1 | Clinical characteristics in kidney transplant recipients with indication biopsy stratified for status of rejection.

Variable	Active rejection	No active rejection	*p*-value
Number of Samples, *N* (%)	36 (33)	72 (67)	
Female, *N* (%)	12 (33)	23 (32)	>0.99
Age at enrollment, Median (IQR)	43 (34–62)	54 (39–62)	0.11
Donor type			**0.003** (**)
Deceased donor, *N* (%)	16 (44)	54 (75)	
Living donor, *N* (%)	20 (56)	18 (25)	
HLA-A+B mismatches, Median (IQR)	2 (1–3)	2 (1–2)	0.28
HLA-DR mismatches, Median (IQR)	1 (0–1)	1 (0–1)	0.16
Months post-transplant at time of biopsy, Median (IQR)	36 (3–135)	28 (3–72)	0.67
DSA MFI > 500, *N* (%)	14 (41)[a]	16 (23)[b]	0.07
DSA MFI > 1,000, *N* (%)	9 (26)[a]	12 (17)[b]	0.31
Presence of non-HLA AB, *N* (%)	7 (19)	28 (39)	0.05
sCD30 > 40, *N* (%)	11 (31)	12 (17)	0.13
S-Creatinine [mg/dL], Median (IQR)	2.5 (1.7–3.2)	2.2 (1.8–3.3)	0.84
eGFR [mL/min/1.73 m^2], Median (IQR)	26.8 (20.6–43.0)	28.3 (17.4–38.8)	>0.99
Proteinuria [g/molCr], Median (IQR)[c]	100.4 (46.4–223.3)	35.6 (17.5–113.4)	**0.002** (**)
dd-cfDNA [%], Median (IQR)	0.6 (0.2–1.7)	0.2 (0.1–0.5)	<**0.001** (***)

*The data includes two patients with re-biopsies after completed follow-up. dd-cfDNA, donor-derived cell-free DNA; DSA, donor-specific anti-HLA antibodies; non-HLA AB, non-HLA antibodies including antibodies targeting the major histocompatibility complex class I-related chain A (MICA), angiotensin II type 1 receptor (AT1R) and endothelin receptor subtype A (ETA); ***p < 0.001; **p < 0.01.*

[a]*Not possible to determine DSA in two patients due to missing data.*

[b]*Not possible to determine DSA in three patients due to missing data.*

[c]*Data on proteinuria were only available in 29 patients with active rejection and 56 patients without active rejection.*

The bold values reflect significance.

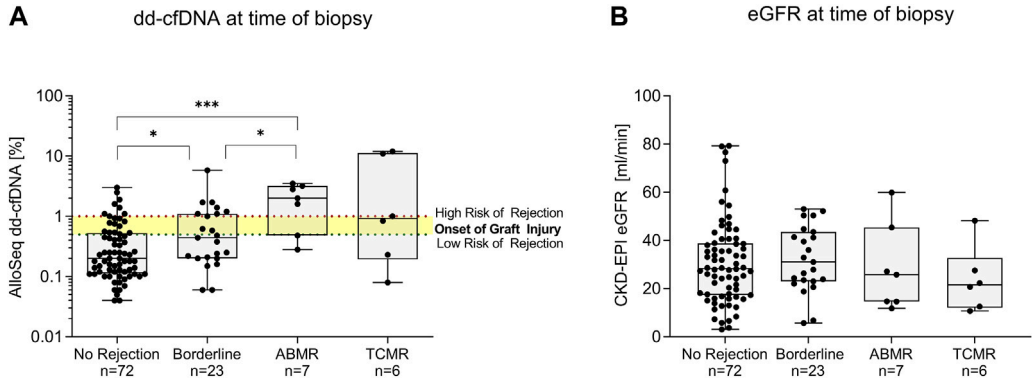


FIGURE 3 | Donor-derived cell-free DNA (dd-cfDNA) and estimated glomerular filtration rate at time of biopsy. **(A)** Donor-derived cell-free DNA was highest in patients with antibody-mediated rejection, compared to patients with T cell-mediated rejection, borderline changes, and patients with no histopathological signs of rejection. The x-axis displays the respective group, dd-cfDNA levels are shown log-transformed on the y-axis. Box plots display the distribution of data, with a horizontal line denoting the median. The bottom and top edges of the box indicate the 25th and 75th percentiles respectively. Individual results are shown as dots. The red dotted line indicates a dd-cfDNA level of 1%, whereas the green dotted line indicates a dd-cfDNA level of 0.5%, corresponding to different cut-points defined in other studies investigating dd-cfDNA as a biomarker for allograft injury. Below the level of 0.5%, the risk of rejection is low. **(B)** Estimated glomerular filtration rate at time of biopsy did not differ significantly between patients with any type of rejection and no rejection. The x-axis displays the respective group, eGFR is shown on the y-axis. Box plots display the distribution of data, with a horizontal line denoting the median. The bottom and top edges of the box indicate the 25th and 75th percentiles respectively. Individual results are shown as dots. ABMR, antibody-mediated rejection; dd-cfDNA, donor-derived cell-free DNA; eGFR, estimated glomerular filtration rate; N, number; TCMR, T cell-mediated rejection; ***$p < 0.001$; *$p < 0.05$.

dd-cfDNA levels were with a median (IQR) % of 2.00 (0.48–3.20) highest in patients with ABMR, followed by 0.92 (0.19–11.25) in patients with TCMR, 0.44 (0.20–1.10) in patients with borderline changes and 0.20 (0.11–0.53) in patients with no signs of rejection (**Figure 3A**). Patients with ABMR had significantly higher dd-cfDNA levels compared to both patients without signs of rejection or those with borderline changes ($p < 0.001$ and $p < 0.05$, respectively, **Figure 3A**). dd-cfDNA levels in patients with borderline changes were also significantly higher compared to patients without rejection ($p < 0.05$, **Figure 3A**). In contrast, eGFR did not differ significantly between the four groups (**Figure 3B**).

To evaluate the diagnostic performance of dd-cfDNA to discriminate acute rejection from no rejection, the area under the ROC curve (AUC) was calculated. The AUC to discriminate any type of rejection including borderline changes from no rejection was at 0.72 (95% CI 0.62–0.83; **Figure 4A**). For the discrimination of only ABMR or only TCMR from no rejection, dd-cfDNA exhibited an AUC of 0.90 (95% CI 0.78–1.00, **Figure 4B**) and 0.73 (95% CI 0.47–0.99, **Figure 4C**), respectively. When only borderline changes vs. no rejection were compared, a lower AUC of 0.66 (95% CI 0.54–0.79, **Figure 4D**) was observed.

The optimal cut point for dd-cfDNA to discriminate active rejection from no rejection as calculated by the Youden index was at a threshold of 0.57, yielding a specificity of 81% (95% CI 70%–88%), a sensitivity of 53% (95% CI 37%–68%), a PPV of 58% (95% CI 41%–73%), and an NPV of 77% (95% CI 67%–85%). **Supplementary Figure S1** displays the values of specificity and sensitivity for different measurements of dd-cfDNA to discriminate acute rejection from no rejection. **Table 2** illustrates the specificity, sensitivity, PPV and NPV when applying different dd-cfDNA levels as established in other studies to our study cohort [7–9].

Twenty-four (22%) patients had dd-cfDNA levels ≥1%, of whom 8 had no histopathological signs of rejection. These patients were diagnosed with BKVAN ($N = 1$), acute tubular injury (ATI; $N = 2$; 8 and 11 days after living donor kidney transplantation), IFTA ($N = 3$, whereof one patient with presence of DSA), or CNI toxicity ($N = 2$, whereof 1 patient with presence of DSA). **Supplementary Figure S2** displays levels of dd-cfDNA in patients with histopathological diagnoses other than rejection.

Donor-Derived Cell-Free DNA in Patients With Borderline Changes

dd-cfDNA levels varied considerably among patients with borderline changes, ranging from 0.06% to 5.80% (**Supplementary Figure S3A**). When categorizing patients with borderline changes based on their dd-cfDNA levels at time of biopsy (either < or ≥ 1% and < or ≥ 0.5%), those with lower dd-cfDNA levels displayed a tendency toward an improvement in eGFR after corticosteroid pulse therapy, in contrast to patients with higher dd-cfDNA levels who exhibited relatively stable or decreasing eGFR over time, albeit not reaching statistical significance (**Supplementary Figure S3B**).

Correlation of Donor-Derived Cell-Free DNA to BANFF Lesion Scores

When calculating the relationship between levels of dd-cfDNA to BANFF lesion scores, a significant moderate correlation for dd-cfDNA was established to ptc (44 patients with ptc ≥ 1; Spearman's rho = 0.34, $p < 0.001$), and to C4d positivity

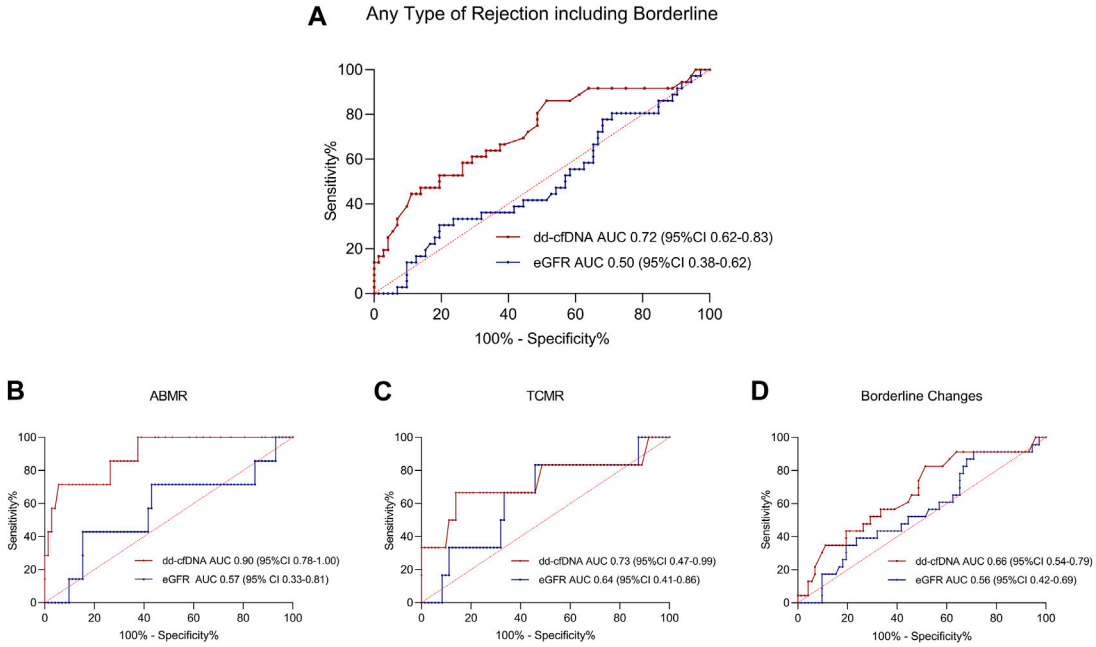


FIGURE 4 | ROC Curves for donor-derived cell-free DNA and estimated glomerular filtration rate to discriminate different types of rejection from no rejection at time of biopsy. ROC curves to discriminate **(A)** any type of rejection, including borderline changes, **(B)** antibody-mediated rejection, **(C)** T cell-mediated rejection, and **(D)** borderline changes from no rejection.100%-specificity in % is displayed on the x-axis, sensitivity in % on the y-axis. dd-cfDNA is plotted in red, whereas the ROC curve for eGFR is plotted in blue for all ROC curves. Respective AUC and 95% CI is given in red for dd-cfDNA and in blue for eGFR at the bottom of each graph. ABMR, antibody-mediated rejection; AUC, area under the curve; CI, confidence interval; dd-cfDNA, donor-derived cell-free DNA; eGFR, estimated glomerular filtration rate; ROC, receiver operating characteristics; TCMR, T cell-mediated rejection.

TABLE 2 | Application of suggested cut points of dd-cfDNA levels in our study cohort of kidney transplant recipients with indication biopsy.

Cut point	Specificity	Sensitivity	Positive predictive value	Negative predictive value
dd-cfDNA ≥ 0.5% [9]	74% (95% CI 62%–82%)	53% (95% CI 37%–68%)	50% (95% CI 35%–65%)	76% (95% CI 65%–84%)
dd-cfDNA ≥ 0.57%	**81% (95% CI 70%–88%)**	**53% (95% CI 37%–68%)**	**58% (95% CI 41%–73%)**	**77% (95% CI 67%–85%)**
dd-cfDNA ≥ 0.74% [8]	82% (95% CI 72%–89%)	48% (95% CI 32%–63%)	57% (95% CI 39%–73%)	76% (95% CI 65%–84%)
dd-cfDNA ≥ 1% [7]	89% (95% CI 80%–94%)	44% (95% CI 30%–60%)	67% (95% CI 47%–82%)	76% (95% CI 66%–84%)

In literature, different cutoffs have been proposed for determining when to assume graft injury and/or rejection. Stites et al. found a 0.5% threshold of dd-cfDNA to be associated with increased risk of eGFR decline, DSA development and future episodes of rejection in patients with borderline and 1A T cell-mediated rejection [9]. Huang et al. introduced a threshold of ≥0.74% for distinguishing between cell-mediated, antibody-mediated, and mixed rejection from cases with no rejection [8]. Of note, similar to Bloom et al., who advocated for a 1% cut-off, they also excluded patients with borderline lesions from their rejection cohort [7, 8].

The bold values indicate the cut-off calculated in our study and the respective sens/Spec/PPV/NPV in contrast to other studies.

(4 patients with C4d ≥ 1; Spearman's rho = 0.30, $p = 0.002$), and a weak correlation to cg (12 patients with cg ≥ 1; Spearman's rho = 0.21, $p = 0.03$) and to PVI (13 patients with PVI ≥ 1; Spearman's rho = −0.26, $p = 0.009$) (**Supplementary Table S3**). The presence of DSA at either cutoff (MFI > 500 or MFI > 1,000) was not significantly associated with higher dd-cfDNA levels, neither was the presence of non-HLA antibodies (Spearman's rho of −0.19, −0.14, 0.11, respectively). Higher sCD30 levels as a marker of an activated immune system were weakly but significantly associated with higher dd-cfDNA levels (Spearman's rho = 0.2; $p = 0.04$). In the presence of DSA, the AUC for discriminating active rejection including borderline changes from no rejection with the help of dd-cfDNA was 0.77 (95% CI 0.60–0.94) when applying a cutoff of MFI > 500 and 0.75 (95% CI 0.53–0.97) when applying a cutoff of MFI>1,000 for determining DSA (**Supplementary Figure S4**).

Changes in Donor-Derived Cell-Free DNA Upon Treatment

In patients with histopathological signs of ABMR or TCMR, dd-cfDNA decreased significantly when comparing levels at time of biopsy to levels at 7, 30, and 90 days of follow-up ($p = 0.04$, $p = 0.02$, and $p = 0.002$, respectively; **Figure 5A**). For patients with borderline changes who received corticosteroid pulse therapy ($N = 19$), dd-cfDNA decreased significantly from a median of 0.4% (0.2–1.1) at time of biopsy to 0.1% (0.1–0.4) 90 days post-biopsy ($p = 0.03$), whereas no significant differences were seen in eGFR when comparing values

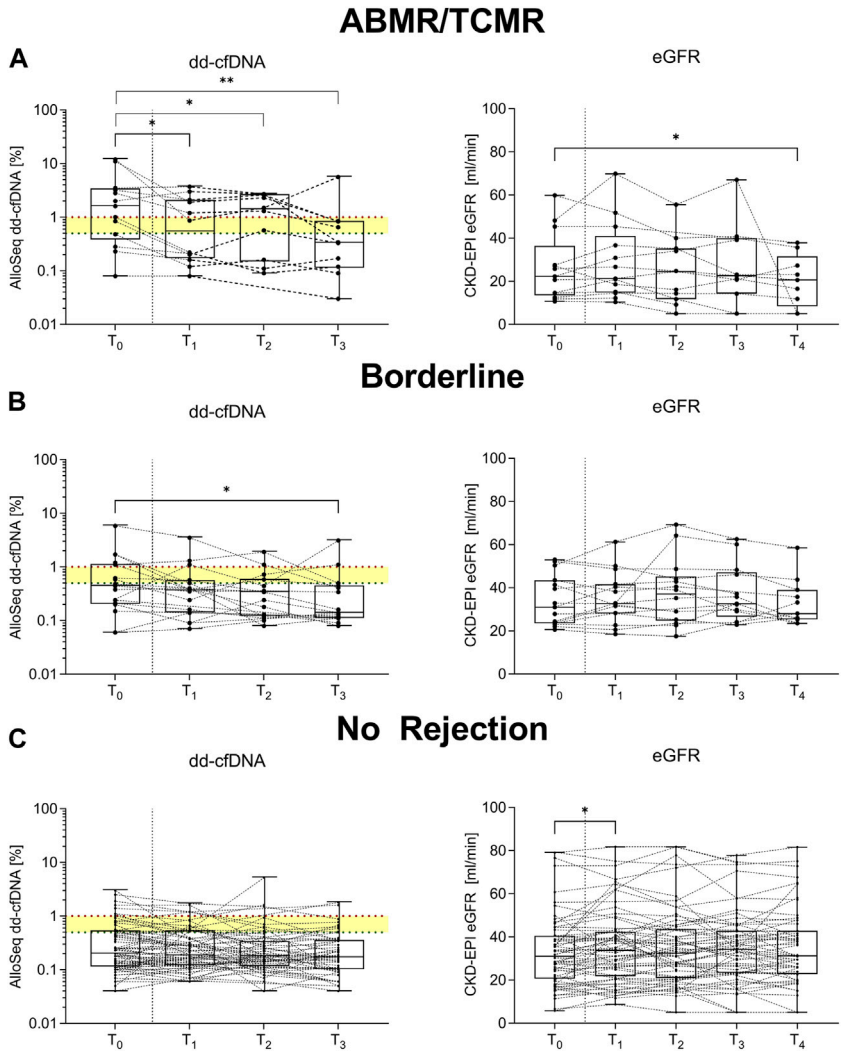


FIGURE 5 | Longitudinal changes in donor-derived cell-free DNA and estimated glomerular filtration rate. **(A)** In patients with antibody-mediated or T cell-mediated rejection ($N = 13$), donor-derived cell-free DNA (dd-cfDNA) decreased comparing levels from time at biopsy (T_0) to 7 days (T_1), 30 days (T_2) and 90 days (T_3) post-biopsy. Estimated glomerular filtration rate (eGFR) remained largely unchanged. **(B)** For patients with borderline changes who received corticosteroid pulse therapy ($N = 19$), dd-cfDNA decreased significantly comparing levels at time of biopsy (T_0) to levels 90 days post-biopsy (T_3), whereas no significant differences were seen in eGFR when comparing values obtained at 7 (T_1), 30 (T_2), 90 (T_3), and 180 days (T_4) follow-up to eGFR at biopsy (T_0). **(C)** No significant differences in dd-cfDNA levels were observed in patients with no histopathological signs of rejection ($N = 72$), whereas eGFR improved slightly in these patients comparing eGFR at biopsy (T_0) to levels at 7 days post-biopsy (T_1). The x-axis displays the respective time point, dd-cfDNA levels are shown log-transformed and eGFR is displayed linearly on the y-axis. Box plots display the distribution of data, with a horizontal line denoting the median. The bottom and top edges of the box indicate the 25th and 75th percentiles, respectively. Individual results are shown as dots. The red dotted line indicates a dd-cfDNA level of 1%, whereas the green dotted line indicates a dd-cfDNA level of 0.5%, corresponding to different cut-points defined in other studies investigating dd-cfDNA as a biomarker for allograft injury. Below the dd-cfDNA level of 0.5%, the risk of rejection is low. ABMR, antibody-mediated rejection; dd-cfDNA, donor-derived cell-free DNA; CKD-EPI eGFR, Chronic Kidney Disease Epidemiology Collaboration estimated glomerular filtration rate; TCMR, T cell-mediated rejection. $^{**}p < 0.01$; $^{*}p < 0.05$.

obtained at 7, 30, 90, and 180 days follow-up to eGFR at biopsy (**Figure 5B**). No significant differences in dd-cfDNA levels were observed in patients without histopathological signs of rejection, whereas eGFR improved slightly in these patients from a median (IQR) of 31.9 mL/min/1.73 m^2 (21.3–40.5) at biopsy to 33.8 (21.8–42.4) 7 days post-biopsy ($p = 0.04$; **Figure 5C**). **Supplementary Tables S4, S5** summarize the changes in dd-cfDNA and eGFR post biopsy, respectively, when analyzing pairs.

DISCUSSION

In this prospective study, we assessed the diagnostic usefulness of dd-cfDNA in a cohort of German kidney transplant recipients with indication biopsy. We found that dd-cfDNA levels were significantly higher in patients with active rejection compared to patients with no rejection. dd-cfDNA discriminated active rejection (including borderline changes diagnosed during allograft dysfunction) from no rejection with an AUC of 0.72.

This is in line with results of Bloom et al. who validated dd-cfDNA in the DART study and found an AUC of 0.74 to discriminate between biopsy showing any rejection (ABMR or TCMR) vs. no histopathological signs of rejection [7]. When excluding borderline changes from the analysis, the AUC for dd-cfDNA to discriminate ABMR or TCMR from no rejection even reached a higher 0.82, albeit including only a small sample size of 13 patients. Huang et al., who reported on their early clinical experience using dd-cfDNA since it became Medicare reimbursable in the United States in October 2017, reported exactly the same AUC of 0.82 for dd-cfDNA to effectively distinguish ABMR from no rejection [8]. Thus, it appears that dd-cfDNA performs particularly well in correctly identifying active ABMR which corresponds to our findings of a significant correlation between increased dd-cfDNA levels to ptc lesion score, matching with findings of Gielis et al. [20]. Since glomerulitis (g) and intimal arteritis (v) were infrequently observed within our cohort, statistical analyses could not be performed for these specific lesions.

Next, we identified that a dd-cfDNA level of 0.57% was best to distinguish rejection (including borderline changes) from no rejection, yielding a specificity of 81%, a sensitivity of 53%, a PPV of 58%, and an NPV of 77%. The cutoffs ≥1.0% and ≥0.74%, as suggested by Bloom et al. [7] and Huang et al. [8], discriminated active rejection from no rejection in our study with specificities of 89% and 82% and sensitivities of 44% and 48%, respectively. It is evident that specificity increases at higher dd-cfDNA thresholds, however, if we used a cutoff of ≥1%, we would have misinterpreted as many as 56% of the 36 patients (2 patients with ABMR, 3 patients with TCMR, and 15 patients with borderline changes) as having no rejection when relying only on the dd-cfDNA levels. It is crucial to highlight that unlike Bloom et al. and Huang et al. we also incorporated patients with borderline lesions into the rejection group which may account for the lower sensitivity and NPV observed at our calculated 0.57% threshold [7, 8]. Specifically, 13 out of 23 (57%) patients with borderline lesions had dd-cfDNA levels below this cut-off and were thus "false negative." In addition, a significant proportion of patients in our study were biopsied at later stages post-transplantation, revealing chronic lesions that were previously shown to be associated with lower dd-cfDNA levels, further impeding sensitivity to correctly identify rejection [21].

On the contrary, 8/24 (33%) patients with dd-cfDNA levels of ≥1% had no histopathological signs of rejection but other causes of graft injury, such as ATI, BKVAN, IFTA, or CNI-Toxicity. Regarding higher levels of dd-cfDNA in patients with no molecular or histologic rejection, Halloran et al. argued that dd-cfDNA may also be released if parenchymal injury is present, such as in acute injury or atrophy fibrosis [21]. The substantial number of patients exhibiting dd-cfDNA levels ≥1% without corresponding histopathological findings for rejection thus emphasizes that dd-cfDNA best serves as an indicator of active graft injury. Evidently, dd-cfDNA cannot differentiate the various causes of acute kidney injury following transplantation, some of which may require opposing treatment approaches. However, as stated by Roy Bloom before, it seems rather unlikely that one biomarker will emerge as a universal solution for diagnosing all kidney transplant-related issues with both high sensitivity and specificity [22]. A more practical approach would involve utilizing a combination of blood and urine biomarkers alongside various clinical parameters to provide comprehensive insights into cellular damage and immune responses [22]. Nonetheless, the expanding body of literature on dd-cfDNA underscores its potential in assisting with the identification of at-risk patients in routine clinical practice.

Another potential benefit of dd-cfDNA lies in its ability to identify patients with rejection in whom injury does not resolve upon corticosteroid pulse therapy, warranting closer monitoring, re-biopsies, and possibly more aggressive therapeutic interventions. This hypothesis is supported by Stites et al. who found that higher levels of dd-cfDNA identified patients with TCMR 1A rejection and borderline changes with more unfavorable clinical outcomes, such as eGFR decline, *de novo* DSA development, and future or persistent rejection [9]. In our study, we observed considerable variability in dd-cfDNA levels among patients with borderline changes (**Supplementary Figure S3**). When we categorized these patients into two groups based on their dd-cfDNA levels at the time of biopsy (either <0.5/1% or ≥0.5/1%) and compared their eGFR trajectories, we observed a tendency towards eGFR improvement in patients with lower dd-cfDNA levels whereas patients with higher dd-cfDNA levels showed stable or declining eGFR, although we could not establish statistical significance. When interpreting these findings, one should consider the controversially discussed pathological relevance of borderline changes. Borderline changes with low dd-cfDNA levels may represent non-pathogenic histological findings that may require no treatment at all. However, this hypothesis is to be tested in future studies.

In addition to helping the clinician to identify patients at risk for rejection or with severe injury, dd-cfDNA may also be of use to assess response to therapy. In agreement with the findings of Wolf-Doty et al. and Hinojosa et al., we observed decreasing levels of dd-cfDNA in patients receiving anti-rejection therapy [23, 24]. However, similar to Wolf-Doty et al., we did not observe any significant changes in eGFR or serum creatinine following treatment [23]. It is important to exercise caution when interpreting these findings as dd-cfDNA primarily serves as an indicator of injury, whereas eGFR reflects graft function. Since we did not routinely conduct re-biopsies, it remains uncertain whether the injury completely resolved with therapy, which is a limitation to our study.

Another limitation of our study is the relatively small number of cases with ABMR ($N = 7$) or TCMR ($N = 6$). However, despite this limitation, our findings align consistently with current literature, supporting the robustness and reliability of the results.

In conclusion, our prospectively designed study verified the good performance of dd-cfDNA to discriminate kidney transplant recipients with active rejection, particularly patients with ABMR, from those with histopathological findings other than rejection. Based on our results, we hypothesize that dd-cfDNA may aid the clinician in monitoring patients at risk, for example, those with *de novo* DSA or previous biopsy-proven rejection, where elevated or increasing dd-cfDNA levels may aid in decision-making regarding the necessity and timing of a graft biopsy. The potential benefit of dd-cfDNA in the assessment of response to therapy and for risk

stratification of patients with borderline changes needs further validation. Additionally, it is yet to be determined whether screening with dd-cfDNA will significantly reduce the number of unnecessary biopsies and can be carried out cost-effectively.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving humans were approved by the Ethikkommission der Universität Heidelberg. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LB, CM, MZ, and CSü designed the study. LB, CM, and TT analyzed and interpreted the data and drafted the manuscript. LB and MR collected the data. AF established and performed the quantification of dd-cfDNA. CSc and RW were responsible for histopathological classification of biopsy specimens. LB, CM, CSp, FK, CN, JB, and MZ were responsible for clinical management of patients. CM, MZ, CSü, and TT supervised the project and revised the manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11899/full#supplementary-material

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MEETING REPORT
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New Approaches to Manage Infections in Transplant Recipients: Report From the 2023 GTI (Infection and Transplantation Group) Annual Meeting

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INTRODUCTION

This year's GTI ("Groupe Transplantation and Infection") annual meeting was held in Paris, France in February 2023. This meeting focused on new approaches to manage infectious complications in solid organ and stem cell transplant recipients.

In this meeting report, we summarize the presentations and discussions from this annual meeting. Covered topics included new anti-infective agents and non-antibiotic approaches to manage infections due to multidrug-resistant Gram-negative bacteria, staphylococci, and fungal infections, as well as new approaches to manage symptomatic urinary tract infections and asymptomatic bacteriuria in kidney

transplant recipients. Innovative approaches are needed to manage infectious complications in transplant recipients, who are at high risk of difficult-to-treat infections and side effects associated with the use of anti-infective agents.

MANAGEMENT OF POST-TRANSPLANT BACTERIAL INFECTIONS

Multidrug Resistant Enterobacterales Infections in Solid Organ Transplantation: Current Situation and New Non-Antibiotic Approaches

Solid-organ transplantation (SOT) is the treatment of choice for patients diagnosed with end-stage organ disease, and the median survival of both recipients and grafts has significantly increased in the last years [1]. While the incidence of infections (including opportunistic ones such as cytomegalovirus [CMV]) is decreasing due to better prevention, the burden of "classical" infections linked to multidrug-resistant (MDR) bacteria especially related to Gram-negative bacilli (GNB) is increasing [2, 3]. Multidrug resistant Enterobacterales are involved in one-third of bacterial infections in SOT recipients [4]. Prior intestinal colonization with ESBL (extended spectrum beta-lactamase)-producing Enterobacterales is an essential prerequisite for the onset of infection among SOT recipients [5]. Furthermore, among patients with intestinal colonisation with MDR (multidrug resistance) Enterobacterales, prior exposure to anti-infectives appears to be a major risk factor for subsequent infection due to the colonizing strain [5]. This can be explained by an increase in intestinal density of resistant Gram-negative bacilli (commonly referred as relative fecal abundance) during antibiotic administration [6]. Antimicrobial stewardship (AMS) programs are designed to improve the quality of prescribing practices in terms of choice of antibiotic, dosage, duration, route of administration and de-escalation. Benoit Pilmis presented innovative AMS strategies aimed at limiting antibiotic-induced dysbiosis, decolonizing patients colonized by MDR Enterobacterales, and restoring a healthy microbiota [7]. The efficacy of oral colistin-neomycin in preventing multidrug-resistant Enterobacterales (MDR-E) infections in solid organ transplant (SOT) recipients have been evaluated previously in a multicentre, randomized, controlled, open-label, parallel-group clinical trial [8] but showed negative results in term of efficacy and tolerance (particularly for colistin).

Among these strategies, the exact benefits of fecal microbiota transplantation (FMT) remain unclear [9]. A multicenter randomized controlled trial (FeCeS study) evaluating the efficacy of FMT in decolonizing carriers of ESBL- or carbapenemase-producing Enterobacterales will provide an answer (NCT05035342). This indication of FMT in decolonizing patients has been evaluated in allo-hematopoietic stem cell transplant (allo-HSCT) recipients a systematic review has been recently published [10]. FMT was performed before or after HSCT but each time on a low number of patients. Decolonization was obtained in 40%–60% of cases. The majority of the included studies report FMT as a generally well tolerated procedure, with no serious adverse events. Interestingly, in the case series of Shouval et al. two patients developed bacteremia after the infusion, but targeted metagenomic sequencing demonstrated that the bacterial strains did not originate from the FMT inoculum [11].

Altogether, FMT seems an interesting option for decolonization, but the safety profile and efficacy of the procedure must be determined more strongly to better assess the role of FMT in allo-HSCT recipients.

One-promising way to protect the gut microbiota is to develop molecules to chelate or degrade the non-absorbed part of orally administered antibiotics and the fraction of oral and parenteral antibiotics excreted in the bile that reach the colon, induce dysbiosis and a decrease in richness and diversity of the microbiota. For example, ribaxamase (an orally administered beta-lactamase hydrolyzing β-lactams in the colon appears promising in Phase 2 studies although limited to β-lactam antibiotics) and DAV-132 which is a millimetric beads consisting of a core of a specific activated charcoal surrounded by a polymer coating that is insoluble during transit. The charcoal is activated in the ileum and adsorbs and thereby inactivates antibiotics in the caecum/colon [12–16]. For now, no investigation of this strategy exist in transplant recipients but its evaluation and implementation are of interest in the TOS patients, a population highly exposed to antibiotics.

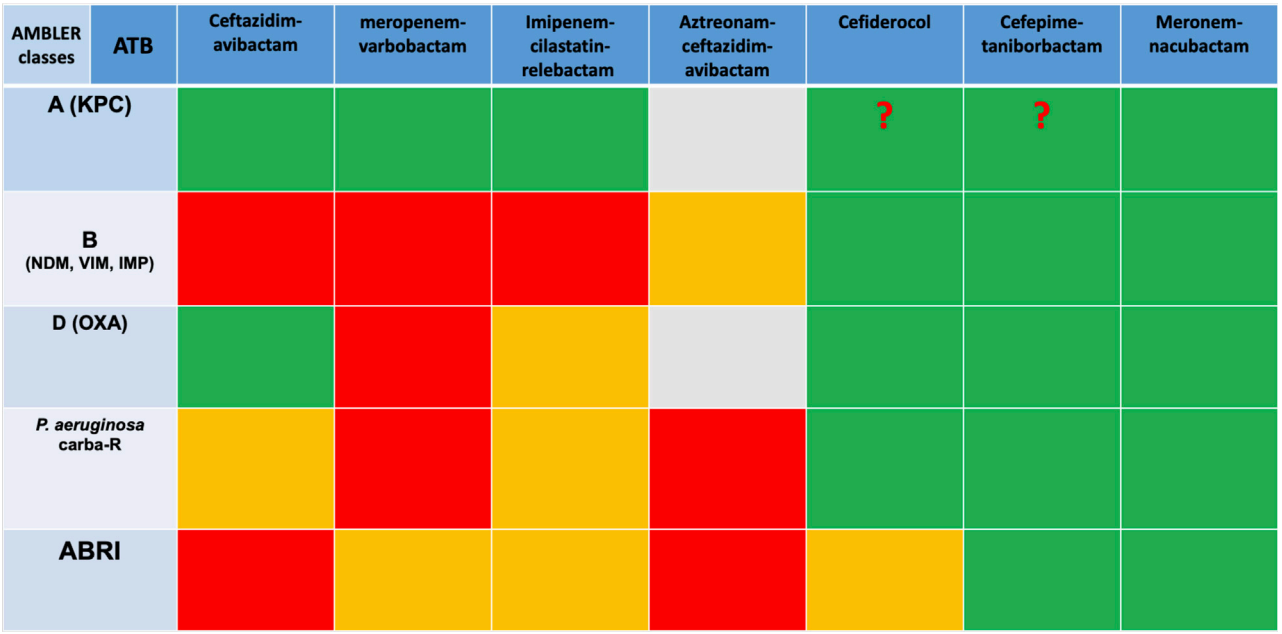
Multidrug Resistant Enterobacterales Infections in Solid Organ Transplantation: New Antibiotics

Antibiotic-resistant Gram-negative bacterial infections are the leading cause of death attributable to antibiotic resistance in Europe and worldwide. This is linked to the epidemic success of 3rd generation cephalosporins (3GC)- resistant Enterobacteriaceae. The widespread use of carbapenems to treat 3GC-resistant strains has led to the emergence of carbapenem-resistant isolates, in particular those secreting carbapenemases, with very limited therapeutic options. New molecules have recently been developed to combat carbapenem-resistant bacteria. Victoire de Lastours summarized the updated antimicrobial management of carbapenem-resistant bacteria related infection.

These include ceftazidime-avibactam, a combination of a 3GC with a new betalactamase inhibitor, avibactam. This combination is effective on strains carrying OXA 48 or KPC, but not metallobetalactamases. This molecule was granted authorization in Europe and the USA following 3 phase 3 trials in complicated intra-abdominal infections versus meropenem, as well as two trials in complicated urinary tract infections yielding non-inferiority. In a retrospective cohort study of 210 SOT recipients with carbapenemase-producing *Klebsiella pneumoniae* blood stream infections, ceftazidime-avibactam significantly increased the probability of 14 and 30 days clinical success, as compared to the best available therapy [17].

A second compound, meropenem-varbobactam, is also active against class A betalactamases (KPC) and cephalosporinases, but inactive against metallobetalactamases and oxacillinases, which limits its interest in some European coutries such as France,

TABLE 1 | Spectrum of new antibiotics regarding the type of resistance.



Abbreviations: ABRI, Acinetobacter *baumani mutli resistant; ATB, antibiotic; carba-R, carbapenem-resistant.*

TABLE 2 | Spectrum of activity, tissue diffusion and drug-drug interactions (DDIs) with immunosuppressive drugs of olorofim, ibrexafungerp and rezafungin.

Molecule	Spectrum of activity	Diffusion	DDIs with immunosuppressive drugs	Potential advantages
Olorofim	*Aspergillus* spp. *Scedosporium* spp. *Lomentospora prolificans Fusarium* spp. *Histoplasma capsulatum Blastomyces dermatitidis Coccidioides* spp.	• Good diffusion in kidney, liver, and lung • Low levels in CNS [54]	• Substrate of several CYP450 enzymes: anticipate dose reduction if given with a strong 3A4 inhibitor (or a moderate dual 3A4+2C9 inhibitor) • Weak inhibitor of CYP3A4: small reductions of tacrolimus and sirolimus might be needed (guided by standard monitoring)	Active against highly resistant molds
ibrexafungerp	*Candida* spp. including echinocandin resistant *C. glabrata* and *C. auris Aspergillus* spp. *Paecilomyces variotii Pneumocystis jirovecii*	• Good diffusion in liver, spleen, lungs, bone marrow, kidney, skin and uvea • Low levels in CNS [65]	• Substrate of CYP3A and P-glycoprotein: avoid coadministration of strong CYP3A inducers • Reversible inhibitor of CYP2C8 and CYP3A4 • interaction with tacrolimus: 1.4-fold increase in AUC; no change in tacrolimus Cmax [66]	• Active against resistant *Candida* species • First orally bioavailable inhibitor of [1(3)- β-D-glucan synthase]
Rezafungin	*Candida* spp. *Aspergillus* spp. *Pneumocystis jirovecii*	Improved drug penetration in liver and kidney abscesses (mouse model of intra-abdominal candidiasis) in comparison with micafungin [67]	Minimal inhibition of CYP450 enzymes [68]: Limited reduction (10%–19%) of the AUC or Cmax of tacrolimus, ciclosporine and mycophenolic acid (probably not clinically meaningful) [69]	• Long half-life allows once weekly dosing • Less hepatotoxicity • May prevent Pneumocystis pneumonia [61, 62]

where KPCs are rare. Non-inferiority has been demonstrated in several trials against optimized treatment. A third molecule, imipenem-relebactam, is also active against KPCs but not against oxacillinases or metallobetalactamases. Imipenem-relebactam is also effective against carbapenem-resistant strains of *Pseudomonas aeruginosa*, but not against carbapenem-resistant *Acinetobacter baumanii* (CRAB). The molecule has been approved in France only as a last resort for the treatment of patients with no other possible therapeutic alternative, and in particular if KPC-type carbapenemase are produced.

Altogether, several choices are now available to treat KPC and OXA-48 oxacillinases which are approved in France and Europe.

For carbapenem-resistant *P. aeruginosa*, ceftolozane-tazobactam is generally effective. Tolerance is generally good (as with beta-lactams), and these molecules are bactericidal. However, these molecules are not effective against metallobetalactamases nor against most CRAB, which poses major therapeutic problems. Its use was reported in a multicenter cohort study of 69 immunocompromised patients including 47 SOT, with multi-drug resistant *P. aeruginosa* infections, mostly respiratory and wound. Clinical cure was achieved in 68% and mortality was 19% [18].

A recently approved molecule, cefiderocol, is a siderophore cephalosporin which uses the bacterial iron entry machinery to achieve high concentrations inside the bacteria. It is unaffected by betalactamases, even metallobetalactamases, and acts as a Trojan horse. In pivotal trials, cefiderocol showed non-inferiority to high-dose meropenem in the treatment of gram-negative nosocomial pneumonia, except for *A. baumanii* infections, a result that remains unexplained. Cefiderocol has been marketed in Europe and the USA only as a last resort for infections caused by multi-resistant gram-negative bacteria, notably in cases of KPC and metallo-betalactamases.

This molecule therefore represents an important therapeutic hope, although it appears to have a relatively significant inoculum effect, which needs to be better studied. Finally, some cefiderocol-resistant strains have been described, combining several resistance mechanisms. To date, very few data are available in specific immunocompromised settings including solid organ transplantation [19], hematological malignancies [20, 21]. Most Cefiderocol prescriptions have primarily targeted multi-resistant severe *P. aeruginosa* infections, but its use has broadened to other difficult-to-treat non-fermentative gram negative bacteria, especially *S. maltophilia* for which its complex virulence and resistance profile drastically limit available antibiotics. Updated clinical and safety outcome data are needed in highly susceptible immunocompromised settings.

Another interesting combination in this context is ceftazidime-avibactam + aztreonam for strains carrying metallo-betalactamases. Several studies have demonstrated the efficacy of the avibactam + aztreonam combination, which is currently being developed by the manufacturer. An inoculum effect could also have an impact on the efficacy of this combination. This combination proved effective and safe in a serie of 4 SOT recipients with metallo-β-lactamase carbapenemase-producing Enterobacteriaceae [22].

Lastly, plazomicin, an aminoglycoside developed for the treatment of carbapenem-resistant Enterobacteriaceae infections, had shown interesting results in the United States, but was not developed in Europe due to its low commercial potential.

Treatment recommendations for carbapenem-resistant infections are summarized in the 2022 ESCMID guidelines [23]. Several new molecules are under development and could be of interest for the treatment of these infections, particularly those due to organisms producing a metallobetalactamase, such as cefepime-taniborbactam and meropenem-nacubactam. Studies are currently underway.

Finally, in the face of this type of infection, optimizing the use of available molecules is a crucial point, including rapid diagnosis of resistance, determination of MICs (minimal inhibitory concentration) for the different molecules and combinations available, and optimization of dosages with the use of high doses and prolonged infusions. Last but not least, multidisciplinary discussions between microbiologists and clinicians and the reduction of bacterial inoculum through drainage are essential. A summary of antibiotics efficiency regarding resistance mutation has been made in **Table 1**.

New Approaches to Manage Urinary Tract Infections in Kidney Transplant Recipients

The management of urinary tract infections (UTIs) in kidney transplant recipients represents a major opportunity for antimicrobial stewardship because kidney transplantation is the most common type of organ transplant worldwide, and because UTI is the most common infection in this population [3, 24]. Julien Coussement summarized the most recent evidence about the management of post-transplant symptomatic UTI and asymptomatic bacteriuria, and identified gaps of knowledge and clinical scenarios that remain understudied.

Asymptomatic bacteriuria, which is generally defined as significant bacteriuria (≥100.000 CFU/mL) without signs or symptoms of UTI (e.g., fever, chills, kidney pain, or symptoms of bladder inflammation), is relatively common after kidney transplantation [24].

Recent randomized trials have shown that the historical practice of screening for and treating asymptomatic bacteriuria is not beneficial in stable kidney transplant recipients [25–28]. A limited-size trial even suggested that asymptomatic bacteriuria might be left untreated in patients who are in the first 2 months post-transplant and have a ureteral stent [29]. Additional opportunities probably exist to improve the care of kidney transplant recipients with pyelonephritis. First, research is needed to determine the benefits and harms associated with the empiric use of very broad-spectrum antibiotics in kidney transplant recipients admitted for presumed pyelonephritis [24]. Second, a randomized trial is starting to determine whether 7 days of antibiotic therapy can be sufficient to treat non-severe episodes of pyelonephritis in kidney transplant recipients who are beyond the first month post-transplant and do not have a urinary catheter [30–32].

