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Kidney procurement: Time is of the essence

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Editorial Office
Rebecca Smith
tieditorialoffice@frontierspartnerships.org

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Kidney procurement: Time is of the essence

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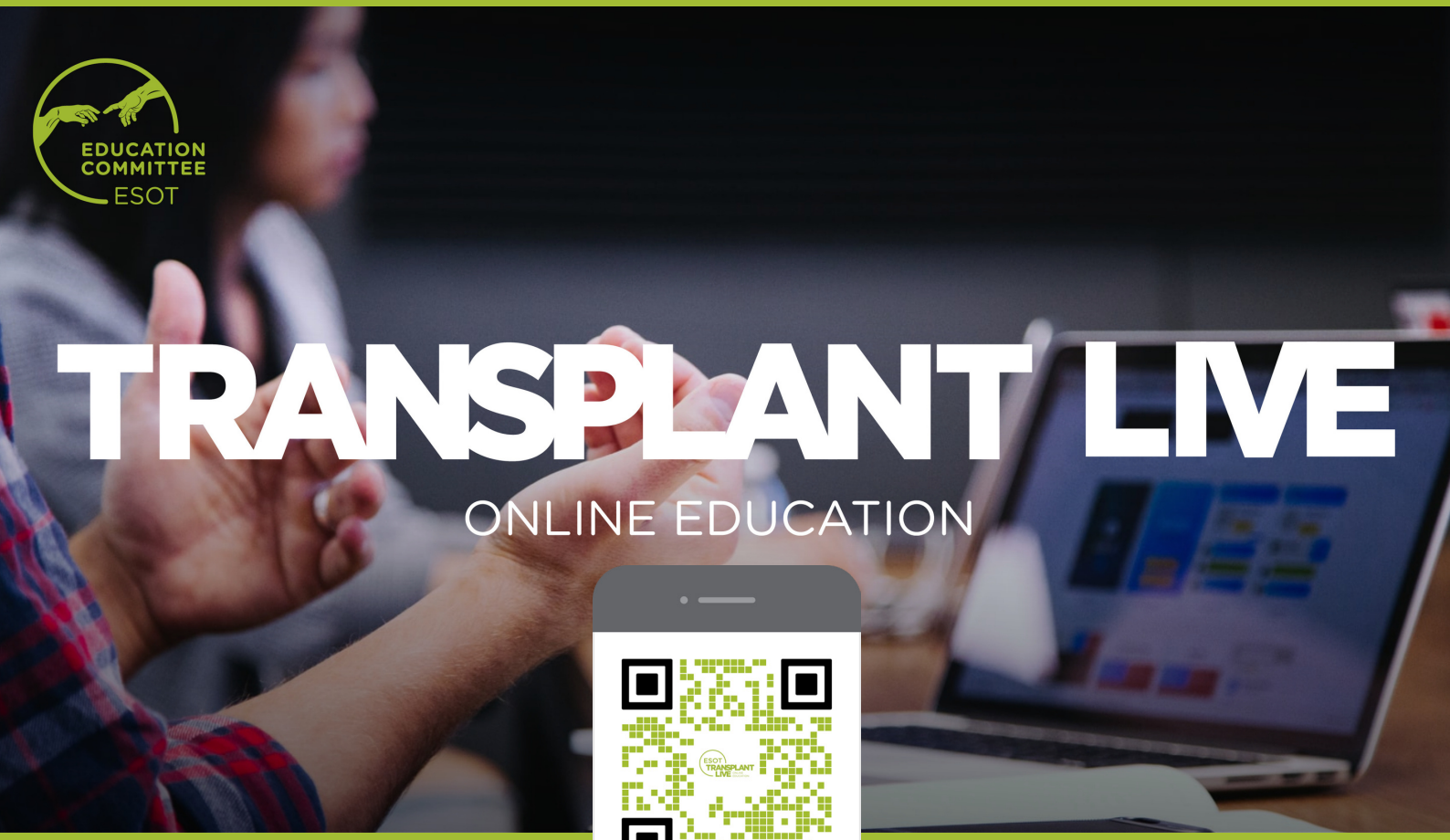

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TRANSPLANT TRIAL WATCH
published: 15 March 2022
doi: 10.3389/ti.2022.10307

Transplant Trial Watch

Simon R Knight[1,2]*

[1]Oxford Transplant Centre, Churchill Hospital, Oxford, United Kingdom, [2]Centre for Evidence in Transplantation, Nuffield Department of Surgical Sciences, University of Oxford, Oxford, United Kingdom

Keywords: liver transplantation, immunosuppression, lung transplantation, induction, pediatric patients

Randomised Controlled Trial 1

Early Everolimus-Facilitated Reduced Tacrolimus in Liver Transplantation: Results from the Randomized HEPHAISTOS Trial
by Nashan, B., et al. Liver Transplantation [record in progress].

Randomised Controlled Trial 2

CTOTC-08: A Multicenter Randomized Controlled Trial of Rituximab Induction to Reduce Antibody Development and Improve Outcomes in Pediatric Lung Transplant Recipients
by Sweet, S. C., et al. American Journal of Transplantation [record in progress].

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.

Transplant Trial Watch

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***Correspondence:**
Simon R Knight
simon.knight@nds.ox.ac.uk

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RANDOMISED CONTROLLED TRIAL 1

Early everolimus-facilitated reduced tacrolimus in liver transplantation: Results from the randomized HEPHAISTOS trial

by Nashan, B., et al. Liver Transplantation [record in progress].

Aims

This study aimed to investigate the outcomes related to early initiation of everolimus-facilitated reduced-exposure tacrolimus (EVR + rTAC) in *de novo* liver transplant patients.

Interventions

Participants were randomised to either the group that received EVR + rTAC or the group receiving standard-exposure tacrolimus (sTAC) with steroids.

Participants
333 *de novo* liver transplant recipients.

Outcomes
The primary outcome was renal function. The secondary outcomes included death, graft loss, acute rejection (AR), treated AR or treated biopsy-proven acute rejection (tBPAR), assessed as composite or individual components at 12 months posttransplant.

Follow-up
12 months.

CET Conclusion
The HEPHAISTOS superiority trial compared everolimus plus reduced exposure tacrolimus versus everolimus with standard exposure tacrolimus in *de novo* liver transplant recipients. The multicentre, German study randomised recipients 7–21 days posttransplant using a validated system that automates random assignment. The power analysis indicated that 105 patients in each group were needed, which was adjusted to 165 patients per group to allow for dropouts. The study randomised 333 patients and the primary full-analysis set, which included all randomised patients who received at least one dose of the study drug, found no statistically significant difference in eGFR at 12 months between groups. A statistically significant difference between groups in eGFR was found for the per-protocol and on-treatment analyses. The composite efficacy-endpoint of graft loss, death or treated BPAR was similar between groups. Treatment-emergent (serious) adverse events were similar between groups but there were more adverse events leading to study drug interruption or adjustment in the reduced exposure tacrolimus group.

Jadad Score
3.

Data Analysis
Intention-to-treat analysis.

Allocation Concealment
Yes.

Trial Registration
ClinicalTrials.gov, NCT01551212; EudraCT, 2011-003118-17.

Funding Source
Industry funded.

RANDOMISED CONTROLLED TRIAL 2

CTOTC-08: A Multicenter Randomized Controlled Trial of Rituximab Induction to Reduce Antibody Development and Improve Outcomes in Pediatric Lung Transplant Recipients

by Sweet, S. C., et al. American Journal of Transplantation [record in progress].

Aims
The aim of this study was to investigate whether rituximab in addition to rabbit anti-thymocyte globulin induction was effective in reducing the development of *de novo* donor-specific human leukocyte antigen antibodies (DSA) and improve outcomes, in paediatric lung transplant recipients.

Interventions
Participants were randomly assigned to either the rituximab group or the placebo group.

Participants
27 paediatric lung transplant patients.

Outcomes
The primary outcome was a composite of chronic allograft dysfunction, listing for re-transplant or death. The secondary outcomes were the incidence of primary graft dysfunction, antibody-mediated rejection and acute cellular rejection.

Follow-up
24 months.

CET Conclusions
This is a good quality randomised controlled trial in paediatric lung transplantation. The study was double-blinded and conducted in multiple centres. Patients were randomised to either standard immune induction with ATG (plus placebo) or to ATG and Rituximab. The primary outcome was composite graft dysfunction, death or re-listing. Unfortunately, only 11 subjects met criteria for the composite primary outcome, so the study was underpowered to demonstrate all but the most drastic of differences between the study arms. Whilst there was no significant difference in the primary outcome, there was a significantly lower generation of *de novo* DSA in the Rituximab arm (21% vs. 73%). There was no significant difference in adverse event rates. A much larger study, and with longer follow up, is required.

Jadad Score
5.

Data Analysis
Intention-to-treat analysis.

Allocation Concealment
Yes

Trial Registration
ClinicalTrials.gov, NCT02266888.

Funding Source
Non-Industry funded.

CLINICAL IMPACT SUMMARY

Most current induction immunosuppression strategies focus on T-cell inactivation or depletion. B-cell activation and donor-specific antibody production also play an important role in allograft damage, which has led to interest in the use of B-cell depleting therapies such as rituximab as induction agents following solid organ transplantation.