Besides, innovative non-antibiotic-based approaches are needed to better prevent symptomatic UTIs, which remain prevalent and detrimental after kidney transplantation. Julien Coussement discussed the potential benefits, harms and applicability of emerging approaches, including anti-adhesion therapies (which aim at preventing bacterial adhesion to host tissues, and therefore decreasing the risk of UTI) [33], intravesical instillation of a low-virulence organism (which aims at promoting bacterial interference) [34], and FMT (which aims at repopulating the gut with a "healthy" microbiome that could outcompete uropathogens) [35–38]. Vaccine candidates that are in development against extra-intestinal pathogenic *Escherichia coli* are also promising [39]. Many challenges, however, exist, including the fact that transplant recipients generally have an

impaired immune response to vaccines, and the fact that around half of the UTI episodes which occur after kidney transplantation are due to microorganisms other than *E. coli.*

New Antibiotics to Treat Infections Due to Gram-Positive Cocci

Aurélien Dinh reminded the drawbacks of vancomycin and daptomycin, before presenting new antibiotics targeting gram-positive cocci.

Vancomycin is a relatively old and difficult-to-manage glycopeptide. Several new antibiotics with activity against methicillin-resistant Staphyloc*occi* are now available.

Daptomycin is bactericidal and as effective as penicillin M against methicillin-susceptible *Staphylococcus aureus* and vancomycin for methicillin-resistant *S. aureus,* according to a randomized controlled trial (RCT) on bloodstream infections (BSI) [40]. Nevertheless, some treatment failures due to inoculum effect have been observed, and bacterial resistance is described, even among patients without previous exposure to this drug, which could be due to *in vivo* exposure to endogenous cationic peptides [41]. In liver transplant recipients, such resistance was indeed associated with prior daptomycin use and increased mortality [42]. In kidney transplant recipients, combinations of daptomycin and other antibiotics have also been suggested for resistant enterococcal infections [43, 44].

Dalbavancin is a new long acting glycolipopeptide, with a half-life of 14 days. MIC of dalbavancin against *S. aureus* and resistant coagulase-negative staphylococci are low. One retrospective cohort compared dalbavancin *versus* standard of care in patients with *S. aureus* bacteremia and found no significant difference [45]. Two RCTs are currently underway to better determine the effectiveness of dalbavancin in patients with *S. aureus* bacteremia [46, 47]. Dalbavancin is of particular interest for patients requiring prolonged antibiotic therapy, such as those with endocarditis or bone and joint infection (BJI) such as prosthetic joint infections. Several cohorts and literature reviews found dalbavancin to be safe, with nearly 80% cure rate in these indications and high level of patient satisfaction, mostly due to early discharge [48].

Ceftaroline and ceftobiprole are new generation cephalosporins with excellent activity against methicillin-resistant staphylococci according to bacterial killing curves [49]. Clinical efficacy during BJI and endocarditis are promising according to cohort studies [50, 51]. The ERADICATE trial comparing ceftobiprole *versus* daptomycin in *S. aureus* bacteremia showed non-inferiority [52].

So far, to our knowledge, no data exist regarding the use of dalvabancin, ceftaroline and ceftobiprole in SOT recipients.

Finally, oritavancin is a recently available lipopeptide, with a semi long-life activity (7 days) and important intra-cellular activity, which could be of interest for device-associated infection with biofilm [53].

These new antibiotics may allow new management and innovative approaches to treat patients with infections due to resistant Staphylococci.

MANAGEMENT OF FUNGAL INFECTIONS

Because of the toxicities of the available drugs and the emergence of resistance caused by an increased use of antifungal agents in the growing population at risk of invasive fungal diseases and in agriculture, there is a pressing need for more antifungal drug options. Recently, several new antifungal drugs have reached late-stage clinical development and obtained a temporary use authorization, as depicted by Alexandra Serris.

Olorofim is the only member of a novel class named orotomide. It inhibits fungal growth through inhibition of the fungal dihydroorotate dehydrogenase enzyme involved in pyrimidine synthesis. It has a good tissue distribution, notably in the kidney, liver, lung, and the brain (although at lower levels) [54]. It is metabolized by several CYP450 enzymes including CYP3A4 and is thus susceptible to strong CYP3A4 inhibitors and inducers. Olorofim exhibits activity *in vitro* against azole-resistant *Aspergillus, Scedosporium*, *Lomentospora*, *Rasamsonia*, dimorphic fungi (notably *Histoplasma*), dermatophytes, but has no activity against yeasts, *Mucorales* and *Alternaria alternata* [55, 56].

Olorofim is currently evaluated in two clinical studies: one open-label, single-arm study including patients with invasive fungal infections due to Lomentospora prolificans, Scedosporium spp., Aspergillus spp., and other resistant fungi with limited treatment options (ClinicalTrials.gov identifier: NCT03583164) and one phase III, randomized study to evaluate the efficacy and safety of olorofim versus liposomal amphotericin B in patients with invasive aspergillosis (ClinicalTrials.gov Identifier: NCT05101187). Published experience is currently limited to case reports (abstracts).

Ibrexafugerp is a first-in-class oral glucan synthase inhibitor, whose mechanism of action is close to the one of echinocandins (but with a different binding site). It is fungicidal against most wild-type, echinocandin or azole-resistant *Candida* spp., including *C. auris*, and fungistatic against *Aspergillus* spp [57]. Based on animal models, ibrexafungerp shows a high tissue penetration in the spleen, liver, lungs, kidney, vaginal tissue, and muscles, but not in the brain [58].

An interim analysis of the phase III FURI study evaluating the efficacy and safety of ibrexafungerp in patients with severe mucocutaneous candidiasis, invasive candidiasis, chronic or invasive aspergillosis reported complete or partial response in 58% of the patients [59]. Inclusion criteria were further expanded to include histoplasmosis, coccidioidomycosis and blastomycosis.

Rezafungin is the first member of second-generation echinocandins with enhanced pharmacokinetic/pharmacodynamic parameters, allowing for a weekly administration and potential less hepatic toxicity [60]. It has potent *in vitro* activity against most *Candida* spp., including *C. auris*, and common dermatophytes [58].

Moreover, rezafungin has shown promising results as prophylactic and curative treatment of pneumocystis *in vivo* by eradicating both the cyst and trophic forms of the fungus [61, 62]. A case report of the successful eradication of a refractory intra-abdominal candidiasis with rezafungin in a liver transplant recipient was published in 2022 [63] and rezafungin was recently

found non-inferior to caspofungine in a Phase 3 trial (ReSTORE) for the treatment of candidemia/invasive candidiasis [64].

These antifungal treatments offer significant improvement in terms of spectrum of activity, tolerability, drug interactions and/or route of administration. Further clinical studies will be needed to evaluate their optimal place in the therapeutic arsenal in the solid organ transplant recipient population, taking into account the emergence of drug-resistant fungi and the problem of drug-drug interactions with immunosuppressants. **Table 2** summarize the Spectrum of activity, tissue diffusion and drug-drug interactions (DDIs) with immunosuppressive drugs of olorofim, ibrexafungerp and rezafungin.

CONCLUSION

During the well-attended "**Infection and Transplantation Group**" day, the major advances in the field of **new anti-infective therapies in transplantation** were presented and discussed. New direct and indirect anti-infective approaches in transplantation are devoted to several improvements:

- decrease antibiotics pressure in our high risk multidrug resistant bacteria population with a better use of already known antibiotics and new original non-antibiotic approaches that have promising usages.
- improve efficacy of bacterial and fungal treatment with antibiotics or antifungal therapy that have a good inoculum effect and a good broadcast
- improve the tolerance of antimicrobial drugs in our polymedicated population with high risk of drugs interactions.

Altogether, those new approaches are likely to feature alternative anti-infective therapies that promise to change patient management.

AUTHOR CONTRIBUTIONS

AlS, JC, BP, VD, AD, and HK wrote the manuscript. AD, AS, FA, OL, EM, NK, EF, DL, JD, FC, AL, and HK revised the manuscript. AS, FA, OL, EM, NK, EF, DL, JD, FC, AL, and HK conceived the manuscript.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Organ Repair and Regeneration During *Ex Situ* Dynamic Preservation: The Future is Nano

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Organ preservation and assessment with machine perfusion (MP) has provided transplant physicians with the ability to evaluate and select grafts suitable for transplantation. Nevertheless, the discard of organs considered too damaged still sustains the imbalance between donor organs supply and demands. Therefore, there is the pressing clinical need for strategies to repair and/or regenerate organs before transplantation, and MP is uniquely positioned to satisfy this need. The systemic administration of mesenchymal stromal cells (MSC) was shown to reduce ischemia-reperfusion injury in pre-clinical organ transplant models but could not be reproduced in clinical transplantation, largely because of inefficient cell delivery. The administration of MSC during MP is one strategy that recently gained much attention as an alternative delivery method to target MSC directly to the donor organ. However, careful reinterpretation of preliminary results reveals that this approach is equally limited by a suboptimal delivery of short-lived MSC to the target organ. In contrast, the use of MSC secretome and/or extracellular vesicles therapy during MP seems to be more efficient in harnessing MSC properties during MP. In this mini review we speculate on the future of the novel niche of *ex situ* organ repair and regeneration before transplantation.

Keywords: organ repair, organ regeneration, machine perfusion, dynamic preservation, organ preservation, mesenchymal stromal cell, extracellular vesicles

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INTRODUCTION

The field of organ preservation for transplantation has undergone significant changes due to the increasing use of grafts from high-risk donors. The need for improved preservation of these organs has prompted a progressive shift from static cold storage to dynamic organ preservation strategies, also known as machine perfusion (MP). Dynamic organ preservation strategies have also moved the field at the intersection with regenerative medicine as they provide a platform for repairing and regenerating organs before transplantation [1]. However, prolonged *ex situ* preservation for multiple

Abbreviations: EV, extracellular vesicles; IRI, ischemia-reperfusion injury; MAPc, multipotent adult progenitor cells; MP, machine perfusion; MSC, mesenchymal stromal cells; NMP, normothermic machine perfusion.

days is likely required to achieve clinically meaningful organ repair and regeneration during MP. Recent advancements in liver normothermic-MP (NMP), which allows for the preservation of the liver for up to 1 week [2], suggest that this may soon be attainable for all other transplantable organs.

To this end, several interventions during MP have been proposed, including cell therapy, pharmacological agents, gene modulation and editing, and nanoparticles [3]. Whereas most of these strategies are still in early stages of investigation, numerous pre-clinical studies have shown that the systemic administration of mesenchymal stromal cells (MSC) reduces ischemia-reperfusion injury (IRI) during organ transplantation [4]. MSC suppress the inflammatory response, downregulate innate and adaptive immunity, and promote organ regeneration, thereby interfering with the major pathophysiological events of IRI in transplantable organs [4]. However, the clinical application of MSC systemic treatment during organ transplantation has failed to replicate these results [5], one of the major putative cause being the inefficient delivery of MSC to the target organ. Therefore, MSC administration during MP has gained interest as an alternative method to deliver the cells directly to an organ and circumvent the shortcomings of systemic administration. Nevertheless, the efficiency of this approach in delivering MSC-therapy to organs for transplantation remains underinvestigated. In this narrative minireview, we summarize results and limitations of MSC-therapy during MP based on available evidence from published studies. To select these studies, we utilized a systematic literature search approach, a rigorous method for minimizing biases during evidence selection (see **Supplementary Material** for additional method information). Additionally, we hypothesize a path towards a cell-free future for *ex situ* organ repair and regeneration.

NOVEL DELIVERY METHOD, SAME SHORTCOMINGS

Unlike preclinical transplant models of MSC-therapy, clinical studies have failed to show significant benefits from systemic MSC administration on post-transplant IRI [6, 7]. This was ascribed to inefficient and off-target delivery of cells to the graft [5]. Systemic infusion of MSC are short-lived as the cells are primarily sequestered in the lungs and eliminated by resident monocytes [5]. Administration of stem cells after organ transplantation has also been associated with a pro-inflammatory effect, which further damages the graft [8]. Lastly, calcineurin inhibitors suppress the immunomodulatory properties of MSC *in vitro* [9]. To overcome these hurdles, it was proposed to deliver the cells directly to an organ during MP, before the full extent of IRI events has occurred and interference with immunosuppressive agents can take place.

However, the currently available pre-clinical evidence shows that MSC delivery during MP presents shortcomings similar to those of systemic therapy, as summarized in **Table 1** and depicted in **Figure 1**. A significant proportion of MSC injected through the vascular cannula during MP are eliminated by a "device barrier," constituted by oxygenator(s) and filter(s), which remove the cells from the perfusate similarly to the "lung barrier" phenomenon in systemic MSC-therapy. In a porcine kidney study, Pool et al. demonstrated that 90% of infused MSC are eliminated from the perfusate in a NMP circuit operated without the organ, and that only a few MSC were retained after the first passage through the kidney [10]. In a porcine lung NMP model, Mordant et al. found MSC sequestered in the leukocyte filter [11]. Similarly, Laing et al. did not observe any cell in left hepatic segments after selectively delivering multipotent adult progenitor cells (MAPc) to the right hemi-liver during NMP of discarded human grafts [12]. These results indicate that, at best, only a (small) fraction of MSC is effectively retained in the perfused organ. Additionally, biodistribution studies have shown that MSC have an inhomogeneous distribution in otherwise well-perfused kidney [10] and liver [13] grafts. In accordance with this, histological studies have shown that <10 cells per high-power field are found outside of the vascular space during MP of the liver [12, 13], lung [11], and kidney [14, 15]. Although in the study by Pool et al. increasing the dose of MSC increased the number of cells observed at histology, a dose of MSC far exceeding the previously suggested range for MSC systemic therapy was needed to visualize the cells in the glomeruli of porcine kidneys [10]. Lastly, five other studies reported that MSC did not leave the perfusate or migrate out of the vascular lumen during MP of human and porcine kidneys [16] and rat livers [17–20] (**Table 1**).

Next to the "device barrier" and low cell retention rates, there are also indications that MSC infused during MP are short-lived, likely due to factors such as mechanical trauma, perfusate toxicity, or phagocytosis by resident monocytes. Pool et al. consistently observed disintegrated MSC in porcine glomeruli colonized by stem cells [10], whereas Thompson et al. reported that at the end of NMP of discarded human kidneys only 21% of the MAPc still circulating in the perfusate were viable (**Table 1**) [15]. Research has shown that, compared to standard culturing medium, suspending MSC in a standard red blood cells-based MP perfusate reduces significantly their survival and adherence to endothelial cells [21]. Additionally, because monocytes were already shown to phagocyte MSC [5], it is plausible that resident monocytes and/or passenger leukocytes will eliminate MSC during MP (**Figure 1**). However, to date this phenomenon has not been investigated yet.

Despite the low cell retention rates, there are indications of significant anti-inflammatory [15, 22], immunomodulatory [18] and pro-regenerative [16, 20] effects of MSC-therapy during MP (**Table 1**). Nevertheless, the clinical relevance and durability remain unclear as the few studies that transplanted MSC-treated grafts have only reported short-term follow-ups with contrasting results (**Table 1**). Rat livers were transplanted after MSC-therapy during MP, showing significant improvement of survival and reduction of the incidence of acute cellular rejection at 14 days post-transplant [18]. Porcine lungs treated with MSC were transplanted and followed up for 4 h after reperfusion,

TABLE 1 | Summary of findings of studies investigating stem cell therapy delivery during *ex situ* dynamic organ preservation identified after systematic search of the literature (details in **Supplementary Material**). Results from preliminary studies investigating extracellular vesicle therapy during machine perfusion of transplantable organs are also summarized.

Studies investigating mesenchymal stromal cell delivery during dynamic organ preservation													
Study	**Subject**	**Organ**	**Organ transplant**	**Type and duration MP**	**MSC type**	**MSC dose**	**MSC paracrine activity during MP**	**Device barrier**	**MSC migration from vascular space**	**MSC engraftment[a]**	**MSC Viability**	**MSC therapeutic effect**	**Effect without engraftment**
[36]	Human	Lungs (discarded)	No	Normothermic, 4 h	Human BM-MSC	$5*10^6$	NA	NA	NA	NA	NA	↑ alveolar fluid clearance	NA
[8]	Human	Lungs (discarded)	No	Normothermic, 4 h	MAPc	10^7	NA	NA	NA	NA	NA	↓ BAL cellularity & histological inflammation	NA
[9]	Pig	Lungs	No	Normothermic, max 12 h	Human UC-MSC	$50*10^6$ $150*10^6$ $300*10^6$	NA	Yes, MSC trapped in filters	Yes, some cells in the lumen at histology	Yes,<10 cells/ HPF	NA	↓ IL-8 perfusate concentration	NA
[10]	Rat	Kidneys	No	Hypothermic, 4 h	Rat BM-MSC	$3*10^6$	NA	NA	Yes	Yes,<10 cells/ HPF	NA	↓ severity histological damage	NA
[37]	Pig	Lungs	No	Normothermic, 6 h	MAPc	$150*10^6$	No	NA	No	No	NA	No significant therapeutic effect	NA
[34]	Mouse	Lungs	No	Normothermic, 1 h	Human UC-MSC	$3*10^6$	NA	NA	NA	NA	NA	↑ compliance ↓ inflammation, neutrophil infiltration & oedema	NA
[38]	Rat	Liver	No	Normothermic, 2 h	Swine AD-MSC	$0.2*10^6$ 10^6	NA	NA	NA	NA	NA	No significant therapeutic effect	NA
[11]	Human	Kidneys (discarded)	No	Sub-normothermic, 24 h	Not specified	$25*10^6$ $50*10^6$ $75*10^6$ $1*10^8$ $2*10^8$	Yes	No	No, 95% MSC still circulating at the end of MP	No	NA	↑ renal cell proliferation & tissue regeneration	Yes
[12]	Pig	Kidneys	No	Normothermic, 7 h	Human AD-MSC & BM-MSC	$1*10^5$ $1*10^6$ $1*10^7$	NA	Yes, Inhomogeneous distribution in well perfused kidneys	No	No	Disintegrated MSC in colonized glomeruli	Study investigating feasibility and biodistribution	NA
[13]	Pig	Lungs	Yes, f-up 4 h	Normothermic, 12 h	Human UC-MSC	$50*10^6$/ Kg	Yes	NA	Yes, Unspecified proportion of MSC remained in the lumen	Yes, alveolar interstitium	Yes, indirect evidence based on production of human cytokines	During MP: ↓ apoptosis & perfusate concentration of IL-18 and IFNγ, ↓ peak airways pressure Post-transplant: ↓ oedema & severity histological injury, f-up limited to 4 h	NA

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TABLE 1 | (*Continued*) Summary of findings of studies investigating stem cell therapy delivery during *ex situ* dynamic organ preservation identified after systematic search of the literature (details in **Supplementary Material).** Results from preliminary studies investigating extracellular vesicle therapy during machine perfusion of transplantable organs are also summarized.

Studies investigating mesenchymal stromal cell delivery during dynamic organ preservation													
Study	**Subject**	**Organ**	**Organ transplant**	**Type and duration MP**	**MSC type**	**MSC dose**	**MSC paracrine activity during MP**	**Device barrier**	**MSC migration from vascular space**	**MSC engraftment[a]**	**MSC Viability**	**MSC therapeutic effect**	**Effect without engraftment**
[14]	Human	Kidneys (discarded)	No	Normothermic, 7 h	MAPc	$50*10^6$	Yes	No	Yes, Unspecified proportion of MSC kept circulating at the end of MP	Yes, glomeruli in the cortex, peritubular space in the medulla	21% of circulating MSC were viable	↑ urinary output & medullar flow ↓ urinary concentration NGAL & perfusate concentration IL-1β ↑ perfusate concentration IL-10	NA
[15]	Human	Liver	No	Normothermic, 6 h	MAPc	$50*10^6$	Yes	Yes, MSC infused via left hepatic vessels did not reach right segments	Yes, only if infused via the hepatic artery	Yes, only if infused via the hepatic artery	NA	↓ perfusate concentration pro-inflammatory cytokines ↑ perfusate concentration anti-inflammatory cytokines	Yes
[16], [39]	Rat	Liver	No	Normothermic, 8 h	Rat BM-MSC	$1–3*10^7$	NA	NA	No	No	NA	↓ perfusate AST/ALT and severity histological damage ↓ mitochondrial injury	Yes
[17]	Pig	Liver	No	Hypothermic for MSC delivery, 30 min Normothermic for functional assessment, 4 h	Human BM-MSC	$5*10^6$ $1*10^7$	Yes	Yes, inhomogeneous distribution in well perfused livers	Yes	Yes	Yes, indirect evidence based on production of human cytokines	Study investigating feasibility and biodistribution	NA
[12]	Pig	Kidneys	No	Normothermic, 7 h	Human AD-MSC & BM-MSC	$1*10^7$	Yes	NA	NA	NA		No significant therapeutic effect	NA
[18] [b]	Pig	Kidneys	Yes, f-up 14 days	Normothermic, 4 h	Human AD-MSC	$1*10^7$	NA	NA	NA	Yes, Y human chromosome detected in parenchyma but circa 20-fold ↓ 14 days post-transplant	NA	No safety concern during perfusion, No significant post-transplant therapeutic effect	NA
[19]	Rat	Liver	Yes, f-up 14 days	Normothermic, 4 h	Rat BM-MSC[c]	$1*10^7$	NA	NA	No	No	NA	↓ post-transplant AST/ALT release & acute cellular rejection	Yes

(Continued on following page)

TABLE 1 | (*Continued*) Summary of findings of studies investigating stem cell therapy delivery during *ex situ* dynamic organ preservation identified after systematic search of the literature (details in **Supplementary Material).** Results from preliminary studies investigating extracellular vesicle therapy during machine perfusion of transplantable organs are also summarized.

Studies investigating mesenchymal stromal cell delivery during dynamic organ preservation

Study	Subject	Organ	Organ transplant	Type and duration MP	MSC type	MSC dose	MSC paracrine activity during MP	Device barrier	MSC migration from vascular space	MSC engraftment[a]	MSC Viability	MSC therapeutic effect	Effect without engraftment
[20]	Rat	Liver	No	Normothermic, 6 h	Rat BM-MSC	$1*10^7$ $3*10^7$	NA	NA	No	No	NA	↓ severity of ferroptosis & perfusate AST/ ALT concentration	Yes
[21]	Rat	Liver	Yes, f-up 14 days	Normothermic, 4 h	Rat	$1–3*10^7$	NA	NA	NA	Unspecified location in the hepatic tissue	NA	During MP: ↑ proliferation cholangiocyte extrahepatic bile duct and preservation of epithelial lining	NA
					BM-MSC[c]							Post-transplant: ↓ AST/ALT/GGT/bili 7 days post-transplant; ↑ proliferation & ↓ apoptosis peribiliary glands	

Studies investigating extracellular vesicles delivery during dynamic organ preservation

Study	Subject	Organ	Organ transplant	Type and duration MP	Source of EV	EV dose	EV uptake confirmed	EV therapeutic effect	Compared to MSC
[22]	Human	Lungs (discarded)	No	Normothermic, 6 h	Human BM-MSC	100–200 µL	*In vitro* only (human alveolar epithelial type 2 cell line)	↑ alveolar fluid clearance & ↓ oedema and weight gain, ↑ compliance	No
[10]	Rat	Kidneys	No	Hypothermic, 4 h	Rat BM-MSC	Concentration not reported, EV released by $3*10^6$ cells	NA	↓ perfusate LDH and MDA, ↑ glucose metabolism,↓ severity histological damage	Yes, magnitude of effects of EV > MSC
[34]	Mouse	Lungs	No	Normothermic, 1 h	Human UC-MSC	Concentration not reported, EV released by $3*10^6$ cells	NA	↑ compliance, ↓ inflammation, neutrophil infiltration & oedema	Yes, magnitude of effects of EV = MSC
[23]	Rat	Liver	No	Normothermic, 4 h	Human liver stem-like cells	$5*10^8$ EV/g of liver	Yes, intracellular localization in hepatocytes	↓ perfusate AST & severity histological damage	No
[24]	Rat	Lungs	No	Normothermic, 3 h	Human BM-MSC	$24.56 \pm 5.53 *10^{10}$ EV/mL, 5 mL were administered	Yes, intracellular localization in alveolar cells	↓ total vascular resistance, ↑ glucose metabolism and tissue content of ATP	No
[25]	Rat	Liver	No	Normothermic, 6 h	Human liver stem-like cells	$5*10^8$ EV/g of liver $25*10^8$ EV/g of liver	Yes, intracellular localization in hepatocytes	↓ perfusate AST/ALT & ↑ bile excretion, ↓ necrosis & ↑ hepatocellular proliferation	No
[26]	Human	Kidneys (discarded)	No	Hypothermic, 4 h	Human BM-MSC	28.5*109	NA	↓ apoptosis & ↑ tubular cells proliferation, ↓ mitochondrial injury	No

[a]*Refers to the visualization of MSC between parenchymal cells (outside of the vascular lining) at histology. When available, the estimated cellular concentration is reported.*

[b]*In this study, porcine kidneys underwent 14 h preservation with hypothermic oxygenated MP, followed by 4 h of normothermic MP with or without MSC infusion.*

[c]*In these studies, MSC were modified to overexpress the enzyme heme oxygenase 1.*

Abbreviations: AD-MSC, adipose-derived mesenchymal stem cells; ALT, alanine transaminase; AST, aspartate transaminase; ATP, adenosine triphosphate; BAL, bronchoalveolar lavage; BM-MSC, bone marrow-derived mesenchymal stem cells; EV, extracellular vesicles; HPF, high-power field; IFNγ, interferone gamma; IL-1β, interleukin 1 beta; IL-8, interleukin 8; IL-10, interleukin 10; IL-18, interleukin 18; LDH, lactate dehydrogenase; MAPc, multipotent adult progenitor cells; MDA, malondialdehyde; MP, machine perfusion; NGAL, neutrophil gelatinase-associated lipocalin; UC-MSC, umbilical cord-derived mesenchymal stem cells.

NA, or not applicable, is assigned when a manuscript reported insufficient details for accurate evaluation.

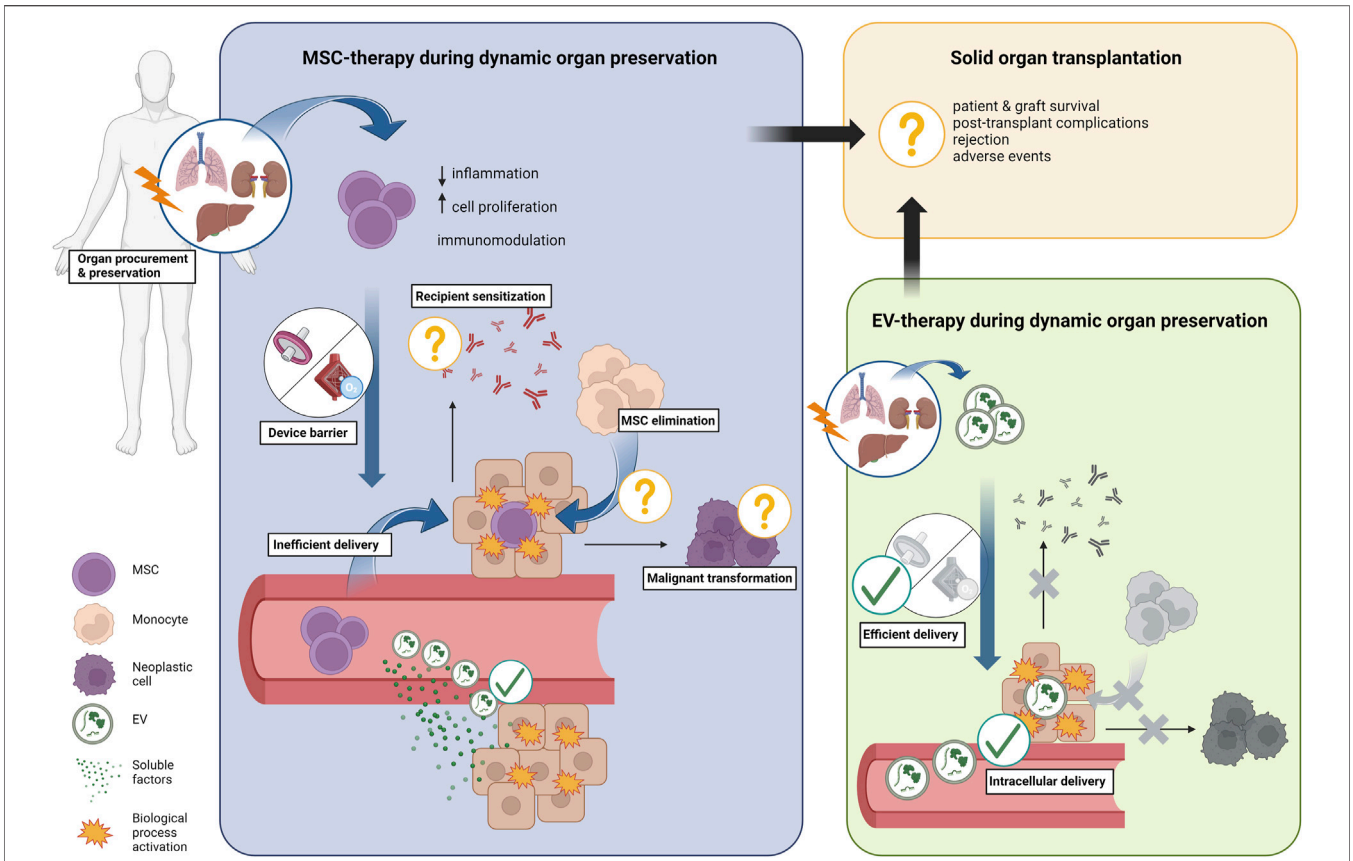

FIGURE 1 | Overview of current knowledge on cell therapy delivery during *ex situ* dynamic preservation. Mesenchymal stromal cells (MSC) are perfect candidates for organ repair and regeneration during *ex situ* dynamic organ preservation due to their anti-inflammatory, regenerative, and immunomodulatory properties. However, a "device barrier" created by components of the perfusion circuit (i.e., oxygenator(s) and filter(s)) sequester the cells from the circulating perfusate, with a bottleneck effect on the number of cells that effectively reach the parenchyma during perfusion. Additionally, migration of stem cells out of the vascular space is infrequent, only few cells are usually observed in the parenchymal space at histology, and parenchymal retention rate of stem cell is rather low. Furthermore, monocytes may actively eliminate successfully engrafted stem cells, similarly to what has been previously observed in pre-clinical studies. Because secretion of soluble factors has been observed during perfusion, it is plausible that the biological effects of stem cells are mostly dependent on paracrine mediators, extracellular vesicles (EV) included. EV delivery during *ex situ* dynamic organ preservation has been shown to circumvent the "device barrier" while intracellular uptake of EV has been demonstrated during lung and liver perfusion specifically, resulting in significant anti-inflammatory and regenerative effects. Therefore, EV therapy may be more effective than MSC therapy in promoting organ repair and regeneration during *ex situ* dynamic organ preservation because of more efficient therapy delivery. Next to a more efficient therapy delivery, cell-free therapy with EV prevents resident monocytes activation and eliminates the risk of malignant transformation and recipient sensitization, which cannot be excluded when allogenic stem cells are administered.

showing reduction of pulmonary oedema and severity of histological injury [23]. In contrast, porcine kidneys that were transplanted after MSC-therapy during NMP showed no relevant therapeutic effect within 14 days after transplantation [24].

Hence, while a direct comparison is lacking, available pre-clinical evidence indicates that MSC-therapy during MP presents similar shortcomings and may not be more effective than MSC systemic therapy in delivering the cells to the graft. Furthermore, allogeneic MSC-therapy during MP does not eliminate the potential for recipient allo-sensitization to cell donor antigens [6, 25] or malignant transformation of (the few) successfully engrafted cells. These are two potential complications that cannot be ruled out when allogeneic stem cells are administered to patients who will receive immunosuppressants after transplantation.

CELL-FREE ORGAN REPAIR AND REGENERATION DURING *EX SITU* DYNAMIC PRESERVATION

While MSC administration during MP does not have high efficiency in cell delivery, the anti-inflammatory [12], immunomodulatory [18], and regenerative [16, 20] effects of MSC, as well as significant reduction in the severity of graft injury [14, 17, 19, 20] have been observed during perfusion. These effects were observed even when MSC remained suspended in the perfusate, did not migrate out of blood vessels, or did not survive (**Table 1**). Most strikingly, Brasile et al. found that renal cell proliferation was significantly enhanced in perfused kidneys despite the fact that 95% of MSC did not migrate in the renal tissue but remained in the perfusate for 24 h [16]. This effect was

attributed to the release of growth factors by MSC [16]. Several other MP studies reported that MSC actively secrete soluble and paracrine factors in the perfusate (**Table 1**) [12, 13, 15, 23, 26]. The frequent observation that MSC have significant detectable effects during MP even when no direct contact between MSC and parenchymal cells has taken place, and that MSC secrete paracrine mediators during MP, strongly suggest that their effects rely mostly on soluble factors and paracrine mediators, such as growth factors, cytokines, chemokines, and extracellular vesicles (EV). This implies that the biological properties of MSC beneficial against organ IRI could be harnessed during MP with a cell-free therapy consisting of MSC secretome and/or purified EV [4, 5].

EV are nano-sized particles released by every (stem) cell. As they contain genetic information, growth factors, and signal transduction molecules [4], they play an important role in (stem) cell-mediated regulation of homeostasis and orchestration of tissue regeneration [27]. Upon internalization by neighboring or distant target cells, EV release their biological active cargo and induce epigenetic modifications of target cell biology, mediating the biological effects of the parent stem cell. During *ex situ* dynamic organ preservation, cell-free therapy with concentrated stem cell-derived EV has already shown encouraging results (**Table 1**). Studies have already demonstrated that EV are taken up by alveolar cells and hepatocytes during perfusion in rodent models of NMP of freshly procured lungs [28] and livers [29], resulting in significant improvements in pulmonary metabolism and adenosine triphosphate content [28], as well as reduction in transaminases and severity of histological injury during perfusion [29]. Additionally, in the study by De Stefano et al., EV from human liver stem-like cells reduced hepatocellular injury and increased cell proliferation during NMP of rat livers that suffered 60 min warm ischemic injury [30]. Gennai et al. showed that EV-therapy during NMP of discarded human lungs significantly improved alveolar fluid clearance, reducing inflammation and pulmonary oedema [31]. Gregorini et al. delivered MSC-EV during hypothermic-MP of rat kidneys, showing a significant reduction in markers of renal injury and oxidative stress [14]. The same group reported similar observations with EV-therapy during hypothermic-MP of discarded human kidneys [32]. If replicated, these findings would indicate that there may be an additional window of opportunity to deliver cell-free therapy during hypothermic dynamic organ preservation. However, transplantation of grafts treated with EV during MP has not been attempted yet, and future studies should focus on testing the hypothesis that EV-therapy at the time of MP affects post-transplant outcomes.

Hypothetically, cell-free therapy during MP could also avail of the delivery of MSC secretome, which contains both soluble factors and EV. To our knowledge, this therapeutic option has not yet been investigated.

DISCUSSION

The Future is Nano

Dynamic organ preservation strategies have entered the clinical arena and are expected to improve the preservation of high-risk organs. MSC-therapy during MP was proposed as an approach to repair high-risk grafts that are deemed too damaged and render them suitable for transplantation [1, 3]. However, there is sufficient accumulated evidence to conclude that MSC are short-lived during MP and poorly delivered to the target organ, similarly to systemic MSC-therapy, while the inherent risks of recipient's sensitization [6] and malignant transformation remain. Therefore, although cell therapy may still play a role for instance in the recellularization of human organ scaffolds, alternative strategies for repairing and regenerating organs *ex situ* should be investigated in the future.

MSC-derived cell-free therapy during MP has several advantages and circumvent the shortcomings of MSC delivery during *ex situ* dynamic preservation. In a recent systematic review of preclinical studies, we examined the efficacy of EV-therapy derived from stem cells in mitigating IRI in transplantable organs. Our findings indicate that EV-therapy significantly enhances post-reperfusion outcomes, histology, and function in the heart, lung, liver, and kidney, regardless of the originating stem cell source [33]. As EV and soluble factors are unaffected by the "device barrier" phenomenon [28–30], it can be hypothesized that the EV delivery during MP will be more efficient than MSC delivery (**Figure 1**). Furthermore, whereas MSC suspended in the perfusate at the end of MP are flushed out of the organ before transplantation, the intracellular localization of EV during MP [30, 31] ensures that they will be readily available at the time of graft reperfusion. The EV intracellular localization also prevents their elimination by resident monocytes, and the absence of human leukocytes antigens on EV membranes minimizes the risk of allo-sensitization in the recipient. Additionally, cell-free therapy during MP eliminates the risk of malignant transformation of engrafted cells. Lastly, the use of concentrated EV may offer a selective advantage because they transfer mRNA and miRNA. This transfer has the potential to induce long-lasting biological changes in target cells, which may persist even after graft reperfusion. For these reasons, and because EV possess biological properties comparable to those of the parental stem cell population, it can be hypothesized that EV-therapy will be safer and more efficient than MSC delivery during MP in harnessing MSC properties for repairing and regenerating organs before transplantation. Treatment with EV during MP has already delivered encouraging preliminary results [29, 31]; nevertheless, this hypothesis must be tested in preclinical transplant models of high translational value, as well as in clinical studies.

To move toward clinical applications, it is crucial to determine whether the therapeutic effects of EV match those of their parent stem cells. Preliminary studies suggest that MSC and EV-therapy during MP yield similar results [14, 34]. Nonetheless, further research is required to validate this hypothesis. Additionally, the mechanisms of protection against organ IRI of soluble factors and EV released by different stem cell types should be thoroughly assessed and compared. Indeed, given the complex pathophysiology of IRI, a combined treatment with soluble factors and EV from multiple sources may deliver superior benefits. Dose-finding studies in a clinically relevant model are also necessary to identify the optimal dose of EV and/or soluble factors needed to yield relevant and durable therapeutic effects [33]. Currently, the dose of EV necessary for treating human organs can only be projected based on small animal studies, and inter-species difference may lead to overestimation of the

therapeutic dose. This is a crucial point since the large scale production of purified EV is currently an unmet need and one of the major impediments to the clinical application of EV-therapy due to technological limitation. Pre-clinical studies with a larger model, phylogenetically closer to the humans may improve the estimation of the therapeutic dose. Next to organ transplantation, EV-therapy may be of benefit in several medical fields, including genetic and oncological diseases. Indeed, EV can be engineered and programmed to interact with specific cell populations to deliver a cargo enriched with gene modulating and editing agents for the treatment of genetic conditions [35], or chemotherapy and other antineoplastic agents for the treatment of malignant diseases. Thus, there seems to be ample convergence of interests for academic centres and industry to engage in research cooperations to foster technological advancements and develop procedures for scalable production and purification of EV compliant with good manufacturing practice. We strongly advocate for this type of cooperation as an essential step towards bringing EV-therapy to clinical practice, in particular to the novel field of *ex situ* organ repair and regeneration before transplantation.

In conclusion, MSC-therapy during MP is burdened by suboptimal delivery of short-lived MSC. However, their therapeutic benefits may be leveraged using a cell-free therapy consisting of concentrated EV and/or MSC secretome administered during MP. This approach resulted in the intracellular delivery of EV during perfusion and yielded therapeutic benefit in non-transplant models. We hypothesize that technology at nanoscale, such as EV, gene editing, and nanoparticles, have the highest likelihood of successfully translating into clinical applications and will shape the future of *ex situ* organ repair and regeneration before transplantation.

AUTHOR CONTRIBUTIONS

NG and JB contributed equally to study design, systematic search planning, articles screening, data analysis, results interpretation, writing of the manuscript. JP RR, and GC participated in results interpretation, critical revision of the manuscript. DM participated in study design, data analysis, results interpretation, and critical revision of the manuscript. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11947/full#supplementary-material

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SYSTEMATIC REVIEW AND META-ANALYSIS
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Psychological Impact of Living Kidney Donation: A Systematic Review by the EAU—YAU Kidney Transplant Working Group

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We performed a systematic literature review of the psychological impact on donors of living kidney donation. We conducted a literature review in PubMed/Medline according to PRISMA guidelines which included both qualitative (based on interviews) and quantitative studies (based on standardized questionnaire). There were 15 quantitative studies and 8 qualitative studies with 2,732 donors. Given that the methodologies of qualitative and quantitative studies are fundamentally different, we narratively synthetized results of studies according to four axes: quality of life, anxiety/depression, consequences of donation on the donor/recipient relationship, overall satisfaction and regret. The quantitative studies reported that donor quality of life remained unchanged or improved. Donor regret rates were very low and donor-recipient relationships also remained unchanged or improved. Qualitative studies reported more complex donation experiences: one can regret donation and still decide to recommend it as in a social desirability bias. In both study types, donor-recipient relationships were closer but qualitative studies reported that post-donation rebonding was required. The qualitative studies therefore highlighted the psychological complexity of donation for donors, showing that living donation impacts the donor's life whether it is successful or not. A better understanding of the impact of donation on donors could provide better care for donors.

Keywords: living kidney donors, quality of life, anxiety, depression, regret

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Abbreviations: QoL, quality of life; LV, living donor; SDS, self-rating depression scale; SAS, self-rating anxiety scale; SSRS, social support rating scale; SF-36, the short-form-36; BDI, the Beck depression inventory; BAI, the Beck anxiety inventory; GAD-2, The 2-item generalized anxiety disorder scale; PHQ-2, the 2-item patient health survey; PHQ-9, the patient health survey questionnaire-9; CSQ-8, the client satisfaction questionnaire; LOT, life orientation test; MMPI-2, the Minnesota multiphasic personality inventory-2; STAI, the state trait anxiety inventory; CES-D, the center for epidemiologic studies depression scale; EULID, European living donation and public health project; ESS, European social survey; ACSA, anamnestic comparative self-assessment; HADS, hospital anxiety depression scale; LOT-R, life orientation test; SOCS, sense of coherence scale; EPQ-RA, Eysenck personality questionnaire-revised-abbreviated.

INTRODUCTION

Kidney transplantation is currently the best treatment for patients with end-stage renal disease [1, 2]. However, the number of available organs is too limited to meet the growing demand for transplantation. This situation has led to the development of living-donor (LD) kidney transplantation, a practice that allows for the transplantation of better-quality kidneys with a longer lifespan than grafts from deceased donors. In an increasing number of countries, living donors who present themselves as potential candidates for donation are no longer only close family members or biologically related to the recipient [3]. Living donation is at a crossroads in medicine with a greater need for organs for patients waiting for transplants. Current studies therefore deal with the psychological repercussions of such a procedure on the donor and on the psychological evaluation of him or her in the living donor process [4]. At present, recommendations do not require living donors to have a psychological evaluation before donation but it is nevertheless "strongly recommended" [5]. The increase in this activity is prompting studies to look more closely at factors that could influence the mental health of living donors.

The psychological impact of donation on donors can be assessed through two different methodologies: quantitative studies assessing the donor with tests and questionnaires as well as qualitative studies that evaluate the donor's subjective experience assessed through research interviews. Quantitative studies based on standardized questionnaires are significantly cited when supporting organ donation since they report an increase in donor quality of life after donation compared with the pre-donation period [6]. Conversely, qualitative studies based on interviews report that donation has an impact on donor lives and that it necessarily induces a renegotiation of their identities, roles and relationships with the recipient [7, 8].

The aim of this study was to review the literature for studies of the psychological experience of donation among living donors. Contrary to previous reviews, we aimed to include both quantitative and qualitative studies in order to better understand the psychological impact of donation on the donor.

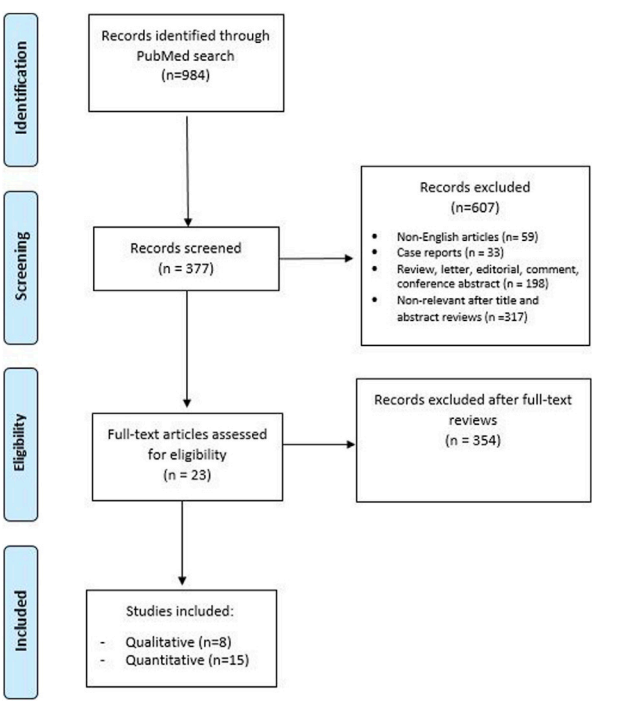

FIGURE 1 | PRISMA flow chart.

MATERIALS AND METHOD

Search Strategy

We conducted a systematic review in line with Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines [9] (**Figure 1**).

A literature search was conducted up to 31 October 2022 in PubMed/Medline. The following keywords were used in our search strategy: (renal transplantation or kidney transplantation) AND (living donor nephrectomy) AND (quality of life OR anxiety OR depression OR regret).

Inclusion and Exclusion Criteria

We included studies which analyzed the psycho-social impact of living renal donation with standardized questionnaires or interviews. In case of duplicate publications, either the higher-quality or the most recent publication was selected. Reviews, meta-analyses, letters, editorials, meeting abstracts, author replies, case reports, and non-English articles were excluded. Studies dealing with living donor nephrectomy which did not consider the postoperative psycho-social impact of donation as a primary endpoint were excluded. Since the impact on the relationship with the donor was part of our aim, studies that included anonymous donation were excluded. No restriction on publication date was applied.

Initial screening was performed independently by two investigators based on the titles and abstracts of articles to identify ineligible reports (VC and RB). Potentially relevant reports were subjected to a full-text review and the relevance of the reports was confirmed after the data extraction process. Disagreements were resolved by consultation with a third co-author (VM).

Data Extraction and Analysis

Two review authors (VC and RB) performed independent initial screening based on the titles and abstracts. Studies were allocated to the group "Quantitative study results" when a standardized questionnaire was used and were allocated to the group "Qualitative study results" when the evaluation was based on interviews.

Both authors independently extracted the following variables from the included studies: first author's name, publication year, country of research, study design, period of patient recruitment, number of patients included, type of evaluation (quantitative vs. qualitative).

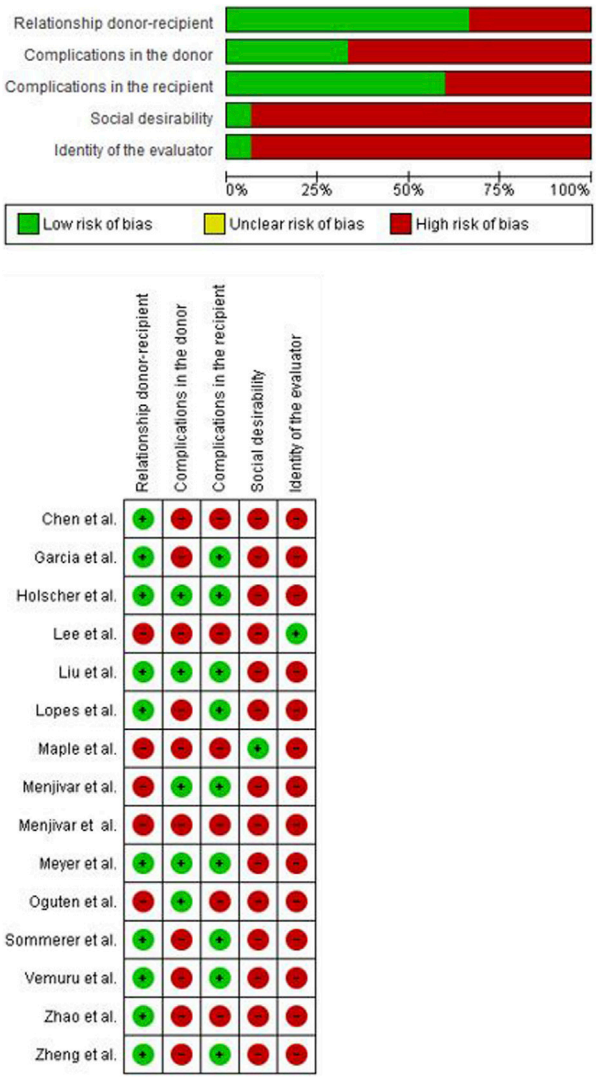

FIGURE 2 | Risk of bias quantitative studies.

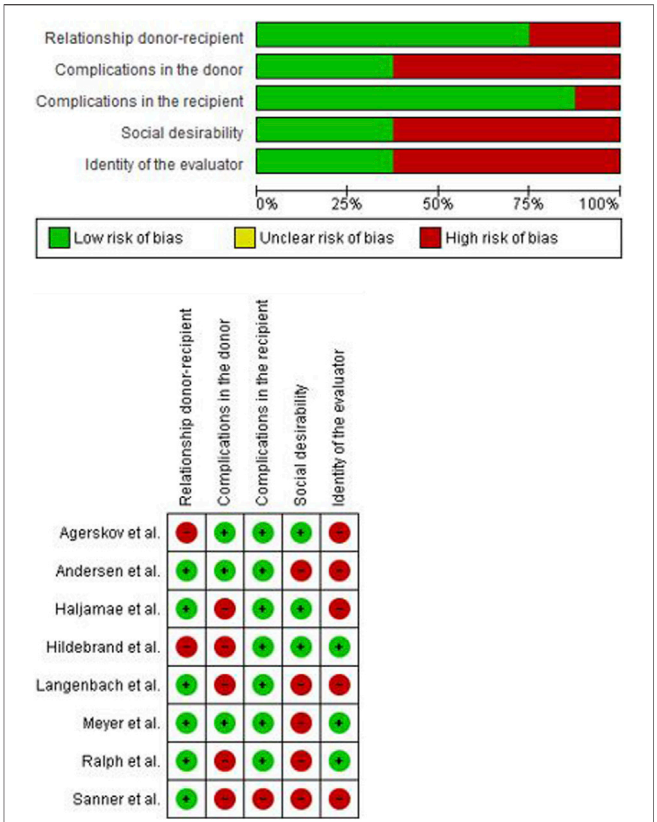

FIGURE 3 | Risk of bias qualitative studies.

Risk of Bias Assessment

The risk of bias (RoB) of the included studies was evaluated according to the "Risk of Bias in Non-Randomized Studies of Interventions (ROBINS-I)" tool [10]. ROBINS-I is the recommended tool for Cochrane Reviews for non-randomized studies of interventions. In addition, two reviewers independently assessed the RoB using five confounding factors which were identified *a priori*: donor-recipient relationship, medical/surgical complications in the donor, medical/surgical complications following renal transplantation in the recipient, social desirability, identity of the evaluator. The RoB summary and graph figures were generated using the Cochrane Review Manager 5.4 (RevMan 5.4; The Cochrane Centre, Copenhagen, Denmark). The overall RoB level was judged as "low," "unclear," or "high" risk (**Figures 2**, **3**).

Analysis

The planned meta-analysis of the psycho-social impact of living donation evaluated quantitatively by standardized questionnaire was not possible owing to the heterogeneity of the questionnaires used in the literature. Since the methodologies of qualitative and quantitative studies are fundamentally different and cannot be compared in a classical systematic review, we choose to narratively synthetize the results of both types of studies according to four axes: quality of life, anxiety/depression, consequences of donation on the donor/recipient relationship, overall satisfaction and regret.

RESULTS

Donor Characteristics

Across all studies, a total of 2,732 donors were assessed. The mean age on donation was 49 years, the majority of donors were female (61%) and employed. None of the studies provided exact descriptions of the donor-recipient relationship but we can observe that the majority of them were genetically related and were parents (22.7%), siblings (19.4%) or spouses/partners (15.0%). Concerning marital status, type of the surgery, religious belief, the studies do not report enough data for analysis (**Table 1**).

TABLE 1 | Demographic data of donors in all studies.

Variables	Statistic *n*, (%)
Age (y)	49
Gender (*n* = 2,732)	
Male	1,060 (38.8)
Female	1,672 (61.2)
Donor-recipient relationship (*n* = 1,899)	
Parent	431 (22.7)
Sibling	368 (19.4)
Spouse/partner	285 (15.0)
Child	48 (2.5)
Friend	39 (2.0)
Third and fourth degree	22 (1.2)
Other unspecified (related)	95 (5.0)
Genetically related (unspecified)	400 (21.1)
Emotionally related (unspecified)	211 (11.1)
Occupation (*n* = 1,785)	
Employed	1,205 (67.7)
Unemployed	325 (18.2)
Retired	240 (13.4)
Student	1 (0.01)
Other	14 (0.7)
Marital status (*n* = 1,215)	
Married/live with a partner	1,108 (91.2)
Single/Divorced/Widow	107 (8.8)
Type of Surgery (*n* = 601)	
Open nephrectomy	233 (38.8)
Laparoscopy	368 (61.2)

n = *number of donors described in the studies. Marital Status and Type of surgery are not always reported in studies. The studies do not report the exact type of donor-recipient relationship and use the categories "Genetically related" and "Emotionally related."*

This review reports 15 articles with quantitative measures and 8 articles with qualitative measures. Fifteen studies were retrospective post-donation and 8 studies were prospective assessing donors before donation and then at 3 months and/or 6 months and/or 1 year and up to 10 years after donation (**Tables 2**, **3**). Among the included quantitative studies, half used the Short-Form Health Survey-36 item (SF-36). Owing to the heterogeneity of the questionnaires used and the limited number of studies that used the most represented test (SF-36), a meta-analysis could not be considered.

Narrative Synthesis of Evidence

Quality of Life

Quantitative Study Results

The concept of quality of life is one of the main concepts used in the evaluation of the psychological and physical impact of donation on a living donor. Studies use quality-of-life measures by assessing it either prospectively or retrospectively. Prospectively, they compare donor outcomes with those of recipients, with general population norms, or compare different types of donors with one another (e.g., type of donor-recipient relationship).

The studies report two types of results. The first finding is that there is no significant difference in quality-of-life scores between pre-donation and 1 year post-donation [16, 18, 25]. The second finding is that some studies observe an increase in quality of life as early as 1 year post-donation compared with the pre-donation period [12, 22–24]. Studies also point to risk factors associated with decreased donor quality of life such as donor fatigue, anxiety depression, lack of social support, the nature of the donor-recipient bond and postoperative complications or recipient graft loss [11, 16, 17, 22, 23] (**Supplementary Table S1**). Socio-demographic data does not impact the quality of life [13, 18, 23]. The studies are not in agreement regarding the impact of transplant failure on quality of life [12, 23] (**Supplementary Table S1**).

Qualitative Study Results

None of the qualitative studies evaluated the concept of quality of life in their results (**Table 3**).

Anxiety/Depression

Quantitative Study Results

Several measures are used to assess anxiety and depression (**Table 2**). After donation, studies generally report a low prevalence of anxiety and depression in donors [13, 18, 21, 24, 25]. However, they also point certain risk factors associated with an increase in symptoms of depression and anxiety. Donors who experienced postoperative complications or recipient graft loss had more anxiety and lower life satisfaction [13, 18, 21]. There was an "emotional contagion" [14] from recipient to donor meaning that recipient anxiety or depression could impact the donor. Chen et al. reported that parent donors showed more anxiety and depression than sibling donors [11] but another study found no impact of the nature of relationship on the donor [21] (**Supplementary Table S1**). Education, marital status and gender also appeared to be risk factors [13, 22].

Qualitative Study Results

The studies noted that the majority of donors reported post-donation depression and anxiety and also great difficulty adjusting after nephrectomy with aggression, hyper-vigilance about wellbeing and fear of rejection by the recipient [26, 27, 30, 32, 33]. The donors also reported feelings of vulnerability associated with intense fatigue [26, 27, 31, 33] after donation. They explained this as a result of having to go from a healthy person to someone who has undergone surgery [26]. It was also disappointed expectations that impacted the donation experience [26, 30, 32] as well as the evolution of the donor-recipient relationship or failure of the transplant or the death of the recipient [27, 28, 32]. One study found that the donation event took a back seat with the passing of time [31].

Study results were diverse concerning the psychological impact on donors vis-à-vis complications, transplant failure or recipient death. Some studies reported that donors experienced feelings of guilt such as not having donated a good enough kidney, grief, depression, a sense of fault and responsibility, disappointment, severe psychological distress, and physical symptoms [27, 28]. Conversely, another study reported that some donors denied feeling guilt or regret over the failure of the transplant because they felt they had done the right thing for their families [27]. Over time, donors appeared to have accepted

TABLE 2 | Characteristics of quantitative studies.

Source	Journal	No. of donors	Country	Years of inclusion	Methods (tests)	Time since donation	Psycho-social outcomes following living donation				
							Quality of life	Anxiety depression	Regret	Impact failure/ death	Donor/recipient relationship
[11]	Asia Pac Psychiatry	98	Asia	2008–2010	SDS-SAS- SSRS -SF-36	5–1 y	Yes	Yes	No	No	Yes
[12]	Clin Transplant	50	Brazil	2007–2009	Donor Questionnaire—SF-36	3–12 m	Yes	No	Yes	Yes	Yes
[13]	BMC Nephrol	825	United States	2011–2017	GAD-2 - PHQ-2 - 1 question regret	3–10 y	No	Yes	Yes	Yes	No
[14]	BMC Nephrol	53	Korean	2008–2019	MMPI-2 - STAI - CES-D	—	No	Yes	No	No	No
[15]	Transplant Proc	41	Taiwan		The Decision Regret Scale - Effective decision subscale - SF-12	>3 m	Yes	Yes	Yes	No	Yes
[16]	Transplant Proc	45	Portugal	2002–2008	Socio-demo-test - SF-36	>12 m	Yes	Yes	No	No	No
[17]	BMC Nephrol	217	Norway	2013	SF-36-MFI and specific questions	8–12 y	Yes	No	Yes	Yes	Yes
[18]	Transpl Int	100	England	2012–2013	Questionnaire by the research team	3 and 12 m	Yes	Yes	Yes	Yes	Yes
[19]	Sci Rep	60	France, Germany, Portugal, Spain, Sweden	2011	ACASA - SF-36 - HADS - LOTR - SOCS - EPQ-RA - ELSA	12 m	Yes	Yes	No	Yes	Yes
[20]	BMC Nephrol	332	Spain	2005–2015	EULID - ESS	>12 m	No	No	Yes	No	No
[21]	Transplant Proc	208	Turkish	2006–2017	BDI - BAI - CLAS	1–12 y	No	Yes	Yes	No	Yes
[22]	BMC Nephrol	211	Germany	1983–2011	SF-36 - MFI-20 - PHQ-9	—	Yes	No	No	No	No
[23]	Indian J Uol	100	India	NE	WHO QoL BREF	6 m	Yes	No	No	Yes	No
[24]	BMC Nephrol	84	China	2002–2007	BDI - SAS - SSR - SF-36 – 22-item sociodemographic	6–12 m	Yes	Yes	Yes	No	Yes
[25]	Transplant Proc	110	China	2002–2012	SAS - SDS – Self-made socio-demographic questionnaire	1–106 m	Yes	Yes	Yes	No	Yes

SDS, self-rating depression scale; SAS, self-rating anxiety scale; SSRS, social support rating scale; SF-36, the short-form-36; BDI, the beck depression inventory; BAI, the beck anxiety inventory; GAD-2, the 2-item generalized anxiety disorder scale; PHQ-2, the 2-item patient health survey; PHQ-9, the patient health survey questionnaire-9; CSQ-8, the client satisfaction questionnaire; LOT, life orientation test; MMPI-2, the Minnesota multiphasic personality inventory-2; STAI, the state trait anxiety inventory; CES-D, the center for epidemiologic studies depression scale; EULID, European living donation and public health project; ESS, European social survey; ACSA, anamnestic comparative self-assessment; HADS, hospital anxiety depression scale; LOT-R, life orientation test; SOCS, sense of coherence scale; EPQ-RA, Eysenck personality questionnaire-revised-abbreviated; y, year(s); m, month(s); —, not evaluated; Yes, evaluated; No, not evaluated; NE, not evaluated.

TABLE 3 | Characteristics of qualitative studies.

Source	Journal	No. of donors	Country	Years of inclusion	Methods	Time since donation
[26]	J Rend Care	18	Denmark	2012–2013	Interview–observation	1 week before donation (do); 3 months after do
[27]	Clin Transplant	12	Norway	2004	Semi-structured telephone interview	1 year after do
[28]	Clin Transplant	10	Sweden	1997	Interview	>3 years after do
[29]	Clin Transplant	76	United States	NE	Telephone survey–interview	1–6 years after do
[30]	Transplant Proc	11	Germany	NE	Semi-structured interview	2–3 years after do
[31]	BMJ Open	16	Norway	2014–2015	Semi-structured interview	>10 years after do
[32]	BMJ Open	16	Australia	2014–2017	Face-to-face semi-structured interview; telephone	Before & 11–14 m after do
[33]	Nephrol Dial Transplant	39	Sweden	2000	Open interview	1 day before–3 weeks after do

the failure of the transplant as their fate or in a less negative manner [27, 28].

Despite the obviousness of the decision [28, 29, 31–33], donors explained the dilemmas, ambivalence and anxiety they faced [26, 32]. Donor dilemmas were sometimes reinforced by close friends or family who showed "overwhelming concern for the donor's health" or questioned the donor's decision. The pre-donation evaluation period is often very distressing for donors [26, 32, 33].

Consequences of Donation on the Donor/Recipient Relationship

Quantitative Study Results

The quality of the donor-recipient relationship was assessed using specific questions about improvement of the relationship after donation (**Table 2**). The majority of studies reported that the relationship of the donor-recipient dyad remained either unchanged [19, 21] or had improved and become "closer" [12, 21, 24, 25]. However, there were a few cases where the relationship deteriorated after donation [17–19, 21, 25] (**Supplementary Table S1**).

Qualitative Study Results

In general, the studies found that the relationship between donors and recipients either remained unchanged or a special bond developed between them. Donors reported a closer and stronger bond, a better understanding of each other, a more dynamic dyad, and a more balanced relationship [26, 27, 29, 32]. However, this closer donor-recipient bond was not always the case. This relationship was associated in many dyads with a renegotiation of roles, expectations of the recipient, conflict, tension owing to disappointment, unmet expectations, broken contact or divorce after donation [27, 29, 31, 32]. The nature of the donor-recipient relationship appeared to impact the type of motivation and secondary benefits expected from the transplant [28, 31–33].

Donor psychological experience appeared to be dependent on the realization of initial expectations and expected benefits from the transplant. The donors who achieved a personal benefit after donation related to the success of the transplant participated in a positive donation experience [27, 32]. Donors reported difficulty in having to fill multiple roles after donation. For parents, there was an increase in tension and stress with other family members or it was difficult for some to balance work and family responsibilities [27]. Some donors hoped that the caregiver role would diminish after donation, especially in the case of spousal dyads. When this did not happen, donors felt disappointed or frustrated [32]. Donors expressed negative donation experiences when recipients were not compliant [27, 29, 32]. The studies showed that the living donor transplant process involved not only the recipient and his or her donor but the entire family [31, 33]. As for the impact of transplant failure on the donor, there was no impact on the donor-recipient relationship [30].

Overall Satisfaction and Regret of the Donation

Quantitative Study Results

The studies were in agreement that in the majority of cases, donors were satisfied with the donation process and remained committed to their decision [12, 15, 20, 21, 25]. The vast majority of donors did not regret the donation [12, 13, 17, 18, 20, 21, 23–25]. Several studies attempted to quantify donation regret with specific questions such as "If you had to do it again, would you?" "Would you recommend it?". The results of the studies showed that it is possible to regret the donation and still recommend it or agree to do it again if possible and *vice versa* [15, 20, 24, 25] (**Supplementary Table S1**).

All of the studies reported a low rate of regret in donors at different times after donation. The authors reported correlations between regret and fatigue rates [17], regret and graft failure or complications in the recipient [17], while other studies found no correlation between donor regret and recipient complications or death [12, 15, 19]. There was also a correlation between donor regret and deterioration of the relationship with the recipient after donation as well as a correlation between donor regret and anxiety and depression [13, 21]. On the other hand, the percentages in the studies showed that the majority of donors expressed that they would donate again if they had to but there was also a decrease in percentages when the donor was asked if he or she "would encourage donation" [15, 24, 25] (**Supplementary Table S1**).

Qualitative Study Results

Donors reported an ambivalent donation experience [26, 33]. Most of them were very satisfied with the donation and the

positive effects observed in the recipient [26, 27, 29, 30]. This feeling was reinforced by the family, social environment, the recipient and sometimes the transplant team, who made them proud of their gesture [27, 29, 31–33]. However, the donation experience was still described as an "overwhelming experience" [26] that was not always positive and was often marked by a feeling of vulnerability in the donor after surgery [26, 27, 29, 30, 32, 33]. The donors who were the least supportive of donation were those who experienced transplant failure in the recipient [29]. However, in the majority of cases, even the least supportive donors reported that they would donate again if necessary [29].

Subjects Added to the Analysis of the Qualitative Studies

The qualitative studies reported other findings that focused on the pre-donation period that appeared to be necessary for outcome analysis.

The Pre-Donation Period

The pre-donation evaluation period is often not a good experience for the donor [26, 32, 33]. Some find it "the worst step" in the donation process. It is a very anxious and uncertain period where the donor is confronted with both his or her own dilemmas, the fear of being rejected as a donor, the long wait for test results, etc. During this period, donors also reported a feeling of being "out of touch" with their families with feelings of abandonment and loss.

The Use of Strategy to Influence the Transplant Team

Several studies reported that some donors used strategies to try to influence transplant teams to select them as donors [26, 29, 33]. These donors tried to convince teams that they were not psychologically fragile, that previous psychological problems would not interfere with donation, and that their physical health was not a barrier. For some, the explanations given concerning their motivation were thought out beforehand so they would not be misinterpreted. Some donors withheld information from the transplant team to increase their chances of being selected as a donor [29].

Sense of Abandonment and Support

Some donors reported feeling forgotten, lost and abandoned after donation whereas they were considered "sensational" prior to donation [27, 30, 33]. They expressed the importance of medical follow-up after donation in order to feel supported and reassured [27, 29, 31, 33]. They also criticized the lack of active approach by transplant teams during post-donation follow-up, especially when the transplant failed or the recipient died [27, 28, 31].

DISCUSSION

Our initial aim was to review recent literature in order to better understand the psychological impact of living donation on the donor in renal transplantation. Living donation is currently the most favorable solution for patients waiting for a kidney transplant. This literature review focused on quantitative and qualitative studies. At first glance, it is not intuitive. However, this is also what gives it a unique approach. We thought it would be of interest to place the results of two very different study methods side by side in the same article. The results are very different and give rise to new questions about living donation.

Quantitative studies have reported that quality of life, anxiety, and depression in donors remained unchanged after donation whereas in prospective studies quality of life improved after donation. The rate of regret among donors was very low and the donor-recipient relationship also remained unchanged or improved after donation. Qualitative studies reported a more complex donation experience that included positive experiences, vulnerability, ambivalence and anxiety. Relationships with the recipient were closer as shown in the quantitative studies but they had to go through a post-donation reshaping of relationships, a renegotiation of roles and expectations.

Analysis of the qualitative literature revealed that the concepts used are not the same as in the analysis of the quantitative literature. Indeed, the method used with semi-structured research interviews allowed donors to disclose their experiences which generated free-flowing and unanticipated commentary, whereas the quantitative studies evaluated precise and predefined concepts based on scales and questionnaires.

The qualitative studies showed that the donors used conscious or unconscious strategies to influence the transplant team to select them as a donor during the pre-donation period. Indeed, they wanted to prove that they were in good mental and physical health and therefore fit to donate their kidney. In this light, the results of the quantitative studies are questionable and comparison of the results of the pre-donation and post-donation periods would appear to be difficult to interpret. In addition, the majority of studies did not take into account the impact of social desirability bias on the results obtained. This bias represents the tendency of individuals to give socially desirable answers when responding to surveys or personality tests [34]. It is a bias that influences the responses to questionnaires or tests administered. The donor answers what he or she thinks is expected and does not want to give answers that would make him or her look bad. In this type of study, it is conceivable that the answers given by the donor evaluated in the pre-donation or post-donation period could be influenced by this bias [18, 28, 35].

This literature review highlighted the difficulty in assessing donor regret after donation. Indeed, the results and scores showed that it is possible to regret the donation and still decide to donate again if possible or to recommend it. The qualitative studies also affirmed that a negative donation experience does not necessarily undermine the donor's decision. The answer to the question "Would you donate again if possible?" does not guarantee whether or not the donor regrets the decision. The concept of regret seems to be much more complex. This is more especially the case if we take into account the impact of social desirability. It appears to be difficult to know whether donors are able to consciously assume regret in this evaluation process or in their lives.

As for the relationship between the donor and the recipient, the quantitative studies showed that overall donation had no impact on the donor-recipient relationship or that the

relationship was often "closer." The qualitative studies reported the same result. However, the relationship and roles often needed to be reshaped [8, 36, 37]. Even if the donor reported that the donation did not have an impact on his or her relationship with the recipient, his or her account suggested that the donation was a very present event in the relationship [7]. The Marcel Mauss theory teaches us that one gift always awaits another: the counter-gift [38]. This is the basis of a social bond. The gift necessarily implies in the relationship to the other the question of symbolic debt and guilt [39, 40].

Quantitative and qualitative studies pay little attention to the psychological consequences on the donor if post-donation surgical complications occur. They are more interested in the consequences that the recipient's complications have on the donor. Donors are very motivated by the idea of helping a family member or a sick relative but at the same time, they feel doubts and fears about the operation that will affect the integrity of their body. Our study reports donors with different cultural background and socio-demographic data (**Tables 2**, **3**).

According to previous studies, healthy donors undergoing nephrectomy are subject to stress events that they must adapt to [36, 41, 42]. This ambivalence towards donation does not in any way call living donation into question. On the contrary, it makes it possible to understand and therefore to accompany in a more precise manner the experience of donors which oscillates between an almost unanimously positive experience and feelings of vulnerability, anxiety, disillusionment and doubt. The results do not call into question the merits of living donation but do allow us to consider donation from another angle. It is no longer a question of identifying the impact of donation on the donor in terms of a positive or negative experience but rather as a singular experience where the donor must co-construct his or her desire to donate, his or her expectations, the reshaping of social and family relationships, the relationship with his or her body. These studies highlighted the importance of the psychological support needed before, during and after the process.

CONCLUSION

In conclusion, this review of the literature showed complementary and sometimes conflicting results between quantitative and qualitative studies. The quantitative studies reported that donor quality of life remained unchanged or improved while this point was poorly evaluated in qualitative studies. Donor regret rates were very low and donor-recipient relationships also remained unchanged or improved. Qualitative studies reported more complex donation experiences, showing it is possible to regret donation but still decide to repeat or recommend it. The concept of social desirability could bias the analysis of psychological outcomes in the donor. In view of these results, it would appear important to remember that living donation has an impact on the donor's life as soon as he or she engages in this type of procedure whether it is successful or not. Qualitative results may be useful to shape future quantitative studies and to interpret past ones. The transplantation team and the psychologist must accompany the donor to reflect on his or her decision to donate, even if it carries implicit constraints, so that the donor is a player in his or her decision.

AUTHOR CONTRIBUTIONS

VC: Contribution to protocol design, initial screening, collection and analysis or interpretation of results + writing of article. VM: Contribution to protocol design, analysis or interpretation of results + help to resolve the various disagreements. TP: Contribution to protocol design + article proofreading and updated/adjusted. APe: Contribution to protocol design + article proofreading and updated/adjusted. APi: Contribution to protocol design + article proofreading and updated/adjusted. RC: Contribution to protocol design + article proofreading and updated/adjusted. VH: Contribution to protocol design + article proofreading and updated/adjusted. AT: Contribution to protocol design + article proofreading and updated/adjusted. RB: Contribution to protocol design, initial screening, collection and analysis or interpretation of results + writing of article + work supervision.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11827/full#supplementary-material

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Impact of Preformed Donor-Specific Anti-HLA-Cw and Anti-HLA-DP Antibodies on Acute Antibody-Mediated Rejection in Kidney Transplantation

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Given the risk of rejection, the presence of preformed donor specific antibodies (DSA) contraindicates transplantation in most allocation systems. However, HLA-Cw and -DP DSA escape this censorship. We performed a multicentric observational study, in which the objective was to determinate risk factors of acute antibody-mediated rejection (aABMR) in recipients transplanted with preformed isolated Cw- or DP-DSA. Between 2010 and 2019, 183 patients were transplanted with a preformed isolated Cw- or DP-DSA (92 Cw-DSA; 91 DP-DSA). At 2 years, the incidence of aABMR was 12% in the Cw-DSA group, *versus* 28% in the DP-DSA group. Using multivariable Cox regression model, the presence of a preformed DP-DSA was associated with an increased risk of aABMR (HR = 2.32 [1.21–4.45 (p = 0.001)]) compared with Cw-DSA. We also observed a significant association between the DSA's MFI on the day of transplant and the risk of aABMR (HR = 1.09 [1.08–1.18], p = 0.032), whatever the DSA was. Interaction term analysis found an increased risk of aABMR in the DP-DSA group compared with Cw-DSA, but only for MFI below 3,000. These results may plead for taking these antibodies into account in the allocation algorithms, in the same way as other DSA.

Keywords: donor-specific antibodies, kidney transplant, acute antibody-mediated rejection, HLA-Cw, HLA-DP

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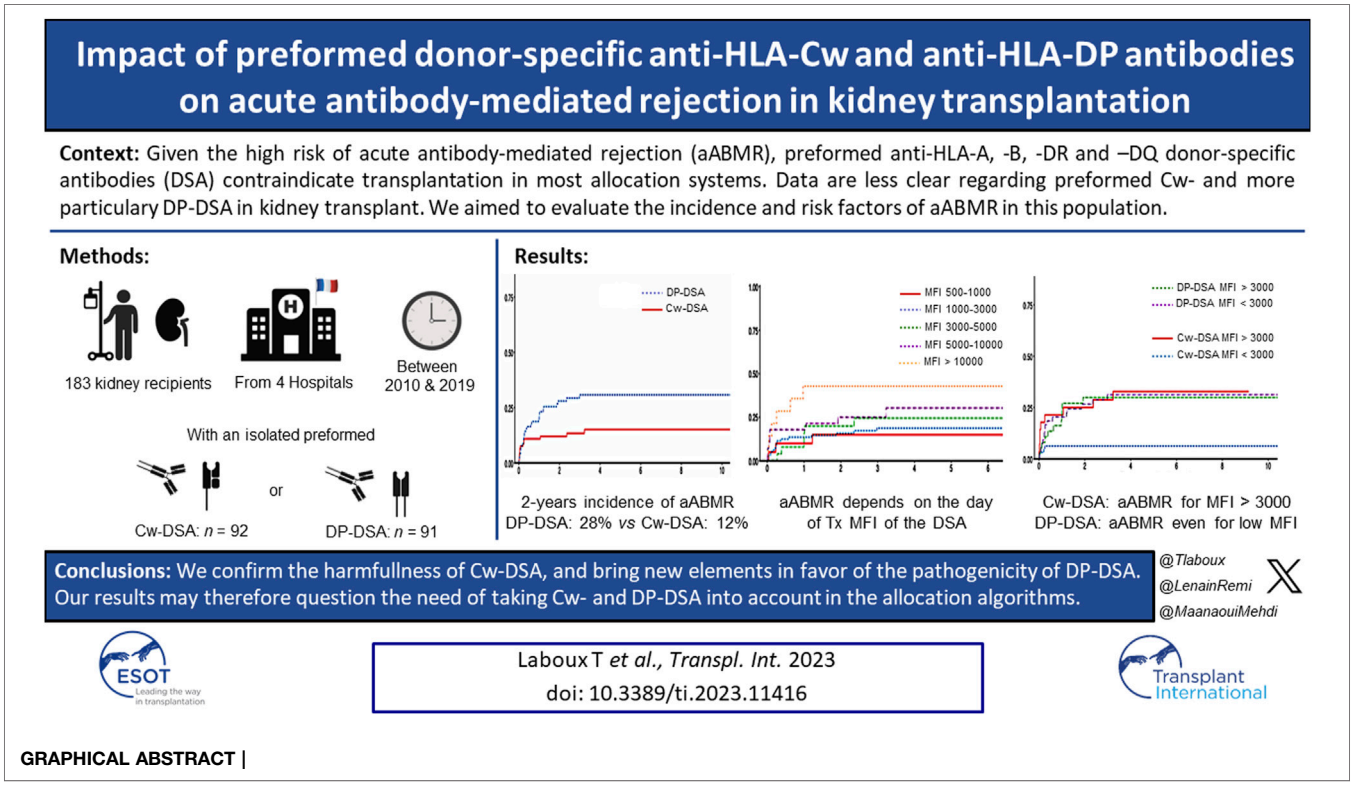
Abbreviations: 95%CI, 95% confidence interval; aABMR, acute antibody-mediated rejection; ABMR, antibody-mediated rejection; aHR, adjusted hazard ratio; BMI, body mass index; CDC, complement-dependent cytotoxicity; cPRA, calculated panel-reactive antibody; DSA, donor specific antibody; HLA, human leukocyte antigens; HR, hazard ratio; IVIGs, intraveinous immunoglobulins; MFI, mean fluorescence intensity; PRA, panel-reactive antibody; Q1, first quartile; Q3, third quartile; RRT, renal replacement therapy.



GRAPHICAL ABSTRACT |

INTRODUCTION

Preformed anti-human leukocyte antigens (HLA) donor-specific antibodies (DSA), especially targeting the A, B, DR and DQ antigens, are reputedly known to be associated with post-transplant rejection [1], with up to 30% of acute antibody-mediated rejection (aABMR) in the first year of transplantation in some series [2–4], and with impaired graft survival [2, 5]. Therefore, most allocation programs introduced the concept of "unacceptable antigens" to avoid kidney transplantation when a preformed DSA is present. However, anti-HLA-Cw and anti-HLA-DP DSA are disregarded in many transplant allocation systems and thus matching algorithms, such as the one provided by the French *Agence de la Biomédecine*, while on the other hand Cw-DSA are mandatory and DP-DSA recommended in the organ allocation system of the United Kingdom for instance [6]. The reasons for this singularity are multiple. First, HLA-Cw and -DP molecules are described as less expressed than other HLA antigens by the endothelial cells [7, 8] and barely immunogenic [9]. Second, the development of bead–based technologies [10] to study anti-HLA antibodies, and more importantly the recent complete and systematic *HLA-C* and *HLA-DP* genotyping of the donor greatly helped to characterize Cw- and DP-DSA. Few clinical cases [11] and retrospective studies [12, 13] have recently provided arguments in favor of a potential pathogenicity of Cw-DSA. However, data are scarce and conflicting regarding isolated preformed DP-DSA. Few small-sized studies did not report any association with aABMR or graft loss with preformed DP-DSA [14, 15], while some cases reported on the contrary hyper-acute ABMR [16, 17]. Some studies also mixed patients with DP- and Cw- DSA, which made their interpretation difficult [18]. As to date, and considering current matching algorithms, the only significant preformed DSA we may face in case of a kidney transplant proposition are Cw- and DP-DSA. The objective of our study was thus to evaluate the incidence of acute ABMR in a multicentric cohort transplanted with either isolated Cw- or DP-DSA, and to identify risk factors of aABMR in this specific population.

MATERIAL AND METHODS

Study Patients

The study included every adult transplanted with a single kidney graft in the presence of an isolated preformed Cw- or DP-DSA between 2010 and 2019 at the French University Hospitals of Amiens, Bordeaux, Lille and Nantes. Criteria of exclusion were: pediatric patients, patients presenting another preformed A-, B-, DR- or DQ-DSA, patients presenting with both anti-Cw and anti-DP DSA, ABO-incompatible transplantation, multiorgan transplantation, and patients pre-treated with desensitization protocols before transplantation. Finally, for DP-DSA, as donors' *HLA-DPA1* genotype was not available for most of the patients, only recipients with at least one anti-DPB1-DSA were included.

Data Source and Ethical Statement

This multicentric observational study conforms to the tenets of the Istanbul Declaration and the ethical guidelines set forth by the Helsinki Declaration and was approved by the local institutional review boards. No organs were procured from prisoners. All participants provided their informed consent. The dataset was processed under French and European Union data protection laws and regulations (reference: #DEC20-002). This study complies with the "Strengthening the Reporting of Observational Studies in Epidemiology" (STROBE) guidelines [19]. Data from Nantes were collected from the French DIVAT multicentric prospective cohort of kidney and/or pancreatic transplant recipients (www.divat.fr, N°CNIL 914184, ClinicalTrials.gov recording: NCT02900040).

Data Collection

For each patient, data regarding donor and recipient age, sex, body mass index (BMI), blood group, rank of transplantation, time spent in dialysis, renal-replacement therapy (RRT) method, initial causal nephropathy, calculated panel-reactive antibody (cPRA, defined as the proportion of incompatible grafts that had unacceptable mismatches among proposed deceased kidney donors in the same blood group over the 5 previous years), pre-transplant sensitization in class I and class II antibodies, donor cause of death, cold ischemia time, conservation method, number of *HLA-A*, *-B*, *-DR* and *-DQ* mismatches, induction and peri-transplant prophylactic therapies, and the result of complement-dependent cytotoxicity (CDC) crossmatch, were collected.

Anti-HLA Antibody Testing

HLA antibodies were detected by single antigen flow beads using a LUMINEX© (LUMINEX 100 or 200) with the LABScreen Single Antigen HLA Class I© and LABScreen Single Antigen HLA Class II© kits (ONE LAMBDA©). The antibody level was approximated by the mean fluorescence intensity (MFI). DSA were considered only if present in the serum the day of transplant with a minimum mean fluorescence intensity of 500. In the presence of two or more anti-DP DSA or anti-Cw DSA, the strongest MFI was considered in the analysis. MFI of the preformed Cw- or DP-DSA were secondly monitored at day 15, in the 3rd, 6th, 12th and 24th months post-transplantation.

Histopathology

The diagnosis of biopsy-proven acute antibody-mediated rejection was performed according to the 2019 Banff classification [20] on "for cause" kidney graft biopsies.

Endpoints

The primary endpoint was to determine the incidence of aABMR when transplanted with an isolated Cw- or DP-DSA, and then to identify risk factors of aABMR. Secondary endpoints included the identification of risk factors associated with death-censored graft loss and to describe the use of additional prophylactic strategies (Rituximab and/or Intravenous Immunoglobulins (IVIGs) and/or plasmapheresis and/or Eculizumab) performed the day of transplant to prevent rejection in the whole Cw- and/or DP-DSA population.

Statistical Analysis

The Cw-DSA and the DP-DSA groups were compared on baseline characteristics by chi-2 (categorical data) or Student's t-tests (continuous data). The Aalen-Johansen estimator was used to estimate event probabilities and to analyze the cumulative incidence of aABMR accounting for the competing risk of death or graft loss for rejection analyses and the competing risk of death for graft loss analysis [21]. Cumulative incidence functions were compared by Gray test when appropriate. Median follow-up times were estimated by a reverse Kaplan Meier method [22]. Hazard ratios for aABMR, and graft loss were computed using Cox proportional hazards modeling. A multivariable backward selection procedure was implemented for the primary endpoint, with a univariate threshold $p < 0.20$ for inclusion and a $p < 0.05$ being defined as statistically significant in the final model. For graft loss, known confounders were included regardless of significance level. An interaction term analysis was performed on the primary endpoint in order to assess the consistency of the effect of MFI on aABMR risk according to the Cw-DSA and the DP-DSA groups. Log-linearity and the proportional hazards assumption were tested using a graphical method. All analyses were carried out in R, version 3.6.3 [23].

RESULTS

Study Population and Baseline Characteristics

Among the 183 patients included, 92 were transplanted with isolated preformed Cw- DSA and 91 with preformed DP-DSA. Anti-Cw5 ($n = 17$), anti-Cw7 ($n = 16$) and anti-Cw2 ($n = 11$) were the most frequent Cw-DSA reported, while anti-DP4 ($n = 21$), anti-DP1 ($n = 13$) and anti-DP2 ($n = 10$) were the more frequently found DP-DSA (**Supplementary Table S1**). The median time of follow-up post-transplant was 4.2 years [Q1: 2.71; Q3: 7.14]. Baseline patients' characteristics are presented in **Tables 1**, **2**. Overall, mean recipients age was 51.5 years old (±13.0), with a slight over-representation of women. More than half of the patients were retransplanted recipients (51.4%). Mean calculated-PRA was 69.3% (±35.1), with anti-class I and anti-class II HLA sensitization occurring for 89.3% and 76.3% of the patients, respectively. The mean immunodominant DSA MFI at the time of transplantation was 3,540 (±3,537) [Cw-DSA: 3,228 (±3,216); DP-DSA: 3,855 (±3,826), $p = 0.231$]. Of note, four patients were transplanted despite a positive CDC crossmatch (2 Cw-DSA for T-cells and 2 DP-DSA for B-cells). Anti-thymocyte globulins was the main induction therapy (86.4%) and 63 patients (34.4%) were treated with an additional prophylactic protocol the day of transplantation: Rituximab ($n = 31$ [17.3%]), IVIGs ($n = 45$ [25.1%]), plasmapheresis ($n = 9$ [5.03%]), and/or Eculizumab ($n = 2$ [1.12%]). Baseline characteristics were similar between Cw- and DP-DSA recipients, except for BMI [25.4 kg/m^2 *versus* 23.9 kg/m^2 respectively ($p = 0.049$)], cPRA [61.5% *versus* 77.3% respectively ($p = 0.002$)], class I HLA sensitization [100% *versus* 77.9% respectively ($p < 0.001$)], class II HLA

TABLE 1 | Baseline characteristics.