In a recent publication in the American Journal of Transplantation, Sweet et al. report a multicentre randomised-controlled trial using rituximab as induction therapy in paediatric lung transplant recipients (1). The study is well designed, with double blinding and allocation concealment ensured by use of placebo and centralised web-based randomisation. Unfortunately, the study failed to recruit the required target sample within the funding time-frame, resulting in a loss of power and shorter follow-up than initially planned. Perhaps as a result, no difference in the primary clinical endpoint [a composite of death, bronchiolitis obliterans syndrome (BOS) and relisting] was seen. However, there was a significantly lower incidence of *de novo* donor specific antibodies (DSA) in the rituximab-treated group, leading the authors to cautiously claim some evidence of benefit.

Whilst it is difficult to draw firm conclusions from an underpowered study, the suggestion of benefit seen in this study is at odds with previous studies in renal and cardiac transplantation. A systematic review of studies in renal transplantation from our own group in 2014 found no clear evidence of benefit to rituximab induction across a small number of studies (2). The authors of the current study postulate that this may be due to a lack of T-cell depleting induction in these earlier studies. Rituximab also depletes regulatory B-cells, and this loss of regulation in the presence of donor-reactive T-cells may increase the risk of T-cell mediated rejection. Combination of B- and T-cell depletion is proposed to overcome this.

One specific area of concern, perhaps not apparent in the current paediatric study, is the impact of rituximab therapy on the risk of cardiovascular disease. Previous studies in both renal transplantation and cardiac transplantation have suggested increased risk of cardiovascular mortality and graft vessel disease, possibly related to the role of B-regulatory cells in atheroprotection (3, 4). Any future studies, especially in adult populations, would need to collect these outcomes and ensure long-enough follow-up to adequately assess the impact on cardiac disease.

Overall, the study does provide some interesting data suggestive of a potential role of B-cell depletion in conjunction with T-cell depleting induction in the reduction of DSA formation and subsequent chronic allograft damage. Further, well-powered studies in adult populations will need to focus on the long-term safety of such a strategy.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

CONFLICT OF INTEREST

The author declares 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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Insights From Transplant Professionals on the Use of Social Media: Implications and Responsibilities

Shaifali Sandal[1], Arvinder Soin[2], Frank J. M. F. Dor[3,4], Elmi Muller[5], Ala Ali[6], Allison Tong[7], Albert Chan[8], Dorry L. Segev[9] and Macey Levan[9]*

[1]Division of Nephrology, Department of Medicine, McGill University Health Centre, Montreal, QC, Canada, [2]Institute of Liver Transplantation and Regenerative Medicine, Medanta-The Medicity, Gurugram, India, [3]Imperial College Renal and Transplant Centre, Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, United Kingdom, [4]Department of Surgery and Cancer, Imperial College, London, United Kingdom, [5]Division of General Surgery, Department of Surgery, University of Cape Town, Cape Town, South Africa, [6]Nephrology and Renal Transplantation Centre, The Medical City, Baghdad, Iraq, [7]Sydney School of Public Health, University of Sydney, Sydney, NSW, Australia, [8]Division of Hepatobiliary and Pancreatic Surgery and Liver Transplantation, Queen Mary Hospital, The University of Hong Kong, Hong Kong, China, [9]Department of Surgery, Division of Transplantation, Johns Hopkins University School of Medicine, Baltimore, MD, United States

Keywords: social media, transplantation, transplant professionals, advocacy, education

INTRODUCTION

Social media (SoMe) is now widely accepted and used in medicine and considered a measure of scholarly output to support academic advancement at some institutions (1–4). In the field of transplantation, SoMe platforms, such as Twitter and Facebook, have been leveraged to promote both living and deceased donation, and for public engagement and outreach (5–11). These platforms are an important source of information on transplantation in many low and middle-income countries (12). Thus, academic institutions, and transplant organizations and journals are increasingly using SoMe to increase their visibility and footprint in the public domain and engage with the transplant community.

Despite this, there is still some hesitation among the transplant community to engage in SoMe. The majority of transplant surgeons in the United States perceive SoMe to be influential in increasing awareness about deceased organ donation and increasing living donation, yet 39% reported no SoMe outreach by their center (7). In Europe, while many transplant professionals reported using SoMe for work-related information, the majority do not engage in transplant-related campaigns (13). Thus, we reached out to eight leaders in transplantation who are known in their respective regions for their SoMe engagement or outreach or their work in the field of SoMe in transplantation. We specifically asked questions related to the opportunities and challenges of SoMe use related to their expertise which are presented below; some of the more general comments are concisely summarized in **Table 1**. For the purposes of this paper we focused on two platforms; one with the widest global reach (Facebook) and one most commonly used to disseminate knowledge (Twitter).

The Prolific and Enthusiastic Users

Dr. Arvinder Soin, a Transplant Surgeon from India, is considered to be a key medical influencer in various public health domains (most notably organ donation), and a leading healthcare figure on Twitter with over 97,000 followers. *Dr. Frank Dor*, a Transplant Surgeon from the United Kingdom is

Abbreviations: SoMe, social media.

News & Views

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Correspondence:
Shaifali Sandal
shaifali.sandal@mcgill.ca

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TABLE 1 | Suggestions on social media (SoMe) use by transplant professionals.

For transplant professionals

Always
- Be professional
- Protect patient confidentiality
- Separate SoMe platforms into personal and professional use
- Carefully review the privacy settings of SoMe platforms to customize the content
- Share content from reputable sources only
- Be upfront about any conflicts of interest
- Be vigilant of transplant tourism and commercialization
- Abide by national laws and institutional policies

Consider
- Training in how to use SoMe platforms
- Sharing content with positive and genuine messages on organ donation and transplantation
- Tackling misinformation about transplantation
- Only sharing politically neutral content
- Engaging with patients but only with an appropriate oversight or ethical approval

Avoid
- Engaging in inflammatory content
- Using SoMe as a medium for personal attacks
- Sharing confidential and sensitive information that infringes on the intellectual property rights of others

For transplant societies and organizations
- Assist and empower transplant professionals in
  - o How to use SoMe to promote the interest of transplantation
  - o Maintain a SoMe presence
  - o Engage with the transplant community including patients and donors
- Explore the short- and long-term repercussions of the digital footprint of transplant professionals
- Explore avenues to assist professionals in tackling cyberbullying and harassment

the current social media ambassador for the European Society of Organ Transplantation and set up the social media editorship of the Transplantation journal.

SS: Please share your overall thoughts on SoMe use by transplant professionals and the opportunities and responsibilities.

AS: SoMe platforms are excellent channels of communication that "democratize" the sharing of research, insights, and innovation; a process that has traditionally been confined to publications and conferences. Hashtags, such as #LiverTwitter, can inform and engage, and help glean breakthroughs. While SoMe engagement is a personal choice, lack of engagement by professionals leads to missed opportunities in expanding the field.

FD: SoMe is an underutilised instrument in professional communication, especially in the modern era of proper patient engagement. I am an enthusiastic SoMe user but establish boundaries across these platforms into personal and professional use. Professionals should engage in SoMe for patient/general public education, raising awareness about organ donation and transplantation, sharing scientific publications, promoting educational opportunities, such as courses and congresses, and influencing societal discussions (13).

SS: Dr. Soin, as a transplant figure with quite possibly the highest Twitter following of anyone in the field, what type of content do you create/share and any personal anecdotes you would like to mention?

AS: After establishing credibility in the field, I found it easier to expand my repertoire and engage on many medical issues, such as to keep the Indian public abreast on COVID-19. Also, I regularly share content with positive and genuine messages on organ donation and other aspects of transplantation. I make every attempt to be factually accurate and balanced when doing this, as the public accepts my transplantation-related content at face value. Also, I recommend avoiding taking a paternalistic stand to assert the superiority of one's expertise; this may be counter-productive, or, at best, give only a short-term yield.

SS: Dr. Dor, as a SoMe ambassador of a leading organization what would you advise those who might be nervous about using SoMe?

FD: I believe that it is our professional duty to advocate for the best options for our patients and SoMe provides us with platforms to do so. However, I advocate for training, as the use of these platforms requires knowledge about how they work and what their potential pitfalls are. SoMe is a powerful tool, but as with all powerful tools, one needs to know how to use them safely and correctly to fit the purpose.

The Prolific but Cautious Users

Dr. Elmi Muller is a prolific figure and Transplant Surgeon from South Africa whose work and profile has been featured in prominent scientific journals. She is involved in many organ transplant-related outreach and education programmes for the public. *Dr. Ala Ali*, a Transplant Nephrologist from Iraq, is an engaged professional and emerging transplant leader from the Middle East.

SS: Based on the comments made above, can you highlight some of the risks of SoMe use by transplant professionals?

EM: While I agree with what has been said, I recommend using SoMe cautiously as the message of a tweet or a story on Facebook, can fragmentize over time, be taken out of context, and create an ever-lasting digital footprint that can impact one's personal and professional life years down the lane. There are many examples of individuals who applied for jobs and their SoMe profiles were scrutinized. In addition, there can be legal ramifications of sharing political views, making political statements, and violating patient confidentiality.