	All	Cw-DSA	DP-DSA		
	n = 183	*n* = 92	*n* = 91	*p*-value	*n*
Transplant centers				0.633	183
Amiens	8 (4.37%)	5 (5.43%)	3 (3.30%)		
Bordeaux	57 (31.1%)	28 (30.4%)	29 (31.9%)		
Lille	72 (39.3%)	33 (35.9%)	39 (42.9%)		
Nantes	46 (25.1%)	26 (28.3%)	20 (22.0%)		
Recipients					
Age (years)	51.5 (±13.0)	51.7 (±13.4)	51.2 (±12.7)	0.809	183
Sex (% of men)	86 (46.9%)	46 (50.0%)	40 (43.9%)	0.502	183
BMI (kg/m^2)	24.7 (±4.85)	25.4 (±5.22)	23.9 (±4.34)	0.049	178
Rank of transplantation				0.090	183
1	89 (48.6%)	52 (56.5%)	37 (40.7%)		
2	73 (39.9%)	30 (32.6%)	43 (47.3%)		
3	20 (10.9%)	10 (10.9%)	10 (11.0%)		
5	1 (0.55%)	0 (0.00%)	1 (1.10%)		
ABO blood group				0.488	183
A	84 (45.9%)	47 (51.1%)	37 (40.7%)		
B	18 (9.84%)	8 (8.70%)	10 (11.0%)		
AB	5 (2.73%)	3 (3.26%)	2 (2.20%)		
O	76 (41.5%)	34 (37.0%)	42 (46.2%)		
Time spent in waiting list (days)	1,128 (±1,320)	1,294 (±1,692)	961 (±758)	0.088	183
RRT technique				0.662	183
Preemptive transplant	18 (9.84%)	9 (9.78%)	9 (9.89%)		
Hemodialysis	156 (85.2%)	80 (87.0%)	76 (83.5%)		
Peritoneal dialysis	9 (4.92%)	3 (3.26%)	6 (6.59%)		
Initial nephropathy				0.697	183
Undetermined	21 (11.5%)	11 (12.0%)	10 (11.0%)		
Glomerular	79 (43.2%)	39 (42.4%)	40 (44.0%)		
Vascular	18 (9.84%)	7 (7.61%)	11 (12.1%)		
Tubulo-interstitial	11 (6.01%)	4 (4.35%)	7 (7.69%)		
Polycystic	27 (14.8%)	16 (17.4%)	11 (12.1%)		
Uropathy	27 (14.8%)	15 (16.3%)	12 (13.2%)		
Other organ transplant				0.617	181
Pancreas	4 (2.21%)	1 (1.09%)	3 (3.37%)		
Liver	2 (1.10%)	1 (1.09%)	1 (1.12%)		
Lung	1 (0.55%)	0 (0.00%)	1 (1.12%)		
cPRA (%)	69.3 (±35.1)	61.5 (±37.3)	77.3 (±31.0)	0.002	183
Anti-HLA classe I	159 (89.3%)	92 (100%)	67 (77.9%)	<0.001	178
Anti-HLA classe II	135 (76.3%)	46 (53.5%)	91 (100%)	<0.001	177
Donors					
Age (years)	53.3 (±16.5)	53.2 (±16.4)	53.5 (±16.7)	0.928	183
Sex (% of men)	99 (54.1%)	53 (57.7%)	46 (50.5%)	0.418	183
BMI (kg/m^2)	26.9 (±5.66)	27.4 (±6.06)	26.3 (±5.18)	0.176	183
Donor type				0.090	183
Vascular brainstem death	95 (51.9%)	53 (57.6%)	42 (46.2%)		
Non-vascular brainstem death	78 (42.6%)	32 (34.8%)	46 (50.5%)		
Living donor	6 (3.28%)	5 (5.43%)	1 (1.10%)		
Maastricht III	4 (2.19%)	2 (2.17%)	2 (2.20%)		
ABO blood group				0.174	183
A	68 (37.2%)	38 (41.3%)	30 (33.0%)		
B	12 (6.56%)	8 (8.70%)	4 (4.40%)		
AB	4 (2.19%)	3 (3.26%)	1 (1.10%)		
O	76 (41.5%)	34 (37.0%)	42 (46.2%)		
Cold ischemia time (min)	1,074 (±490)	1,015 (±536)	1,133 (±435)	0.106	181
Perfusion machine use	58 (31.7%)	32 (34.8%)	26 (28.6%)	0.457	183

BMI, body mass index; cPRA, calculated panel-reactive antibody; RRT, renal replacement therapy.

sensitization [53.5% *versus* 100% respectively ($p < 0.001$)], HLA-DR mismatch [0.84 (±0.72) *versus* 0.54 (±0.62) respectively ($p = 0.003$)], and the use of peri-operative Rituximab [9 (10%) *versus* 22 (24.7%) respectively ($p = 0.016$)].

Primary Endpoint

Biopsy-Proven Acute Antibody-Mediated Rejection

During follow-up, 41 of the 183 patients (22.4%) presented a biopsy-proven aABMR including 14 in the Cw-DSA group and

TABLE 2 | Histocompatibility and peri-operative prophylactic strategies.

	All	Cw-DSA	DP-DSA		
	n = 183	*n* = 92	*n* = 91	*p*-value	*n*
Histocompatibility					
DSA MFI day of transplant	3,540 (±3,537)	3,228 (±3,216)	3,855 (±3,826)	0.231	183
Positive CDC crossmatch	4 (2.19%)	2 (2.17%)	2 (2.20%)	1.000	183
HLA-A mismatch number	0.99 (±0.75)	0.95 (±0.69)	1.03 (±0.81)	0.432	183
HLA-B mismatch number	1.29 (±0.69)	1.37 (±0.66)	1.21 (±0.72)	0.118	183
HLA-DQ mismatch number	0.73 (±0.69)	0.75 (±0.72)	0.72 (±0.65)	0.807	181
HLA-DR mismatch number	0.69 (±0.68)	0.84 (±0.72)	0.54 (±0.62)	0.003	183
A, *B*, *DR*, *DQ* mismatch number	3.70 (±1.94)	3.91 (±1.91)	3.49 (±1.61)	0.148	181
Induction and desensitization					
Induction therapy				0.787	183
Thymoglobulin	158 (86.3%)	78 (84.8%)	80 (87.9%)		
Anti-CD25	24 (13.1%)	13 (14.1%)	11 (12.1%)		
Alemtuzumab	1 (0.55%)	1 (1.09%)	0 (0.00%)		
Prophylactic treatment					
None	120 (65.6%)	66 (71.7%)	54 (59.3%)	0.108	183
Rituximab	31 (17.3%)	9 (10.0%)	22 (24.7%)	0.016	179
IVIGs	45 (25.1%)	21 (23.3%)	24 (27.0%)	0.698	179
Plasmapheresis	9 (5.03%)	3 (3.33%)	6 (6.74%)	0.330	179
Eculizumab	2 (1.12%)	1 (1.11%)	1 (1.12%)	1.000	179

CDC, complement-dependent cytotoxicity; DSA, donor-specific antibodies; IVIGs, Intravenous Immunoglobulins; MFI, mean fluorescence intensity.

27 in the DP-DSA group. AABMR occurred within a median time of 92 days [Q1: 25; Q3: 370]. Of note, no difference in aABMR prevalence emerged between first and retransplanted-patients (**Supplementary Table S2**). The 6 months, 1 and 2 years probabilities of aABMR were 10.9% [95% CI 6.0–19.3], 10.9% [95% CI 6.0–19.3] and 12% [95% CI 6.8–20.6] in the Cw-DSA group respectively, *versus* 16.5% [95% CI 10.3–25.8], 22.0% [95% CI 14.8–32.0] and 28% [95% CI 19.8–38.6] in the DP-DSA group, respectively (**Figure 1**). Multivariable Cox regression showed that preformed DP-DSA were associated with an increased risk of aABMR compared with Cw-DSA, with an adjusted Hazard Ratio (aHR) of 2.25 [1.17–4.31] ($p = 0.015$) (**Table 3**). Regardless of the nature of the preformed DSA, day of transplant MFI was independently associated with the risk of aABMR, with an aHR of 1.09 [1.08–1.18] ($p = 0.032$) per 1000 MFI increment (**Table 3**). Other variables associated with the risk of aABMR were recipient age [aHR = 0.76 [0.60–0.97] ($p = 0.026$)] and a positive CDC crossmatch the day of transplant [aHR = 4.59 [1.03–20.38] ($p = 0.045$)]. For MFI below 3,000, the risk for aABMR was increased in the DP-DSA group compared with Cw-DSA group, with an aHR of 4.69 [1.68–13.08]. Conversely, there was no significant difference between the groups for MFI greater than 3,000 (aHR 1.05 [0.43–2.57]), suggesting that the increased risk observed of aABMR in the DP-DSA group compared with Cw-DSA mostly concerned DSA with MFI < 3,000 (**Table 4**).

Post-Transplant DSA Monitoring

To ensure the plausibility of the effect of preformed Cw- and/or DP-DSA on the occurrence of aABMR, we monitored the post-transplant evolution overtime of the preformed DSA's MFI. In patients who experienced aABMR, mean MFI decreased from 4,446 (±3,898 SD) at the day of transplant, to 4,175 (±4,729 SD) at day 15, 2,916 (±4,934 SD) at 3 months, 2,487 (±4,191 SD) at 6 months, 1,758 (±3,139 SD) at 12 months and finally to 1,506 (±3,295 SD) at 24 months. However, strikingly, mean MFI of the preformed DSA was still at 4,463 (±5,257 SD) on the onset of aABMR. In patients who did not experience aABMR, mean MFI decreased as well from 3,191 (±3,239 SD) at the day of transplant, to 2,707 (±3,652 SD) at day 15, 1,698 (±2,589 SD) at 3 months, 1,866 (±2,424 SD) at 6 months, 1,350 (±2,324 SD) at 12 months and finally to 1,127 (±2,187 SD) at 24 months. Mean DSA's MFI follow-up is presented in **Supplementary Figure S1**. Thirty-eight *de novo* DSA (*dn*DSA) appeared during the follow-up in 25 out of the 183 (13.7%) patients (13 in the Cw-DSA group, and 12 in the DP-DSA group). The median time to onset of *dn*DSA was 494 days [Q1: 101; Q3: 882]. *De novo* DSA were directed against the *loci* A ($n = 3$), B ($n = 10$), Cw ($n = 5$), DR ($n = 11$), DQ ($n = 5$), and DP ($n = 4$). Ten of these 25 patients will present aABMR during follow-up. The onset of *dn*DSA appeared to be attributable to aABMR in 6 of these 10 aABMR-patients (14.4% of the whole aABMRs observed here), with a concomitant or shortly appearance of *dn*DSA preceding the acute rejection episode (two patients in the Cw-DSA group, and four patients in the DP-DSA group). For the other four patients, the *dn*DSA appeared largely after the occurrence of aABMR (median time between rejection and the onset of *dn*DSA (in this order): 1860 days [Q1: 481; Q3: 2,701]), and were therefore considered as unrelated to the development of aABMR (**Supplementary Table S3**). Taken together, *dn*DSA emergence may therefore interfere here with 14.4% of the aABMR onset, letting the 85.6% other aABMR+ patients with no other DSA than the preformed Cw- or DP-DSA.

Secondary Endpoints

Graft Loss

Considering graft loss, death-censored graft loss occurred for 41 of the 183 patients (Cw-DSA: 18; DP-DSA: 23). The median time until death-censored graft loss was 2.3 years [Q1: 0.4; Q3:

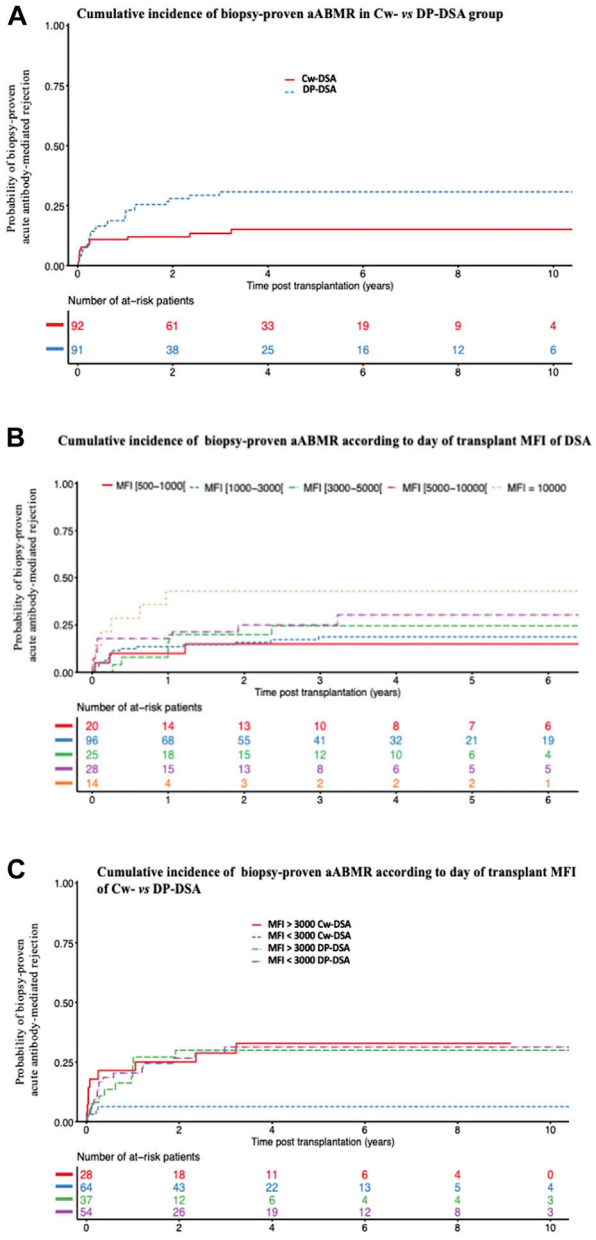


FIGURE 1 | Cumulative incidence of biopsy-proven acute antibody-mediated rejection. **(A)** Cumulative incidence of biopsy-proven aABMR according to Cw- and DP-DSA groups **(B)** Cumulative incidence of biopsy-proven aABMR according to the day of transplant DSA's MFI **(C)** Cumulative incidence of biopsy-proven aABMR according to the day of transplant MFI of Cw- *versus* DP-DSA. aABMR, acute Antibody-mediated rejection; DSA, Donor Specific Antibody; MFI, Mean Fluorescence Intensity.

6.3]. Probabilities of death-censored graft loss at 1, 3, and 5 years were 5.4% [95% CI 2.3–12.6], 9.1% [95% CI 4.7–17.4] and 16.0% [95% CI 9.2–26.8] in the Cw-DSA group *versus* 11.0% [95% CI 6.1–19.5], 15.9% [95% CI 9.7–25.5] and 19.5% [95% CI 12.3–30.3] in the DP-DSA group (**Figure 2**), respectively. Multivariable Cox regression model did not find any significant association between the type of DSA or the level of MFI with death-censored graft loss (**Table 5**).

TABLE 3 | Multivariable Cox model for the risk of biopsy-proven acute antibody-mediated rejection.

	Biopsy-proven acute ABMR	
	Multivariate	*p*-value
	HR [95% CI]	
Preformed DP- *vs* Cw-DSA	2.25 [1.17–4.31]	0.015
Day of transplant DSA's MFI (/1,000 MFI increment)	1.09 [1.01–1.18]	0.032
Recipient age (per 10 years)	0.76 [0.60–0.97]	0.026
CDC crossmatch positivity (vs. negativity)	4.59 [1.03–20.4]	0.045

ABMR, antibody-mediated rejection; CDC, complement-dependent cytotoxicity; DSA, donor-specific antibodies; MFI, mean fluorescence intensity.

Additional Prophylactic Treatment the Day of Transplant

A total of 63 patients (34.4%) were treated with an additional prophylactic treatment on the day of transplant, in addition to conventional induction, as previously described. None of the treatments had any significant effect in univariate analyses (**Figure 3**). Overall, among the 63 patients who received any additional prophylactic therapy on the day of transplant (Rituximab and/or IVIGs and/or plasmapheresis and/or Eculizumab), 15 experienced aABMR (23.8%), *versus* 26 out of the 120 patients who received the standard of care treatment (21.6%) (p = 0.65). AABMR occurred in 9 out of 31 patients treated with Rituximab (29%), *versus* 32 out of 131 other patients who were not (24.4%). Twelve out of 45 patients treated by IVIGs (26.6%) experienced aABMR, *versus* 29 out of 134 other patients who were not (21.6%). Two of the 9 patients treated by plasmapheresis (22.2%), compared with 39 out of 170 (22.9%) presented with aABMR. None of the two patients who received Eculizumab experienced rejection.

DISCUSSION

In this study, we described the incidence and risk factors associated with aABMR in a multicentric observational study of recipients transplanted in the presence of isolated preformed Cw- or DP-DSA. Two years after transplantation, the probability of developing an aABMR was 12% and 28% for patients transplanted with a preformed Cw- or DP-DSA, respectively. In multivariate analysis, the presence of a preformed DP-DSA was associated with approximately twice the risk of aABMR compared with Cw-DSA. We also found that the MFI of the DSA at the time of transplantation was significantly associated with aABMR, whatever the DSA was, and that there was a significant interaction between the nature of the DSA and the MFI. The increased risk associated with DP-DSA, compared with Cw-DSA, was significant only for MFI below 3,000. No difference was found between the groups in terms of death-censored graft loss. Finally, the use of a prophylactic therapy the day of

TABLE 4 | Multivariate interaction term model for the risk of biopsy-proven acute antibody-mediated rejection.

	Biopsy-proven acute ABMR	
	Multivariate	*p*-value*
	HR [95% CI]	
Preformed DP- *vs.* Cw-DSA/MFI < 3,000	4.69 [1.68–13.1]	0.033
Preformed DP- *vs.* Cw-DSA/MFI > 3,000	1.05 [0.43–2.57]	

This model was adjusted for recipient age and CDC crossmatch positivity. ABMR, antibody-mediated rejection; DSA, donor-specific antibodies; MFI, mean fluorescence intensity.

** The calculated p-value stands for the whole interaction term multivariate analysis.*

transplantation to prevent rejection did not seem to be associated with a lower incidence of aABMR.

Twelve percent of patients transplanted in the presence of a Cw-DSA presented in our study an aABMR at 2 years of follow-up. This incidence is lower than those reported in previous reports for Cw-DSA, between 20% and 30% [13, 24]. These results may have been impacted by a non-estimated proportion of denatured anti-HLA-Cw. Like all class I molecules, HLA-Cw can lose its β2m-chain, leading to the denaturation of the HLA molecule. Sensitization against cryptic antigens of these denatured class I molecules is frequent, yet clinically irrelevant [25]. Compared with anti-HLA-A and -B, denatured anti-HLA-Cw are particularly prevalent, corresponding to 10% of antibodies from pre-transplant patients and up to 40% of DSA in sensitized kidney transplant recipients [24, 26]. Using acid-treated Luminex beads (iBeads®, One Lambda) recognizing only native class I anti-HLA, Visentin et al., showed a prevalence of nearly 45% of denatured anti-HLA-Cw (23 of 52 patients with isolated preformed Cw-DSA). The authors revealed then a 2 years incidence of aABMR of 55% (16/29 patients) in the native Cw-DSA group, compared with 8.7% (2/23 patients) in the denatured Cw-DSA group ($p = 0.006$). This increase in aABMR was clinically reflected by a significant decrease in graft survival in the native Cw-DSA group [12]. Interestingly, in this study, mean baseline MFI of native Cw-DSA were significantly and importantly higher than of denatured anti-HLA-Cw antibodies (5,503 [1,655–8,198] *versus* 998 [742–2,140]) [12]. We may assume then that in our population, denatured DSA may be present in the lowest categories of Cw-DSA MFI, which would explain the difference of risk of aABMR associated with Cw-DSA below and over the 3,000 threshold. Considering that nowadays the probability of being transplanted in the presence of a preformed DSA is almost exclusively limited to Cw- and DP-DSA, the challenge in this population remains therefore to successfully identify pathogenic Cw-DSA, in order to help further stratify the risk. In addition to iBeads mentioned above [12], other tools such as the ability of the DSA to bind C1q [27], C3d [28], or the identification of DSA's IgG subclass [29] could be useful, and deserve to be tested specifically in this population.

We report here the largest cohort to our knowledge of patients transplanted in the presence of an isolated preformed DP-DSA, confirming the alleged association of these antibodies with aABMR. The pathogenicity of DP-DSA has indeed been raised by several case-reports [16, 17, 30–33] and clearly suggested by Bachelet et al., which provided a pooled analysis of Cw- and DP-DSA preformed DSA [18]. We report here a 2 years-incidence of aABMR of 28% in the presence of a preformed DP-DSA at the time of kidney transplantation. This is consistent with the recent report from the Swiss transplant cohort study, who also found a 2 years prevalence of aABMR of around 25% in 33 recipients transplanted in the presence of an isolated preformed DP-DSA, results of note no different from those observed in DR- or DQ-

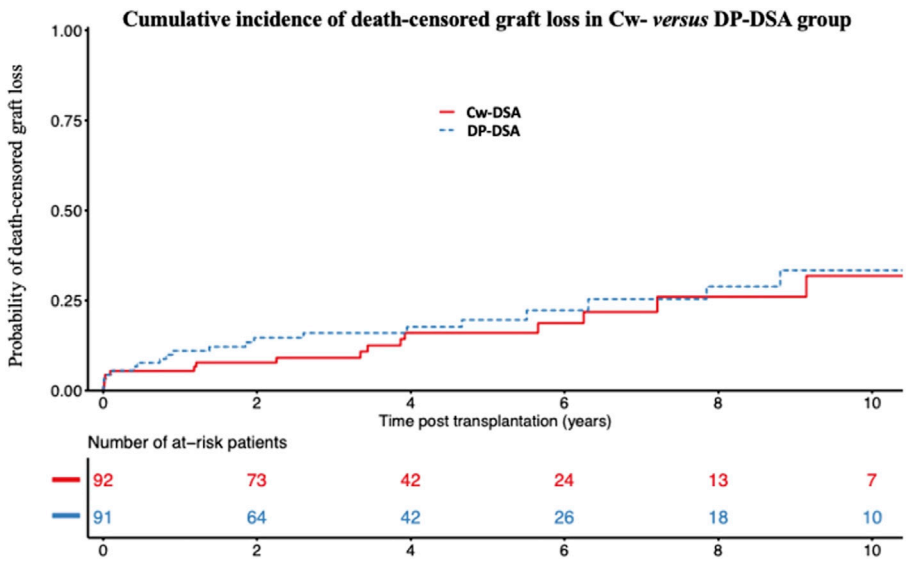


FIGURE 2 | Cumulative incidence of death-censored graft loss. Cumulative incidence of death-censored graft loss in the Cw- *versus* DP-DSA group. DSA, Donor Specific Antibody.

TABLE 5 | Multivariable Cox model for the risk of development of death-censored graft loss.

	Death-censored graft loss	
	Multivariate	***p*-value**
	HR [95% CI]	
Preformed DP- vs. Cw-DSA	1.10 [0.55–2.23]	0.786
Day of transplant DSA's MFI (/1,000 MFI increment)	1.04 [0.95–1.14]	0.358
Recipient age (/10 years)	0.87 [0.65–1.17]	0.368
Recipient sex (male vs. female)	1.27 [0.60–2.69]	0.532
Recipient BMI (per 1 kg/m^2)	1.06 [0.98–1.15]	0.127
Rank of transplantation (one vs. several)	1.90 [0.75–4.80]	0.174
Waiting time on list (per day)	1.00 [1.00–1.00]	0.411
Cold ischemia time (per minute)	1.00 [1.00–1.00]	0.202

BMI, body mass index; DSA, donor-specific antibodies; MFI, mean fluorescence intensity.

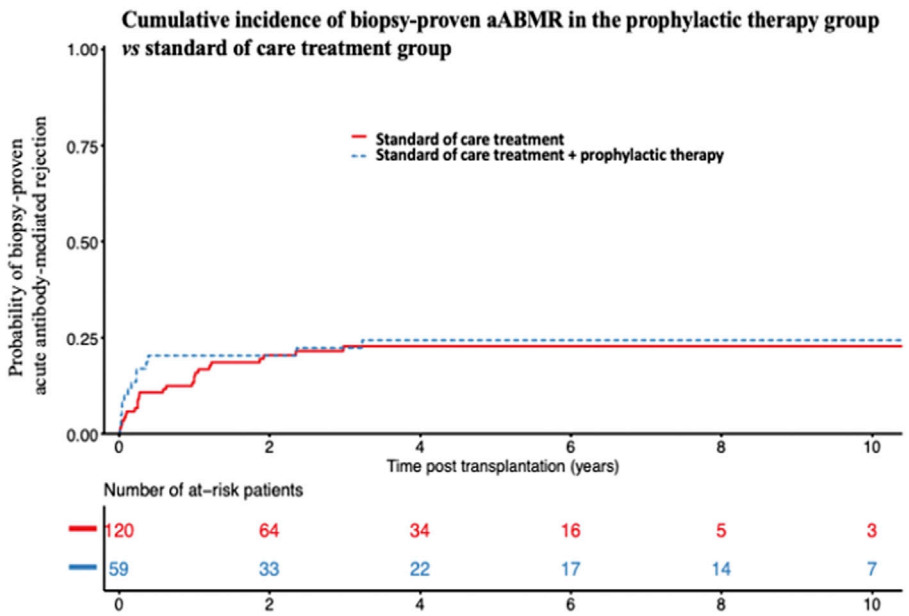


FIGURE 3 | Cumulative incidence of biopsy-proven acute ABMR after supplemental prophylactic therapy on the day of transplant. Cumulative incidence of biopsy-proven aABMR in the prophylactic therapy-group (Rituximab and/or IVIGs and/or plasmapheresis and/or Eculizumab) *versus* standard of care alone-treated group. aABMR, acute Antibody-mediated rejection; IVIGs, Intravenous Immunoglobulins.

DSA patients in this same study [34]. The nature of the rejection (aABMR), the persistence of significant post-transplant MFI of the preformed DP-DSA at the time of aABMR [3,178 (±4,618 SD)] despite natural post-transplant decrease, the relatively low proportion of *de novo* DSA appearance who could have interfered with the onset of aABMR [only 4 out of 27 (14.8%) aABMR attributable to a *dn*DSA emergence in the DP-DSA group], and finally the significant association between the MFI of the preformed DP-DSA and aABMR, are all together strong arguments in favor of the pathogenicity of these antibodies. Despite quite early onset of aABMR, anamnestic B-cell response did not seem to be the main immunological pathway here, as DSA's MFI did not strongly increase at day 15, and as no difference was observed here between first and retransplanted-patients. Noteworthily, distribution of antigenic specificities of DP-DSA matched here with the prevalence of the different *HLA-DPB1* alleles expressed by the general populations [35]. In our cohort of 183 sensitized patients transplanted with a preformed DSA, the presence of a DP-DSA was associated with a two-fold increased risk compared with Cw-DSA. Using interaction analyses, we also showed that the risk was dependent of the DSA's MFI. Below an MFI of 3,000, DP-DSA had approximately a 4-fold increased risk compared with Cw-DSA, but this risk disappeared for MFI over 3,000. Taken together, these results could suggest that the Cw-DSA pathogenicity would be proportional to its MFI on the day of transplantation for values greater than 3,000, whereas the pathogenic effect of DP-DSA would be constant, and would appear whatever the MFI is. Conversingly, the recent Swiss transplant cohort study already discussed above found similar

results for all classe II-DSA, including DP-DSA, demonstrating an association with aABMR and death-censored graft loss even for MFI < 1,000, while classe I-DSA (including 28 Cw-DSA patients) where associated with aABMR only for MFI > 1,000 [34]. In a historical cohort, Lefaucheur et al., demonstrated a prevalence of aABMR in a sensitized cohort of patients without DSA of only 0.94% (3/319 patients) at 8 years, a result largely below the 28% of aABMR observed in our study at 2 years in the DP-DSA group [2]. The prevalence of aABMR in this same study was conversely 34.9% (29/83 patients) in the group transplanted in the presence of a preformed anti-A, -B, -DR or -DQ DSA [2], results close to those observed in our study in the DP-DSA group. Taken together, these results suggest that DP-DSA may exhibit a pathogenicity at least similar to other DSA included in "unacceptable antigens" allocation programs.

Finally, our study did not show any trend in favor of a reduction of aABMR after peri-operative prophylactic treatment to prevent rejection. In a small prospective cohort, Akalin et al., showed a mean decrease in the MFI of preformed DSA in the group treated with IVIGs and plasmapheresis of 38% (n = 14), compared with a decrease of 24% in the group receiving only IVIGs (n = 9). The prevalence of aABMR was 44% (4 patients out of 9) in the IVIGs alone group *versus* 7% (1 patient out of 14) in the IVIGs + plasmapheresis group [36]. In a prospective uncontrolled study, Jin et al., reported no episode of acute rejection in 7 HLA-incompatible transplant-recipients (presence of a DSA on transplant day) treated with peri-operative low-dose IVIGs, plasmapheresis and Rituximab over a mean follow-up of 3 years [37]. Finally, in a retrospective cohort of 50 sensitized recipients transplanted in the presence of a preformed DSA, treated (n = 25) or not treated (n = 25) with Rituximab in addition to treatment with IVIGs and peri-operative plasmapheresis, the Rituximab-treated patients had less DSA rebound during follow-up. However, there was a similar proportion of biopsy-proven acute rejection and especially aABMR (4 *versus* 6, p = 0.23), with similar graft survival between the two groups [38]. It is important to emphasize that none of these studies, retrospective and/or with small number of patients, included Cw- or DP-DSA. Although no trend in favor of an aggressive prophylactic strategy emerged from our observation, our retrospective study was not designed to evaluate the efficacy of such therapies. Further randomized studies would be warranted then to assess the validity of such treatments.

Our findings need to be interpreted in the context of some caveats. First, the retrospective nature of the study could be associated with information bias. Another limitation pertains to the absence of a control group without Cw- or DP-DSA. Including a control group, while essential for drawing meaningful conclusions, poses challenges related to potential biases that could compromise the validity of our findings. To navigate this issue, we deliberately refrained from introducing such a control group. On the one hand, a control group comprising exclusively non-sensitized recipients would not permit us to distinguish between the impact of HLA sensitization and the effect of preformed DSA. HLA sensitization is recognized to influence the likelihood of acute rejection and graft loss, even in the absence of DSA [39]. Therefore, introducing this group would confound our ability to isolate the specific effects of DSA. On the other hand, forming a control group solely consisting of sensitized recipients without preformed DSA introduces biases associated with prevailing definitions of HLA sensitization in allocation systems. In France, as in many other countries, HLA sensitization is assessed using the cPRA, which is contingent upon the prevalence of HLA antigens within the allocation population. Comparing outcomes between sensitized recipients with equivalent cPRA values assumes uniform levels of sensitization, disregarding the nuanced nature of HLA antibodies according to the prevalence of HLA antigens in the French population. Considering the current debate surrounding cPRA's effectiveness in stratifying immune risk [40], we opted to restrict our analyses to a specific population in the French allocation system that can be transplanted with preformed DSA—namely, those with Cw- or DP-specific DSA. This approach allows us to more directly assess the impact of these specific DSA while minimizing potential biases inherent in broader control groups.

In conclusion, the 2 years incidence of acute ABMR in this multicentric study was 12% and 28% for patients transplanted in the presence of a preformed Cw- or DP-DSA, respectively. The pathogenicity of Cw-DSA was MFI-dependent, and appeared essentially for MFI superior to 3,000, while the increased risk of aABMR occurred even for low-MFI value DP-DSA. Taken together, these results suggest that Cw- and DP-DSA might present a pathogenicity at least equivalent to other DSA included in "unacceptable antigens" program. Today, no consensual attitude exists in most allocation systems regarding Cw-DSA and DP-DSA. Our results may therefore question the need of taking these antibodies into account in the allocation algorithm, in the same way as the other anti-HLA antibodies.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

This multicentric observational study conforms to the tenets of the Istanbul Declaration and the ethical guidelines set forth by the Helsinki Declaration and was approved by the local institutional review boards, and authorized with the n° DEC20-002 by French authorities (Comission Nationale de l'Informatique et des Libertés, CNIL). No organs were procured from prisoners. All participants provided their informed consent.

AUTHOR CONTRIBUTIONS

Study conceptualization: TL, RL, MH, and MM. Data acquisition: TL, JV, GF, and PC. Statistical Analysis: RL and MM. Manuscript drafting: TL, RL, and MM. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11416/full#supplementary-material

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Measurement of the Immunosuppressant Possession Ratio by Transplant Clinical Pharmacists Captures a Non-Adherence Associated With Antibody-Mediated Rejection

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Our objective was to calculate an immunosuppressant possession ratio (IPR) to diagnose non-adherence at the time of antibody-mediated rejection (ABMR). IPR was defined as the ratio of number of pills collected at the pharmacy to the number of pills prescribed over a defined period. In a first cohort of 91 kidney transplant recipients (KTRs), those with an IPR < 90% had more frequently a tacrolimus through level coefficient of variation >30% than patients with an IPR = 100% (66.7% vs. 29.4%, $p = 0.05$). In a case-control study, 26 KTRs with ABMR had lower 6 months IPRs than 26 controls (76% vs. 99%, $p < 0.001$). In KTRs with ABMR, non-adherence was more often diagnosed by a 6 months IPR < 90% than by clinical suspicion (73.1% vs 30.8%, $p = 0.02$). In the multivariable analysis, only *de novo* DSA and 6 months IPR < 90% were independently associated with ABMR, whereas clinical suspicion was not (odds ratio, 4.73; 95% CI, 1.17–21.88; $p = 0.03$; and odds ratio, 6.34; 95% CI, 1.73–25.59; $p = 0.007$, respectively). In summary, IPR < 90% is a quantifiable tool to measure immunosuppressant non-adherence. It is better associated with ABMR than clinical suspicion of non-adherence.

Keywords: kidney transplantation, clinical pharmacist, adherence, immunosuppresant possession ratio, antibody-mediated rejection

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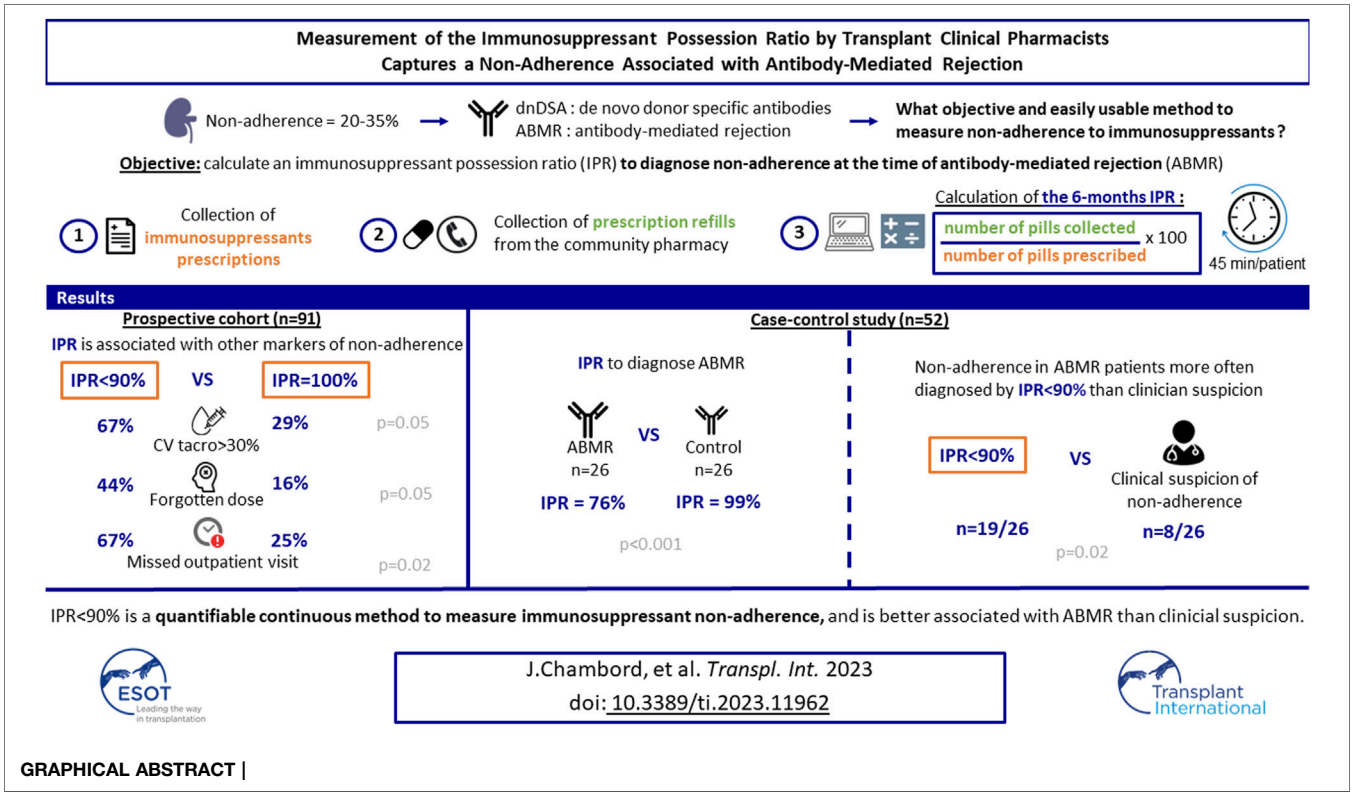
Abbreviations: ABMR, antibody-mediated rejection; CV_{TAC}, tacrolimus trough levels coefficient of variation; CV_{CsA}, cyclosporine trough levels coefficient of variation; DSA, donor-specific antibodies; g, glomerulitis; hABMR, histological antibody-mediated rejection; IFTA, interstitial fibrosis and tubular atrophy; IPR, immunosuppressant possession ratio; KTR, kidney transplant recipients; ptc, peritubular capillaritis; SAFB, single-antigen flow beads assays.



GRAPHICAL ABSTRACT |

INTRODUCTION

The prevalence of non-adherence to immunosuppressants in renal transplant recipients is between 20%–35% in adults [1–3]. This is a continuous process which increases during the first 2 years post-transplantation [1, 4, 5], and is associated with *de novo* donor-specific antibodies occurrence (DSA) [6–8], antibody-mediated rejection (ABMR) [7, 9], T-cell mediated rejection [6, 10], and graft loss [3, 11–16]. Proactive interventions to improve adherence are essential for the prevention of allograft loss. However, before designing a suitable multi-dimensional intervention, the key question is how immunosuppressant non-adherence can be diagnosed prospectively [17].

Subjective methods to assess non-adherence include clinical suspicion and self-administered questionnaires. Suspicion by the clinician of medication non-adherence underestimates this phenomenon and is frequently influenced by a poor outcome or non-adherence to follow-up [18]. Self-reported measurement of medication non-adherence is easily distorted by patients, explaining why its ability to predict rejection and graft loss is equivocal [19–23]. Objective methods for the measurement of non-adherence include calcineurin inhibitor trough levels and electronic monitoring. Both low calcineurin inhibitor trough levels [24–27] and intra-patient variability of tacrolimus are associated with *de novo* DSA, rejection, and graft loss [28–31]. However, interactions with a drug or food can give a false impression of non-adherence. The ability of electronic monitoring to measure non-adherence to immunosuppressants is also debated [17, 21, 22, 32–35]. In addition, this tool is very costly and restrictive, which could prevent its implementation in a clinical setting.

Therefore, transplant physicians do not yet have an objective and easily usable method for measuring non-adherence to immunosuppressants [1]. The Immunosuppressant Possession Ratio (IPR) is the number of therapeutic units collected at the pharmacy divided by the number of therapeutic units prescribed over the same period of time [12]. Retrospective studies using Medicare data in the United States reported that low IPRs were associated with graft failure [11, 12, 15, 36]. In these studies, IPR thresholds used to determine non-adherence varied between 80% and 99%. In France, the rate of prescription refill can be easily retrieved through pharmacy management software. No special authorization is required.

The objectives of this study were: 1) to test the feasibility of prospectively calculating the IPR in a first cohort of kidney transplant recipients (KTR), 2) to determine its association with other markers of non-adherence, 3) to determine a standardized period for its calculation, 4) to analyze whether the IPR-based non-adherence diagnosis was associated with ABMR in a second cohort of KTR, and 5) to compare the IPR-based non-adherence diagnosis with our standard method based on clinical suspicion.

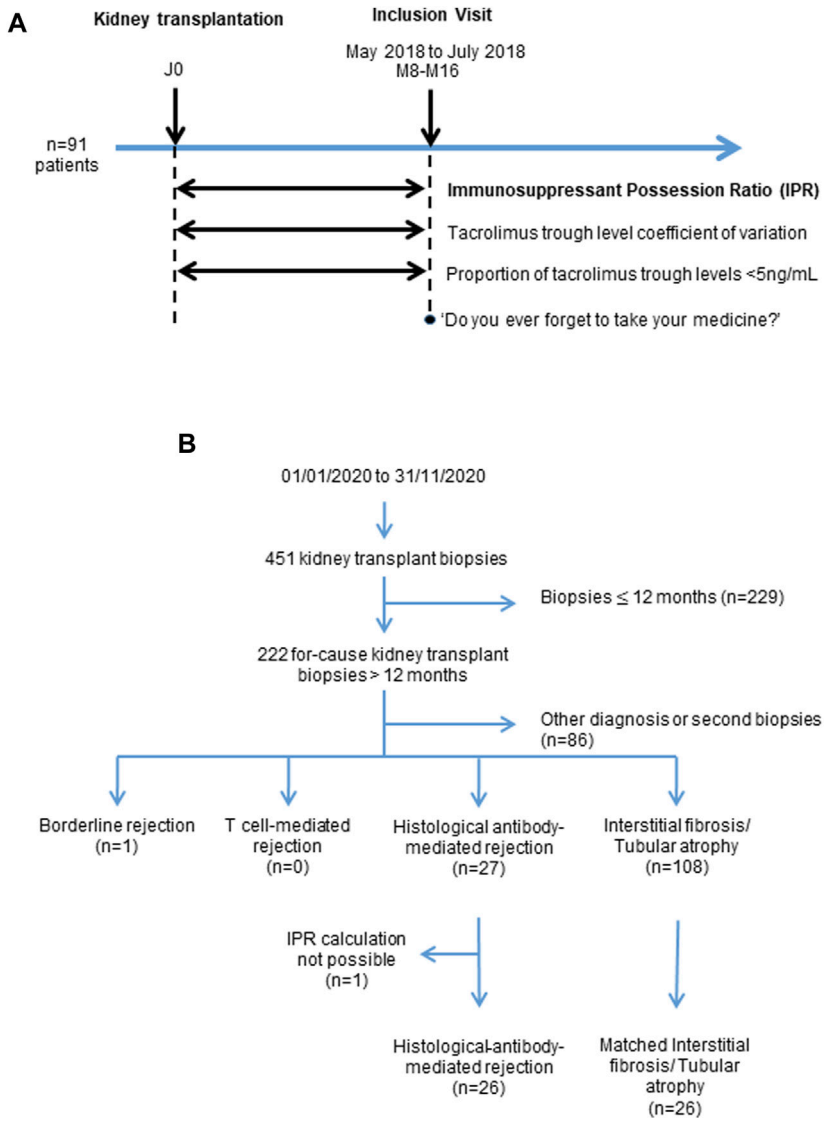


FIGURE 1 | Description of the two Cohorts. Study design of the first prospective Cohort 1 **(A)**. Flow chart of the retrospective Cohort 2 **(B)**.

PATIENTS AND METHODS

Study Design and Patients

First Prospective Cohort

We conducted a non-interventional study at Bordeaux University Hospital between May and July 2018 on a first cohort to test the feasibility of prospectively calculating the IPR. Ninety-one consecutive kidney transplant recipients coming for an outpatient visit between 8 and 16 months post-transplantation were included (**Figure 1A**). During this inclusion visit, the following patient information was collected: treatments doses, prescription refills and hospitalization stays since transplantation for calculating the IPR, calcineurin inhibitor trough levels, missed outpatient visits, and patient-reported drug side effects. Patients were also asked if they had forgotten to take their medication at least once since transplantation. No pill count was carried out.

Second Retrospective Cohort

We tested the association of IPR with the occurrence of clinically apparent histological ABMR (hABMR) in a second retrospective cohort of patients. Between January and December 2020, 451 kidney transplant biopsies were performed at our institution. We excluded 229 biopsies performed during the first 12 months post-transplantation, because the calculation of the IPR required a 6 or 12 months period. Among the 222 remaining for-cause biopsies, we identified 27 patients with a diagnosis of clinical hABMR and 108 with interstitial fibrosis and tubular atrophy (IFTA) without additional specific lesion. The IPR was not available for one ABMR patient because this patient used many community pharmacies and

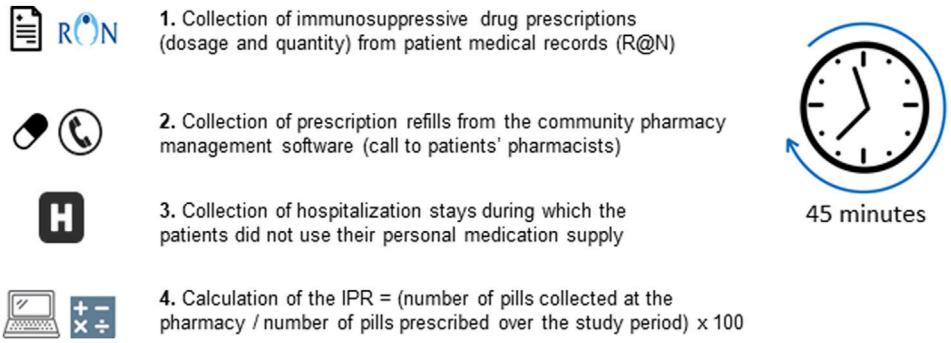


FIGURE 2 | Measurement of the immunosuppressant possession ratio (IPR).

we were not able to recover prescription refills from all of them. The IPR was then compared between the clinical hABMR group ($n = 26$) and an IFTA control group of 26 patients that were matched 1:1 for age and year of transplantation (**Figure 1B**). In this cohort we compared the IPR-based non-adherence diagnosis with our standard method based on clinical suspicion.

The study was approved by the local Ethics Committee and conducted in accordance with the Declaration of Helsinki. Our clinical database had a French CNIL final agreement, decision 2009-413, n° 1357154, 2 July 2009.

Measurement of the Immunosuppressant Possession Ratio

IPR since transplantation was calculated by two transplant clinical pharmacists, as follows: data related to immunosuppressant prescriptions such as dosage and quantity were collected from our patient medical records (R@N); data related to prescription refills and patient dispensing were provided by the patients' pharmacists using their community pharmacy management software. IPR was then calculated according to the following formula: IPR = (number of pills collected at the pharmacy/number of pills prescribed over the study period) × 100. Importantly, IPR was calculated taking into account hospitalization stays, during which the patients did not use their personal medication supply (**Figure 2**).

The most frequently used drug for calculating IPR was mycophenolate, in 71 patients (78.0%) in Cohort 1 and 34 patients (65.4%) in Cohort 2, because its dose did not change frequently. If the mycophenolate was discontinued, steroids, everolimus, azathioprine and tacrolimus were used in 17 patients (18.7%), 1 (1.1%), 1 (1.1%) and 1 patient (1.1%), in Cohort 1, respectively, and steroids, azathioprine, tacrolimus and cyclosporine were used in 14 patients (26.9%), 1 (1.9%), 2 (3.8%) and 1 patient (1.9%) in Cohort 2, respectively.

Measurement of Drug Exposure: Calcineurin Inhibitor Trough Levels

Intra-patient variabilities of tacrolimus and cyclosporine were calculated using the coefficient of variation (CV_{TAC}; CV_{CsA}). The CV was calculated using the following formula: (standard deviation/mean trough levels of tacrolimus or cyclosporine) × 100. The mean and standard deviation were calculated using all available plasma concentrations. Patients with a $CV_{TAC} > 30\%$ were considered to have experienced varying levels of exposure to tacrolimus and were described as being at higher risk of *de novo* DSA and graft loss [30]. In cohort 2, we also measured the last tacrolimus and cyclosporine trough level because values <5 ng/mL, are known to be associated with higher *de novo* DSA incidence [26].

Clinical Suspicion of Non-Adherence

Clinical suspicion of non-adherence was documented by clinic staff and registered in the patients' medical records.

Definition of Histological ABMR

The 222 for-cause biopsies performed were reviewed according to the Banff 2019 classification [16].

We used the term "histological ABMR" (hABMR) proposed by Senev et al. [37] for cases that met the first two Banff 2019 criteria for histology of ABMR. Criterion 1 included one or more of the following lesions: glomerulitis (g), peritubular capillaritis (ptc), arteritis, or thrombotic microangiopathy. Criterion 2 included a microvascular inflammation score (g+ptc) ≥ 2 and/or linear C4d staining on peritubular capillaries [16]. This definition of histological ABMR was made regardless of the third criterion (serological evidence of DSA). Cases with histological ABMR could then be anti-HLA DSA positive or without detectable anti-HLA DSA.

Identification of Anti-HLA Donor-Specific Antibodies

Sera were tested at the time of each biopsy with single-antigen flow beads assays (SAFB) (One Lambda, Inc., Canoga Park, CA) in accordance with the manufacturer's recommendations for routine assay use, with ethylenediaminetetraacetic acid in order to avoid the complement interference phenomenon [38–40]. The SAFB were acquired on a Luminex 100® analyzer (Luminex, Austin, TX). Mean fluorescence intensity (MFI) values were normalized using the baseline formula (Fusion®

TABLE 1 | Patients' characteristics in prospective cohort 1, according to the immunosuppressant possession ratio.

	All (*n* = 91)	IPR < 90% (*n* = 9)	*p* (vs. IPR = 100%)	IPR = 90–94% (*n* = 6)	*p* (vs. IPR = 100%)	IPR = 95–99% (*n* = 25)	*p* (vs. IPR = 100%)	IPR = 100% (*n* = 51)
Baseline characteristics								
Age (years, IQR)	57 (47–65)	54 (35–71.5)	0.91	60.5 (52–70.3)	0.19	60 (49–64.5)	0.14	55 (43–65)
Female (%)	29 (31.9%)	3 (33.3%)	>0.99	1 (16.7%)	0.65	8 (32.0%)	>0.99	17 (33.3%)
Time since transplantation (months, IQR)	12.7 (10.2–15.6)	13.2 (11.4–15.5)	0.68	17.1 (13.4–17.9)	**0.01**	12.1 (10.1–15.5)	0.82	12.7 (9.6–15.5)
≥2 transplantations (%)	13 (14.3%)	2 (22.2%)	0.66	1 (16.7%)	>0.99	1 (4.0%)	0.15	9 (17.3%)
Hemodialysis (%)	63 (69.2%)	7 (77.8%)	0.71	5 (83.3%)	0.65	17 (68.0%)	>0.99	34 (66.7%)
Peritoneal dialysis (%)	13 (14.3%)	1 (11.1%)	>0.99	1 (16.7%)	>0.99	2 (8.0%)	0.32	9 (17.6%)
Post-transplant educational program (%)	37 (40.7%)	2 (22.2%)	0.29	3 (50.0%)	>0.99	10 (40.0%)	>0.99	22 (43.1%)
Treatment								
Number of medications a day (IQR)	10 (8–13)	9 (8–11)	0.48	11.5 (8–17)	0.50	10 (7–11)	0.42	10 (8–14)
Pillbox use (%)	57 (62.0%)	8 (88.9%)	0.14	3 (50.0%)	>0.99	17 (68.0%)	0.46	29 (56.9%)
Tacrolimus ER (%)	84 (92.3%)	6 (66.7%)	0.06	6 (100%)	>0.99	25 (100%)	0.16	47 (92.2%)
Corticosteroids (%)	62 (68.1%)	7 (77.8%)	>0.99	5 (83.3%)	0.32	14 (56.0%)	0.30	36 (70.6%)
Mycophenolate (%)	71 (78.0%)	5 (55.6%)	0.23	5 (83.3%)	>0.99	22 (88.0%)	0.36	39 (76.4%)
Everolimus (%)	12 (13.2%)	0	0.33	0	0.58	3 (12.0%)	0.74	9 (17.6%)
Azathioprine (%)	2 (2.2%)	0	>0.99	1 (16.7%)	0.20	0	>0.99	1 (2.0%)
Side effects (%)	19 (20.9%)	4 (44.4%)	0.21	1 (16.7%)	>0.99	3 (12.0%)	0.36	11 (22.0%)
Immunosuppressant possession ratio (IPR, median, IQR)	100% (98–100)	85% (76–89)		93% (92–94)		98% (97–99)		100% (100–100)
Tacrolimus exposure								
Tacrolimus trough levels coefficient of variation (CV_{TAC}, median, IQR)	26.2 (20.8–30.7)	32.0 (24.7–36.6)	0.06	27.3 (23.2–28.7)	0.98	22.0 (19.0–28.4)	**0.05**	26.3 (21.5–30.7)
Number of patients with a CV_{TAC} > 30% (%)	26 (28.6%)	6 (66.7%)	**0.05**	1 (16.7%)	0.67	4 (16%)	0.27	15 (29.4%)
Number of patients who claimed having forgotten to take their medicine since transplantation at least once (%)	14 (15.4%)	4 (44.4%)	**0.05**	0	>0.99	3 (13.6%)	>0.99	7 (15.9%)
At least one missed outpatient visit since transplantation (%)	25 (27.5%)	6 (66.7%)	**0.02**	2 (33.3%)	0.65	4 (16%)	0.40	13 (25.5%)

ER, extended-release; IQR, interquartile range; IPR, immunosuppressant possession ratio; SR, standard-release. Quantitative variables are reported as: median (IQR). Results in bold are the number of patients, column labels and significant p-values (<0,05).

software, One Lambda, Inc.). The positivity threshold was set at MFI ≥ 500.

Statistical Analysis

The groups were compared using Fisher's exact test or McNemar's test for the qualitative variables and Student's t-test and the Mann–Whitney test for the quantitative variables. The relationship between different computations of the IPR were assessed with Spearman's correlation (rho). Patient characteristics and pharmacokinetics data were expressed as medians with the interquartile range (IQR). A p-value ≤ 0.05 was considered to represent statistical significance. Factors associated with hABMR in cohort 2 were identified using logistic regression. Risk factors with a p-value lower than 0.2 in the univariable analysis were included in two multivariable models that were simplified by iterative backward elimination, only keeping the covariables with a p-value below or equal to 0.05. A ROC curve analysis was performed to identify an optimal threshold of IPR to predict hABMR. Finally, we used the net reclassification improvement (NRI) to compare the clinical utility of the 6 months IPR < 90% with the clinical suspicion of non-adherence, for the hABMR prediction [41]. The GraphPad Prism v8® software was used for statistical analyses.

RESULTS

Prospective Calculation of the Immunosuppressant Possession Ratio

The baseline characteristics of the 91 patients of the first cohort at inclusion are presented in **Table 1**. All patients received tacrolimus, and 84 (92.3%) were treated with an extended-release formulation (**Table 1**). Tacrolimus was given in association with mycophenolate in 71 patients (78.0%), everolimus in 12 patients (13.2%) and azathioprine in 2 patients (2.2%). Steroids were given to 62 patients (68.1%).

At inclusion, we were able to calculate the IPR since transplantation in all these patients, and the mean time needed to calculate was approximatively 45 min per patient (**Figure 2**). IPR ranged from 49% to 100% with a median (IQR) of 100% (97–100).

Immunosuppressant Possession Ratio Since Transplantation Is Associated With Other Markers of Non-Adherence

In the first cohort, patients were divided into three groups according to their IPR (<90%, 90%–94%, 95%–99%) and compared to the patients with an IPR = 100%, in order to determine an optimal non-

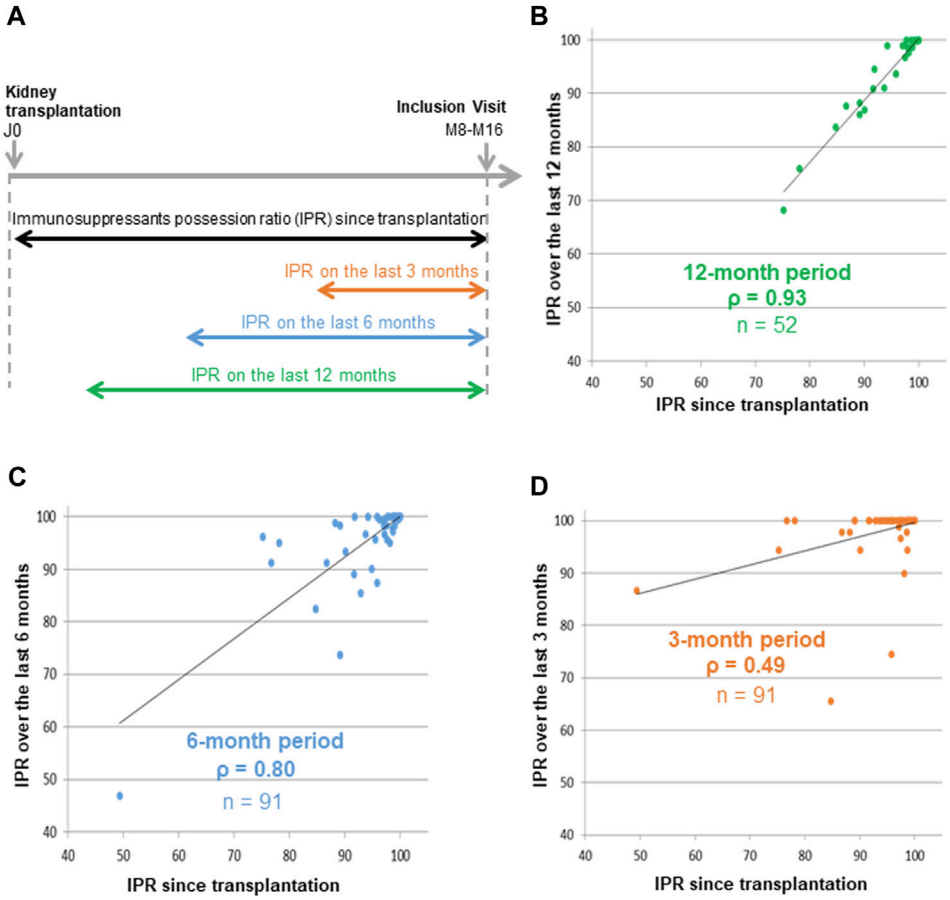


FIGURE 3 | Correlations between immunosuppressant possession ratios since transplantation and three standardized periods The immunosuppressants possession ratio (IPR) was calculated since transplantation for all 91 patients, over the previous 12 months in 52 patients, over the previous 6 months in 91 patients, and over the previous 3 months in 91 patients **(A)**. Spearman correlations were performed between the IPRs calculated since transplantation and the IPRs calculated over the previous 12 months **(B)**, 6 months **(C)** and 3 months **(D)**.

adherence threshold. Nine patients had an IPR<90% (9.9%) 6 patients an IPR of 90%–94% (6.6%), 25 patients an IPR of 95%–99% (27.5%), and 51 patients an IPR = 100% (56.0%) (**Table 1**).

Patients with an IPR < 90% had more frequently a CV_{TAC} > 30% (66.7% vs. 29.4%, $p = 0.05$), and claimed to have forgotten to take their medication more frequently (44.4% vs. 15.9% $p = 0.05$) than patients with an IPR = 100%. Patients with IPRs<90% were also more likely to miss at least one outpatient visit (66.7% vs. 25.5%, $p = 0.02$) than patients with IPRs = 100% (**Table 1**). Patients with an IPR of 95%–99% had a lower CV_{TAC} (22.0% vs 26.3%, $p = 0.05$) than patients with IPRs = 100% (**Table 1**).

In summary, patients with an IPRs < 90% exhibited more frequently other markers of non-adherence.

Calculation of the Immunosuppressant Possession Ratio Over a Standardized Period

We then tried to determine the optimal duration for calculating the IPR in order to standardize the measurement of this variable. We observed a poor correlation between the IPR calculated over the previous 3 months period and the IPR calculated since transplantation ($\rho = 0.49$). We observed a very good correlation between the IPR calculated over the previous 12 months and the previous 6 months period and the IPR calculated since transplantation ($\rho = 0.93$, and $\rho = 0.8$, respectively) (**Figure 3**). In summary, the IPR seemed to be calculated reliably over a period of 6 or 12 months. However, the 6 months IPR was used for the rest of the study because it is faster to calculate and more representative of current adherence than the 12 months IPR.

Six-Month Immunosuppressant Possession Ratio at the Time of Clinical Histological Antibody-Mediated Rejection Diagnosis

We next tested the association of 6 months IPR with the occurrence of clinical hABMR in the retrospective cohort 2. Among 222 for-cause biopsies for the period considered, 26 patients with clinical hABMR were compared to

TABLE 2 | Patients' characteristics in retrospective cohort 2.

	All (*n* = 52)	Histological antibody-mediated rejection (*n* = 26)	Interstitial fibrosis and tubular atrophy (*n* = 26)	*p*
Baseline characteristics				
Age (years, median, IQR)	49 (42–62)	50 (41–63)	49 (45–59)	0.89
Female (%)	24 (46.2%)	12 (46.2%)	12 (46.2%)	>0.99
Time since transplantation (months, IQR)	81 (41.3–173.3)	88.5 (41.3–155.3)	72.5 (40.8–192.3)	0.96
Treatment				
Tacrolimus ER (%)	29 (55.8%)	14 (53.9%)	15 (57.7%)	0.78
Tacrolimus SR (%)	8 (15.3%)	5 (19.2%)	3 (11.5%)	0.44
Cyclosporine (%)	13 (25.0%)	6 (23.1%)	7 (26.9%)	0.75
Corticosteroids (%)	35 (67.3%)	19 (73.0%)	16 (61.5%)	0.38
Mycophenolate (%)	34 (65.4%)	17 (65.4%)	17 (65.4%)	>0.99
Everolimus (%)	4 (7.7%)	0	4 (15.4%)	**0.04**
Azathioprin (%)	1 (1.9%)	1 (3.8%)	0	0.31
Sirolimus (%)	1 (1.9%)	1 (3.8%)	0	0.31
Renal injury				
Microvascular inflammation (g + ptc, median, IQR)	1 (0–3)	3 (2–3)	0 (0–0)	< **0.001**
C4d graft deposition (%)	12 (23.1%)	12 (46.2%)	0	< **0.001**
Transplant glomerulopathy (cg, median, IQR)	0 (0–2)	1 (0–2)	0 (0–0)	< **0.001**
Interstitial inflammation and tubulitis (i + t, median, IQR)	0 (0–1)	0 (0–1)	0 (0–1)	0.14
Interstitial fibrosis and tubular atrophy (ct + ci, median, IQR)	4 (2–4)	4 (2–6)	3.5 (2–4)	0.69
Arteriosclerosis (cv, median, IQR)	1 (0–2)	1 (0–2)	2 (1–3)	0.34
De novo anti-HLA donor specific antibodies (DSA)				
DSA (%)	19 (38%)	15 (57.7%)	4 (15.4%)	**0.03**
Only class I DSA (%)	1 (1.9%)	1 (3.8%)	0	>0.99
Only class II DSA (%)	10 (19.2%)	7 (26.9%)	3 (11.5%)	0.29
Class I + II DSA (%)	8 (15.4%)	7 (26.9%)	1 (3.8%)	**0.05**
Sum of DSA MFI (arbitrary unit, median, IQR)	0 (0–4,767)	3,542 (0–18,985)	0 (0–0)	< **0.001**
Treatment exposure				
Last tacrolimus trough level (ng/mL, IQR)	***n* = 32**[a] 6.3 (5.5–7.9)	***n* = 14** 6.6 (6.0–8.5)	***n* = 18** 5.9 (5.1–7.8)	0.29
Tacrolimus trough level coefficient of variation (CV_{TAC}, median, IQR)	***n* = 31**[b] 24.4 (14.2–34.7)	***n* = 13** 17.5 (12.8–37.8)	***n* = 18** 30.0 (16.6–35.2)	0.92
Last cyclosporine through level (ng/mL, IQR)	***n* = 13** 121 (92–152)	***n* = 6** 103 (65–147)	***n* = 7** 143 (104–178)	0.23

DSA, donor-specific antibodies; ER, extended-release- IQR, interquartile range-SR, standard-release; MFI, mean fluorescence intensity. Quantitative variables are reported as: median (IQR).

[a]*15 patients were not treated with tacrolimus and 5 patients had no tacrolimus trough level available on the last year.*

[b]*A minimum of three available plasma concentration values was required to calculate the tacrolimus coefficient of variation: incomplete data for one patient.*

Results in bold are the number of patients, column labels and significant p-values (<0,05).

26 patients with IFTA (**Figure 1B**). The patients' characteristics at the time of the for-cause biopsy are depicted in **Table 2**. No patients had preformed anti-HLA DSA. *De novo* anti-HLA DSA were found in 15/26 patients (57.7%) with clinical hABMR and in 4/26 patients (15.4%) with IFTA ($p = 0.03$).

The 6 months IPR was calculated from the day of the biopsy for the 52 patients. Patients with clinical hABMR had a lower 6 months IPR than patients with IFTA (76% vs. 99%, $p < 0.001$) (**Figure 4A**). Univariable analysis identified only *de novo* DSA and 6 months IPR as risk factors for hABMR. In a first multivariable analysis (model 1), these two variables were independently associated with hABMR (odds ratio, 4.66; 95% CI, 1.19–20.94; $p = 0.03$, and odds ratio, 0.73 per 10% increase; 95% CI, 0.51–0.98; $p = 0.05$, respectively) (**Table 3**).

Diagnosis of Non-Adherence Based on 6 Month Immunosuppressant Possession Ratio Below 90%

ROC curve analysis showed that the IPR was a good predictor of hABMR (AUC = 0.79). The optimal predictive threshold of IPR for clinical hABMR occurrence was 92% with a 77% sensitivity and 77% specificity (**Supplementary Figure S1**). This threshold value was very close to 90% found in cohort 1. Therefore, we chose to compare the

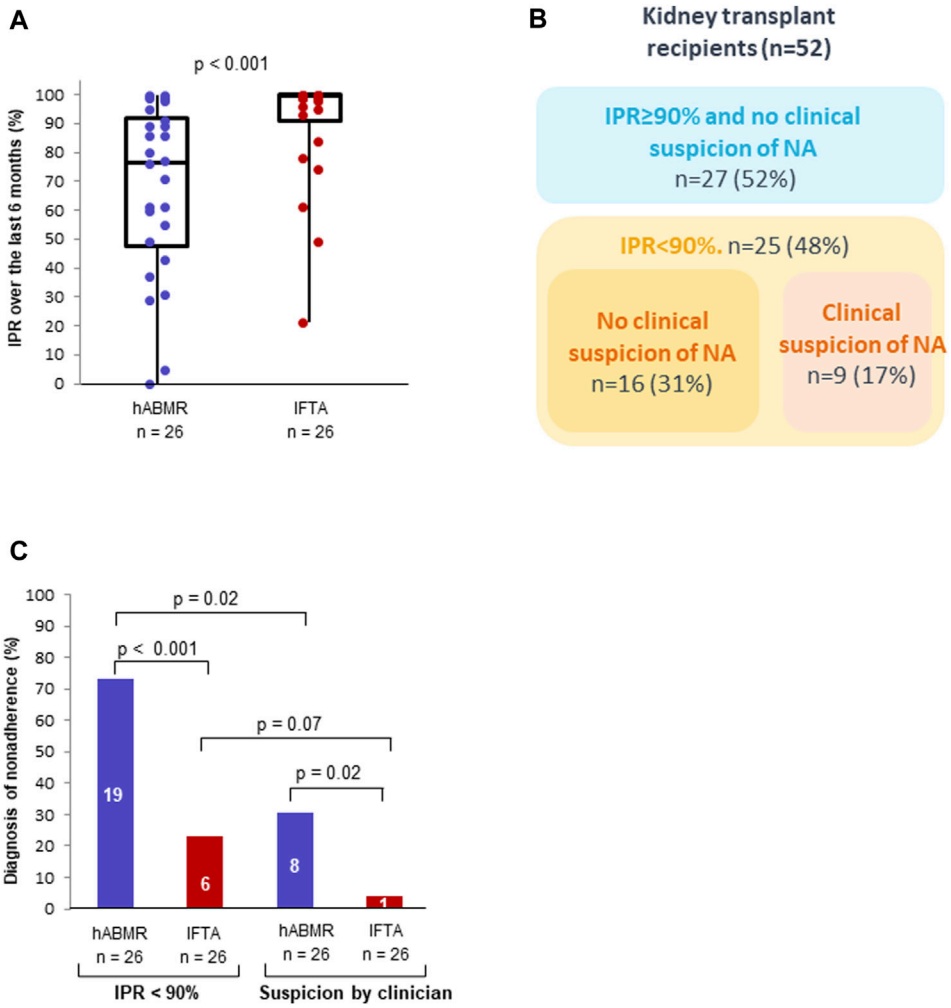


FIGURE 4 | Immunosuppressant possession ratio and clinical suspicion of non-adherence in patients with clinical histological antibody-mediated rejection (hABMR) or interstitial fibrosis with tubular atrophy (IFTA). Description of the immunosuppressant possession ratios calculated over the previous 6 months in patients with a clinical hABMR and IFTA **(A)**. Contingency table of non-adherence identified by immunosuppressant possession ratio <90% over the previous 6 months and clinical suspicion **(B)**. Non-adherence identified by clinical suspicion and immunosuppressant possession ratio <90% over the previous 6 months in patients with a clinical hABMR and IFTA **(C)**.

TABLE 3 | Factors associated with histological antibody-mediated rejection.

Variable	Univariable analysis		Multivariable analysis model 1		Multivariable analysis model 2	
	Unadjusted OR (95% CI)	*p*-value	Adjusted OR (95% CI)	*p*-value	Adjusted OR (95% CI)	*p*-value
Age (year)	0.99 (0.96–1.04)	0.84				
Male sex (male *versus* female)	1.00 (0.33–3.00)	>0.99				
Time since transplantation (months)	1.00 (0.99–1.01)	0.96				
Tacrolimus versus cyclosporine	1.23 (0.35–5.51)	0.75				
Mycophenolate versus other	1.00 (0.29–3.46)	>0.99				
Corticosteroids	1.00 (0.58–1.73)	>0.99				
Tacrolimus trough level coefficient of variation >30%	0.58 (0.17–1.93)	0.38				
De novo DSA[1, 2]	7.50 (2.15–31.53)	0.003	4.66 (1.19–20.94)	0.03	4.73 (1.17–21.88)	0.03
6-month IPR (10% increase)[1]	0.67 (0.47–0.87)	0.008	0.73 (0.51–0.98)	0.05		
Non-adherence based on 6-month IPR <90%[2]	9.05 (2.72–34.46)	0.0006			6.34 (1.73–25.59)	0.007
Non-adherence based on clinician suspicion[2]	11.11 (1.81–215.6)	0.03				

IPR, immunosuppressant Possession ratio. Covariates with p*-values < 0.2 on univariable analyses were included into a multiple logistic regression then iteratively removed retaining only those with a* p*-value ≤ 0.05. Variables with the index [(1)] were used in the model 1. Variables with the index [(2)] were used in the model 2.*

diagnosis of non-adherence based on the 6 months IPR < 90% with our standard method based on clinical suspicion. Across the whole cohort, non-adherence was more often diagnosed with the definition based on a 6 months IPR < 90% (25/52 patients) than with the clinical suspicion (9/52 patients) (48.1% vs. 17.3%, $p < 0.001$). The diagnosis of non-adherence was achieved by the two methods in 9 KTR (17.3%) and by the IPR < 90% alone in 16 KTR (30.8%). All the patients with a clinical suspicion of non-adherence had an IPR < 90% (**Figure 4B**).

The proportion of non-adherent KTRs, based on a 6 months IPR < 90% was much higher in the clinical hABMR group (19/26 patients) than in the IFTA group (6/26 patients) (73.1% vs. 23.1%, $p < 0.001$) (**Figure 4C**). In KTRs with clinical hABMR, the percentage of non-adherent KTRs was higher with the definition based on an IPR < 90% (19/26 patients) than with the clinical suspicion (8/26 patients) (73.1% vs. 30.8%, $p = 0.02$) (**Figure 4C**). In KTRs with IFTA, the percentage of non-adherence was also higher with the definition based on an IPR < 90%, but the difference was not significant (23.1% vs. 3.8%, $p = 0.07$). Similar results were observed with the 12 months IPR (**Supplementary Figure S2**).

Univariable analysis also identified 6 months IPR < 90% and non-adherence based on clinical suspicion as risk factors for hABMR (**Table 3**). In a second multivariable analysis including these two variables and *de novo* DSA (model 2), only *de novo* DSA and 6 months IPR < 90% were independently associated with hABMR (odds ratio, 4.73; 95% CI, 1.17–21.88; $p = 0.03$; and odds ratio, 6.34; 95% CI, 1.73–25.59; $p = 0.007$, respectively).

We finally used the NRI to compare the clinical utility of the 6 months IPR<90% with the clinical suspicion, for the prediction of hABMR. Compared with clinical suspicion, a 6 months IPR < 90% adequately reclassified 42% of patients within the hABMR group, but misclassified 19% of patients of the IFTA group, resulting in a non-significant overall NRI of 0.23 (95% CI −0.07–0.53; $p = 0.13$).

Immunosuppressant Possession Ratio in Clinical Histological Antibody-Mediated Rejections Related to Anti-HLA DSA

Regardless of histological lesions, *de novo* anti-HLA DSA-positive patients, had a lower 6 months IPR than anti-HLA DSA-negative patients (71% vs. 98%, $p = 0.004$) (**Figure 5A**). Patients with a *de novo* anti-HLA DSA-positive clinical hABMR had a lower 6 months IPR than patients with anti-HLA DSA-negative clinical hABMR (61% vs. 89%, $p = 0.03$). Patients with anti-HLA DSA-negative clinical hABMR also had a lower 6 months IPR than patients with IFTA (89% vs. 99%, p 0.02) (**Figure 5B**). Moreover, the proportion of KTRs with a 6 months IPR < 90% was much higher in the anti-HLA DSA-positive clinical hABMR group than in the anti-HLA DSA-negative clinical hABMR and IFTA groups (86.7%, 54.5% and 23.1%, respectively, $p < 0.001$) (**Figure 5C**). Similar results were observed with the 12 months IPR (**Supplementary Figure S3**).

DISCUSSION

This study showed that the prospective calculation of a real-time IPR after transplantation is feasible thanks to a close collaboration between transplant clinical pharmacists and community pharmacists. This IPR was correlated with a high intra-patient variability of tacrolimus trough level and outpatient visit non-adherence. Low IPRs were found at the time of clinical hABMR, especially in patients with *de novo* DSA. This tool was better associated with hABMR than clinical suspicion.

Nowadays, pharmacy management software packages are exhaustive and contain all prescription refill data. These data can be obtained very easily thanks to a close collaboration between the transplant clinical pharmacist and the patient's pharmacist. By combining them with a reliable collection of each dose change and hospitalization days in the patient's medical record, it is simple to calculate a very precise IPR for each patient.

We chose to use mycophenolate as a priority for IPR calculation because variations in dosage are infrequent and there are only two commercially available dosages. This method works for brand-name and generic drugs, regardless of the formulation. For those who did not receive mycophenolate, we chose another immunosuppressant with which the calculation of the IPR was simple. We avoided using calcineurin inhibitors because patients must regularly use several different pills of tacrolimus and the dose can vary very frequently. These variations make IPR analysis for calcineurin inhibitors more difficult.

The IPR calculated over a 3 months period was poorly correlated with the IPR calculated for a period of between 8 and 16 months. Some drug packages allow the patient to collect their treatment for 2 or 3 months in a single dispensing. Some patients therefore could have a high IPR over a 3 months period, based on a single dispensing of medication. Choosing to calculate the IPR over a 6 months period seems to be a good compromise because it allows to obtain a reliable, fast and representative calculation of the current adherence.

Patients with IPRs < 90% had higher tacrolimus trough level coefficients of variation compared to patients with IPRs = 100%. This could be explained by a correlation between the mycophenolate possession ratio and the tacrolimus possession ratio. Nevertheless, the latter was not calculated due to its complexity. Patients with an IPR < 90% also claimed to take their medicine less frequently. We also showed that patients with IPRs < 90% were more likely to have had at least one missed visit (66.7% vs. 23.2%, $p = 0.01$). These results are in line with the study of Taber et al. which showed that non-adherence to outpatient visits was strongly correlated with non-adherence to treatment, and both were predictive of adverse clinical consequences [16]. Based on these results, we defined the non-adherent patients as those having a threshold of IPR < 90%. Only 10% of the patients in Cohort 1 had an IPR < 90%. This can be explained by the fact that our patients were adults who had recently been transplanted and because the French health system covers the full cost of immunosuppressants.

It has been reported in previous retrospective studies that non-adherence to immunosuppressants was associated with *de novo* DSA and ABMR [7, 26]. In these studies, non-adherence to immunosuppressants was suspected by transplant physicians. Our study shows that the 6 months IPR < 90% was the only non-adherence measurement tool independently associated with hABMR. It allowed to identify 42% of hABMR patients who had been misclassified by clinical suspicion, confirming the low

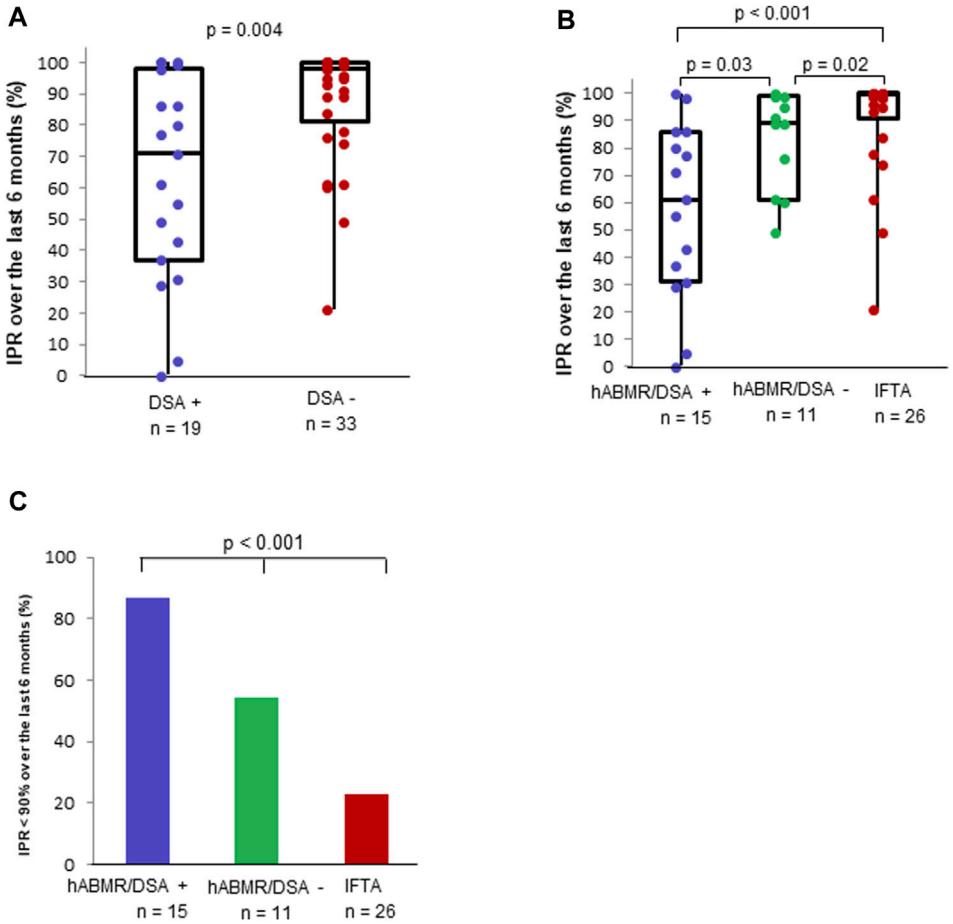


FIGURE 5 | Immunosuppressant possession ratio in patients with or without *de novo* anti-HLA donor-specific antibody (DSA). Description of the immunosuppressant possession ratios calculated over the previous 6 months in patients with or without anti-HLA DSA **(A)**. Description of the immunosuppressant possession ratios calculated over the previous 6 months **(B)** in anti-HLA DSA-positive hABMR, anti-HLA DSA-negative hABMR and IFTA groups. Immunosuppressant possession ratio <90% over the previous 6 months **(C)** in anti-HLA DSA-positive hABMR, anti-HLA DSA-negative hABMR and IFTA groups.

sensitivity of this latter method [18]. It is also worth noting that measurement of non-adherence with the tacrolimus trough level coefficients of variation was not possible for around half of the patients because they were non-adherent to the recommended biological follow-up. Moreover, the tacrolimus trough level coefficients of variation was not associated with hABMR.

The overall NRI showed only a trend toward a better prediction of hABMR by the IPR < 90% when compared to clinical suspicion. The better identification of hABMR in patients with an IPR < 90% comes at a price of 23.1% of false positive, namely, patients with an IPR < 90% in the IFTA group. A low IPR necessarily implies poor adherence to immunosuppressants, because a patient cannot take treatments he has not collected. Therefore, these 23.1% of false positive patients could be at risk of developing rejection in the future. They may also have acquired operational tolerance, but these two hypotheses deserve to be explored.

The IPR was the lowest in positive anti-HLA DSA-ABMR, but negative anti-HLA DSA-ABMR also had a lower IPR than the control group. Negative anti-HLA DSA-ABMR is an entity caused by non HLA-DSA or missing-self induced microvascular rejection [42, 43]. Our data suggest that non-adherence could also be associated with these recently identified rejections.

One of the limitations of the IPR measurement is that it may be biased if the patient visits different pharmacies without informing medical staff. This phenomenon is rare in France because pharmacies order these expensive treatments only for their usual patients. In addition, patients were asked to report any pharmacy changes. Additionally, patients with IPR = 100% were considered as adherent, but it does not determine whether patients were taking the right dose, even if they had collected their medication from the pharmacy. Another limitation of our study was the small sample size of the two cohorts. However, this did not prevent us from achieving the objectives of the study.

In summary, IPR calculation by transplant clinical pharmacists can be used to diagnose immunosuppressant non-adherence in patients with hABMR. This tool could allow continuous monitoring of adherence and thus take into account the dynamic and individual nature of non-adherence over time. In addition, it

could generate a red flag for transplant physicians and pharmacists about patients who are non-adherent to their outpatient visits. Prospective studies are urgently needed to determine its ability to predict all kinds of rejection and graft losses. At the same time, the optimal threshold of IPR associated with the onset of *de novo* DSA and ABMR will have to be determined. An automatic calculation could be envisioned by aggregating the prescription refills which are stored in the national health data system and patients' medical records in order to save pharmacists time.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving humans were approved by French CNIL final agreement, decision 2009-413, n° 1357154, 2 July 2009. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JC: designed study, performed study, collected and analyzed data, wrote the paper; FX and KM: designed study, revised the paper; JeV, BT, BC, and JoV: collected and analyzed data, revised the paper; PM and SD: designed study, analyzed data, revised the paper; LC: designed study, collected and analyzed data, wrote the paper. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11962/full#supplementary-material

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A Decade of Experience With Alemtuzumab Therapy for Severe or Glucocorticoid-Resistant Kidney Transplant Rejection

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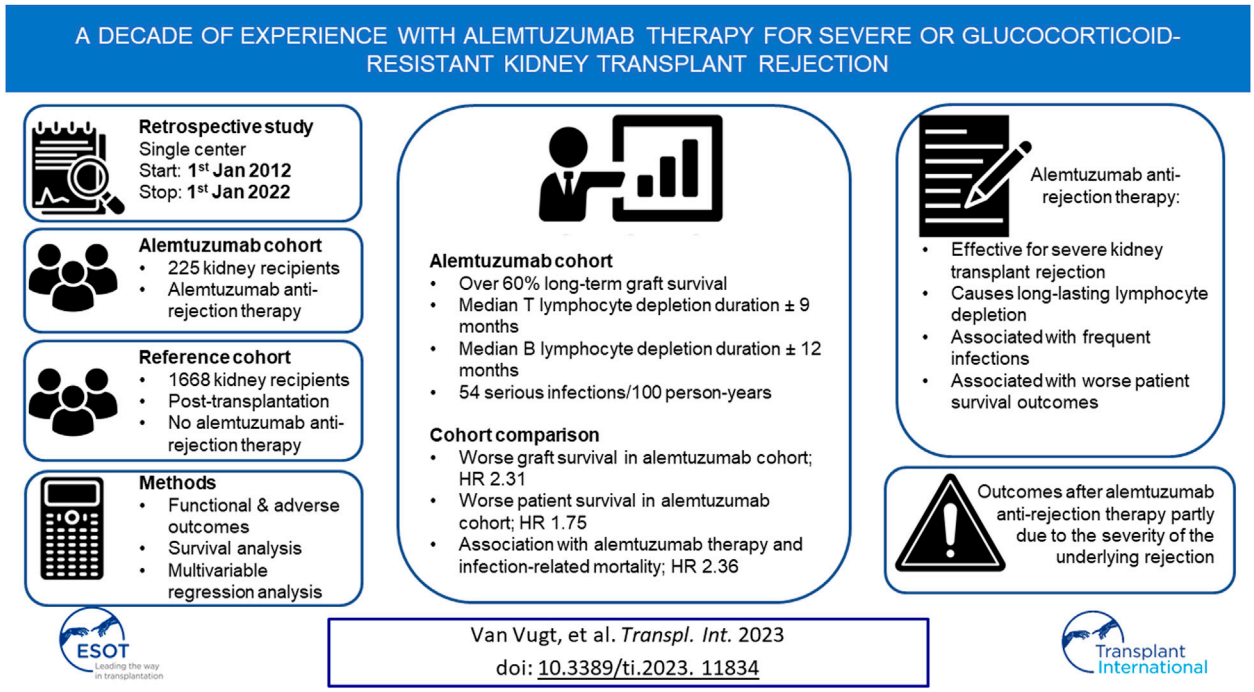
Alemtuzumab is used as lymphocyte-depleting therapy for severe or glucocorticoid-resistant kidney transplant rejection. However, the long-term efficacy and toxicity of alemtuzumab therapy are unclear. Therefore, all cases of alemtuzumab anti-rejection therapy between 2012 and 2022 in our institution were investigated. Graft survival, graft function, lymphocyte depletion, serious infections, malignancies, and patient survival were analyzed and compared with a reference cohort of transplanted patients who did not require alemtuzumab anti-rejection therapy. A total of 225 patients treated with alemtuzumab were identified and compared with a reference cohort of 1,668 patients. Over 60% of grafts was salvaged with alemtuzumab therapy, but graft survival was significantly poorer compared to the reference cohort. The median time of profound T- and B lymphocyte depletion was 272 and 344 days, respectively. Serious infection rate after alemtuzumab therapy was 54.1/100 person-years. The risk of death (hazard ratio 1.75, 95%-CI 1.28–2.39) and infection-related death (hazard ratio 2.36, 95%-CI 1.35–4.11) were higher in the alemtuzumab-treated cohort. In conclusion, alemtuzumab is an effective treatment for severe kidney transplant rejection, but causes long-lasting lymphocyte depletion and is associated with frequent infections and worse patient survival outcomes.

Keywords: adverse effects, alemtuzumab, kidney transplant rejection, efficacy, kidney transplantation

INTRODUCTION

Alemtuzumab is a monoclonal antibody directed against CD52 that causes depletion of T- and B lymphocytes, monocytes, and NK cells [1]. Alemtuzumab is prescribed off-label for both the prevention and treatment of acute kidney transplant rejection [2].

Rabbit anti-thymocyte globulin (rATG) is a lymphocyte-depleting antibody registered for the treatment of severe or glucocorticoid-resistant T cell-mediated rejection (TCMR) and may be used for treating severe antibody-mediated rejection (ABMR) [3, 4]. However, the requirement of a high-



GRAPHICAL ABSTRACT

flow venous access for rATG administration and its associated infusion reactions have instigated the search for alternative therapies [5]. Previous studies demonstrated that alemtuzumab is a safe and efficacious alternative for rATG [5–9]. Notably, alemtuzumab is nearly devoid of infusion-related side effects when administered subcutaneously [10]. Therefore, since 2012, alemtuzumab has been the lymphocyte-depleting antibody of choice for treating severe or glucocorticoid-resistant kidney transplant rejection in our hospital [11].

Despite its efficacy and apparent short-term safety, concerns remain about its long-term adverse effects. Alemtuzumab causes profound and long-lasting lymphocyte depletion, which puts patients at risk for infection and malignancy. Furthermore, rare cases of autoimmunity have been linked to alemtuzumab therapy [12, 13].

Here, the long-term safety and efficacy of alemtuzumab was investigated in a large cohort of patients who received alemtuzumab to treat severe or glucocorticoid-resistant kidney transplant rejection.

PATIENTS AND METHODS

Study Design

This was a retrospective cohort study that included all consecutive adult kidney transplant recipients who were treated with alemtuzumab for acute kidney transplant rejection (AR) between 1st January 2012, and 1st January 2022, at the Erasmus MC, University Medical Center Rotterdam, the Netherlands. The study was approved by the local medical ethical review board (protocol number MEC-2021-0924). Alemtuzumab-treated patients were identified via the hospital pharmacy records.

To interpret patient survival, graft survival, and the risk of malignancy of alemtuzumab-treated patients, they were compared to a reference cohort that consisted of all adult patients that received a kidney transplant in our hospital between 1st January 2012, and 1st January 2022, but were not treated with alemtuzumab for rejection. This reference cohort was identified through the Dutch Organ Transplant Registry ("Nederlandse Orgaantransplantatie Registratie" (NOTR)) database and included some patients with non-depleting anti-rejection therapy and some who received induction therapy with lymphocyte-depleting agents. To account for the effects of alemtuzumab induction therapy, comparative analyses were repeated after exclusion of reference patients who received alemtuzumab induction therapy.