AA: We should consider shouldering responsibilities to advance the field of transplantation ethically and righteously and to help our patients as suggested by Drs. Dor and Soin. SoMe is being used to spread misinformation and as potential channels for transplant tourism and commercialization (14). For professionals there are several opportunities to deliver high-quality information to counter some of this misinformation but professional, ethical, and legal complexities exist in the developing world; the pandemic exaggerated these complexities.

SS: How do you recommend we address these emerging issues?

EM: Professionals need more training and guidance on how to use SoMe to our benefit and how to safely use it. There are opportunities for professional development, but how much it helps is not known. For example, it is very easy to re-tweet a paper and comment on its findings, but the repercussions of this in the short- and long-term need more exploration.

AA: I absolutely agree; we need more guidance that is tailored to the local context of practice and policy. While avoiding direct communication with patients, I recommend engaging in a

medium, such as a patient's education group, that permits professional responses only.

The Transplant Researchers

Dr. Allison Tong is a Transplant Researcher from Australia and *Dr. Albert Chan* is a Transplant Surgeon from Hong Kong. Both are known for using SoMe for promoting their research endeavors and are well cited for their research contributions. Dr. Tong has over 24,000 citations of her scientific work with an h-index of 60 and Dr. Chan has over 4,500 citations of his scientific work with an h-index of 36.

SS: Can you share your thoughts on the use of SoMe for research in transplantation?

AT: SoMe is an important platform for sharing scientific information with opportunities for collaborations, exchanging opinions, and gaining different insights and perspectives. The impact on research findings has been demonstrated; a tweeted article was three times more likely to be downloaded compared with those that were not tweeted (2). SoMe is a great platform for patient engagement, especially for research conducted under the oversight of Institutional Review Boards.

AC: I agree. Given the transformation in information technology, SoMe use is inevitable and it can play some part in the promotion of transplantation and finding living donors. SoMe is useful in knowledge dissemination of novel surgical techniques, and an effective way to allow knowledge exchange and communication among professionals, or between professionals and the community.

SS: What are some of the risks that you have experienced?

AT: There can be a risk of oversimplifying or sensationalizing findings of a study or the "science," which is open to public scrutiny. There is a need to ensure that one is making informed commentary and I caution against sharing confidential and sensitive information that infringes the intellectual property rights of others.

AC: I would add that there is a lack of verification mechanism, which substantially increases the risk of misinformation. Knowledge dissemination should be substantiated by publications in peer-reviewed journals with the link to citations in the postings or next to a "hashtag. " Also, I recommend SoMe engagement to be politically neutral and restricted to purely scientific comments based on factual findings.

The Political Advocates

Dr. Dorry Segev is a Transplant Surgeon from the United States who is a prominent transplant figure, researcher and globally known for his expertise in transplantation. His research has informed congressional bills and the HIV Organ Policy Equity Act that was signed into law. *Dr. Macey Levan* is a lawyer and living kidney donor recognized for her advocacy and ethics in living donation. Both have published extensively on SoMe use in transplantation.

SS: As someone who has been quite vocal on SoMe platforms, can you share your positive and negative experiences?

DS: SoMe engagement is rewarding professionally and personally that carries a risk of public scrutiny and occasional criticism and negative comments. Despite this, we should not silence professionals as these platforms can create dialogue and movements that can positively impact medicine and transplantation. I strongly recommend that professionals advocate for causes relevant to transplantation, including political conversations as it has significantly, sometimes negatively, intersected with the care of our patients, such as masking during the pandemic. There are opportunities to tackle misinformation and perpetuate new research and science.

ML: I find that SoMe can be seen as both disruptive and opportunistic as it allows quick and active communication but can also create conversations that can be superficial and passive. The American public craves information and access to it through social media channels, but attention spans are very short, with adults typically being able to pay attention to one task for 8 s.

SS: Dr. Henderson-Levan, as an ethicist can you provide any unique comments for SoMe use by transplant professionals?

ML: Organs from deceased donors are considered to be a national resource, and we should encourage transplant professionals to elevate organ donation and transplantation as part of an elevated public health conversation. We need to meet people where they are as we are in a public field that relies on the public to make it work, so we need to communicate with people in a quick and impactful way. Social media channels are great examples of ways to do this.

SS: Dr. Segev, any quick shot way to stay out of trouble when engaging on SoMe?

DS: First, set boundaries on SoMe platforms use into personal and professional. Second, pause before posting using the "front page of the New York times" litmus test. If the content and the message were to appear on the front page of any important newspaper, it must be acceptable to one's personal and professional image.

Implications of SoMe Use by Transplant Professionals for Patients

SS: With respect to SoMe use by transplant professionals, please share your thoughts on the implications to patients?

AS: SoMe conversations have the potential to change or garner public opinions that can perpetuate or debunk myths and fuel mass movements. I have had excellent engagement with sharing uplifting patient stories via SoMe (with informed consent of course), thus opening up avenues to apprise the masses about the benefits of organ donation and transplantation. I believe this has helped many of my patients and acknowledged the contributions of living donors.

FD: It is important that professionals engage in SoMe for patient/general public education and raising awareness about organ donation and transplantation. SoMe can reach a lot of people that professionals can't reach normally in such magnitude. It is important to create and enlarge networks to increase the impact on our field. With proper training and guidance, I strongly believe our patients will benefit from transplant professional engagement.

EM: Transplant professionals are always exposed and always expected to maintain the highest level of ethics and professionalism. Comments can be taken out of context, and

there may be legal ramifications. There are risks of violating patient confidentiality. I recommend transplant leadership considers exploring the risks and benefits to patients, donors and the public. This will better help assess how to engage in SoMe that benefit our patients and how to minimize the associated risks.

AA: SoMe provides opportunities for transplant professionals to deliver high-quality information to patients. I would consider engaging in a medium, such as a patient's education group, that permits professional responses only. One should consider shouldering responsibilities to advance the field of transplantation in an ethical and righteous manner, and this can be of direct and indirect benefits to patients. In addition, the Declaration of Istanbul may consider updating its preamble surrounding SoMe use by professionals.

AT: SoMe can be useful for connecting with and engaging patients or other stakeholders in research; research benefits patients and improves their experiences, outcomes and health. However, there are risks. For recruitment, one should always go through approval by an Institutional Review Board to ensure messages are appropriate (i.e., not coercive). As long as we refrain from giving personal medical advice, promoting treatments, asking for personal medical history/information and similar unethical practices, I do think our patients will benefit from SoMe engagement by transplant researchers.

AC: SoMe is helpful to patients and to increasing organ donation. Maintaining patient confidentiality is the main risk to patients.

DS: I recommend setting boundaries on SoMe usage into personal and professional use. My patients follow me on Twitter but I never engage with them directly and instead will have a private conversation with them. I share my updated research outputs, ongoing studies and other exciting news. I have had a tremendous response to my work on COVID-19 vaccination to transplant patients. This has been of direct benefit to patients, especially in the midst of a pandemic.

ML: It is common now to use SoMe as a tool for research participation. Meeting these participants where they are, provides us insight into patient-oriented questions, and helps them feel the benefit and purpose from their participation. However, it is important to be mindful of what patients perceive from your SoMe persona while still ensuring you are able to hold your own professional and personal boundaries. When communicating with patients, it also reflects on associated institutions. In the Twitter era, if a patient does not like an interaction in a care facility, they can share these opinions both with the institution and with the public.

CONCLUSION

Our collective expertise suggests that a lack of SoMe engagement leads to several missed opportunities in advancing the interests of our patients, our field, and our careers. We believe transplant professionals should consider maintaining a SoMe presence, engaging with the transplant community, and debating hot topics while seeking guidance on how to do so safely and effectively. We have summarized our collective suggestions in **Table 1**.

AUTHOR CONTRIBUTIONS

SS: Conceived and designed the work; collected and summarized the expert insights; drafted the manuscript; approved the final version AS, FD, AT, AC, AA, EM, and DS: Provided their insight; critically revised the manuscript; approved the final version ML: Helped design the work; provided her insight; critically revised the manuscript, approved the final version.

CONFLICT OF INTEREST

ML is the Social Media Editor for Transplantation. FD was the previous social media editor for Transplantation, and currently leading social media ambassador programme for the European Society for Organ Transplantation.

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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ABO-Incompatibility: Time to Challenge the Paradigm of Equivalence in Live-Donor Kidney Transplantation?