Data was extracted from the electronic patient files and the NOTR. Data was collected after pseudonymization and stored in a protected hospital database. Collected data included patient and transplantation characteristics, pathology data, medication use, information on kidney outcomes, lymphocyte repopulation, and various clinical outcomes, including serious infections and malignancies. "Graft failure" was defined as return to dialysis, transplantectomy or re-transplantation. "Delayed graft function" was defined as the need for dialysis within the first post-transplant week. Primary non-function was determined at 3 months post-transplantation, unless transplantectomy or re-transplantation occurred earlier. "Insufficient treatment response" was defined as the need to treat the same graft with

any additional anti-rejection therapy within 6 months after alemtuzumab therapy. "Serious infections" were defined as infections occurring during hospitalization or an infection that required hospital admission. Malignancies were counted from the year of alemtuzumab therapy in the alemtuzumab cohort and from the year of transplantation for reference patients. If multiple dermatologic malignancies were diagnosed within 1 year, they were counted as a single occurrence.

Outcomes and Follow-Up

Transplant-specific outcomes such as graft survival and function and alemtuzumab-specific outcomes such as post-treatment infections and lymphocyte recovery, were analyzed per kidney transplantation case. Patient-specific outcomes such as patient survival and the occurrence of malignancies were analyzed per patient case.

For transplant-specific outcomes, follow-up started at transplantation until graft loss, death or right censoring by loss to follow-up or treatment with rATG occurred. For patient-specific outcomes, follow-up started at first transplantation in the study period until death or right censoring by loss to follow-up or treatment with rATG occurred. For alemtuzumab-specific outcomes, follow-up started on the day of alemtuzumab treatment until death or right censoring by loss to follow-up, treatment with rATG or re-transplantation occurred.

Pathology

Kidney transplant biopsies of all alemtuzumab-treated patients were reviewed and reclassified according to the Banff 2019 classification by a nephro-pathologist (M.C.C–v.G.). No protocol biopsies were performed and only for-cause biopsies were analyzed. When multiple follow-up biopsies were performed after alemtuzumab therapy, only the first was revised. Rejections were considered biopsy-proven acute rejection (BPAR) if the diagnostic criteria of the Banff 2019 classification were fulfilled. Cases classified as presumed ABMRs demonstrated histologic signs of acute tissue injury (e.g., acute tubular necrosis or thrombotic microangiopathy) without C4d positivity or donor-specific anti-HLA antibodies (DSA). Cases demonstrating microvascular inflammation but without C4d and DSA were primarily classified as ABMR, but the functional outcomes were also analyzed without these cases in anticipation of the upcoming Banff 2021 classification. The presence or absence of DSA was assessed within 3 months before and up to 6 months after AR. Patients who were treated with alemtuzumab for non-BPAR were not included in the analysis of the functional outcomes of different types of BPAR.

Immunosuppressive Therapy

The typical immunosuppressive regimen in our center comprises induction therapy with 20 mg intravenous (IV) basiliximab (days 0 and 4) and 100 mg IV prednisolone (days 0–2) for both recipients of a living and deceased donor kidney, followed by an immunosuppressive maintenance regimen consisting of tacrolimus, mycophenolate mofetil (MMF), and glucocorticoids. Target tacrolimus pre-dose concentrations were 10–15 μg/L (weeks 1 and 2), 8–12 μg/L (weeks 3 and 4), 5–10 μg/L (weeks 5–16), and 5–8 μg/L thereafter [11]. MMF was started at a dose of 1,000 mg twice daily and was subsequently adjusted to target pre-dose concentrations of 1.5–3.0 mg/L. A 20 mg daily dose of prednisolone was started on day 3 and then tapered. Except for immunologically high-risk recipients, prednisolone was completely withdrawn around week 16 [11].

Anti-Rejection Therapy

The first-line treatment for TCMR and empirical treatment for suspected AR consisted of 1,000 mg IV methylprednisolone for three consecutive days. ABMR and mixed-type rejections were treated with methylprednisolone plus two doses of intravenous immunoglobulin (IVIG; 1 g/kg) with or without plasma exchange [14]. Alemtuzumab was prescribed for glucocorticoid-resistant, severe (Banff IIA or worse), or recurrent AR at the discretion of the treating nephrologist. The standard alemtuzumab dose was a single 30 mg dose administered subcutaneously. Premedication consisted of 50 mg IV prednisolone, acetaminophen, and clemastine. Patients received sulfamethoxazole/trimethoprim and valganciclovir prophylaxis until their T lymphocyte count exceeded 200×10^6/L.

Statistical Analysis

Statistical analysis was performed with the R statistical software (v4.3.0) [15], using the cmprsk (v2.2.11), ggeffects (v1.1.4), ggsurvfit (v0.2.1), icenReg (v2.0.15), interval (v1.1.0.8), kidney.epi (v1.2.0), MASS (v7.3.55), nlme (v3.1.155), survival (v3.4.0) and tidycmprsk (v0.2.0) packages. A two-sided p-value <0.05 was considered statistically significant. Continuous variables were expressed as means with standard deviations or medians with interquartile ranges (IQRs) when not normally distributed. Normality was assessed by visual inspection. The Mann-Whitney U and Kruskal–Wallis tests were used to compare continuous variables between groups. Categorical variables were reported as proportions with percentages, and differences between groups were assessed using the Fisher's exact test.

Graft survival was analyzed with death as a competing risk and the non-parametric estimate of the cumulative incidence was plotted for its visualization. Patient survival was analyzed as a definitive endpoint and infection-free survival was analyzed as the time to first serious infection. Both were visualized with Kaplan-Meier survival curves. To correct for differences between the alemtuzumab and reference cohorts, while accounting for the time-dependent exposure of certain covariates, multivariable time-varying Cox proportional hazard models were used for the analysis of graft and patient survival. When a separation problem occurred, this was resolved with a ridge regression term. Multivariable Cox proportional hazard models were also used to evaluate associations between patient characteristics and survival outcomes from the initiation of therapy in the alemtuzumab cohort solely, and the cumulative incidence function between alemtuzumab-treated rejection subgroups was compared using the Gray's test [16, 17]. To analyze interval-censored

TABLE 1 | Patient baseline characteristics.

		Patient group		Statistic[a]
		Alemtuzumab (n = 225)	Reference (*n* = 1,668)	*p*-value
Recipient age at transplantation	Median (IQR)	55.0 (38.0–64.0)	60.0 (49.0–67.0)	<0.01
Recipient gender	Female/Male (%)	88/137 (39.1%/60.9%)	636/1,032 (38.1%/61.9%)	0.77
Recipient BMI	Median (IQR)	26.9 (23.4–31.9)	26.5 (23.5–30.2)	0.11
	Unknown or missing	1 (0.4%)	0 (0.0%)	
Diabetes mellitus prior to transplantation	No/Yes (%)	155/69 (69.2%/30.8%)	1,169/499 (70.4%/29.6%)	0.82
	Unknown or missing (%)	1 (0.4%)	0 (0.0%)	
Cardiac event prior to transplantation	No/Yes (%)	196/29 (87.1%/12.9%))	1,378/288 (82.7%/17.3%)	0.11
	Unknown or missing (%)	0 (0.0%)	2 (0.1%)	
Vascular event prior to transplantation	No/Yes (%)	206/19 (91.6%/8.4%)	1,536/131 (92.1%/7.9%)	0.79
	Unknown or missing (%)	0 (0.0%)	1 (0.1%)	
CVA prior to transplantation	No/Yes (%)	201/24 (89.3%/10.7%)	1,480/187 (88.8%/11.2%)	0.91
	Unknown or missing (%)	0 (0.0%)	1 (0.1%)	
Primary underlying kidney disease	Hypertension (%)	7 (3.1%)	133 (8.0%)	0.01
	Diabetes (%)	15 (6.7%)	98 (5.9%)	0.65
	Glomerulonephritis (%)	26 (11.6%)	139 (8.3%)	0.13
	PKD (%)	13 (5.8%)	77 (4.6%)	0.41
	Reflux nephropathy (%)	11 (4.9%)	26 (1.6%)	<0.01
	Other (%)	142 (63.1%)	1,137 (68.2%)	0.13
	Unknown (%)	11 (4.9%)	658 (3.5%)	0.26

[a]*Mann-Whitney U (continuous variables) or Fisher's exact (categorical variables) test statistic.*

BMI, body mass index; CVA, cerebrovascular accident; IQR, interquartile range; PKD, polycystic kidney disease.

lymphocyte recovery data, the nonparametric maximum likelihood estimators of the survival functions were calculated to construct interval-censored survival curves [18]. Negative binomial regression models, where follow-up time was used as offset, were applied to assess covariate associations with malignancy and infection events.

To compare the median values of paired measurements of estimated glomerular filtration rate (eGFR), lymphocytes and urinary creatinine-protein ratios, the paired Wilcoxon signed rank test was used. To analyze the evolution of eGFR over time, we considered a linear mixed-effects model, with a linear fixed effect of time and an individual-specific random intercept.

RESULTS

Patient, Transplant, and Rejection Characteristics

Between 1st January 2012, and 1st January 2022, 236 rejections were treated with alemtuzumab in 225 patients. Alemtuzumab was prescribed as second-line therapy for 174 glucocorticoid-resistant rejections (73.7% of 236 cases), and as first-line therapy for 62 severe rejections (26.3% of 236 cases). The reference cohort consisted of 1,732 kidney transplantations performed in 1,668 patients. This reference cohort included 53 transplantations in 46 patients in whom alemtuzumab was given as induction therapy. Alemtuzumab-treated patients were younger than reference patients (**Table 1**), had higher panel reactive antibodies (PRA) and were more likely to be repeat transplantations (**Table 2**). Of the 236 alemtuzumab-treated rejections, 226 were biopsy-proven. Details of these rejection episodes and their treatment are provided in **Tables 3**, **4**.

Functional Outcomes

For better estimates of graft loss over time, the cumulative incidence of graft loss with death as competing risk was calculated (**Figure 1**). The cumulative incidence of graft loss at one, three and five years after alemtuzumab therapy was 21.7% (95%-CI 16.3–27.1), 32.3% (95%-CI 26.2–38.5), and 37.4% (95%-CI 31.1–43.8), respectively. The cumulative incidence of graft loss at one, three and five years after transplantation was 4.1% (95%-CI 3.2–5.0), 5.4% (95%-CI 4.4–6.5), and 7.0% (95%-CI 5.9–8.2), respectively. Alemtuzumab-treated patients also had a higher risk of graft loss after correcting for other covariates, including the start of any rejection treatment (hazard ratio [HR] 2.31, 95%-CI 1.72–3.10, **Supplementary Table S1**). These conclusions were not altered after exclusion of reference patients who received alemtuzumab induction.

Graft loss was compared between different BPAR subtypes with a competing risk analysis for death (**Supplementary Figure S1**). The overall cumulative incidence of graft loss was not significantly different between TCMR, ABMR, and mixed-type rejection ($p = 0.12$). The cumulative incidence of graft loss associated with TCMR, ABMR and mixed-type rejection at 5 years after alemtuzumab therapy was: 36.1% (95%-CI 27.6–44.7), 44.0% (95%-CI 28.7–59.3) and 56.7% (95%-CI 38.7–74.6), respectively. Rejection type was not significantly associated with an increased risk of graft loss in multivariable analysis (**Supplementary Table S2**). Exclusion of C4d and DSA-negative rejections did not alter these conclusions.

The eGFR of patients not on dialysis are depicted in **Figure 2**. Kidney function improved significantly within 2 weeks after treatment with alemtuzumab and remained significantly better compared to baseline at all other time points ($p < 0.01$), with median values of 25–35 mL/min per 1.73 m^2. A linear mixed-effects model was generated to model the trend of eGFR over time

TABLE 2 | Transplant characteristics.

		Patient group		Statistic[a]
		Alemtuzumab (*n* = 225)	Reference (*n* = 1,668)	*p*-value
Number of transplantations	1 (%)	179 (79.6%)	1,477 (88.5%)	<0.01
	2 (%)	31 (13.8%)	143 (8.6%)	0.02
	3 or more (%)	15 (6.7%)	48 (2.9%)	0.01
Pre-emptive kidney transplantation	No/Yes (%)	160/65 (71.1%/28.9%)	1,086/582 (65.1%/34.9%)	0.08
PRA actual	0–10 (%)	196 (87.1%)	1,554 (93.2%)	<0.01
	10–50 (%)	22 (9.8%)	68 (4.1%)	<0.01
	50–100 (%)	7 (3.1%)	46 (2.8%)	0.67
PRA peak	0–10 (%)	158 (70.2%)	1,346 (80.7%)	<0.01
	10–50 (%)	15 (7.7%)	128 (7.7%)	0.69
	50–100 (%)	52 (23.1%)	194 (11.6%)	<0.01
CMV IgG serostatus recipient	Negative/Positive (%)	64/160 (28.6%/71.4%)	582/1,084 (34.9%/65.1%)	0.06
	Unknown or missing (%)	1 (0.4%)	3 (0.2%)	
EBV IgG serostatus recipient	Negative/Positive (%)	12/212 (5.4%/94.6%)	120/1,547 (7.2%/92.8%)	0.40
	Unknown or missing (%)	1 (0.4%)	1 (0.1%)	
Donor age	Median (IQR)	55.0 (44.0–63.0)	57.0 (46.0–65.0)	0.06
Donor type	DBD/DCD/Living (%)	25/56/144 (11.1%/24.9%/64.0%)	264/443/961 (15.8%/26.6%/57.6%)	0.11
CMV IgG serostatus donor	Negative/Positive (%)	94/129 (42.2%/57.8%)	562/630 (47.1%/52.9%)	0.19
	Unknown or missing (%)	2 (0.9%)	476 (28.5%)	
HLA A mismatch	0/1/2 (%)	57/108/59 (25.4%/48.2%/26.3%)	438/859/346 (26.7%/52.3%/21.1%)	0.20
	Unknown or missing (%)	1 (0.4%)	25 (1.5%)	
HLA B mismatch	0/1/2 (%)	21/106/97 (9.4%/47.3%/43.3%)	234/790/619 (14.2%/48.1%/37.7%)	0.07
	Unknown or missing (%)	1 (0.4%)	25 (1.5%)	
HLA DR mismatch	0/1/2 (%)	37/118/69 (16.5%/52.7%/30.8%)	329/844/470 (20.0%/51.4%/28.6%)	0.44
	Unknown or missing (%)	1 (0.4%)	25 (1.5%)	

[a]*Mann-Whitney U (continuous variables) or Fisher's exact (categorical variables) test statistic.*

CMV, cytomegalovirus; DBD, donation after brain death; DCD, donation after circulatory death; EBV, Epstein–Barr virus; HLA, human leukocyte antigen; PRA, panel reactive antibody.

TABLE 3 | Characteristics of biopsy-proven, alemtuzumab-treated rejections.

		Rejection subtype			Statistic[a]
		TCMR (n = 142)	ABMR (*n* = 49)	Mixed (*n* = 35)	*p*-value
Time to rejections (days)	Median (IQR)	10.0 (6.0–159.8)	11.0 (6.0–94.0)	194.0 (9.5–749.0)	<0.01
Timing of rejection	Early rejection/Late rejection (%)	94/48 (66.2%/33.8)	36/13 (73.5%/26.5%)	15/20 (42.9%/57.1%)	0.01
Delayed graft function at moment of rejection	No/Yes (%)	90/52 (63.4%/36.6%)	32/17 (65.3%/34.7%)	29/6 (82.9%/17.1%)	0.08
Donor-specific antibodies during rejection	No/Yes (%)	126/16 (88.7%/11.3%)	27/22 (55.1%/44.9%)	22/13 (62.9%/37.1%)	0.51[b]
DSA Type 1	No/Yes (%)	139/3 (97.9%/2.1%)	37/12 (75.5%/24.5%)	32/3 (91.4%/8.6%)	0.08[b]
DSA Type 2	No/Yes (%)	128/14 (90.1%/9.9%)	32/17 (65.3%/34.7%)	23/12 (65.7%/34.3%)	1[b]
Blood-group incompatible transplantation	No/Yes (%)	139/3 (97.9%/2.1%)	43/6 (87.8%/12.2%)	28/7 (80.0%/20.0%))	<0.01
C4d in biopsy	Negative/Positive (%)	–	19/30 (38.8%/61.2%)	6/29 (17.1%/82.9%)	0.05[b]

[a]*Kruskal-Wallis (continuous variables) or Fisher's exact (categorical variables) test statistic.*

[b]*Only tested between ABMR, and mixed-type rejection, as DSA, and C4d are part of the diagnostic criteria for rejection subtyping.*

ABMR, antibody-mediated rejection; C4d, fragment of complement component C4; DSA, donor-specific antibodies; Early rejection, within three months; IQR, interquartile range; Late rejection, after three months or more; mixed, mixed-type rejection; TCMR, T cell-mediated rejection.

(**Figure 3**). eGFR tended to increase in the first year after alemtuzumab treatment, gradually decreased between 1 to 3 years after treatment, and then stabilized after 3 to 5 years. After 5 years, eGFR gradually declined. No significant differences were modelled for the different rejection subtypes (**Supplementary Figure S2**). The urinary protein-creatinine ratio was increased at the start of therapy (median 56.7 mg/mmol) and decreased significantly at three, six and twelve months after therapy (**Supplementary Figure S3**).

Follow-Up Biopsies

In 109 cases (46.2% of 236 cases), for-cause follow-up biopsies were obtained. Of these, 50 (45.9% of 109 biopsies) showed no rejection but another diagnosis such as recurrent, primary disease or infection. 59 (54.1% of 109 biopsies) showed TCMR (n = 19), ABMR (n = 21), or mixed-type (n = 19) rejection. Twenty biopsies demonstrated ABMR or mixed-type rejection after an initial diagnosis of TCMR. An overview of rejection type at diagnosis and during the first follow-up biopsy is provided in **Supplementary Table S3**.

Insufficient Treatment Response

During the first six months after alemtuzumab treatment, additional anti-rejection therapy was prescribed for 25 rejections (10.6%). Methylprednisolone was administered in 18 cases, IVIG in ten cases, a second

TABLE 4 | Immunosuppression and additional anti-rejection therapy.

		Non-BPAR	BPAR			Statistic[a]
		pABMR (*n* = 8)	TCMR (*n* = 142)	ABMR (*n* = 49)	Mixed (*n* = 35)	*p*-value
		No biopsy (*n* = 2)				
Induction therapy	Alemtuzumab (%)	0 (0.0%)	0 (0.0%)	6 (12.2%)	2 (5.7%)	<0.01
	Basiliximab (%)	9 (90.0%)	135 (95.1%)	37 (75.5%)	26 (74.3%)	<0.01
	ATG (%)	0 (0.0%)	0 (0.0%)	4 (8.2%)	1 (2.9%)	0.01
	Rituximab (%)	1 (10.0%)	4 (2.8%)	2 (4.1%)	6 (17.1%)	0.01
	None (%)	0 (0.0%)	3 (2.1%)	0 (0.0%)	0 (0.0%)	0.77
Maintenance immunosuppression	TAC/MMF/ glucocorticoids (%)	5 (50.0%)	102 (71.8%)	40 (81.6%)	19 (54.3%)	0.02
	TAC/MMF (%)	1 (10.0%)	21 (14.8%)	5 (10.2%)	8 (22.9%)	0.245
	TAC + other (%)	2 (20.0%)	3 (2.1%)	1 (2.0%)	4 (11.4%)	0.01
	MMF + other (%)	1 (10.0%)	15 (10.6%)	1 (2.0%)	4 (11.4%)	0.21
	TAC & MMF–free regimen (%)	1 (10.0%)	1 (0.7%)	2 (4.1%)	0 (0.0%)	0.06
Co-treatment with methylprednisolone	No/Yes (%)	0/10 (0.0%/ 100.0%)	8/134 (5.6%/94.4%)	3/46 (6.1%/93.9%)	1/34 (2.9%/97.1%)	0.90
Co-treatment with IVIG	No/Yes (%)	3/7 (30.0%/70.0%)	114/28 (80.3%/19.7%)	13/36 (26.5%/73.5%)	17/18 (48.6%/51.4%)	<0.01
Co-treatment with antibody removal	No/Yes (%)	10/0 (100.0%/0.0%)	140/2 (98.6%/1.4%)	41/8 (83.7%/16.3%)	34/1 (97.1%/2.9%)	<0.01

[a]*Kruskal-Wallis (continuous variables) or Fisher's exact (categorical variables) test statistic. (p)ABMR, (presumed) antibody-mediated rejection; ATG, anti-thymocyte globulin; IVIG, intravenous immunoglobulin; mixed, mixed-type rejection; MMF, mycophenolate mofetil; TAC, tacrolimus; TCMR, T cell-mediated rejection.*

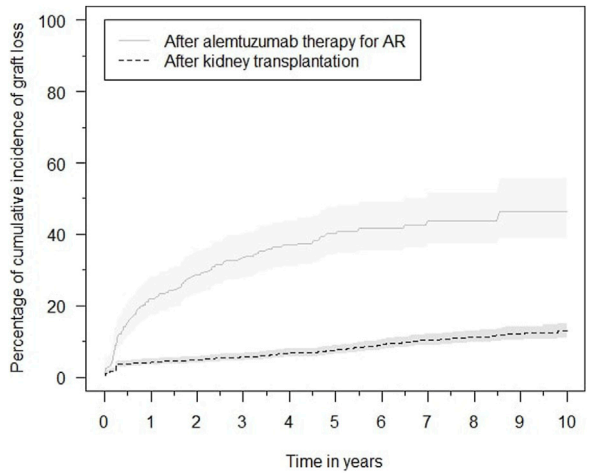


FIGURE 1 | Cumulative incidence functions of graft loss in the alemtuzumab and reference groups, with associated 95% confidence intervals.

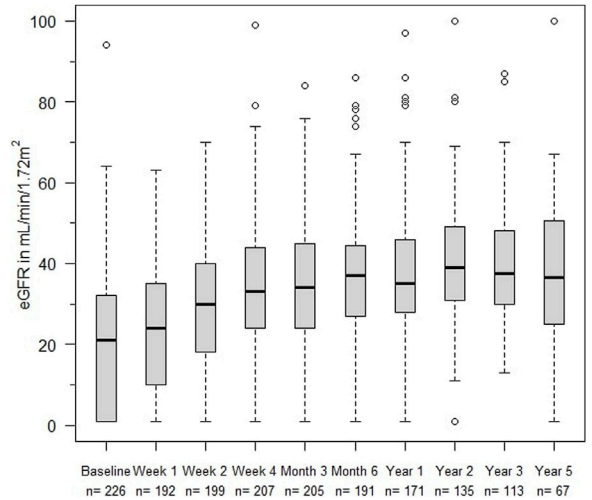


FIGURE 2 | eGFR (mL/min/1,72 m^2) at alemtuzumab therapy initiation and subsequent time points. Box indicates 25th–75th percentiles with medians. Whiskers indicate the value of 1.5 times the IQR below the 25th percentile or above the 75th percentile respectively. Dots indicate outliers.

course of alemtuzumab in ten cases and both tocilizumab and rATG in one case. Fifteen out of these 25 rejections were lost after additional therapy after a median of 419 days (IQR 133–980 days).

Hematologic Effects

Rapid and profound depletion of both T and B lymphocytes occurred after treatment and was not fully restored after 18 months (**Figure 4**). The baseline median T lymphocyte count was 627×10^6/L, and after 18 months, it was 201×10^6/L ($p < 0.01$). The baseline median B lymphocyte count was 140×10^6/L, and after 18 months, it was 97.5×10^6/L ($p = 0.03$). **Figure 5** shows the interval-censored survival curve of lymphocyte recovery. The median time of T lymphocyte depletion, defined as a T lymphocyte count below 200×10^6/L, was 272 days. The median time of B lymphocyte depletion, defined as a B lymphocyte count below 100×10^6/L, was 344 days.

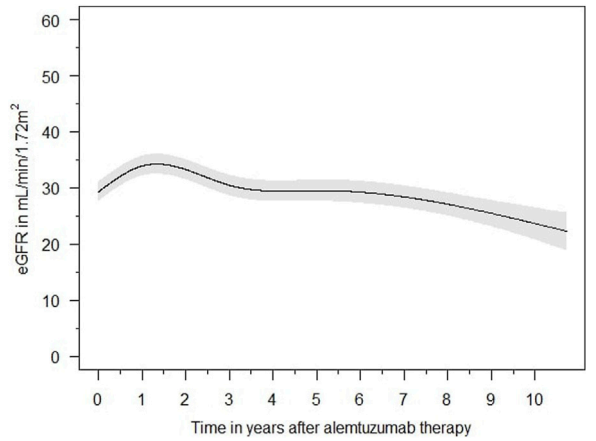


FIGURE 3 | Averaged estimated effect of time on eGFR (mL/min/1,72 m^2) progression after alemtuzumab initiation, with associated 95% confidence intervals.

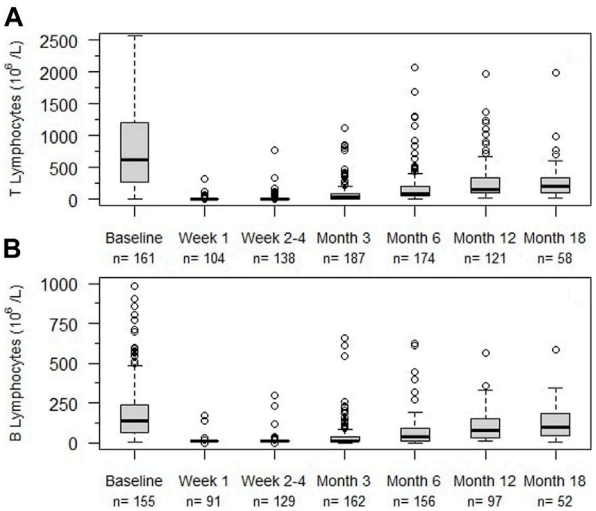


FIGURE 4 | Lymphocyte counts (10^6/L) at different times after alemtuzumab initiation. **(A)** T lymphocytes, **(B)** B lymphocytes, n: number of individuals. Box indicates 25th – 75th percentiles with medians. Whiskers indicate the value of 1.5 times the IQR below the 25th percentile or above the 75th percentile respectively. Dots indicate outliers.

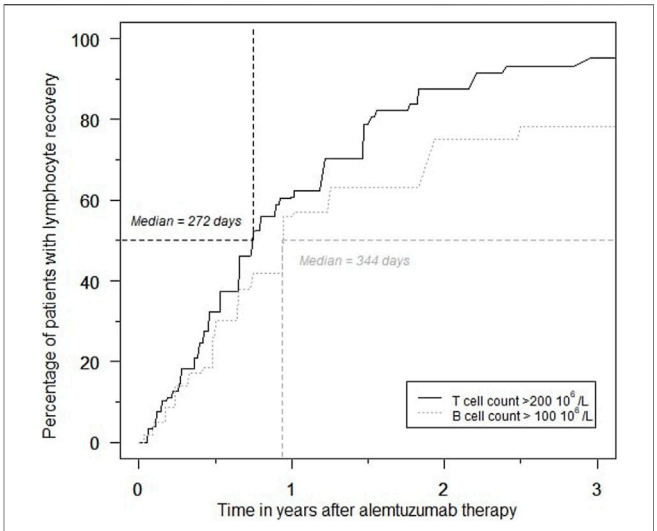


FIGURE 5 | Percentage of patients with T- and B lymphocyte recovery over time during the first 3 years after alemtuzumab therapy.

TABLE 5 | Incidence of serious infections in alemtuzumab-treated patients per 100 person-years.

Infection type	Incidence
Total serious infections	54.1
Urinary tract infections	20.7
Pulmonary infections	12.9
Gastrointestinal infections	5.0
Infections of skin and soft tissues	3.4
Opportunistic infections	5.0
Peritoneal dialysis-related infections	1.8
Other (including vascular catheter-related infections)	5.4

Infections

A total of 512 serious infections occurred in 236 alemtuzumab-treated cases. The overall infection rate was 54.1 infections per 100 person-years (**Table 5**). Urinary tract infections were the most common (20.7 per 100 person-years), followed by pulmonary infections (12.9 per 100 person-years). The incidence of primo- and reactivation infection with BK virus (BKV), cytomegalovirus (CMV) and Epstein–Barr virus (EBV) was 22.5% ($n = 53$), 26.7% ($n = 63$), and 3.0% ($n = 7$), respectively. Serious infection-free survival is depicted in **Supplementary Figure S4**. Almost half of the alemtuzumab-treated patients experienced at least one serious infection within the first year after treatment. However, serious infections did not affect all patients to a similar degree. In approximately one-third of alemtuzumab-treated rejections ($n = 73$), no serious infections occurred. The infection count or time to first infection was not related to the duration of T- and B-cell depletion, but this explorative analysis was limited by missing repopulation data. The infection-free survival decreased and number of infections increased for older age at alemtuzumab initiation and with the presence of cardiovascular disease in medical history (**Supplementary Tables S4, S5**).

Malignancies

74 malignancies were diagnosed in 42 patients in the alemtuzumab cohort (18.7%), while 460 malignancies were diagnosed in 330 patients in the reference cohort (19.6%). Total malignancy counts and incidence rates are provided in **Table 6**. The incidence rates of overall, solid, dermatologic and hematologic malignancy counts were higher in the alemtuzumab cohort than the reference cohort, but only the overall malignancy incidence rate differed significantly. In multivariable count regression, however, alemtuzumab was not significantly associated with higher malignancy risk. This finding was not

TABLE 6 | Overview of malignancies. Data in absolute counts with incidence rates per 100 person-years.

	Alemtuzumab	Reference
Total malignancy count	74 (7.0)	460 (4.9)
Solid malignancies – overall	20 (1.9)	141 (1.5)
Breast	2	16
Digestive tract	5	42
Lung cancer	6	24
Urogenital tract	4	37
Other solid	3	22
Dermatologic malignancies – overall	49 (4.7)	289 (3.1)
Melanoma	1	10
Non-melanoma skin cancer	48	279
Hematologic malignancies – overall	5 (0.5)	30 (0.3)
PTLD	4	20
Other hematologic	1	10

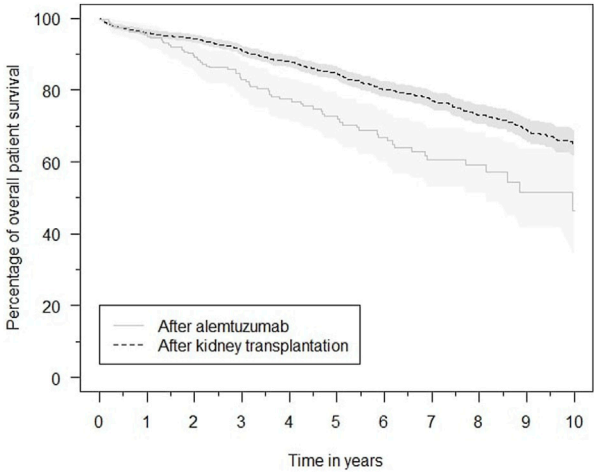


FIGURE 6 | Kaplan–Meier estimates of the survival probability after kidney transplantation in general and after initiation of alemtuzumab therapy, with associated 95% confidence intervals.

altered after exclusion of reference patients who received alemtuzumab induction.

Autoimmunity

Three cases of suspected alemtuzumab-related autoimmunity occurred among 225 patients (1.3%): one case of acquired hemophilia A [12], one case of Guillain-Barré syndrome [13] and one case of chronic inflammatory demyelinating polyradiculoneuropathy [13]. Furthermore, five autoimmune-related phenomena of unknown etiology were observed (2.2%): one case of vitiligo, one case of Raynaud's phenomenon, one unexplained case of pericarditis, peritonitis and axonal polyneuropathy without demyelination, one case of recurrent pericarditis (which necessitated anakinra treatment), and one case of pulmonary granulomas.

Patient Survival

Patient survival after alemtuzumab treatment was inferior to overall post-transplantation survival (**Figure 6**). The survival probability one, three and 5 years after transplantation was 96.1%, 91.2%, and 84.7%, respectively. Comparatively, after alemtuzumab treatment, patient survival was 95.4%, 83.1%, and 72.7%, respectively. Alemtuzumab-treated patients had a significantly higher risk of death (HR 1.75, 95%-CI 1.28–2.39). Other baseline variables significantly associated with death were the start of any treatment for rejection, older age, diabetes mellitus, and having a medical history of at least one cardiac, peripheral vascular or cerebrovascular event at the time of transplantation (**Supplementary Table S6**). Alemtuzumab-treated patients also had a higher risk of infection-related death (HR 2.36, 95%-CI 1.35–4.11; **Supplementary Table S7**). However, they did not have a higher risk of malignancy-related death (**Supplementary Table S8**). These conclusions remained unaltered after excluding reference patients who received alemtuzumab induction.

DISCUSSION

Here the efficacy and safety of alemtuzumab therapy for glucocorticoid-resistant or severe kidney transplant rejection is reported for the largest cohort described in the literature. The present findings demonstrate that alemtuzumab is an effective therapy to counter severe kidney transplant rejection. However, it leads to a profound, long-lasting lymphocyte depletion and is frequently complicated by serious infections. Furthermore, patient survival after alemtuzumab therapy is worse compared to the general post-transplant population.

Limitations

The major limitation of this study is the absence of a control group treated with rATG. A prospective comparison between alemtuzumab and rATG would be ideal for determining the superiority of one therapy over the other. Although we feel it is unlikely that such a head-to-head comparison will be performed anytime soon, the present data may serve as a power calculation basis for such a trial.

The aim of this study was to report the outcomes after alemtuzumab anti-rejection therapy and how these relate to the outcomes in a general transplantation cohort (our "reference" cohort). One should realize that the outcomes after alemtuzumab therapy are not solely dependent of the biological effects of alemtuzumab itself, but also of the effects of the severe rejection that prompted this therapy.

Another limitation was the presence of missing data due to the retrospective study design. Furthermore, bias may have been introduced due to incomplete outcome reporting, especially for the recording of serious infections and malignancies.

Graft Survival

Not surprisingly as AR is associated with a higher risk of graft loss [19], graft prognosis was worse for patients who required alemtuzumab treatment compared to the reference group. However, despite the severity of the rejection, over 60% of kidney transplants functioned for at least 5 years after alemtuzumab. The renal function was acceptable, ranging

around 30 mL/min/1.72 m^2. Clatworthy et al. reported a higher death-censored graft survival of 75% after 10 years in 15 patients [7], but these patients received alemtuzumab as a first-line treatment. In contrast, here, it was primarily used as a second-line therapy for glucocorticoid-resistant rejections. Our findings are in line with those of Basu et al., who reported a graft survival rate of 73.5% after 453 ± 163 days of follow-up in 40 patients treated with alemtuzumab for glucocorticoid-resistant rejection.

Most of the available data of rATG was published before 1998 [20], which complicates the comparison with a recent cohort. Van der Zwan et al. previously reported a death-censored graft survival of 60% 5 years after rATG therapy in patients treated between 2002 and 2012 in our center, which was comparable to alemtuzumab [11]. They therefore concluded that alemtuzumab and rATG probably have similar efficacy, although they could not correct for all potential confounders that arose from the comparison of two cohorts that were treated during different decades [11]. Without a contemporary cohort of rATG-treated patients as control group however, whether alemtuzumab outperforms rATG remains an unanswered question.

Patient Survival

Overall patient survival was worse in the alemtuzumab cohort. AR is associated with an increased mortality risk [19]. Increased mortality after AR probably stems both from both the loss of transplant function as complications from anti-rejection therapies. To what extend alemtuzumab therapy contributes to the increased mortality in this cohort, cannot be determined. Nevertheless, there is no evidence that alemtuzumab is associated with lower patient survival compared with rATG, as Van der Zwan et al. previously reported equal allograft survival between rATG- and alemtuzumab-treated patients [11].

Infections, Malignancies and Auto-Immunity

Unfortunately, data of serious infections were not available for the reference cohort and therefore the incidence rates could not be compared. Infections seem to occur regularly in other alemtuzumab-treated cohorts as well. Basu et al. and Clathworthy et al. also reported frequent infectious complications and an excess of early infection-related deaths after alemtuzumab therapy [6, 7]. Again, it is unclear if infections occur more frequently after alemtuzumab than rATG. A high incidence of opportunistic infections has also been observed after rATG anti-rejection therapy [20, 21], and van der Zwan et al. reported significantly shorter infection-free survival and a higher number of serious infection after rATG compared to alemtuzumab [11]. If the duration of lymphocyte depletion and excess susceptibility to infection are correlated, could not be determined in the present study because of missing data. Possibly, lower doses of alemtuzumab may result in more rapid recovery of lymphocyte counts and reduce excess infection.

Treatment with T cell-depleting antibodies for AR is a significant risk factor for the development of post-treatment malignancy in general [22]; however, this risk is not specified per type of T cell-depleting antibody. The present study did not find a significantly increased risk of malignancies or malignancy-related death in the alemtuzumab cohort. Nevertheless, we cannot state with any certainty that alemtuzumab does not lead to an increased incidence of malignancies, considering the higher incidence rates of all malignancies in the alemtuzumab cohort and the fact that the present cohort was relatively small from a cancer epidemiology perspective.

The incidence of autoimmunity after alemtuzumab was low, with an incidence of 1.3% of proven cases. Concerns regarding autoimmune complications after alemtuzumab treatment originate from studies in patients who were treated for multiple sclerosis, where thyroid autoimmunity and immune thrombocytopenia occurred frequently [23, 24]. The current study observed neither type of autoimmunity. However, several other cases of suspected autoimmune disease did occur. Differences in autoimmunity risk between the transplant and neurologic populations may be explained by differences in concomitant immunosuppression and baseline risks.

Lymphocyte Repopulation

Lymphocyte repopulation in the present cohort exceeded the recovery times in multiple sclerosis [25] and transplant trials with alemtuzumab induction therapy [26, 27]. This might be due to differences in the concomitant use of other myelosuppressive therapies and comorbid conditions. Nonetheless, the observed long-lasting lymphocyte depletion is unwanted and is likely a sign of alemtuzumab overdosing. In other studies, a lower dose of alemtuzumab has been applied in kidney transplant induction and demonstrated equal efficacy but faster lymphocyte recovery and fewer infection-related side effects [28, 29]. A recent pharmacokinetic study reported supra-therapeutic concentrations and long periods of lymphocytic drug exposure after 30 mg of alemtuzumab induction therapy [30], which delayed lymphocyte repopulation [31], suggesting that a fixed dose of 30 mg is sub-optimal. These findings and the fact that the current dosing strategy of alemtuzumab anti-rejection therapy is not supported by dose-finding studies [2] indicate the need for alternative dosing strategies [32, 33]. We suggest a stepwise dosing strategy, starting with a lower dose of alemtuzumab with the possibility of a repeated dose in case of incomplete lymphocyte depletion or fast lymphocyte recovery.

Without a clear graft and patient survival benefit of alemtuzumab over rATG and considering the possible risks alemtuzumab, the question remains if rATG should be the preferred treatment. Although contemporary reports of rATG-anti-rejection therapy are scarce in terms of number and follow-up, the available data show this therapy also has substantial risks [20]. Alemtuzumab does have benefits over rATG in terms of mode of administration and fewer infusion reactions [11]. We are currently planning future studies to resolve this matter.

In summary, alemtuzumab is an effective therapy to counter severe kidney transplant rejection. However, the current dose leads to a profound, long-lasting depletion of both B and T lymphocytes, frequent serious infections, and is associated with increased patient mortality. Further research is necessary to both determine the additional risks of alemtuzumab over alternative treatment strategies, and to optimize alemtuzumab therapy.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, upon reasonable request.

ETHICS STATEMENT

The studies involving humans were approved by the Medical-Ethical Board Erasmus MC, Rotterdam, the Netherlands. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because the data was obtained from the NOTR database, for which patients consented at the time of kidney transplantation.

AUTHOR CONTRIBUTIONS

LV participated in the research design, performance of the research, data-analysis and writing of the paper. MC-vG participated in the performance of the research and writing of the paper. PA participated in data analysis and provided statistical consultation. DH participated in the research design, performance of the research and writing of the paper. MZ, MA, DH-P, BW, and MR participated in writing of the paper.

CONFLICT OF INTEREST

DH received lecture and consulting fees from Astellas Pharma, Astra Zeneca, Chiesi Pharma, Medincell, Novartis Pharma, Sangamo Therapeutics, and Vifor Pharma. He received grant support from Astellas Pharma, Bristol-Myers Squibb and Chiesi Pharma (paid to his institution). DH does not have employment or stock ownership at any of these companies, nor does he have patents or patent applications. MC-vG received consulting honoraria from Sangamo Therapeutics and project support from Astellas Pharma (paid to her institution).

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11834/full#supplementary-material

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Impact of Infection-Related Immunosuppressant Reduction on Kidney Transplant Outcomes: A Retrospective Study Considering the Temporal Dynamics of Immunosuppressive Requirements

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Immunosuppressant reduction (ISR) is a common treatment for kidney transplant recipients experiencing infections, but its impacts on kidney transplant outcomes remains unclear. This retrospective single-center study included 300 patients who underwent kidney transplantation between January 2017 and April 2020. The post-transplant timeline was divided into four distinct phases: ≤1 month, 2–6 months, 7–12 months, and >12 months. Patients were categorized based on the presence of clinically relevant infections and whether they received ISR. Significant differences were observed in the spectrum of clinically relevant infections across the post-transplant phases. During the ≤1 month phase, primary infections were associated surgical operation, such as urinary tract infections involving *Enterococcus* spp. and *Candida* spp. Cytomegalovirus and BK polyomavirus (BKPyV) infections increased during the 2–6 months and 7–12 months periods. Approximately one-third of patients experienced ISR due to infection, with BKPyV infections being the primary causes. Recipients who experienced their first ISR due to infection between 2–6 months and 7–12 months had worse graft survival comparing with patients without any infections. ISR due to infections between 2 and 6 months was associated with a higher risk of rejection. Tailored ISR strategies should be developed according to temporal dynamics of immunosuppressive intensity to prevent rejection.