Farsad Eskandary and Georg A. Böhmig*

Department of Nephrology and Dialysis, Division of Medicine III, Medical University of Vienna, Vienna, Austria

Keywords: ABO-incompatible kidney transplantation, living donation, graft survival, induction, propensity score

Over the past decades, ABO blood group-incompatible live-donor kidney transplantation (ABOi-LDKT) has evolved significantly. Initial reports of—sometimes even inadvertent—crossing of non-permissive ABO blood group barriers have led to a broad use of such transplants as part of the clinical routine on numerous transplant units (1). In 2001, antigen-specific immunoadsorption, a highly efficient method for selective anti-AB antibody depletion was introduced by Tydén et al. (2) and this has led to the set-up of successful ABOi transplant programs in many European countries. Another major improvement was the use of CD20 antibody rituximab; this replaced splenectomy, which sometimes put recipients at significant risk of bleeding (3). Reports of their mid-to long-term results, accompanied by those from two other innovative European transplant centres, suggested excellent results with respect to patient- and graft survival rates, and noted that these were comparable with ABO-compatible live-donor kidney transplantation (ABOc-LDKT) (4–6). Since then, several large meta-analyses and registry studies have reported differing results regarding the equivalence of ABOi- and ABOc-LDKT. These have included information regarding the choice of the ideal induction regimen, as well as the decision to preferentially refer such donor/recipient pairs to national or international kidney paired exchange programs (7-10).

In this issue of Transplant International, de Weerd et al. (11) analyzed a large and well-characterized multicentric cohort of ABOi-LDKT from six different Dutch transplant centres, spanning a period of 14 years. They applied propensity score matching and used cause-specific Cox models to compare ABOi-LDKT with ABOc-LDKT and ABO-compatible deceased-donor (ABOc-DDKT) transplant outcomes.

A key finding was that patient survival was comparable between ABOi-LDKT and ABOc-LDKT, but was better than ABOc-DDKT. However, when looking at death-censored graft survival, ABOi-LDKT was associated with a higher risk for allograft loss, with a hazard ratio of 2.63 [95% CI: 1.72–4.01] when compared to ABOc-LDKT, and revealed results comparable with ABOc-DDKT. The authors applied a well-developed causal model to detect associations between potential confounding variables that they adjusted for in their final model.

The increased risk for graft loss in ABOi-LDKT versus ABOc-LDKT still remained, even when applying sensitivity analysis where dialysis duration prior to transplantation, diabetic nephropathy and use of rituximab as induction agent were excluded. Interestingly, inclusion of dialysis duration prior to transplantation in the model reduced differences regarding the observed benefit in patient survival between ABOi-LDKT versus ABOc-DDKT. One may argue that the patient-mortality benefit of ABOi-LDKT versus ABOc-DDKT observed in the other models could at least in part be explained by the fact that ABOc-DDKT patients had spent—not unexpectedly—a longer time on dialysis when compared to ABOi-LDKT (median 1,152 versus 216 days, respectively).

When comparing this study to a published cohort of equal granularity, the findings are, to an extent, in contrast to an analysis of >100 ABOi-LDKT performed in Freiburg, Germany (12). In this

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Correspondence:
Farsad Eskandary
farsad.eskandary@meduniwien.ac.at

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monocentric cohort study, Langhorst et al. (12) found no differences in patient mortality and graft survival rates when compared to those recorded for a well-matched cohort of ABOc-LDKT.

Looking at rejection rates after ABOi-LDKT, the study by de Weerd et al. (11) did reveal somewhat higher numbers compared to the study from Freiburg (Overall rejection rate 29% vs. 25%, respectively). A strength of their study is the additional reporting of recipient blood groups, as blood group O is—as has been pointed out by the authors—overrepresented in the Dutch population (about 66% of ABOi-LDKT recipients in their study had blood group O). Recipients with blood group O were shown to be associated with higher anti-AB antibody levels, which may account for the reported high rejection rates (13). As mentioned by the authors, reporting of recipient blood groups should be a pre-requisite in publications about ABOi-LDKT.

One aspect of the study by de Weerd et al. (11) which merits further discussion is the lack of data regarding calcineurin-inhibitor (CNI) levels. These might have some impact on outcomes in the immunologically demanding setting of ABOi-LDKT versus ABOc-LDKT. Although CNI trough level goals were reported, the earlier trough level goals of 10–15 ng/ml were later lowered to 8–12 ng/ml after introducing basiliximab or alemtuzumab as induction agents, but were set identical in both LDKT cohorts. It would be interesting to see the median CNI trough-level corridor achieved by clinicians in the six centres. This information might help increase clinicians' confidence when working with ABOi-LDKT recipients. The knowledge that target-CNI goals were mostly achieved in the present study could then also be interpreted in the context of pre-transplant anti-AB IgG and IgM titer subgroups, as higher titers were associated with higher rejection frequency (11).

Lastly, the study sheds light on the effects of different induction regimens used in ABOi-LDKT. Their study clearly shows that sole use of rituximab was associated with a much higher rejection rate compared to combined rituximab/basiliximab or alemtuzumab treatment. Although use of rituximab only was also reported in the early studies by Tydén et al. (2) , most centres in the study by de Weerd et al. (11) included additional basiliximab in their later protocols. This was also the case in centres not discussed in the study. Their findings however, strongly support the use of combined rituximab/basiliximab or—if available—alemtuzumab as induction agents in ABOi-LDKT. The question whether pre-transplant antibody depletion and rituximab is necessary in all patients prepared for ABOi-LDKT is, however, still up for discussion, since the study by Masterson et al. (14) from Melbourne, Australia has shown favorable results in the presence of low anti-AB antibody titers where rituximab and immunoadsorption were omitted.

In conclusion, the study by de Weerd et al. (11) does confirm the suspicion that ABOi-LDKT might not be as beneficial as ABOc-LDKT, but still shows a clear benefit over ABOc-DDKT. If ABOi donor/recipient pairs do have the option to enter kidney-paired donation programs and receive an ABOc-LDKT, this might be a better alternative with respect to long-term graft survival, but there is a need for careful evaluation and counseling by the treating physicians on an individual level.

AUTHOR CONTRIBUTIONS

FE and GAB wrote the commentary, both revised the final 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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SYSTEMATIC REVIEW AND META-ANALYSIS
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Donor-Transmitted Cancer in Orthotopic Solid Organ Transplant Recipients: A Systematic Review

George H. B. Greenhall[1,2*†], *Maria Ibrahim*[1,2], *Utkarsh Dutta*[3†], *Carolyn Doree*[4†], *Susan J. Brunskill*[4], *Rachel J. Johnson*[1], *Laurie A. Tomlinson*[5†], *Chris J. Callaghan*[2,6†] *and Christopher J. E. Watson*[7,8,9†]

[1]*Department of Statistics and Clinical Research, NHS Blood and Transplant, Bristol, United Kingdom,* [2]*School of Immunology and Microbial Sciences, King's College London, London, United Kingdom,* [3]*GKT School of Medical Education, King's College London, London, United Kingdom,* [4]*Systematic Review Initiative, NHS Blood and Transplant, Oxford, United Kingdom,* [5]*Department of Non-Communicable Disease Epidemiology, London School of Hygiene and Tropical Medicine, London, United Kingdom,* [6]*Department of Nephrology and Transplantation, Guy's Hospital, Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom,* [7]*Department of Surgery, University of Cambridge, Cambridge, United Kingdom,* [8]*The Cambridge NIHR Biomedical Research Centre, Cambridge, United Kingdom,* [9]*NIHR Blood and Transplant Research Unit in Organ Donation and Transplantation, University of Cambridge, Cambridge, United Kingdom*

Donor-transmitted cancer (DTC) has major implications for the affected patient as well as other recipients of organs from the same donor. Unlike heterotopic transplant recipients, there may be limited treatment options for orthotopic transplant recipients with DTC. We systematically reviewed the evidence on DTC in orthotopic solid organ transplant recipients (SOTRs). We searched MEDLINE, EMBASE, PubMed, Scopus, and Web of Science in January 2020. We included cases where the outcome was reported and excluded donor-derived cancers. We assessed study quality using published checklists. Our domains of interest were presentation, time to diagnosis, cancer extent, management, and survival. There were 73 DTC cases in liver (n = 51), heart (n = 10), lung (n = 10) and multi-organ (n = 2) recipients from 58 publications. Study quality was variable. Median time to diagnosis was 8 months; 42% were widespread at diagnosis. Of 13 cases that underwent re-transplantation, three tumours recurred. Mortality was 75%; median survival 7 months. Survival was worst in transmitted melanoma and central nervous system tumours. The prognosis of DTC in orthotopic SOTRs is poor. Although re-transplantation offers the best chance of cure, some tumours still recur. Publication bias and clinical heterogeneity limit the available evidence. From our findings, we suggest refinements to clinical practice.

Systematic Review Registration: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020165001, Prospero Registration Number: CRD42020165001.

Keywords: liver transplantation, cancer, heart transplantation, lung transplantation, donor-transmitted disease, deceased organ donors

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Correspondence:
George H. B. Greenhall
george.greenhall@nhsbt.nhs.uk

†ORCID:
George H. B. Greenhall
orcid.org/0000-0002-4816-1474
Carolyn Doree
orcid.org/0000-0001-6955-7497
Utkarsh Dutta
orcid.org/0000-0001-5733-3250
Chris J. Callaghan
orcid.org/0000-0003-3334-4152
Laurie A. Tomlinson
orcid.org/0000-0001-8848-9493
Christopher J. E. Watson
orcid.org/0000-0002-0590-4901

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Abbreviations: CI, confidence interval; CNS, central nervous system; DDC, donor-derived cancer; DTC, donor-transmitted cancer; HCC, hepatocellular carcinoma; IQR, interquartile range; JBI, Joanna Briggs Institute; NET, neuroendocrine tumour; NR, not reported; PTLD, post-transplant lymphoproliferative disorder; SCC, squamous cell carcinoma; SD, standard deviation; SOTR, solid organ transplant recipient.