Keywords: kidney transplantation, graft survival, infection, immunosuppressant reduction, rejection

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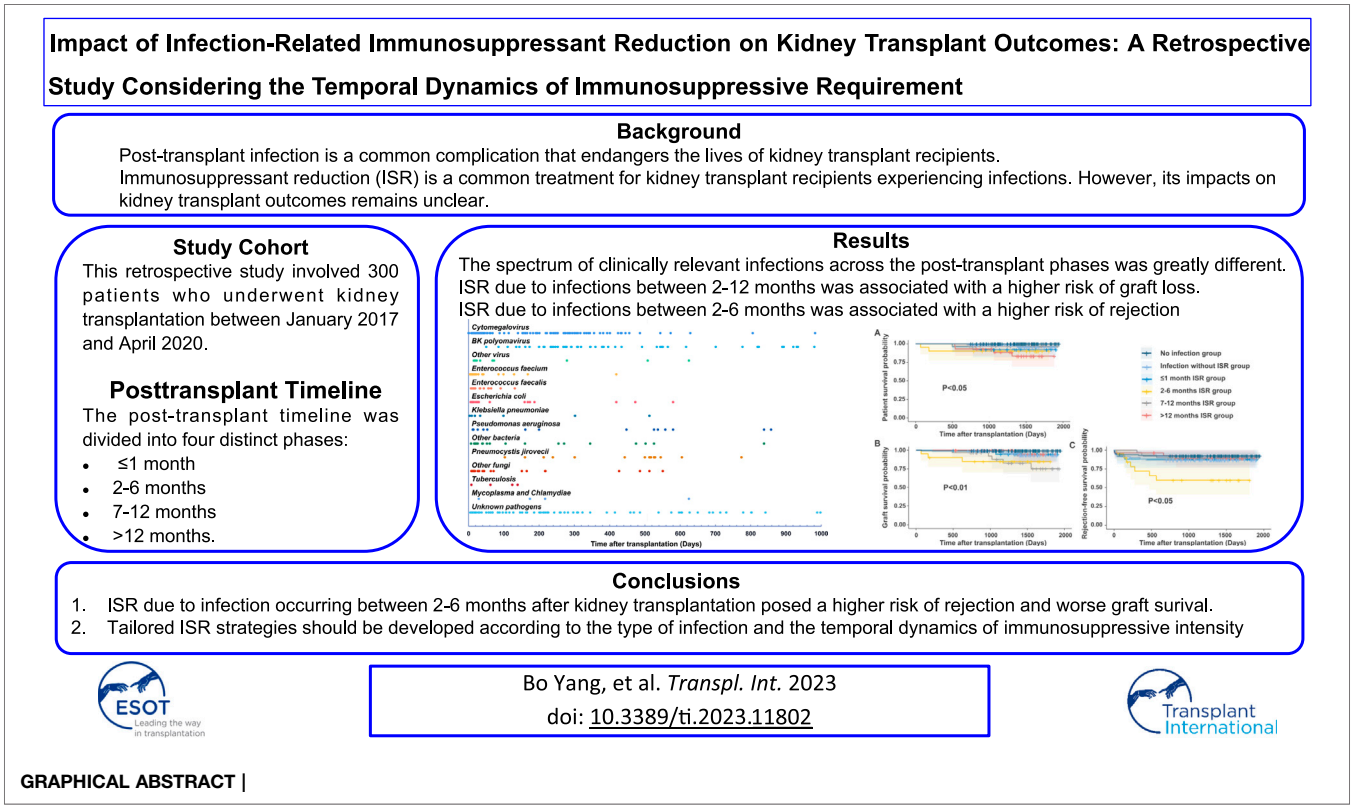

GRAPHICAL ABSTRACT |

INTRODUCTION

Post-transplant infection is a common complication that endangers the lives of kidney transplant recipients. Numerous studies have reported that up to 80% of patients experience at least one episode of infection during the first year following transplantation [1, 2]. Despite the administration of post-operative prophylaxis, infections still account for approximately 21% of deaths during long-term follow-up and remain the most common non-cardiovascular cause of death after kidney transplantation [3, 4].

Successful treatment of post-transplant infections requires accurate diagnosis, targeted antimicrobial therapy, and effective critical care support. Additionally, reducing the intensity of immunosuppression through immunosuppressant reduction (ISR) has been proposed to improve patient's recovery from post-transplant infections [5, 6]. Nevertheless, this strategy poses the risk of acute rejection, as the appropriate extent and duration of ISR are difficult to determine. Previous studies examining the relationship between ISR due to infection and the risk of rejection have yielded conflicting results. While some studies suggest that ISR does not increase the risk of graft loss or acute rejection in patients with bacterial infections, severe pneumonia, or BK polyomavirus (BKPyV) infection [7–10], others suggest that kidney recipients with ISR due to infection may be more susceptible to rejection [11, 12]. Moreover, the impacts of clinical factors such as the time, duration, and methods of ISR on the risk of rejection are not fully understood.

It is generally acknowledged that higher concentrations of immunosuppressants are required in the early phase after transplantation to prevent rejection. Earlier studies that aimed at reducing the toxicity of immunosuppressants indicated that early reductions in tacrolimus (TAC) or mycophenolate mofetil (MMF) levels after kidney transplantation were linked to a higher rejection risk [13–15]. Conversely, late-phase reductions in TAC or MMF were relatively safer [16, 17]. Based on these findings, we hypothesize that patients who receive ISR due to infection early after kidney transplantation may be at a higher risk of rejection. To explore this hypothesis, we conducted a retrospective study on a cohort of consecutive patients who underwent deceased donor kidney transplantation in our center. The objective of this study is to identify the association between ISR due to infection at different phases after transplantation and the risk of rejection.

MATERIALS AND METHODS

Study Design

The organ donation and procurement protocols were approved by the ethics committee of Xiangya Hospital, Central South University. Written, informed consent was obtained from all donors and recipients. No executed prisoners' donations were

TABLE 1 | The baseline characteristics and post-transplant complications of different groups.

Patients' characteristics	No infection (group 1, *n* = 129)	Infection (*n* = 171)					*p*-value
		Without ISR (group 2, *n* = 68)	Time phases of first ISR due to infection (*n* = 103)				
			≤1 month (group 3, *n* = 25)	2–6 months (group 4, *n* = 20)	7–12 months (group 5, *n* = 30)	>12 months (group 6, *n* = 28)	
Age (years), mean ± SD	42.3 ± 10.8	38.8 ± 10.2	45.0 ± 11.9	41.3 ± 13.3	40.3 ± 10.5	42.9 ± 12.1	0.152
Male sex, *n* (%)	100 (77.5)	47 (69.1)	16 (64.0)	17 (85.0)	13 (43.3)[a,b,c]	19 (67.9)	<0.01
PRA positive, *n* (%)	3 (2.3)	3 (4.4)	0 (0.0)	2 (10.0)	2 (6.7)	1 (3.6)	0.317
HLA mismatches, mean ± SD	3.8 ± 1.3	3.6 ± 1.4	3.6 ± 1.2	3.3 ± 1.2	3.8 ± 0.9	3.7 ± 1.3	0.517
First Transplantation, *n* (%)	120 (93.0)	65 (95.6)	23 (92.0)	19 (95.0)	29 (96.7)	27 (96.4)	0.957
Leading causes of ESRD, *n* (%)							
Chronic nephritis[d]	85 (65.9)	57 (83.8)	18 (72.0)	17 (85.0)	24 (80.0)	18 (64.3)	0.057
Diabetic nephropathy	11 (8.5)	2 (2.9)	2 (8.0)	0 (0.0)	1 (3.3)	1 (3.6)	0.545
IgA nephropathy	9 (7.0)	2 (2.9)	1 (4.0)	0 (0.0)	2 (6.7)	4 (14.3)	0.326
Hypertensive nephrosclerosis	8 (6.2)	1 (1.5)	2 (8.0)	1 (5.0)	2 (6.7)	0 (0.0)	0.364
Polycystic kidney	5 (3.9)	2 (2.9)	1 (4.0)	1 (5.0)	0 (0.0)	2 (7.1)	0.717
History of blood transfusion, *n* (%)	7 (20.9)	13 (19.1)	5 (20.0)	4 (20.0)	6 (20.0)	7 (25.0)	0.993
History of smoking	18 (14.0)	19 (27.9)	5 (20.0)	6 (30.0)	3 (10.0)	6 (21.4)	0.132
Pre-transplant comorbidities, *n* (%)							
Essential hypertension	37 (28.7)	13 (19.1)	10 (40.0)	6 (30.0)	8 (26.7)	7 (25.0)	0.469
Type 2 diabetes	17 (13.2)	4 (5.9)	5 (20.0)	2 (10.0)	1 (3.3)	1 (3.6)	0.156
Coronary heart disease	5 (3.9)	3 (4.4)	3 (12.0)	1 (5.0)	1 (3.3)	4 (14.3)	0.185
Hepatitis B viral infection	16 (12.4)	7 (10.3)	4 (16.0)	0 (0.0)	4 (13.3)	3 (10.7)	0.595
History of tuberculosis	2 (1.6)	1 (1.5)	2 (8.0)	1 (5.0)	1 (3.3)	1 (3.6)	0.250
Hemoglobin (g/L), mean ± SD	111.7 ± 21.8	109.8 ± 20.7	107.2 ± 22.4	104.5 ± 14.7	104.7 ± 15.5	108.2 ± 22.3	0.452
Cold ischemia time (h), mean ± SD	10.4 ± 3.5	10.5 ± 3.4	11.1 ± 2.9	11.3 ± 4.6	10.1 ± 4.1	10.4 ± 3.6	0.835
Induction therapy, *n* (%)							
Basiliximab	73 (56.6)	38 (55.9)	15 (60.0)	8 (40.0)	19 (63.3)	17 (60.7)	0.687
ATG	51 (39.5)	25 (36.8)	9 (36.0)	11 (55.0)	11 (36.7)	10 (35.7)	0.766
Baciliximab + ATG	5 (3.9)	5 (7.4)	1 (4.0)	1 (5.0)	0 (0.0)	1 (3.6)	0.707
Post-transplant complications, *n* (%)							
DGF	18 (14.0)	13 (19.1)	6 (24.0)	8 (40.0)	6 (20.0)	3 (10.7)	0.101
Urinary fistula	0 (0.0)	8 (11.8)[a]	3 (12.0)[a]	4 (20.0)[a]	1 (3.3)	0 (0.0)[c]	<0.01
Ureteral stenosis	0 (0.0)	1 (1.5)	0 (0.0)	1 (5.0)	0 (0.0)	1 (3.6)	0.081
NODAT	5 (3.9)	6 (8.8)	5 (20.0)	2 (10.0)	3 (10.0)	4 (14.3)	0.051
All Rejections (*n*, %)	10 (7.8)	9 (13.2)	3 (12.0)	7 (35.0)[a,b]	3 (10.0)	3 (10.7)	0.049
Post-infection rejection	—	6 (8.8)	3 (12.0)	7 (35.0)[b]	3 (10.0)	2 (7.1)[c]	0.038
TCMR	8 (5.6)	2 (2.9)	2 (8.0)	2 (10.0)	1 (3.7)	0 (0.0)	0.624
ABMR	2 (1.4)	3 (4.4)	0 (0.0)	3 (15.0)[a]	1 (3.7)	2 (11.1)	0.020
Mixed rejection	0 (0.0)	1 (1.5)	1 (4.0)	2 (10.0)[a]	1 (3.7)	0 (0.0)	0.015

Abbreviations: ISR, immunosuppressants reduction; CMV, cytomegalovirus; PRA, panel reactive antibodies; HLA, human leukocyte antigen; ESRD, end stage renal disease; ATG, antithyroglobulin; DGF, delayed graft function; NODAT, new onset diabetes after transplantation; TCMR, T cell-mediated rejection; ABMR, antibody-mediated rejection.

[a]*Significant different from group 1.*

[b]*Significant different from group 2.*

[c]*Significant different from group 4.*

[d]*Diagnosed based on clinical manifestation and laboratory findings, without renal biopsy.*

used following international human rights guidelines of the Declaration of Helsinki and the Declaration of Istanbul.

This study enrolled a total of 300 consecutive patients who underwent deceased donor kidney transplantation at our center between January 2017 and April 2020. The perioperative clinical and laboratory data of both donors and recipients were obtained from their medical records and the Chinese Scientific Registry of Kidney Transplantation. Patients with pre-existing donor-specific antibodies (DSA) or those who received multiple organ transplants were excluded. Additionally, we excluded two patients who experienced graft loss shortly after transplantation due to vascular thrombosis, and two patients with primary graft non-function.

According to the protocols of our immunosuppressive therapy and previous studies on the timeline of post-transplant infections [18], we categorized the post-transplant timeline into four phases: ≤1 month, 2–6 months, 7–12 months, and >12 months. Patients were grouped based on the presence of post-transplant infections and whether they received ISR for the infection during different phases (i.e., no infection, infection without ISR, or ISR due to infection within each timeline phase). Patients who had multiple infections with ISR were grouped based on the time of their first infection requiring ISR. **Table 1** provides an overview of the baseline and post-transplant clinical characteristics of patients.

The Protocols of Immunosuppressive Induction and Maintenance Therapy

Anti-thymocyte globulin (ATG) induction therapy involved administering 50 mg/day of ATG at the time of transplantation and for the next 2 days; alternatively, two doses of 20 mg basiliximab were injected during the operation and on the fourth day post-transplant. In some patients with delayed graft function (DGF), basiliximab was initially given upon transplantation but then switched to ATG within the following 3 days to mitigate ischemia-reperfusion injury. Maintenance immunosuppressive therapy consisted of a TAC-based regimen in combination with prednisone and MMF. The TAC target concentration was 10–12 ng/mL within the first month, 8–10 ng/mL between the 2nd and 6th month, 6–8 ng/mL from the 7th to the 12th month, and above 5 ng/mL thereafter. The initial dose of MMF was 1.5 g/day and tapered to 1 g/day after 1 year. Methylprednisolone (500 mg/day) was administered on the day of the procedure and for the next 2 days, followed by oral prednisone starting at 40 mg/day and gradually tapered to 10 mg/day at 1 month and 5 mg/day at 1 year.

The Prophylaxis Protocols for Post-Transplant Infections

Cefoperazone-sulbactam or piperacillin-tazobactam was routinely administered at the time of transplantation and was discontinued within 5 days if there was no sign of infection. Trimethoprim/Sulfamethoxazole (SMZ) was used for prophylaxis (0.48 g/day) against *Pneumocystis jiroveci* pneumonia (PJP) for 6–12 months after transplantation. Since valganciclovir was not covered by most health insurance in China, ganciclovir (1.5 g/day) was given to most patients for 3–6 months to prevent Cytomegalovirus (CMV) infection. For patients who had DGF or could not tolerate ganciclovir, CMV viremia was monitored monthly for 6 months, and then every 3–6 months thereafter. Pre-emptive treatment was initiated when CMV viremia was detected. Among patients with rejection, SMZ and ganciclovir were used for 3 months to prevent infections after antirejection treatments.

A Double J stent was routinely inserted during transplantation to prevent urinary complications and was typically removed after 3–4 weeks, with the aim of reducing the risk of urinary tract infections (UTI). In cases involving patients with a urinary fistula, the removal of the stent was deferred until the leakage had healed.

The Diagnosis of Post-Transplant Clinically Relevant Infections and Rejection

The clinically relevant infection was defined as previously described with minor modification [19]. Briefly, bacterial infections require the isolation of a bacterial pathogen, clinical signs/symptoms, and specific antibiotic treatment. Clinically relevant fungal infections require histopathology of a tissue biopsy showing invading fungal hyphae or yeasts, or clinical and microbiological criteria (probable invasive fungal infection by European Organization for Research and Treatment of Cancer definition) [20, 21]. CMV infection was considered clinically relevant when viremia or CMV disease was evidenced [22]. BKPyV infection was classified as clinically relevant if it was confirmed through biopsy as BKPyV nephropathy or presumed BKPyV nephropathy based on viral load criteria [23]. Alternatively, patients with BKPyV infection were subjected to pre-emptive ISR treatment [9]. Other clinically relevant viral infections are defined by the detection of viral replication together with clinical signs/symptoms. Infections with unknown pathogens were considered clinically relevant when they were symptomatic, evidenced by imaging and/or other laboratory examinations, and required antibiotic treatment.

The indication biopsy was performed on patients with allograft dysfunction, and the pathological diagnosis of rejection and BKPyV nephropathy was based on Banff criteria.

The Strategy of Reduction and Resumption of Immunosuppressants During the Treatment of Infection

The ISR due to infection was defined as any immunosuppression reduction as a part of treatment for any post-transplant infection. The criteria for applying ISR in patients with pulmonary infection is based on the Infectious Diseases Society of America/American Thoracic Society (IDSA/ATS) 2007 guidelines for severe pneumonia [24]. In cases where patients do not meet IDSA/ATS criteria, ISR may be applied if potential life-threatening infections are suspected, or if there is no improvement in key symptoms and indicators, including fever, dyspnea, and PaO2/FIO2 ratio, after 48 h of antibiotic treatment. The approach to reinstating immunosuppressants was tailored to the type of

pathogen and individual patient's condition. Standard prerequisites for reintroducing immunosuppressants include an absence of fever for at least 72 h, significant improvements in pulmonary function, and improved chest X-ray results. The initial step typically involves gradually resuming the calcineurin inhibitor (CNI) to its prescribed concentration. Once a sustained decrease or cessation of infection is confirmed, MMF may be reintroduced progressively at its customary dose.

For patients with simple CMV viremia or UTI, reductions in immunosuppressants were considered if over-immunosuppression was suspected based on clinical experience and laboratory test results. Tapering of MMF was typically the first step, and it was resumed when the infection had been cured for at least 1 week. For patients with complicated conditions such as repeated infections due to multi-drug-resistant strains, mixed fungal and bacterial infections, etc., the strategy of ISR was determined by physicians according to their clinical judgment of the patient's condition.

BKPyV replication was monitored monthly for 6 months after transplantation, followed by subsequent monitoring every 3–6 months in the absence of BKPyV infection. Detection of active viral infection, defined as a viral load of $>2 \times 10^3$ copies/mL in urine, prompted a thorough review of the patient's immunosuppressive regimen, and appropriate adjustments were made according to our center's established practices. Moreover, urine and blood BKPyV detection was repeated every 3 weeks to monitor trends and inform further decisions. If urine BKPyV replication increased rapidly, or blood BKPyV viremia was detected, ISR was applied to the affected patients as needed. ISR involved halving the dosage of MMF and decreasing CNI levels to 4–5 ng/mL for TAC or 80–100 ng/mL for cyclosporine (CsA). For patients with declining graft function due to BKPyV nephropathy or no improvement in BKPyV replication following CNI and MMF reduction, MMF was replaced by Leflunomide at a dose of 20 mg/day. Additionally, intravenous immunoglobulins were administered monthly at a dosage of 0.1–0.2 g/kg for at least 4 months. After BKPyV infection, we considered increasing the intensity of immunosuppression among patients with stable graft function. However, this was only done when their viral load was undetectable in blood and $<1 \times 10^4$ copies/mL in urine for at least two consecutive months. In such cases, the CNI level could be increased to 5–6 ng/mL for TAC or 100–120 ng/mL for CsA. If the graft function remained stable and the viral load continued to improve, MMF could replace Leflunomide.

Follow-Up

All patients regularly visit our clinics as required for monitoring graft function and drug concentration. Whenever major complications were suspected, the patients were admitted to the hospital for further examination. To minimize the impact of COVID-19 on our study, the follow-up date was set to 30 April 2022.

Statistical Analysis

Continuous variables were analyzed by Analysis of Variance and summarized as mean ± standard deviation (SD). Categorical variables were analyzed by chi-square or Fisher's exact tests with Bonferroni correction and described using frequencies and percentiles. Accumulate survival rates were calculated by the life table. Survival curves were estimated by the Kaplan–Meier method and compared with the log-rank tests. Analysis of risk factors was determined by binary logistic regression. SPSS software 23.0 (IBM, United States) was used for data analysis, and p-value < 0.05 was considered to be statistically significant. All tests were 2-tailed.

RESULTS

The Baseline Demographic and Clinical Characteristics of Patients in Different Groups

A total of 171 patients (57.0%) experienced 413 clinically relevant infections during the follow-up period of this study. Among the infection group, 103 out of 171 patients (60.2%) received ISR due to infection. **Table 1** showed that the baseline characteristics of the groups were largely similar, except for a lower proportion of male patients in the 7–12 months ISR group (43.3%) compared to the no infection group (77.5%, $p < 0.05$), infection without ISR group (69.1%, $p < 0.05$), and the 2–6 months ISR group (85.0%, $p < 0.05$). Regarding post-transplant complications, the incidence of urinary fistula was significantly lower ($p < 0.05$) in the no infection group (0.0%, 0/129) compared to the infection without ISR group (11.8%, 8/68), the ≤1 month ISR group (12.0%, 3/25), and the 2–6 months ISR group (20.0%, 4/20). Furthermore, the incidence of urinary fistula in the >12 months ISR group (0.0%, 0/28) was significantly lower than that in the 2–6 months ISR group ($p < 0.05$). The incidence of rejection was significantly higher ($p < 0.05$) in the 2–6 months ISR group (35%, 7/20) compared to the no infection group (7.8%, 10/129) and the infection without ISR group (13.2%, 9/68). Majority of these rejections (60.0%, 21/35) occurred after post-transplant infections.

The Characteristics of Post-Transplant Infections at Different Phases After Transplantation

Figure 1 and **Table 2** present a detailed timeline and characteristics of infections following kidney transplantation. Within the first month post-transplantation, there were 106 clinically relevant infections in 80 patients, with UTIs accounting for 49.1% of all infections, significantly higher than other time phases ($p < 0.05$). However, no BKPyV infections were identified during this phase. Between 2 and 6 months post-transplantation, 62 patients experienced 95 clinically relevant infections, with the proportion of UTIs to all infections decreasing to 29.5% compared to the first month (49.1%, $p < 0.05$), while the incidence of clinically relevant BKPyV infection increased to 7.4%. During the period between 7 and 12 months post-transplantation, 67 patients experienced 88 clinically relevant infections, with BKPyV infection constituting 25.0%

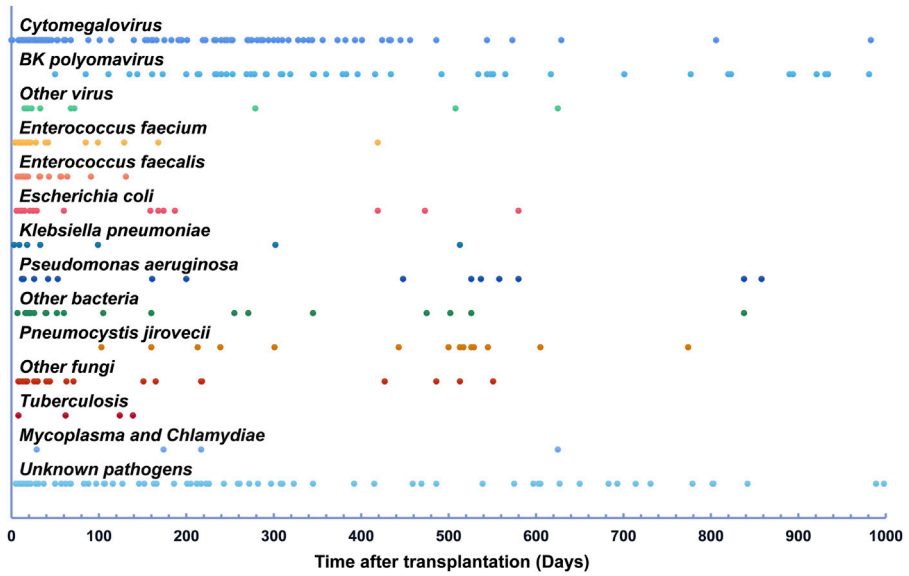

FIGURE 1 | Infections detected within 1,000 days after renal transplantation.

TABLE 2 | The types of infections in different time phases after transplantation.

Types of infection	Infection cases at different post-transplant time phases				*p*-value
	≤1 month (Group 1, *n* = 106)	2–6 months (Group 2, *n* = 95)	7–12 months (Group 3, *n* = 88)	>12 months (Group 4, *n* = 124)	
Pulmonary infections, *n* (%)	21 (19.8)	25 (26.3)	22 (25.0)	31 (25.2)	0.697
Urinary tract infections, *n* (%)	52 (49.1)	28 (29.5)[a]	8 (9.1)[a,b]	20 (16.3)[a,b]	<0.001
BKPyV infection, *n* (%)	0 (0.0)	7 (7.4)[a]	22 (25.0)[a,b]	35 (28.5)[a,b]	<0.001
CMV viremia, *n* (%)	19 (17.9)	29 (30.5)[a]	33 (37.5)[a]	21 (17.1)[b,c]	0.001
Other sites, *n* (%)	10 (9.4)	5 (5.3)	3 (3.4)	16 (13.0)	0.050
Surgical wound, *n* (%)	4 (3.8)	1 (1.1)	0 (0.0)	0 (0.0)[a]	0.032

Abbreviations: BKPyV, BK polyomavirus; CMV, cytomegalovirus.
[a]*Significantly different from Group 1.*
[b]*Significantly different from Group 2.*
[c]*Significantly different from Group 3.*

of all infections, which was much higher than the first month (0.0%, $p < 0.01$) and 2–6 months post-transplantation (7.4%, $p < 0.05$). The proportion of UTI was lowest in the 7–12 months phase (9.1%), and this difference was statistically significant when compared to previous time phases ($p < 0.05$). From the 13th month to the end of the follow-up, 82 patients experienced 124 clinically relevant infections. Pulmonary infection (25.2%) and BKPyV infection (28.5%) were the two most common infections during this period.

The proportion of CMV infection among all infections was highest in the 7–12 months phase (37.5%) and lowest in the >12 months phase (17.1%), and this difference was statistically significant ($p < 0.05$). The proportion of pulmonary infections among all infections ranged from 19.8% to 26.3%, and the difference was not statistically significant ($p > 0.05$).

The pathogens identified in post-transplant infections were summarized in **Supplementary Table S1**. Regarding gram-positive bacterial pathogens, *Enterococcus faecalis* and *Enterococcus faecium* were frequently identified, accounting for 43.4% and 46.9% of all bacterial pathogens isolated during the ≤1 month and 2–6 months post-transplant phases, respectively. These *Enterococcus spp*. were mainly implicated in UTIs within the first 3 months after transplantation but were rare thereafter. The commonly isolated gram-negative bacterial pathogens following kidney transplantation were *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli*. *Pseudomonas aeruginosa* was mainly found in patients with pulmonary infections and accounted for 6.3%–16.7% of all isolated bacterial pathogens in each post-transplant phase, respectively. *Klebsiella pneumoniae* was a substantial pathogen for both pulmonary infections and UTIs,

TABLE 3 | The characteristics of ISR due to infection in different time phases after transplantation.

Characteristics	Patients with ISR due to infection (n = 103)				p-value
	≤1 month (group 1, n = 25)	2–6 months (group 2, n = 20)	7–12 months (group 3, n = 30)	>12 months (group 4, n = 28)	
Infections leading to first ISR, *n* (%)					
Pulmonary infection	10 (40.0)	12 (60.0)	16 (53.3)	11 (39.3)	0.410
Urinary tract infection	12 (48.0)	5 (25.0)	2 (6.7)[a]	1 (3.6)[a]	<0.001
BKPyV infection	0 (0.0)	2 (10.0)	11 (36.7)[a,b]	13 (46.4)[a,b]	<0.001
Other infections	3 (12.0)	1 (5.0)	1 (3.3)	3 (10.7)	0.590
Patients with ISW, *n* (%)	3 (12.0)	5 (25.0)	6 (20.0)	8 (28.6)	0.498
Patients with repeated ISR, *n* (%)	8 (32.0)	6 (30.0)	12 (40.0)	6 (21.4)	0.507
Infections leading to repeated ISR, *n* (%)					
Pulmonary infection	3 (12.0)	1 (5.0)	4 (13.3)	1 (3.6)	0.534
Urinary infection	1 (4%)	1 (5.0)	0 (0.0)	0 (0.0)	0.343
BKPyV infection	6 (24.0)	4 (20.0)	7 (23.3)	4 (14.3)	0.822
Other infections	0 (0.0)	1 (5.0)	1 (3.3)	1 (3.6)	0.881
Duration of first ISR (days), mean ± SD	35.12 ± 78.1	54.6 ± 141.6	221.9 ± 328.9[a]	278.9 ± 345.7[a,b]	0.002
Pulmonary infection	26.3 ± 30.9	30.3 ± 29.1	43.1 ± 70.7	26.27 ± 21.6	0.748
Urinary tract infection	15.6 ± 13.4	9.6 ± 7.0	6.5 ± 3.5	4	0.546
BKPyV infection	—	336.5 ± 439.1	496.7 ± 394.2	560.9 ± 326.8	0.702
Other sites	142.7 ± 218.7	7.0	491.0	74.7 ± 44.1	0.248

Abbreviations: ISR, immunosuppressants reduction; ISW, immunosuppressants withdrawal (completely stopping TAC and MMF); BKPyV, BK polyomavirus.
[a]*Significantly different from group 1.*
[b]*Significantly different from group 2.*

accounting for 7.5%–16.7% of all isolated bacterial pathogens in ≤1 month, 2–6 months, and 7–12 months post-transplant phases, but comprised 45% of all bacterial pathogens isolated in the >12 months post-transplant phase. In contrast, *Escherichia coli* emerged as a major pathogen for UTIs, responsible for 22.6% of all bacterial pathogens isolated within the first month after transplantation, significantly higher than *Pseudomonas aeruginosa* (7.5%) and *Klebsiella pneumoniae* (7.5%). *Candida spp*. were primarily found in the early post-transplant phases, representing 87.5% of fungal infections within the first month after transplantation, but became less prevalent after 3 months. *PJP* reached its peak during 12–24 months after transplantation, accounting for 66.7% of all fungal infections between 13 months to the end of follow-up and was mostly following cessation of SMZ.

The Characteristics of ISR Due to Infections at Different Phases After Transplantation

Among the 103 patients with infection-triggered ISR, 29 had experienced two episodes of ISR, and 3 patients had three episodes (**Table 3**). The initial episode of ISR was attributed to pulmonary infections in 47.6% (49/103) of cases, while BKPyV infections and UTIs accounted for 25.2% (26/103) and 19.4% (20/103) of cases, respectively. Only one patient experienced initial ISR as a result of CMV infection. In contrast, BKPyV infection was the predominant cause (60.0%, 21/35) of repeated ISR (detailed in **Table 3** and **Supplementary Table S2**) while UTIs were responsible for only two cases of repeated ISR.

A total of 22 patients discontinued both CNI and MMF during infection treatment. Among them, one patient halted both CNI and MMF due to sepsis resulting from a UTI, and another patient suspended treatment due to HBV infection, which led to fulminant hepatitis. The remaining 20 patients temporarily suspended CNI and MMF due to severe pulmonary infections. The proportion of patients who temporarily discontinued both medications was 12.0% (3/25), 25.0% (5/20), 20% (6/30), and 28.6% (8/28) in the ≤1 month ISR, 2–6 months ISR, 7–12 months ISR, and >12 months ISR groups, respectively, with no significant differences observed. Additionally, the duration of ISR was significantly shorter in the ≤1 month ISR group and 2–6 months ISR group compared to later time periods. However, the durations of ISR were similar across different groups when categorized by infection type (**Table 3**). No substantial distinctions in ISR duration were observed between patients with and without rejection (191.3 ± 291.1 vs. 153.8 ± 279.6 days, $p = 0.63$) and between patients with and without graft loss (162.1 ± 284.0 vs. 133.1 ± 252.0 days, $p = 0.77$).

ISR Due to Infections Was Associated With a Higher Risk of Rejection and Inferior Patient and Graft Survival After Transplantation

Figure 2 illustrates the impact of ISR due to infection on patient and graft survival. The 3 years patient survival rates showed a significant difference among the subgroups. Specifically, the no infection group and 7–12 months ISR group had a 100% survival rate, while the infection without ISR group had a 98.5% survival rate, the ≤1 month ISR group had a 92% survival rate, the 2–6 months ISR group had a 90% survival rate, and the >12 months ISR group had an 88.6% survival rate. At

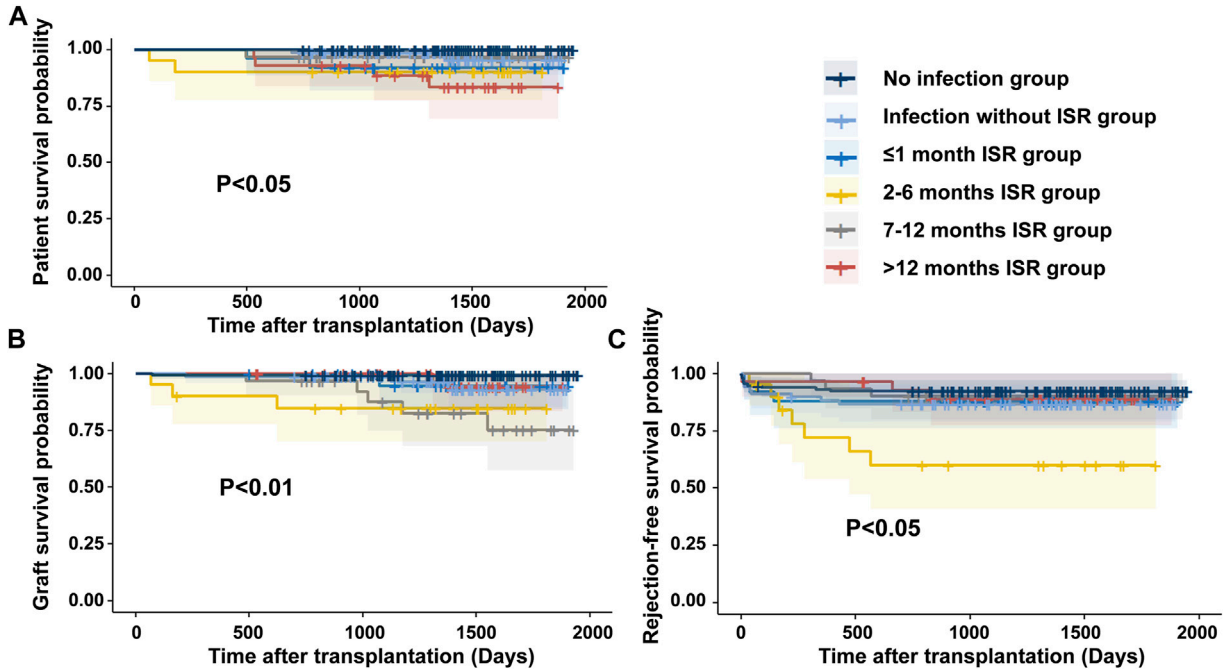

FIGURE 2 | The patient and graft outcomes were associated with ISR due to infection **(A)** The patient survival rate was significantly lower in the >12 months ISR group compared to the no infection group ($p < 0.01$), Infection without ISR group ($p = 0.049$), and the 7–12 months ISR group ($p = 0.046$). **(B)** The death censored allograft survival significantly lower in 2–6 months ISR group and 7–12 months ISR group compared with no infection group. **(C)** Rejection-free survival was inferior in the 2–6 months ISR group compared to all other groups ($p < 0.05$), except for the ≤1 month ISR group ($p = 0.059$).

5 years, the survival rates decreased to 83.4% in the >12 months ISR group, while the patients' survival rates in all other groups remained unchanged. Notably, the no infection group demonstrated significantly higher patient survival rates compared to most subgroups (log-rank $p < 0.05$) except for the 7–12 months ISR group. The 5 years patient survival rate was significantly lower in the >12 months ISR group compared to the no infection group ($p < 0.01$), infection without ISR group ($p = 0.049$), and the 7–12 months ISR group ($p = 0.046$).

Regarding death-censored graft survival rates, at 3 years, the rates were 99.2%, 98.5%, 94.4%, 84.7%, 90.5%, and 100% in the no infection group, infection without ISR group, ≤1 month ISR group, 2–6 months ISR group, 6–12 months ISR group, and the >12 months ISR group, respectively. At 5 years, the graft survival rates decreased to 93.0% in the infection without ISR group, 77.7% in the 6–12 months ISR group, and 94.1% in the >12 months ISR group, but remained unchanged in other groups. The death-censored graft survival rates were significantly lower in the 2–6 months ISR group and 7–12 months ISR group compared to the no infection group ($p < 0.01$), while there was no significant difference between all other subgroups.

During follow-up, the incidence of rejection in the infection group (14.6%, 25/171) was higher than that in the no infection group (7.8%, 10/129; $p = 0.071$). Of the four patients who experienced rejection before infection, three belonged to the infection without ISR group. Among the patients who experienced rejection after infection, 71.4% (15/21) occurred following ISR due to infection. Regarding rejection-free graft survival rates, at 3 years, the rates were 92.2%, 86.7%, 88.0%, 60.1%, 90.0%, and 88.5% in the no infection group, infection without ISR group, ≤1 month ISR group, 2–6 months ISR group, 6–12 months ISR group, and the >12 months ISR group, respectively. At 5 years, rejection-free graft survival rates remained unchanged. The 2–6 months ISR group showed significantly lower rates of rejection-free graft survival compared to all other groups ($p < 0.05$), except for the ≤1 month ISR group ($p = 0.059$).

Multivariate Cox regression analysis identified several factors associated with patient and graft survival. Pulmonary infection ($p = 0.004$), coronary disease ($p = 0.008$), and new onset diabetes after transplantation ($p = 0.013$) were identified as factors associated with patient death (**Supplementary Table S3**). Moreover, overall infections ($p = 0.029$) were found to be associated with death-censored graft survival (**Supplementary Table S4**), while PRA positive ($p = 0.006$), ISR due to infections between 2 and 6 months post-transplantation ($p = 0.035$), and smoking history ($p = 0.012$) were identified as factors associated with rejection-free graft survival (**Supplementary Table S5**).

DISCUSSION

Currently, there is limited data on the incidence rates of infection-driven ISR. In our study, we observed that 57.0% of patients developed clinically relevant infections, and 34.3% of patients experienced ISR due to infection during follow-up. In Posadas

Salas et al.'s study, ISR was defined as sustained TAC levels <8 ng/mL and MMF dosage <1 g/day for at least 1 month within 1 year after transplantation [11]. They reported that 16% of patients had ISR due to infection within the first year after transplantation. As the duration of ISR can vary greatly based on the type, severity, and timing of infections, and physicians often prefer shorter durations of ISR in the early phases after transplantation to minimize the risk of rejection, we did not require a minimum duration of sustained ISR to define ISR, resulting in a higher incidence of ISR due to infection compared to the previous report. Nevertheless, both studies indicate the frequent occurrence of ISR due to infection after transplantation, and further investigation is necessary to understand its impact on patient and graft survival.

To investigate the temporal dynamics of infections and their correlation with rejection, we divided the post-transplant timeline into four phases based on established immunosuppression protocols, antibiotic prophylaxis strategies, and previous research. Our findings revealed significant differences in the spectrum of infections across these phases. In the first month, surgical complications and nosocomial infections were the primary causes of infections, predominantly urinary tract infections involving *Enterococcus* spp. and *Candida* spp. This observation is consistent with prior studies indicating that *Enterococcus* spp. and *Candida* spp. are major infectious pathogens during the early post-transplantation period [2]. Notably, one study found that Beta-lactam antibiotics significantly increase relative gut abundance of *Enterococcus* spp., posing an independent risk factor for *Enterococcal* bacteriuria in kidney recipients [25]. Therefore, our use of Beta-lactam antibiotics for perioperative antibacterial prophylaxis might escalate the likelihood of *Enterococcus* spp. infection. As many of the predisposing factors, such as urinary fistula, prolonged Double J stent placement, and inappropriate antibiotic usage, can be prevented by enhancing surgical techniques and optimizing treatment protocols. Efforts should be made to address these predisposing factors in order to minimize early post-transplant infections and the subsequent need for unnecessary ISR. We observed that CMV and BKPyV infections increased during the 2–6 months and 7–12 months periods, which may be attributed to the maintenance of high immunosuppressive intensity during these periods and cessation of CMV prophylaxis three to 6 months post-transplantation. After 12 months, the incidence of CMV and BKPyV infections declined, likely due to our planned reduction of maintenance immunosuppressive intensity at that point. Similarly, we administered SMZ prophylaxis for 6–12 months, and we noticed an increase in PJP incidence during the 13–24 months post-transplantation following its discontinuation. Our results suggest that the intensity of immunosuppression and antibiotic prophylaxis significantly influence the frequency and timeline of post-transplant infections. As the pharmacokinetic monitoring alone is insufficient for estimating the intensity of immunosuppression after transplantation, researchers have developed some new techniques such as measuring virus-specific T cell levels in addition to pharmacokinetic monitoring, measuring the viral load of torque teno virus, monitoring the intracellular tacrolimus concentration in T-lymphocytes and other immune cells, et al., to solve this problem [26–28]. Optimizing the immunosuppressive protocol based on these new techniques might help us to further reduce the risk of infections and unnecessary ISR in future.

ISR was recommended as the standard treatment for BKPyV infection since no effective antiviral drugs are available [23]. Pre-emptive ISR has demonstrated excellent long-term results for BKPyV infection [9], and our center has implemented a similar strategy. Although some studies have suggested initiating ISR for BKPyV infection upon detection of BKPyV-DNAemia [23], our center aligns with previous findings that indicate sustained BKPyV viruria as an early marker for the development of BKPyV-associated nephropathy. Therefore, our center opts to initiate ISR when the urine BKPyV load is high or shows an increasing trend in subsequent surveillance BKPyV tests [9, 29]. Our data suggest that current ISR strategy for BKPyV infection is effective and does not significantly increase the risk of rejection.

Our results verified the findings of previous research that infection was a major risk for patient and graft survival. Moreover, the survival was worst in patients who had ISR due to infection after 12 months. This could be explained by the fact that more patients had life-threatening pneumonia due to *PJP*, *Klebsiella pneunoniae* infection et al. in the >12 months ISR group. We analyzed the characteristics, including the causes, time, extent, duration, and repeated episodes of ISR due to infection, and investigated their relationship with rejection. We identified that graft survival rates were significantly lower in the 2–6 months ISR group and 7–12 months ISR group compared to the no infection group. ISR during the 2–6 months period due to infection is an independent risk factor for rejection. Our finding did not completely fulfill the previous hypothesis as the earlier time phase (within 1 month after transplantation) was not associated with rejection and a worse prognosis. This could partly be explained by the fact that physicians prefer less extent and shorter durations of ISR in the very early phases after transplantation to minimize the risk of rejection, therefore fewer patients completely stopped TAC and MMF due to infection within 1 month after transplantation. Moreover, the protective effect of induction agents is dose-dependent and weakens over time. Previous studies have shown that induction with low dose ATG (1 mg/kg/day for 3 consecutive days) would result in excellent T cell depletion, but the T lymphocytes will back to normal levels within 1 month after transplantation [30]. As we also use low dose ATG for induction, it is reasonable to think that only patients who had ISR due to infection within 1 month after transplantation might benefit from the rejection-preventing effect of immunosuppressive induction agents, which partly balanced the risk of rejection.

Our study had several limitations. First, due to the retrospective nature of our study, we lacked sufficient data to compare the incidence rates of *de novo* donor specific antibodies (DSAs) between the ISR group and other groups. As DSAs are a major cause of antibody-mediated rejection, we provided detailed pathological diagnostic information in **Table 1** to elucidate the link between ISR due to infection and rejection pathology. Additionally, our study cohort was relatively young, potentially limiting its applicability to elderly recipients, who exhibited a lower risk of acute rejection but a higher susceptibility to mortality related to infectious and cardiovascular diseases [31].

In conclusion, our study revealed that ISR due to infection occurring between 2 and 6 months after transplantation may pose a higher risk of rejection, which provides valuable evidence for physicians to adjust their ISR strategy in infection treatment while minimizing the risk of rejection. A tailored ISR strategy should be designed for kidney transplant recipients with post-transplant infections, considering the type of infection and the temporal dynamics of immunosuppressive requirements.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: All the data were supplied by our single-central follow-up study. Requests to access these datasets should be directed to the corresponding author.

ETHICS STATEMENT

The studies involving humans were approved by the ethics committee of Xiangya Hospital, Central South University. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

XD were involved in planning and supervised the work; BY, QY and CH processed the data and performed the analysis; BY drafted the manuscript and designed the figures. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11802/full#supplementary-material

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ORIGINAL RESEARCH
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The Yield of Routine Post-Operative Doppler Ultrasound to Detect Early Post-Liver Transplantation Vascular Complications

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Early detection of liver transplantation (LT) vascular complications enables timely management. Our aim was to assess if routine Doppler ultrasound (rDUS) improves the detection of hepatic artery thrombosis (HAT), portal vein thrombosis (PVT) and hepatic venous outflow obstruction (HVOO). We retrospectively analysed timing and outcomes, number needed to diagnose one complication (NND) and positive predictive value (PPV) of rDUS on post-operative day (POD) 0,1 and 7 in 708 adult patients who underwent primary LT between 2010–2022. We showed that HAT developed in 7.1%, PVT in 8.2% and HVOO in 3.1% of patients. Most early complications were diagnosed on POD 0 (26.9%), 1 (17.3%) and 5 (17.3%). rDUS correctly detected 21 out of 26 vascular events during the protocol days. PPV of rDUS was 53.8%, detection rate 1.1% and NND was 90.5. Median time to diagnosis was 4 days for HAT and 47 days for PVT and 21 days for HVOO. After intervention, liver grafts were preserved in 57.1%. In conclusion, rDUS protocol helps to detect first week's vascular events, but with low PPV and a high number of ultrasounds needed.