INTRODUCTION

Donor-transmitted cancer (DTC) occurs when a tumour is transferred from an organ donor to the recipient via the transplanted organ. Improvements in cancer care and an ageing population have led to an increase in the proportion of donors with a history of cancer, which may put more solid organ transplant recipients (SOTRs) at risk of DTC (1–4).

A diagnosis of DTC has major implications. Survival is often poor and treatment options may be limited (5–8). The optimal treatment in heterotopic SOTRs (e.g., kidney or pancreas transplant recipients) usually comprises discontinuation of immunosuppression followed by allograft removal. This can lead to cancer remission, even in cases with widespread dissemination (9, 10). However, this option is not readily available to orthotopic SOTRs (e.g., heart, lung, liver recipients), so these patients and their clinicians face difficult decisions and significant uncertainty. A transmission event also has implications for other recipients of organs from the same donor, who may consider pre-emptive re-transplantation. Previous reviews in this area have shown variable outcomes in SOTRs with DTC. However, these included recipients with donor-derived cancer (DDC), which results from neoplastic transformation of donor cells following transplantation and often has different treatment implications for affected patients (11, 12). There are no reviews of DTC across all types of orthotopic SOTRs. Guidance on surveillance or treatment of SOTRs with or at risk of DTC is lacking (4, 13–15).

Given the paucity of information on DTC in orthoptic SOTRs, we systematically reviewed the published literature in this area. Our review addressed the following questions: (1) how and when does DTC present in orthoptic SOTRs? (2) what treatment strategies have been used? (3) what are the outcomes after treatment, including re-transplantation? We aimed to synthesise the available evidence in this area in order to suggest refinements to clinical practice.

MATERIALS AND METHODS

We undertook a prospectively registered systematic review (PROSPERO ID CRD42020165001) (16). We followed the PRISMA and "Synthesis Without Meta-analysis" guidelines for study reporting (17, 18).

Inclusion and Exclusion Criteria

Our review population was orthotopic SOTRs with DTC. In accordance with published guidelines, we defined DTC as a cancer of donor origin in an SOTR, which was known or assumed to be present in the donor at the time of transplantation (8). Importantly, we excluded cases of DDC. Studies were eligible if they described recipients of liver, heart, lung, or intestinal transplants with DTC, and reported transplant type, transmitted cancer type, presentation or management, and patient survival (i.e., vital status at the time of reporting). We included any publication type except review articles and editorials.

Search Strategy

We searched MEDLINE (1946 to present), EMBASE (1974 to present), PubMed (e-publications ahead of print only), Scopus, and Web of Science Core Collection. Our search terms included "cancer," "tumour," "transplant," "donor," "transmission," and all related terms. We limited our search to human studies but did not apply date or language restrictions. We used publicly available search filters to restrict our search to cohort studies, case-control studies, case series and case reports, because we did not expect to find any interventional studies (19). We then searched "grey" literature sources including non-indexed conference proceedings, thesis repositories, and the World Health Organisation "NOTIFY" library (20). Lastly, we hand-searched reference lists of included articles. We executed our search on January 16, 2020. Our full search strategy is in the **Supplementary Material**.

Study Selection and Quality Grading

Two reviewers (GG, MI) independently screened titles and abstracts followed by full-text review to determine study eligibility. We resolved disagreements by discussion. Where cancer origin was unclear (DTC vs. DDC) we involved a senior author (CW) or contacted authors for clarification. We cross-checked all included cases to identify duplicates between publications and included the report with the most complete information on each case.

Two reviewers (GG, MI) independently scored the quality of each included study using tools published by the Joanna Briggs Institute (JBI), with resolution of disagreements by discussion (21, 22). These are tools designed to assess the methodological quality of a study objectively, using categorical responses (yes/no/unclear/not applicable) to questions on key domains (e.g., "was the current clinical condition of the patient on presentation clearly described?"). We assessed case reports and registry studies against the JBI checklists for case reports and prevalence studies, respectively. We did not exclude any studies on the basis of quality. Because we did not find any reports with a comparator group, we were unable to assess the risk of bias.

Data Extraction

Two reviewers (GG, UD) independently extracted data from included studies using a pre-piloted proforma (see **Supplementary Material**), creating a separate record for each case included in our review. Our five main domains of interest were: mode of presentation, time to diagnosis, tumour extent, treatment, and survival time.

We recorded the publication type, year, and total number of DTC cases (including heterotopic transplants) in each article. We considered reports of multiple transplants from a single donor as case reports. For each DTC case, we recorded recipient demographics, transplant type, mode of presentation (symptoms, graft dysfunction, surveillance, *post-mortem*), time to diagnosis, primary tumour site and histology, and cancer extent at diagnosis (confined to allograft/distant metastases). Donor variables were: demographics, history of cancer, and time from cancer diagnosis to donation. Information on management comprised cancer-specific treatment (e.g., chemotherapy, radiotherapy, loco-regional therapy, tumour resection), re-transplantation (and time from diagnosis),

and modification of immunosuppression. We recorded all time intervals in days if less than 1 month and in whole months if more than 1 month.

Our outcomes were patient death, cause of death, cancer remission and cancer recurrence (and time since remission). Unless stated otherwise, we assumed that treatment procedures with curative intent (re-transplantation, resection) achieved remission. Where articles reported death only, we assumed that remission was not achieved. Where information on these outcomes was missing from case reports, we contacted study authors by email.

Statistical Analysis

We first examined data completeness across our main domains of interest (mode of presentation, time to diagnosis, tumour extent, treatment, survival time). We then tabulated donor, recipient, and tumour-related characteristics of all included cases. After analysing all cases, we stratified our dataset, first by transplant type and then by cancer type. We did this because both domains are relevant to scenarios encountered in clinical practice. We grouped cancer type by the site of the primary tumour (e.g., lung), unless we found only one histological type in a particular site (e.g., melanoma). We did not group our data by study type because we analysed information at individual case level.

We determined the range, median and interquartile range (IQR) of the time from transplantation to DTC diagnosis. We then calculated the proportion of tumours with spread beyond the allograft at diagnosis. We compared tumour extent between transplant types using the chi-squared test. We tabulated the treatment modalities reported. Among cases that received a second allograft, we determined the median time from diagnosis to re-transplantation. We then calculated the proportion of cases that achieved cancer remission. Among these, we summarised the treatment modalities received, the proportion that recurred, and the proportion that died.

Our main outcome was all-cause mortality, calculated as the proportion of cases that died after a diagnosis of DTC. Since we only included cases where survival was reported, the denominator here was all cases (or all within a group). We used all-cause mortality because some treatment modalities (e.g., re-transplantation) confer substantial risk, so this is the most relevant patient-related outcome. The lack of comparator groups in each study precluded meta-analysis of treatment effects. Due to the size and heterogeneity of our study, multivariable analyses were not appropriate (23).

To analyse survival time, we restricted our dataset to cases with follow-up of at least 6 months, or to death. We assessed the heterogeneity of cases included in this analysis by summarising the range of follow-up time. We determined the median survival time following DTC diagnosis in all cases, then stratified by transplant and cancer type (for the commonest cancers).

Post hoc, we explored factors that may influence survival among cases with sufficient follow-up (6 months, or to death). Due to substantial variation in follow-up between studies, we censored this analysis at 3 years from DTC diagnosis. We examined the relationship between survival time and (1) transplant type (2), tumour extent at diagnosis, and (3) re-transplantation, using Kaplan-Meier curves and log-rank tests. To reduce confounding, we restricted our analysis of re-transplantation to cases with tumour confined to the allograft at diagnosis. We did this because patients with disseminated cancer would not ordinarily be considered suitable for re-transplantation, making them an inappropriate comparator group. Owing to data sparsity, it was not appropriate to test for an association between cancer type and survival time. Lastly, we tallied the number of cases of DTC in heterotopic SOTRs that received organs from the same donors as our included cases, and the proportion that died.

We performed study screening with Covidence software (Veritas Health Innovation, Australia), data extraction with EpiData v4.6 (EpiData Association, Denmark), and data analysis with Stata v15.1 (StataCorp, United States).

RESULTS

Study Selection

Our search retrieved 2,308 articles. After title and abstract screening, we assessed 223 full texts against our inclusion criteria. Fifty-eight articles (49 case reports, 9 registry studies) published between 1987 and 2019 were eligible for inclusion (**Figure 1**). Our review population comprised 73 cases of DTC in orthotopic SOTRs (52 from case reports and 21 from registry studies). These 73 cases originated from 69 donors and were reported from North America (n = 37), Europe (n = 33), Asia (n = 1), Australia (n = 1) and South America (n = 1). **Supplementary Table S1** shows the characteristics of all included studies.

Study Quality

Overall, the quality of included articles was acceptable. However, there was substantial variation between studies and across quality domains. Among case reports, the domains with the lowest quality were the clinical condition of the patient at presentation and after treatment. The quality of registry studies was lower; most provided insufficient information on study size, case identification methods and sample coverage. **Supplementary Figure S1**, and **Supplementary Figure S2** summarise study quality scoring against the JBI checklists.

Among the 73 cases in our study, data completeness varied across our five domains of interest (**Supplementary Table S2**). The proportion of cases with information in each domain was: tumour extent, 84% (61/73); time to diagnosis, 89% (65/73); presentation, 73% (50/73); treatment, 67% (49/73); survival time, 60% (44/73). We contacted the study authors of eight cases with incomplete outcome data; four replied with supplementary information which was added to the dataset for analysis.

DTC Presentation

Table 1 summarises the characteristics of included cases. There were 52 liver (including one liver-intestine-pancreas transplant), 10 heart, and 11 lung recipients (including one heart-lung transplant). Median (IQR) recipient age at diagnosis was 53 (41–60) years; 51% (37/73) were male. Median (IQR) donor age was 50 (39–62) years. In 29/73 (40%) cases, a cancer had been found in the donor. Six of these were diagnosed before donor assessment (between 4 months

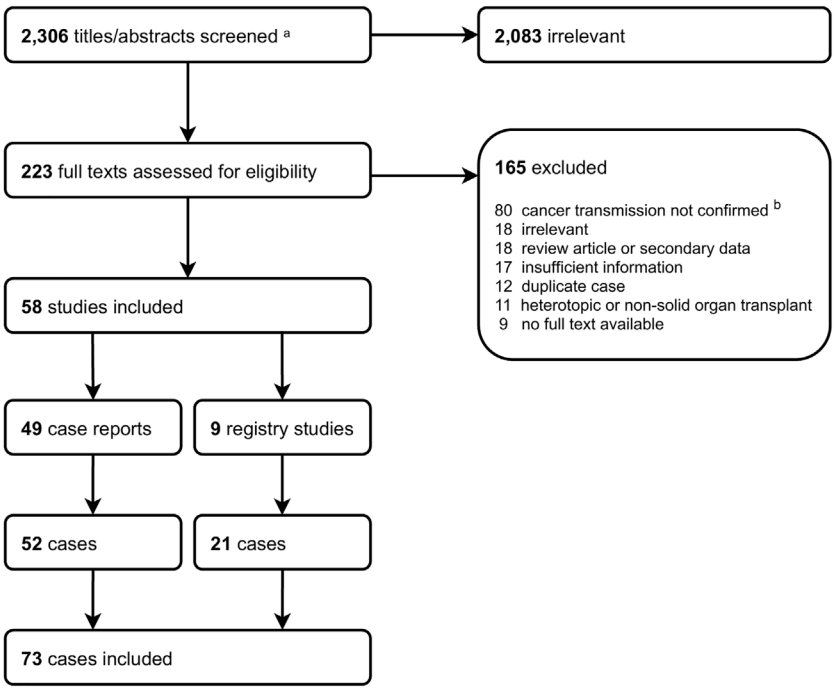

FIGURE 1 | Study screening flowchart. [a]Includes 2,298 from search strategy and 8 from hand searches (5 from WHO NOTIFY Library, 3 from reference lists). [b]Includes donor-derived cancer.

TABLE 1 | Characteristics of cases of donor-transmitted cancer included in review, by transplant type

Total cases	Organ transplanted			All cases
	Liver[a]	Heart	Lung[b]	
	52	10	11	73
Tumour identified in donor	16 (31%)	7 (70%)	6 (55%)	29 (40%)
Time to cancer diagnosis (months)	8 (4–12)	10 (5–12)	9 (3–14)	8 (4–12)
Tumour spread beyond allograft at diagnosis	14 (27%)	10 (100%)	7 (64%)	31 (42%)
Re-transplanted	13 (25%)	0	0	13 (18%)
Survival after DTC diagnosis (months)[c]	9 (2–36)	6 (3–23)	2 (1–5.5)	7 (2–31)

[a]*includes 1 liver-intestine-pancreas.*
[b]*includes 1 heart-lung.*
[c]*restricted to cases with follow-up of at least 6 months, or to death (n = 49; 36 liver, 5 heart, 8 lung).*
Numbers are n (%) or median (IQR).

and 32 years prior to death), while 23 were discovered after organ implantation.

Table 2 summarises the types of transmitted malignancies included in our study. The commonest histological types were melanoma (n = 10) and choriocarcinoma (n = 7). **Supplementary Table S3** shows the histology of all included cases. The most frequent mode of presentation was with symptoms, in 24/73 (33%) cases. Other methods of case detection were surveillance imaging (either routine or targeted because of transmission risk, n = 14), graft dysfunction (n = 4), tumour markers (elevated β-human chorionic gonadotropin in transmitted choriocarcinoma, n = 3), or retrieval or implantation biopsy (n = 5). Four cases were diagnosed at recipient *post-mortem* only.

Time from transplantation to DTC diagnosis ranged from 0 days to 6 years. In total, 48/73 (66%) cases were diagnosed within 1 year, and 60/73 (82%) within 2 years. Median (IQR) time to diagnosis was 8 (4–12) months; this was similar across transplant types (**Table 1**). The cancer types with the shortest time to diagnosis were choriocarcinoma [median (IQR) 1.5 (1 to 3) months] and sarcoma [2.5 (1 to 8) months; **Table 2**].

At the time of diagnosis, 29/73 (40%) tumours were confined to the allograft while 31/73 (42%) had disseminated. Twelve cases (eight heart, two liver, one heart-lung) had distant metastases only, with no tumour in the allograft. There was strong evidence of an association between tumour extent and transplant type; 27% (14/52), 100% (10/10) and 64% (7/11) of liver, heart, and lung recipients, respectively, had tumour dissemination at diagnosis ($\chi^2 = 15.2$, $p = 0.001$; **Table 1**). It also varied between cancer types; all cases of choriocarcinoma had spread beyond the allograft at diagnosis, whereas all intestinal tumours and 6/7 neuroendocrine tumours (NETs) were confined to the allograft (**Table 2**).

DTC Management

Excluding palliative management, 43/73 (59%) cases included treatment details (**Table 3**). The commonest treatment was systemic chemotherapy; this was used in 20 cases and was the main treatment in 14. Seven cases underwent tumour resection and seven received loco-regional therapies, comprising radio/chemo-embolisation (n = 3), radiofrequency ablation (n = 2),

TABLE 2 | Characteristics of cases of donor-transmitted cancer included in review, by primary cancer type.

Primary tumour	Cases	Transplant type (n)	Time to diagnosis (m)	Spread beyond allograft at diagnosis	Re-transplanted	Died
Melanoma	10	Liver (6), Heart (2), Lung (2)	11 (9–13)	6	0	10/10
Choriocarcinoma	7	Liver (5), Heart (2)	1.5 (1–3)	7	0	5/7
CNS tumours	7	Liver (4),[a] Heart (1), Lung (2)	4.5 (4–9)	5	0	7/7
Genitourinary tumours	7	Liver (3), Heart (2), Lung (2)[b]	11 (9–14)	4	1	5/7
Haematological malignancies	7	Liver (6), Heart (1)	12 (1–18)	3	1	6/7
Neuroendocrine tumours[c]	7	Liver (7)	9 (8–36)	1	2	4/7
Lung tumours	6	Liver (3), Heart (1), Lung (2)	6 (4–9)	2	1	5/6
Sarcomas	6	Liver (4), Lung (2)	2.5 (1–8)	1	1	4/6
Tumours of unknown primary site	6	Liver (5), Heart (1)	6 (6–12)	1	3	3/6
Intestinal tumours	5	Liver (5)	11 (6–13)	0	2	4/5
Other tumours[d]	5	Liver (4), Lung (1)	5 (0–16)	1	2	2/5

[a]*includes 1 liver-pancreas-intestine transplant.*
[b]*includes 1 heart-lung transplant.*
[c]*includes 1 small cell neuroendocrine tumour of lung origin.*
[d]*breast (2), hepatocellular (2), pancreas (1).*
Numbers are n or median (IQR); m, months. See ***Supplementary Table S2*** *for full histological details of cases included.*
CNS, central nervous system.

TABLE 3 | Treatment modalities for cases of donor-transmitted cancer included in review.

	Cases
Total cases with treatment reported	43
Cancer treatment	
Chemotherapy[a]	20 (47%)
Tumour resection[b]	7 (16%)
Loco-regional therapy[c]	7 (16%)
External beam radiotherapy	6 (14%)
Immunosuppression management	
Reduction	14 (33%)
Cessation	3 (7%)
Drug change[d]	6 (14%)
Re-transplantation	13 (30%)

[a]*includes 1 patient treated with chemotherapy and hormone therapy for prostate cancer.*
[b]*excludes re-transplantation.*
[c]*radio/chemo-embolisation (3), radiofrequency ablation (2), brachytherapy (1), extracorporeal proton therapy (1).*
[d]*calcineurin inhibitor switch to sirolimus (4) or everolimus (2).*
Numbers are n (%). Some cases received more than one treatment. Excludes cases with only palliative management.

brachytherapy (n = 1), and extracorporeal proton therapy (n = 1). There were six reports of altered immunosuppressive regimens, comprising a switch from calcineurin inhibitors to sirolimus (n = 4) or everolimus (n = 2).