Keywords: routine Doppler ultrasound, hepatic artery thrombosis, portal vein thrombosis, outflow obstruction, liver transplantation

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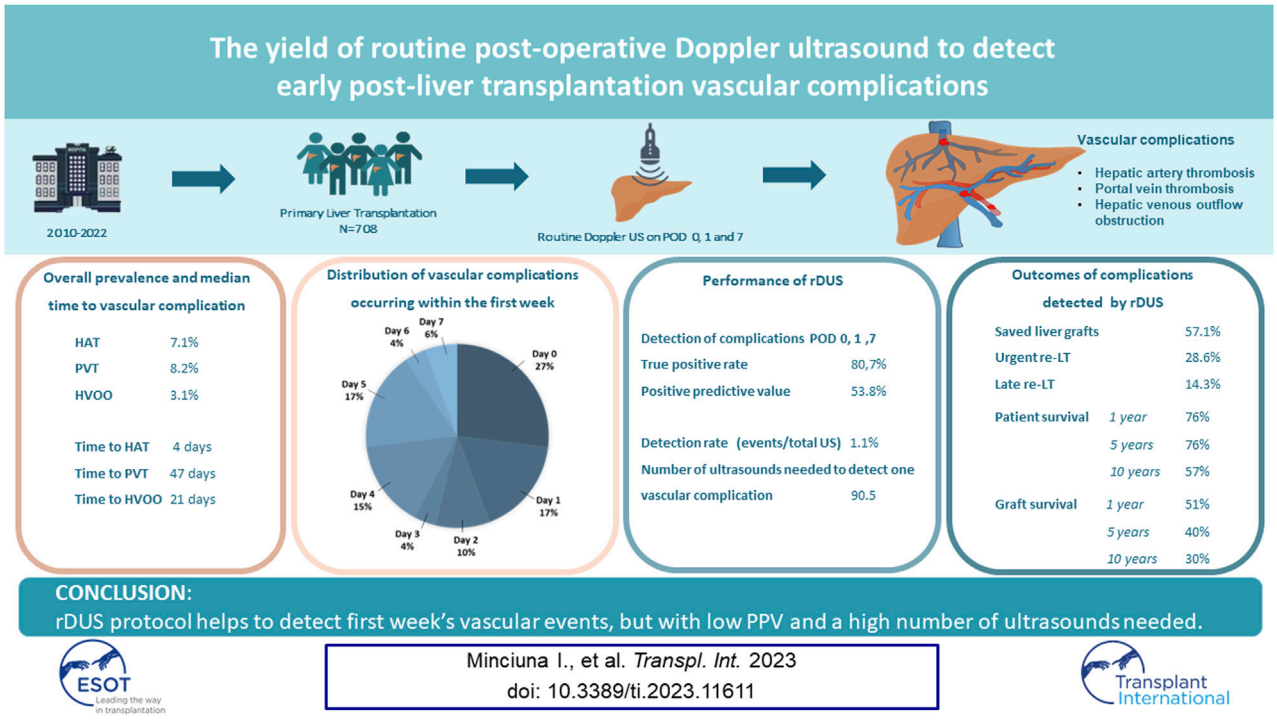
Abbreviations: DUS, Doppler ultrasound; rDUS, routine Doppler ultrasound; HAT, hepatic artery thrombosis; PVT, portal vein thrombosis; HVOO, hepatic venous outflow obstruction; iCT, imaging upon indication; CT, computer tomography; LT, liver transplantation; BMI, body mass index; ALD, alcohol related liver disease; SLD, steatotic liver disease; MELD, model for end-stage liver disease; DBD, donation after brain death; DCD, donation after cardiac death.



GRAPHICAL ABSTRACT |

INTRODUCTION

With an impressive evolution over the past 50 years, liver transplantation (LT) has changed the quality of life and survival of many patients with acute liver failure, end-stage liver disease and hepatocellular carcinoma. The continuous improvements in patient selection, surgical techniques, perioperative management and immunosuppression have led to an increased liver graft and patient survival over time. Although the increased experience and proficiency has resulted in a change of the incidence, nature and outcome of vascular complications, they remain the most feared complications of LT as they can lead to graft dysfunction or even graft loss and patient death.

Hepatic artery thrombosis (HAT) is an infrequent (incidence around 5%) but potentially devastating [1] complication with high morbidity and likelihood of graft failure or even mortality in the early post-LT setting. Moreover, as the bile ducts are particularly susceptible to hypoxia, HAT often leads to ischemic biliary complications, with subsequent secondary infection, abscesses and necrosis [2]. With an incidence of almost 3%, portal vein thrombosis (PVT) can cause graft failure, graft ischaemia, intestinal ischaemia, and persistence or recurrence of portal hypertension with ascites and variceal bleeding [3]. Hepatic venous outflow obstruction (HVOO), due to thrombosis or stenosis at the level of the hepatic veins or cavo-cavostomy, is an infrequent complication (incidence less than 3%) [3] that can lead to graft dysfunction and graft failure, with mortality rates reaching up to 24% [4, 5].

Early detection of these vascular complications is of paramount importance for timely management in order to achieve favourable outcomes in LT patients. Hereto, there are well-accepted standards including early post-LT sequential ultrasound evaluation of the vascular patency. The specifics regarding implementing them, however, differ according to centre as there are ongoing debates regarding the frequency and use of routine liver vessel assessment.

The aim of this study was to assess if routine Doppler ultrasound (rDUS), performed at day 0, 1 and 7 at our institution, improves the detection rate of early post LT vascular complications (i.e., HAT, PVT and HVOO).

PATIENTS AND METHODS

Study Population

All adult patients who underwent LT between January 2010 and September 2022 at our transplant centre were included. A routine Doppler ultrasound protocol was implemented in 2010 and consists of performing abdominal Doppler ultrasound by hepatologists with extensive ultrasonography experience during day 0 (usually within 1 h post-surgery), day 1 and day 7 post LT. We used the Hitachi Hi Version Preirus® from 2010–2018 and the Philips Epiq 7G® from 2018–2022. Ultrasound examination included the evaluation of the hepatic artery by colour Doppler to assess patency and by pulse-wave technology to evaluate the wave pattern and calculate the resistive index (RI) of the hepatic artery

at the level of the hilum and the right and left branch. The arterial anastomosis was considered patent when there was a normal wave pattern (i.e., no parvus tardus) and the RI was greater than 0.5 at all locations. The portal vein patency and flow direction (hepatofugal/hepatopetal) were evaluated by colour Doppler and the flow velocity by pulse-wave technology. The portal vein anastomosis was considered patent when the flow direction was hepatopetal, there was no intraluminal material and the acceleration between the pre-anastomotic and post-anastomotic portal vein velocity was less than three-fold. The patency and phase of three hepatic veins and the cavo-cavostomy were evaluated by colour Doppler. The outflow was considered sufficient when there was flow in all three hepatic veins and the cavo-cavostomy, the wave pattern in the hepatic veins were either triphasic or biphasic and the diameter of the hepatic veins was <10 mm. Whenever the patency of the hepatic artery, portal vein or hepatic vein was not considered sufficient or was debated, patients underwent a confirmatory CT (Computed Tomography). After discharge, all patients underwent life-long complete follow-up at our centre. All participants signed an informed consent before LT for retrospective data collection. The study adhered to the Declaration of Helsinki and is in concordance with the principles of the Declaration of Istanbul on Organ Trafficking and Transplant Tourism.

Data Collection and Definitions

The following recipient variables were collected at time of transplantation: age, gender, BMI, indication for LT, calculated Model for End-Stage Liver Disease (MELD) score at time of LT, type of graft [i.e., donation after brain death (DBD), donation after cardiac death (DCD) or living donor liver transplantation (LDLT)]. We collected data on the occurrence of HAT, PVT and HVOO during the first 7 days post-LT and at any time thereafter. HAT was defined as a thrombotic occlusion of hepatic artery that led to the absence of hepatic artery signal at the hilum or the intrahepatic arterial branches on Doppler Ultrasound and/or a non-enhancing filling defect on contrast-enhanced CT scan. PVT was defined as thrombotic occlusion of the portal vein that led to a filling defect of portal blood flow on Doppler Ultrasound and/or a portal non-enhancing filling defect on contrast enhanced CT. HVOO included either thrombosis or stenosis at the level of caval anastomosis or hepatic veins diagnosed by US, CT or venography. For each vascular complication, we collected data on the first radiological imaging technique used to diagnose the complication (i.e., DUS or CT) as well as the main indication for performing the imaging. These indications were either routine imaging on postoperative day 0, 1 or 7 or a clinical indication, defined as clinical deterioration, worsening graft function, ascites or other signs of portal hypertension, unexplained fever or abdominal pain, or hemodynamic changes. All patients with a rDUS suggestive of a vascular complication underwent subsequent CT to confirm the diagnosis. Furthermore, data on type of therapeutic intervention (i.e., surgical revascularization, endovascular radiological treatment, anticoagulation/antiplatelet treatment only and conservative treatment), total duration of hospitalization, need for re-LT within 7 days from liver vascular complication (i.e., urgent re-LT), and re-LT at any time point (i.e., re-LT) were obtained.

TABLE 1 | Baseline characteristics of 708 patients undergoing primary liver transplantation at our institution between 2010–2022.

Variables	Total population *n* = 708
Recipient sex (male)	445 (62.9%)
Recipient age at LT (years)	55 (17, 72)
BMI (kg/m^2)	25.5 (15.36–46.78)
Liver disease aetiology	
Viral	117 (16.5%)
Alcohol related liver disease	106 (15%)
Steatotic liver disease	70 (9.9%)
Biliary	178 (25.1%)
Autoimmune	20 (2.8%)
Acute liver failure	52 (7.3%)
Inherited metabolic liver diseases	36 (5.1%)
Vascular liver diseases	7 (1%)
Cryptogenic	28 (4%)
Other	94 (13.3%)
Hepatocellular carcinoma	239 (33.8%)
MELD Score at LT	22 (6–40)
Pre-LT PVT	73 (10.3%)
Type of graft	
DBD	419 (59.2%)
DCD	256 (36.2%)
Living Donor	31 (4.4%)
Domino	2 (0.3%)
Use of machine perfusion	108 (15.3%)
Surgical characteristics	
PVT at time of implantation	67 (9.5%)
Performance of portal thrombectomy	57 (8.1%)
Use of portal conduits	11 (1.6%)
Portal vein reconstruction	21 (3%)
End-to-end portal anastomosis	678 (98.7%)
Hepatic artery reconstruction	118 (16.7%)
Arterial re-do	35 (4.9%)
Intraoperative hepatic artery thrombosis	23 (3.2%)
Piggy-back caval anastomosis	681 (99.3%)

LT, liver transplantation; ALD, alcoholic liver disease; MEL, model for end-stage liver disease; DBD, donation after brain death; DCD, donation after cardiac death; rDUS, routine Doppler ultrasound.

Statistical Analysis

Statistical analyses were descriptive. Quantitative variables were expressed as medians with extreme values (range) and compared using Student's t-test or Wilcoxon test as appropriate. Qualitative variables were expressed as numbers and percentages and compared using Chi-square or Fisher's exact tests, as appropriate.

Primary outcomes were the frequency of HAT, PVT and HVOO at different time points within the first week. Secondary outcomes were the use of surgical or interventional therapies as well as graft and patient survival.

The diagnostic performance of protocol routine Doppler ultrasound in detecting HAT, PVT and outflow obstruction was expressed in terms of positive predictive value with CT as gold standard. Detection rate of rDUS was defined as number of vascular events detected/total number of ultrasounds performed. The number needed to image in order to detect one complication was calculated as (1/detection rate of rDUS). Due to the fact that a negative rDUS was not routinely followed by CT, it was not

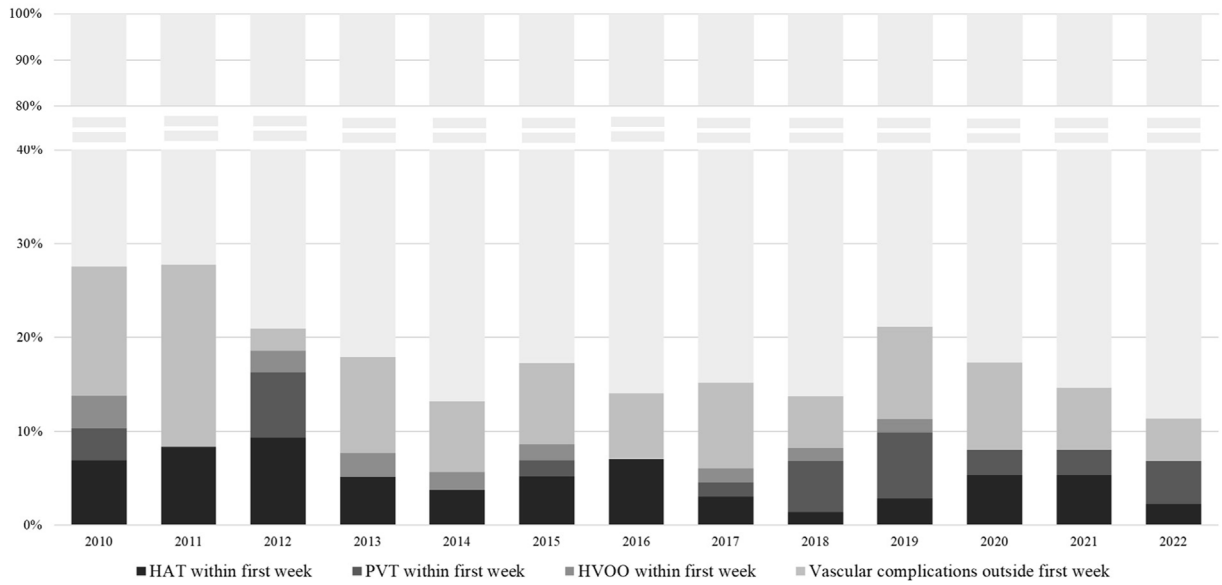


FIGURE 1 | Percentage of vascular complications at any time, HAT within the first week, PVT within the first week and HVOO within the first week, per calendar year of transplantation. rDUS, routine Doppler ultrasound; HAT, hepatic artery thrombosis; PVT, portal vein thrombosis; HVOO, hepatic venous outflow obstruction.

possible to calculate sensitivity, specificity and negative predictive value. All statistical analyses were performed using commercially available statistical software (SPSS Inc., Chicago, IL). A p-value of <0.05 was considered statistically significant.

RESULTS

Patient and Liver Transplantation Characteristics

In total, 708 patients underwent primary liver transplantation and were followed for a median of 3.69 years (range 0–12.4). The baseline characteristics of the study population are presented in **Table 1**.

Most of the patients were male (n = 445; 62.9%), with a median age of 55 (17–72) years, BMI of 25.5 kg/m^2 (15.3–46.7) and laboratory MELD score of 22 (6,40) at the time of first LT. Main LT indications were HCC (n = 239; 33.8%) and cholestatic liver disease (n = 178; 25.1%), followed by viral hepatitis in 117 (16.5%) and alcoholic liver disease in 106 (15%) patients. Machine perfusion was performed in 15.3%. Overall, 1, 5 and 10 years patient survival was 92.1% (95% CI: 91.1–93.1), 80.8% (95% CI: 79.1–82.5), and 68.2% (95% CI: 65.2–71.2). Graft survival was 87.5% (95% CI: 86.2–88.8), 73.9% (95% CI: 72–75.8), and 62.8% (95% CI: 60–65.6), respectively.

Vascular Complications Following Liver Transplantation

Of the entire population, 112 (15.8%) patients developed at least one vascular complication within a median of 0.33 months (range 0–95.4) after LT, of whom 52 (46.4%) within the first week. The rate of vascular complications during the study time period is shown in **Figure 1**. The rate of any vascular complication was 18% in 2010–2016, and 14.7% in 2017–2022 (p = 0.28). Median time to diagnosis of any vascular event was 10 days (0–2,864), for HAT was 4 days (0–1,382), for PVT 47 days (0–2,864) and HVOO 21 days (0–1933). In total, 50 (7.1%) patients developed HAT, of whom 34 (68%) within the first week. PVT was found in 58 patients (8.2%), 21 of whom during the first week (36.2%), and HVOO was identified in 22 patients (3.1%) (i.e., n = 4 caval stenosis, n = 16 thrombosis in hepatic vein(s), n = 2 thrombosis in the IVC), 8 of those (36.3%) within the first week.

The indication for the diagnostic CT within the first 7 days was a suspected vascular complication by rDUS in 40.4% of cases and laboratory changes in 34.6% of cases. Outside of the first week, imaging was either driven by clinical symptoms, laboratory changes or incidental, i.e., during re-evaluation for other indications (e.g., follow-up CT for HCC recurrence) (see **Table 2**).

In the first week, 34 patients were diagnosed with HAT (68% of all vascular events), 21 with PVT (36.2%) and 8 with HVOO (36.3%). Eleven of these (21.1%) had more than 1 vascular complication at the same time. Most of the vascular complications during the first week were diagnosed on post-operative day (POD) 0 (n = 14; 26.9%), 1 (n = 9; 17.3%) and 5 (n = 9; 17.3%), followed by POD 4 (n = 8; 15.4%), while at POD 7, only 3 patients were diagnosed (5.8%) (see **Figure 2**).

Performance of the rDUS Protocol

In total, 26 patients (3.7%) had a vascular event on day 0, 1 and 7, which included 18 (69.2%) HAT, 10 (38.5%) PVT and 2 (7.7%) patients with HVOO. Twenty-one vascular events (80.8%) were detected by rDUS, and the remaining five were initially missed

TABLE 2 | Vascular complications following liver transplantation in the overall population.

Details on vascular complications	Total population *n* = 708
Vascular complications at any time point	112 (15.8%)
Vascular complications on Day 0, 1, 7	26 (3.7%)
Vascular complications within the first week	52 (7.3%)
Indication for diagnostic CT within first week	*n* = 52
rDUS	21 (40.4%)
Laboratory changes	18 (34.6%)
Abdominal complaints	10 (19.2%)
Fever/infection	3 (5.8%)
Indication for diagnostic CT outside first week	*n* = 60
Laboratory changes	15 (2%)
Abdominal complaints	17 (28.3%)
Fever/infection	14 (23.3%)
Incidental on imaging for other reasons	14 (23.3%)
Duration of hospitalization for patients with complications within the first week (days)	25 (10, 186)
Time to HAT diagnosis (days)	4 (0, 1,382)
Time to PVT diagnosis (days)	47 (0, 2,864)
Time to HVOO diagnosis (days)	21 (0, 1,933)
Vascular complications within 7 days to 3 months	30 (4.2%)
Vascular complications within 3 months to 1 year	10 (1.4%)
Vascular complications outside first year	20 (2.8%)

rDUS, routine Doppler ultrasound; iCT, imaging upon indication; HAT, hepatic artery thrombosis; PVT, portal vein thrombosis; HVOO, hepatic venous outflow obstruction; CT, computer tomography.

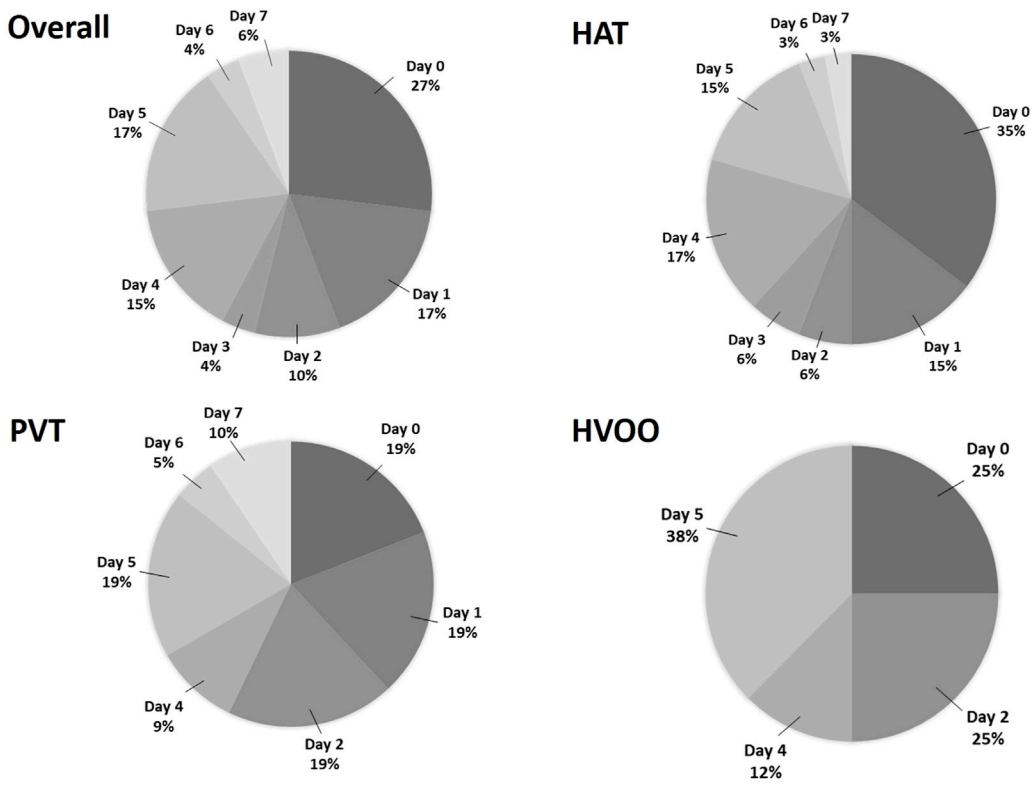


FIGURE 2 | Pie chart showing all (*n* = 52) complications, all HAT (*n* = 34), all PVT (*n* = 21) and all HVOO (*n* = 8), occurring within the first week separated by postoperative day. HAT, hepatic artery thrombosis; PVT, portal vein thrombosis; HVOO, hepatic venous outflow obstruction.

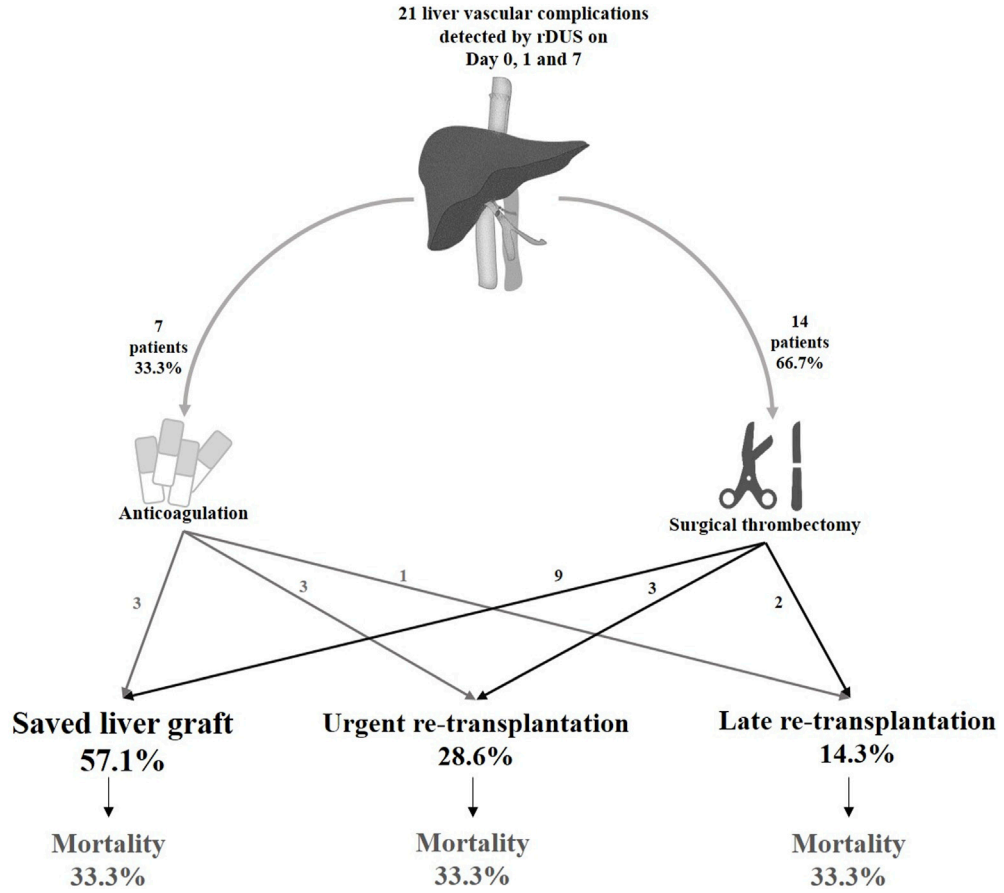


FIGURE 3 | Clinical outcomes of vascular complications diagnosed on days 0, 1 and 7 in the rDUS group. rDUS, routine Doppler ultrasound; iCT, imaging upon indication.

but detected on CT by indication the same day (n = 3 for laboratory changes and n = 2 for abdominal complaints). Therefore, the diagnostic yield of rDUS was 80.7% [i.e., 21 (positive on rDUS and confirmatory CT)/26 (total positive on CT)]. In 18 patients, a vascular complication was suspected on rDUS, but not confirmed on the following CT (i.e., false positive), hence the positive predictive value was 53.8% (21/39). The total number of routine Doppler ultrasounds performed in the 708 patients was 1900 (i.e., median 3 per patient; range 1–3). Therefore, the vascular complication detection rate of rDUS was 21/1900 (1.1%) and the minimal number of DUS needed to detect one vascular complication was 90.5 (1900/21).

Treatment and Outcomes of Vascular Complications Detected by rDUS

The diagnosis of vascular events during rDUS protocol days led to surgical re-intervention (i.e., thrombectomy and a redo of the anastomosis) in 14 patients (66.7%), while 7 (33.3%) were treated by anticoagulant therapy alone (**Figure 3**). This approach preserved the graft in 57.1% patients while 28.6% proceeded to urgent re-LT and 14.3% underwent late re-LT.

Among the 14 patients who underwent surgical intervention, nine (64.3%) had a successful outcome (i.e., preserved graft function), and five (35.7%) underwent re-transplantation (3 HAT, 1 PVT and 1 HAT + PVT). Of these, three patients needed urgent re-LT due to unsuccessful portal thrombectomy (n = 1), or recurrent occlusion of the hepatic artery (n = 2), while two patients required re-LT later due to ischemic biliopathy despite achieving initial arterial recanalization. Among the 7 patients who received anticoagulation alone (n = 4 HAT, n = 1 PVT, n = 1 HAT + PVT and n = 1 HAT + HVOO), three (42.9%) patients with HAT had restored graft function, and other four (57.1%) underwent re-LT. Therefore, the majority of all re-transplantations for vascular complications were due to HAT (n = 5, 55.5%), followed by PVT (n = 2, 22.2%), HVOO + HAT (n = 1, 11.1%) and HAT + PVT (n = 1, 11.1%).

In total, 7 (7/21; 33%) patients died, of whom only 1 because of early graft failure within 2 weeks post LT. All others died within a median time of 7.32 months (0.96–108.8) for causes unrelated to the vascular event, i.e., acute on chronic renal failure (n = 1), uncontrolled sepsis after second LT (n = 2), severe acute pancreatitis (n = 1) and malignancy (n = 2). Thus, in this group of vascular complications detected by rDUS, the 1,

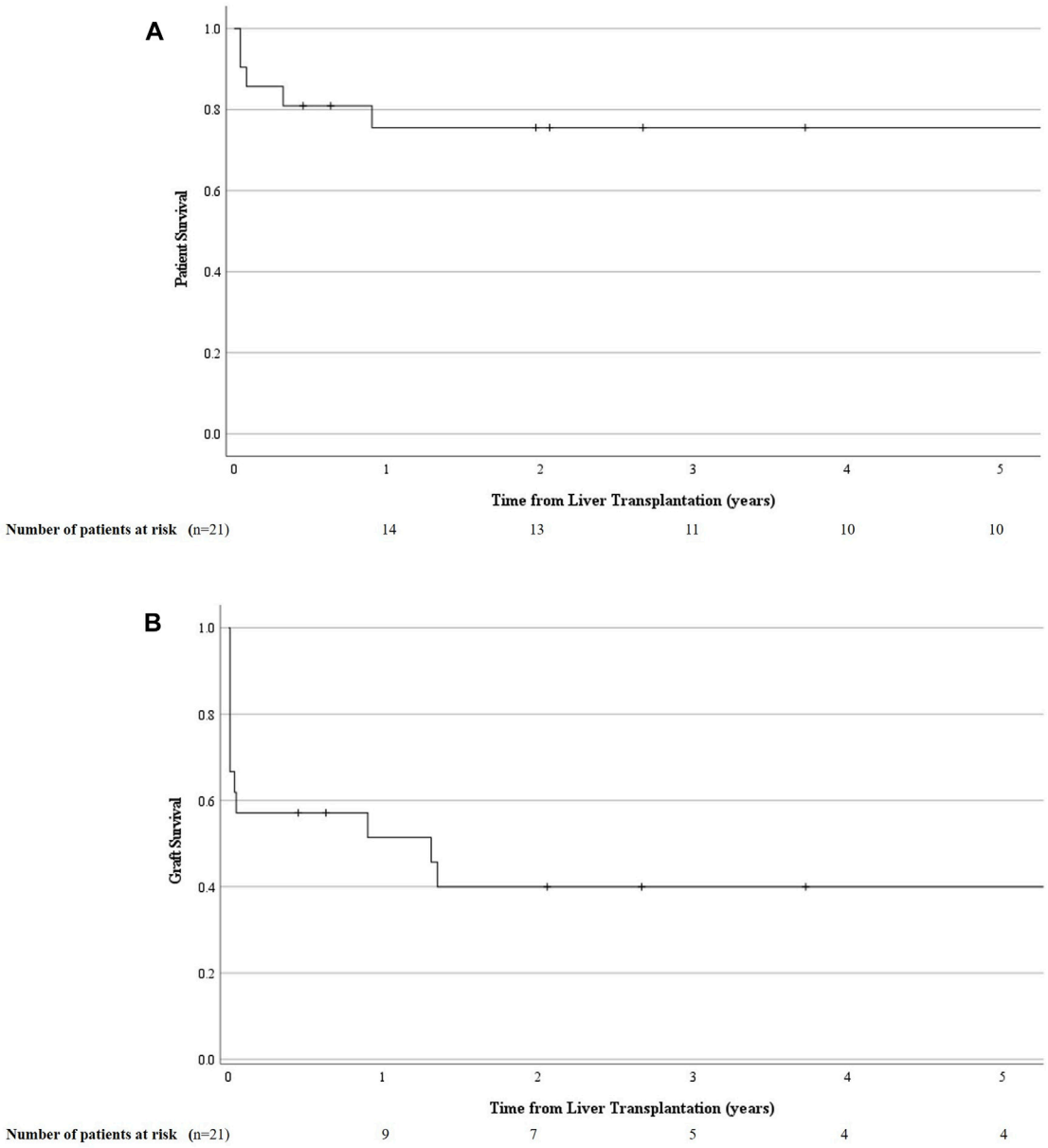


FIGURE 4 | Cumulative patient **(A)** and graft survival **(B)** in those who underwent a primary liver transplantation and developed a liver vascular complication detected by routine Doppler ultrasound (rDUS). rDUS, routine Doppler ultrasound; iCT, imaging upon indication; POD, postoperative day.

5 and 10 years patient survival was 76% (95% CI 66.1–85.1), 76% (95% CI 66.1–85.1) and 57% (95% CI: 42.8–70.6); and graft survival was 51% (95% CI: 40.4–62.4), 40% (95% CI: 29–51) and 30% (95 %CI: 17.9–42.1), respectively (**Figure 4**).

DISCUSSION

The current study shows that rDUS performed on POD 0, 1 and 7 has a detection rate of 80.7% for vascular complications occurring on these days. However, rDUS is associated with a relatively high number of false positive results (PPV 53.8%) and likewise, a high number of ultrasounds needed to detect one complication (90.5). Given the fact that the vast majority of cases occurred within the first 5 days, our rDUS protocol can be further optimised to include POD 0, 1 and 5 instead.

Early detection of post LT vascular complications has always been considered important as these early complications can lead to fulminant graft failure [6]. Re-transplantation, which usually follows, is an undesired event which puts a further strain on the global shortage of organs and is associated with increased recipient morbidity and mortality [7]. Despite CT angiography

being the gold standard for diagnosing HAT and PVT, Doppler ultrasonography is still the most widely used screening imaging technique for assessing the development of hepatic vascular complications in the direct post-LT period because of its non-invasive nature, lack of contrast exposure, accessibility and affordability. In the postoperative period, DUS screening has an important role of detecting liver vascular complications that are still clinically asymptomatic but at the same time may also provide useful information about other, non-vascular complications such as biliary strictures, leakage and fluid collections [8, 9].

Among studies, the highest incidence of early HAT varies from day 1 to day 2 post LT [10, 11], with a median time to detection ranging from 2.5 to 4.9 days [11]. Therefore, increased vigilance is needed during the first week post-LT. Although postoperative DUS is part of the standard screening protocol for vascular complications in many transplant centres, both intraoperatively and postoperatively, the specifics vary considerably [12, 13]. The reported frequency and interval of rDUS range from close monitoring—twice daily for the first week and once daily for the next 7 days [11], to every 3 days for 2 weeks [10], to once on day 1 and once on day 5 [14]—to even continuous monitoring using an implantable DUS for 10 days, with six check-ups per day [8]. In our study population, we showed that most of vascular complications occur during the first week and we have identified the highest number of early post-LT vascular complications at day 0 and 1, followed by day 4 and 5 (totalling up to 90% of the first week complications). Based on the hypothesis that earlier intervention for a vascular complication may benefit graft survival and patient outcome, we considered that rDUS would be better applied at day 0, 1 and 5 instead of 7. This protocol change has now been implemented in our centre based on the current study.

To put some perspective to our findings, we applied the concept of number needed to treat (NNT) to the diagnostic setting. To identify one vascular complication, we needed 91 DUS examinations. Also, the positive predictive value of the DUS was rather disappointing, as almost half of the cases were found to be false positive at the confirmatory CT. Two other studies identified a much higher PPV of 92.3% and 88.9% for rDUS performed daily for either the first 7 days [12] or for the first 2 weeks, respectively [15]. However, it is important to note that we had a low threshold for performing a confirmatory CT. Indeed, we performed a CT not only to confirm an US diagnosis of a clear occlusion or thrombosis but also in situations in which there was any doubt, i.e., when not all vessel patency criteria were met (defined in Methods). This practice may explain our lower positive predictive value. Also, 2 out of 3 days from our rDUS protocol are immediately post-operative and this timeframe is characterized by increased hemodynamic changes due to low cardiac output, arterial spasm and parenchymal oedema [15]. This may hamper DUS visualization of the arterial flow, especially considering the increased portal flow early after LT. As we did not do routine CT in those labelled as negative on DUS, we could not calculate the negative predictive value nor give estimates of sensitivity or specificity. Performing a cost-effectiveness analysis was beyond the scope of our study, however, given the profound impact of re-LT on the patient, on healthcare costs and on the donor organ capacity in general, one could argue that the cost of a relatively cheap DUS examination, despite the high numbers thereof, would still be worthwhile. Ideally, in order to have a definite answer to the diagnostic yield of rDUS compared to performing imaging upon clinical indication only, a randomized trial is needed. Given the now widespread implementation of routine imaging in most transplant centres, and the possibility of missing an important diagnosis, such a trial would be a complicated, and perhaps even unethical, undertaking.

Across time, we identified variations, albeit not significant, in the incidence of vascular complications during the first week. There may be several explanations for this variation. For one, this may be a reflection of variations in the use of more extended criteria grafts including DCD grafts (indeed our centre uses 36.2% DCD's) or increasing acceptance of candidates with pre-LT PVT or other vascular complexities, which may change the risk of early vascular complications. However, it may also be that the implementation of an rDUS protocol contributed to an increased number of detected complications, like we observed in the early years. Most of these patients were clinically asymptomatic at the time of DUS. It may therefore well be that if we went by clinical judgment alone, these may not have been diagnosed at that time, but perhaps (much) later. This is especially true for days 0 and 1, during which time it is very challenging to know when early signs such as abdominal discomfort or laboratory changes are worrisome and when they are just part of the normal postoperative course. Despite the risk of false positive results during those early days, it seems reasonable to suggest that some form of diagnostic monitoring would still be desirable.

One of the main strengths of our study was that we had a homogeneous single centre cohort in which patients were treated according to predefined protocols and no one was lost to follow-up. Another strength was that we not only examined the diagnostic ability of DUS to detect vascular complications (HAT, PVT and HVOO) but also included data on the clinical consequences of their detection. One of the main limitations of our study is, however, that the true performance of the rDUS protocol could not be determined. This is due to the retrospective nature of the study and the fact that DUS is a standalone imaging modality not routinely requiring the confirmation in case of a normal result. Therefore, we could not calculate the true negative and false negative results.

In conclusion, the majority of post-LT vascular complications occur during the first week. rDUS detects the majority of events during protocol days, but with low PPV and a high number of ultrasounds needed. However, a true clinical benefit in terms of graft and patient's survival has yet to be shown.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Medical Ethical Committee of the Erasmus MC Rotterdam. The patients provided written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conceptualization: SDM and IM; Collection of data: IM and SDM; performing Doppler ultrasounds: SDM, CdH, AvdM, MS, DS, RdK, and RM; statistical analysis: IM, SDM, AvdM, and MS; writing—original draft preparation: IM and SDM; writing–review and editing: SDM, IM, CdH, AvdM, MS, DS, RdK, JdJ, RM, and WP; supervision: SDM. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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LETTER TO THE EDITOR
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Women Referred for Liver Transplant Are Less Likely to Be Transplanted Irrespective of Socioeconomic Status

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Keywords: disparities, liver transplant, social vulnerability index, sex disparities, public policy

Dear Editors,

Liver transplantation is the standard of care for end-stage liver disease (ESLD) and transplant oncology patients. Given the organ shortage, equitable organ distribution is key. Recent studies have repeatedly reported that, in the US, waitlisted patients of female sex are less likely to be transplanted and more likely to die awaiting a liver transplant [1, 2]. This has been largely attributed to an imperfect model for end-stage liver disease (MELD) scoring systems and donor-recipient size mismatch [1, 3, 4].

After obtaining institutional board review exemption (IRB 275415), we explored socioeconomic and sex-related disparities of patients referred for liver transplant at Arkansas' single liver transplant institution. The Centers for Disease Control and Prevention (CDC)/Agency for Toxic Substances and Disease Registry (ATCSDR) Social Vulnerability Index (SVI) was employed as surrogate indicator of socioeconomic status [5]. Social vulnerability refers to the resilience of a population when confronted by a health stressor, be it a disease outbreak or a natural or human-caused disaster. CDC/ATSDR SVI database "*can help communities prepare for and recover from public health emergencies, and prevent adverse effects among socially vulnerable populations, such as emotional distress, loss of property, illness, and death*" [5]. The SVI calculation encompasses parameters reflecting a community's *socioeconomic* (e.g., poverty, unemployment, *per capita* income, education, and health insurance), *population* (e.g., children or elderly, disability, single parent, minority, limited English), and *housing/transportation* (e.g., mobile homes, crowding, no vehicle, living in group quarters) *vulnerability*. Data was sourced from the Arkansas Clinical Data Repository.

Patients with less than 1 year follow-up or missing data were excluded. SVI scores were assigned by patient's ZIP code, which reflects the patient's location of residence. The patients were split into SVI quartiles, based on SVI median and interquartile range. Logistic regression was performed for enlisting, adjusted for SVI quartile, age, sex, body mass index, and insurance payor. A Fine-Gray survival model was built, with liver transplant as the primary outcome and death a competing event controlled for sex, SVI quartile, and insurance. Analyses were conducted using R software (4.1.0) and STATA version (17.0).

Study period was from 1st January 2019 to 31st December 2022. The study population included $N = 779$ patients who had been referred to our center during that time for liver transplant evaluation.

Abbreviations: ATCDR, agency for toxic substances and disease registry; CDC, centers for disease control and prevention; ESLD, end-stage liver disease; MELD, model for end-stage liver disease; NASH, non-alcoholic steatohepatitis; SVI, social vulnerability index.

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TABLE 1 | Multivariate analysis of liver transplant outcome.

	Odds ratios (OR)	95% CI	*p*
Male Sex	**2.73**	**1.70–4.52**	**<0.001**
Private Insurance payor	**2.2**	**1.35–3.70**	**0.002**
SVI quartile			
(Intercept)	0.16	0.03–0.78	0.025
2	0.56	0.27–1.12	0.108
3	1.09	0.63–1.92	0.756
4	1.09	0.60–1.99	0.769
Age	0.98	0.96–1.00	0.061

Bold value indicates the male sex and private insurance independently favored liver transplant (odds ratio [OR] 2.73; 95% CI, 1.70–4.52, and 2.2; 95% CI, 1.35–3.70, respectively).

TABLE 2 | Fine gray competing risk survival analysis of patients referred for liver transplant.

	OR	95% CI	*p*
Medicare/Medicaid	**0.48**	**0.30–0.76**	**0.002**
Male Sex	**2.38**	**1.53–3.70**	**<0.001**
SVI quartile			
2 (0.53–0.75)	0.59	0.30–1.13	0.112
3 (0.76–0.81)	1.04	0.64–1.71	0.864
4 (≥0.81)	1.00	0.59–1.69	0.994

Bold value indicates the male sex favored liver transplantation (OR 2.38; 95% CI, 1.53–3.70). Medicare/Medicaid insurance payor decreased the odds getting a liver transplant (OR 0.48; 95% CI, 0.30–0.76).

43.2% ($N = 336$) of these patients were female. Logistic regression analysis indicated that, irrespective of SVI quartile, male sex and private insurance were independent predictors favoring liver transplantation (odds ratio [OR] 2.73; 95% CI, 1.70–4.52, and 2.2; 95% CI, 1.35–3.70, respectively; **Table 1**). Likewise, on Fine-Gray analysis adjusted for SVI quartile, male sex and Medicare/Medicaid insurance payor were independent risk factors (OR 2.38; 95% CI, 1.53–3.70, and 0.48; 95% CI, 0.30–0.76, respectively) (**Table 2**). *Waitlisted* male patients with private insurance were more likely to get transplanted and survive after a liver transplant. What is more, male sex patients *referred* for liver transplant were found more likely to be *evaluated* (OR 1.76, $p < 0.001$), *enlisted* (OR 2.07, $p < 0.001$) and *transplanted* (OR 2.55, $p < 0.001$) compared to their female counterparts (**Supplementary Data**).

In conclusion, our study indicates that, in the population and period studied, there are sex related barriers in the liver transplant process. These obstacles may prevent female sex patients from entering and completing liver transplant evaluation. This gap may be ascribed to *functional status assessment* barriers [2], e.g., higher perceived frailty among females, particularly elderly; *clinical*, e.g., higher female prevalence of nonalcoholic steatohepatitis (NASH), with NASH known to be associated with higher surgical risk; *social* [1, 2], e.g., work or family obligations preventing completion of the evaluation process; the *stigma* of alcohol excess [1, 2]; or *geographic*, i.e., within minority groups residing in remote locations. Beyond introducing remedies such as scoring system upgrades accounting for patient's sex [1, 2], it is also necessary to address sex-based barriers presenting early on in the liver transplant referral and evaluation process [2]. A good start may be the 1) creation of national or regional liver disease/ESLD registries in order to achieve better data granularity; 2) introduction of transplant referral and evaluation efficiency metrics (e.g., time from referral to decision over enlisting) [2]; 3) implementation of objective frailty testing methods [2]; and 4) provisions for a more flexible evaluation process, tailored to individual socioeconomic, geographic, and cultural needs.

Limitations of this pilot study were its limited sample, retrospective nature, and the inclusion of liver transplant referrals to a single US transplant institution.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving humans were approved by UAMS Institutional Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

Conceptualization, EG and MR; methodology, EG and AW; software, EG and AW; formal analysis, AW; data curation, EG and AW; writing-original draft preparation, EG; writing-review and editing, EG, ME, LB, RP, MG, MD, GB, and MR; visualization, EG; supervision, EG. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11667/full#supplementary-material

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