Thirteen cases underwent re-transplantation. All of these were liver recipients that had no tumour dissemination at diagnosis, including one case pre-emptively re-transplanted after a *post-mortem* donor cancer diagnosis (DTC from the first donor subsequently recurred) (24). Re-transplantation was performed at a median (IQR) of 4 months (4 days–6 months) following DTC diagnosis. Treatments received prior to re-transplantation were loco-regional therapy (n = 3), tumour resection (n = 1), and chemotherapy (n = 1). Following re-transplantation, 3/13 (23%) tumours recurred between 2 weeks and 3 years later, and three patients died (**Table 4**).

In total, 19/73 (26%) cases achieved cancer remission following treatment. The main treatment modalities in these cases were: re-transplantation (n = 12; one case with recurrence 2 weeks after re-transplantation was not considered to have achieved remission), tumour resection (without subsequent re-transplantation, n = 5), loco-regional therapy alone (n = 1), and chemotherapy alone (n = 1). Of the 19 cases with cancer remission, six (33%) subsequently experienced a recurrence between 10 months and 3 years later, and five (26%) died (three of which had recurrent cancer).

DTC Outcomes

In total, 55/73 cases (75%) died. This includes four cases diagnosed at *post-mortem*. Forty-seven deaths were due to cancer, three were due to other causes (sepsis, pneumonia, variceal bleed), and in five cases the cause of death was not evident. All-cause mortality was 69% (36/52), 80% (8/10), and 100% (11/11) in liver, heart, and lung recipients, respectively. Mortality by cancer type ranged from 50% in tumours of unknown origin to 100% in melanoma and central nervous system (CNS) tumours (**Table 2**).

There were 49 cases (36 liver, 5 heart, 8 lung) with follow-up of at least 6 months or to death. Among these, survival after DTC diagnosis ranged from 5 days to 13 years. Overall, 1-year survival was 39% (19/49). Overall median (IQR) survival was 7 (2–31) months and 9 (2–36), 6 (3–23) and 2 (1–5.5) months in liver, heart, and lung recipients, respectively. There was some evidence of an association between transplant type and survival time (log-rank χ^2 8.3, $p = 0.02$; **Figure 2**), with the shortest survival in lung recipients. Survival time varied between the commonest cancer types. Median (IQR) survival was 2 (1–7) months in melanoma, 2 (1–2) months in CNS tumours, 9 (3–36) months in NETs and 26 (2–48) months in genitourinary tumours.

Median (IQR) survival was 16 (7–37) months in tumours confined to the allograft (n = 25) and 2 (1–9) months in disseminated cancers (n = 22). There was strong evidence

TABLE 4 | Cases of donor-transmitted cancer undergoing re-transplantation (all liver recipients).

Transmitted cancer (References)	Time from transplantation to diagnosis	Time from diagnosis to re-transplantation	Cancer recurrence (time from re-transplantation)	Died	Total follow-up[a] (months)
NET (29)	8 months	5 months	Yes (17 days)[b]	Yes	9
NET (30)	36 months	24 months	No	No	36
Colonic adenocarcinoma (31)	13 months	9 months	No	No	33
Colonic adenocarcinoma (32)	4 months	4 months[c]	No	Yes[d]	40
Lung adenocarcinoma (24)	11 months	-[e]	Yes (11 months)	Yes	13
Urothelial tumour (33)	14 months	7 days	No	No	48
Sarcoma (34)	0 days	4 days	No	No	76
Plasmacytoma (35)	0 days	9 days	Yes (36 months)	No	42
Pancreatic adenocarcinoma (36)	0 days	1 days	No	No	12
HCC (37)	1 days	3 days	No	No	36
SCC, unknown primary (38)	6 m	6 m	No	No	6
Adenocarcinoma, unknown primary (39)	12 m	NR	No	No	8
Adenocarcinoma, unknown primary (40)	6 months	6 months	No	No	31

[a]*from DTC diagnosis.*
[b]*pancreatic metastases found 2 weeks following re-transplantation; cancer remission not achieved.*
[c]*initially resected, subsequently re-transplanted.*
[d]*died of pneumonia.*
[e]*pre-emptive re-transplantation on day 7 after donor cancer found at autopsy—recurrence 11 months later.*
SCC, squamous cell carcinoma; NET, neuroendocrine tumour; NR not reported; HCC, hepatocellular carcinoma.

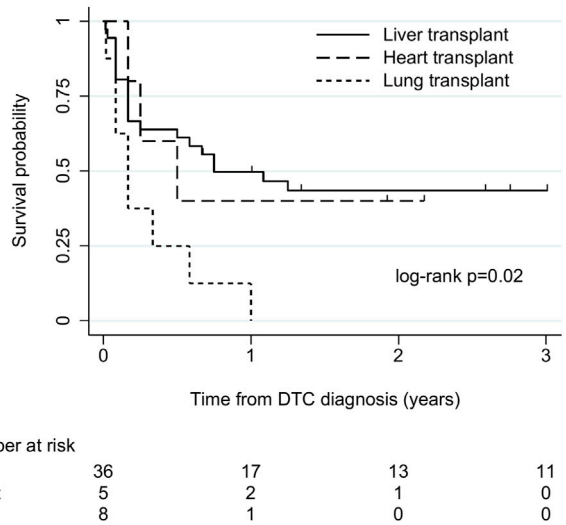

FIGURE 2 | Kaplan-Meier plot of patient survival after donor-transmitted cancer diagnosis, by transplant type. Restricted to cases with follow-up of at least 6 months, or to death (n = 49). Follow-up censored at 3 years. Liver includes liver-pancreas-intestine (1), lung includes heart-lung (1). DTC, donor-transmitted cancer.

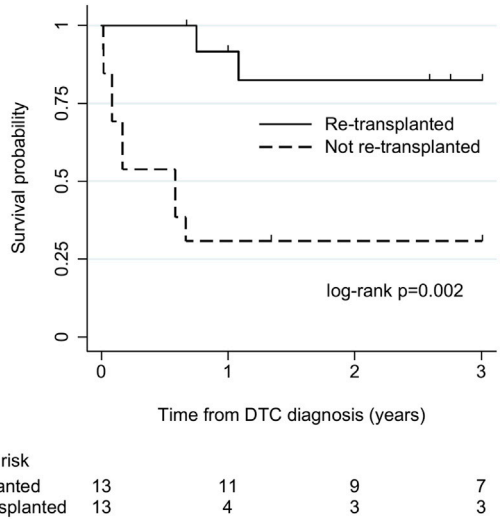

FIGURE 3 | Kaplan-Meier plot of patient survival after donor-transmitted cancer diagnosis, by re-transplantation. Restricted to cases with follow-up of at least 6 months, or to death, and no tumour beyond allograft at diagnosis (n = 26). Follow-up censored at 3 years. Re-transplanted cases: liver recipients (n = 13); cases not re-transplanted: liver (n = 10), lung (n = 3) recipients. DTC, donor-transmitted cancer.

of shorter survival in cases with tumour dissemination at diagnosis (log-rank χ^2 9.9, p = 0.002; **Supplementary Figure S3**).

There were 26 cases (23 liver, 3 lung) without tumour dissemination at diagnosis and with sufficient follow-up for survival analysis. Among these, 13 (all liver) underwent re-transplantation, and 13 (10 liver, 3 lung) did not. All-cause mortality was 23% (3/13) in re-transplanted cases and 85% (11/13) in cases that were not re-transplanted. Median (IQR) survival was 36 (13–40) months and 7 (1–16) months in cases that did and did not undergo re-transplantation, respectively. There was strong evidence of longer survival in re-transplanted cases (log-rank χ^2 9.3, p = 0.002; **Figure 3**).

There were 44 cases of DTC in recipients of heterotopic transplants from the donors of the cases included in our review (42 kidney, 1 pancreas, 1 kidney-pancreas); 21 (48%) of these died.

DISCUSSION

In this systematic review of confirmed cases of donor-transmitted malignancies, we identified 73 orthotopic SOTRs with DTC in the published literature. The commonest malignancies were melanoma and choriocarcinoma. Most presented within 2 years of transplantation and nearly half had spread beyond the allograft at the time of diagnosis. Mortality was high: three-quarters died overall, 60% within a year.

Some characteristics varied between the cancer types that we identified. Choriocarcinoma appeared to be the most aggressive tumour, with early presentation and dissemination at diagnosis in all cases. Conversely, intestinal tumours presented later and were all confined to the allograft. We found the worst outcomes in melanoma, CNS tumours and haematological malignancies. Unsurprisingly, tumour dissemination at diagnosis conferred shorter survival.

We also found some variation between transplant types. Compared to heart and lung recipients, liver recipients were less likely to have tumour beyond the allograft at diagnosis and survived longer. This might be due to the lower level of immunosuppression that these patients require, or their suitability for re-transplantation; all re-transplanted cases in our review were liver recipients, and these had substantially better survival. Tumours recurred in nearly a quarter of re-transplanted cases.

Our study contains fewer cases than previous reviews in this area (11, 12). There are two main reasons for this. The first is our minimum data set for inclusion, which excluded cases with less detail reported. As a result, most cases in our review are from case reports, since most registry studies contain minimal individual-level clinical details. Second, we excluded cases of DDC. We did this because DTC is theoretically preventable, usually has a narrow window of presentation, and often has implications for other recipients of organs from the same donor, whereas DDC tends to present later and may have more favourable outcomes (5). The most striking consequence of this is in relation to lymphoma. We identified four recipients with donor-transmitted lymphoma, all of whom died, contrasting sharply with the 80% survival in 30 cases in a previous review of liver recipients (11). Although the origin of donor-related post-transplant lymphoproliferative disorders (PTLD) is controversial, outcomes in donor-derived PTLD may in fact be better compared to cancers of recipient origin (25, 26). Although our inclusion criteria focussed this review on one patient group, we acknowledge that differentiating between DTC and DDC is subjective; some excluded cases could have influenced our results.

Taken in the context of existing research, our study confirms that the prognosis of DTC in orthotopic SOTRs is worse than in heterotopic transplant recipients (12, 27). The outcomes of the heterotopic SOTRs with DTC from the same donors as our included cases appear to confirm this. However, this is most likely to reflect the optimal treatment strategy—cessation of immunosuppression, allograft removal and systemic anti-cancer therapy—which is available to heterotopic SOTRs, confounding any direct comparison with orthotopic SOTRs.

This is the first study to summarise the experience of DTC across all orthotopic SOTRs and compare outcomes between transplant types. We specifically examined the rate of cancer remission and recurrence, which have not been studied previously. We followed a prospectively registered protocol and identified cases according to international criteria. We took all possible steps to exclude duplicate cases from our review. There were several duplicated reports in the published literature. However, we cannot completely eliminate the possibility that some duplicates remain. Our study grading tools provided objective measures of quality and our minimum dataset for inclusion gave acceptable data completeness.

Our review confirms that the quality of evidence in this area is generally low. Published data are largely limited to anecdotal reports. This is unlikely to change. By definition, these studies do not include comparator groups, so it is difficult to judge the impact of patient or treatment factors in each study reliably. The most important limitation of the available evidence is publication bias. This limits direct comparisons between cases. It would be inappropriate to infer that the risk of transmission mirrors the frequency of cases in our study. Similarly, cases with a favourable outcome are more likely to be reported, which could bias our results; actual outcomes may be worse than our results suggest. Even compulsory reporting of transmission events, as mandated by many national transplant authorities, is prone to under-recognition or biased reporting. Registry linkage is one method to minimise biased case detection and outcome reporting.

There is also a significant amount of clinical heterogeneity in the published evidence. We acknowledge that treatments reported were chosen on a case-by-case basis and may have been published for their novelty, limiting interpretation of our findings. Variable follow-up may have biased our survival analyses. Although we mitigated this by restricting our analyses to cases with sufficient follow-up (at least 6 months) and censoring follow-up at a reasonable point, selection bias remains likely. Assessing survival from the time of DTC diagnosis may also have introduced immortal time bias. The wide time span of publications in our review meant that we could not account for temporal changes in therapeutic options.

The size of our review population limits the power of our analyses; this is inevitable with such a rare condition. Anticipating this, we avoided multivariable analyses which could have introduced more uncertainty. This means we were unable to address the possibility of other factors confounding our results. Grouping cancers by primary site resulted in significant heterogeneity within some groups; histological type may be a more important determinant of tumour behaviour in the host environment. Our main outcome (all-cause mortality) may have been vulnerable to bias because the review population were likely to be at increased risk of death from other causes (e.g., infection), as a result of immunosuppressive therapy, complications of organ failure, or anti-cancer treatment. However, since most deaths were due to cancer, this is unlikely to have changed our findings meaningfully. There was also a certain amount of missing data.

Within the limits of the evidence base, we feel it is reasonable to make some suggestions for practice improvement. Firstly, our findings support surveillance of orthotopic SOTRs at increased risk of DTC for at least 2 years, because approximately 80% of cases present during this time. We suggest this applies to

recipients of transplants from donors with tumours that have more than a "minimal" transmission risk, as defined by international guidelines (14, 15, 28), or those notified of a transmission event from their donor. Tumour characteristics should dictate the type of surveillance; imaging or laboratory studies may be more appropriate. It is notable that one in six cases in our review presented without tumour in the allograft, meriting careful consideration of surveillance imaging. Secondly, monitoring for allograft dysfunction does not appear to be a reliable means of detecting DTC in this population, since only a minority of the cases included in our review presented in this way. Thirdly, if a transmitted tumour is confined to an orthotopic allograft, re-transplantation should be considered. However, the physiological state of the recipient and the availability of a suitable organ will influence this decision since it carries substantial morbidity. Tumour resection or loco-regional therapy may achieve remission while avoiding a second major operation; further experience of these treatments in the context of DTC will benefit the transplant community. There are other important knowledge gaps, including the role of tumour markers in donor assessment or recipient surveillance, optimal management of immunosuppression before/after re-transplantation, and longer-term outcomes in re-transplanted patients.

In summary, this review confirms the poor prognosis of DTC in orthotopic SOTRs. Re-transplantation appears to offer the best hope of survival, but some tumours recur despite this. Further studies using prospectively collected data and disease registry linkage could shed more light on the diagnosis and treatment of this condition and inform guidance on surveillance of patients at risk.

AUTHOR CONTRIBUTIONS

GG: conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing—original draft, writing—review and editing. MI: conceptualization, data curation, investigation, writing—review and editing. UD: investigation, writing—review and editing. CD: conceptualization, resources, data curation, writing—review and editing. SB: conceptualization, resources, writing—review and editing. RJ: supervision, writing—review and editing. LT: conceptualization, supervision, writing—review and editing. CC: conceptualization, supervision, writing—review and editing. CW: conceptualization, supervision, writing—review and editing.

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AUTHOR DISCLAIMER

The views expressed are those of the authors and not necessarily those of the NHS, NHSBT, or Department of Health.

CONFLICT OF INTEREST

CW has no direct conflict of interest related to this work. In the last 3 years his department has received consultancy fees on his behalf from Nefro Health and GlaxoSmithKline, and speaker fees from OrganOx.

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.2021.10092/full#supplementary-material

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Role of Fractalkine-CX3CR1 Axis in Acute Rejection of Mouse Heart Allografts Subjected to Ischemia Reperfusion Injury

Taichi Kanzawa[1], Daisuke Tokita[1,2], Kan Saiga[1,3]*, Takafumi Yamakawa[1], Hidetoshi Ishigooka[1], Hironori Fukuda[1], Haruki Katsumata[1], Satoshi Miyairi[1], Rumi Ishii[1], Toshihito Hirai[1], Toshio Imai[4], Masayoshi Okumi[1] and Kazunari Tanabe[1]*

[1]Department of Urology, Tokyo Women's Medical University, Tokyo, Japan, [2]Clinical and Academic Research Promotion Center, Tokyo Women's Medical University, Tokyo, Japan, [3]Department of Urology, Jyoban Hospital of Tokiwa Foundation, Fukushima, Japan, [4]KAN Research Institute Inc., Kobe, Japan

Transplantation outcomes are affected by the increase in rejection associated with ischemia reperfusion injury (IRI). Fractalkine (FKN), a chemokine for recruitment of $CX3CR1^+$ leukocytes, contributes to the pathogenesis of various inflammatory diseases. Herein, we evaluated the importance of the FKN-CX3CR1 axis during IRI-related rejections using a mouse heterotopic heart transplantation model. FKN expression and graft survival was compared between wild-type C57BL/6 recipients transplanted with BALB/c hearts preserved for 8 (WT-IRI) and 0.5 h (WT-control) at 4°C. Graft survival of WT-IRI was shorter than that of WT-control. FKN was expressed on the vascular endothelium in WT-IRI allografts, but minimally in WT-control. The role of the FKN-CX3CR1 axis in IRI-related rejection was directly investigated using the transplant model with CX3CR1-deficient recipients (CX3CR1 KO-IRI) or treatment with anti-mouse FKN monoclonal antibodies. Graft survival of CX3CR1 KO-IRI was longer than that of WT-IRI; antibody treatment prolonged graft survival. The contribution of $CX3CR1^+$ monocytes to IRI-related rejection was evaluated by adoptive transfer to CX3CR1 KO-IRI. Adoptive transfer of $CX3CR1^+$ monocytes attenuated the effect of prolonged graft survival in CX3CR1 KO-IRI. Overall, the FKN-CX3CR1 axis plays a major role during IRI-related rejection; its blockade has the potential to improve the outcomes of deceased donor transplantation.

Keywords: transplantation, rejection, ischemia-reperfusion injury, fractalkine, CX3CR1, monocyte

Correspondence:
Daisuke Tokita
tokita.daisuke@twmu.ac.jp
Kan Saiga
saiga.kan@twmu.ac.jp

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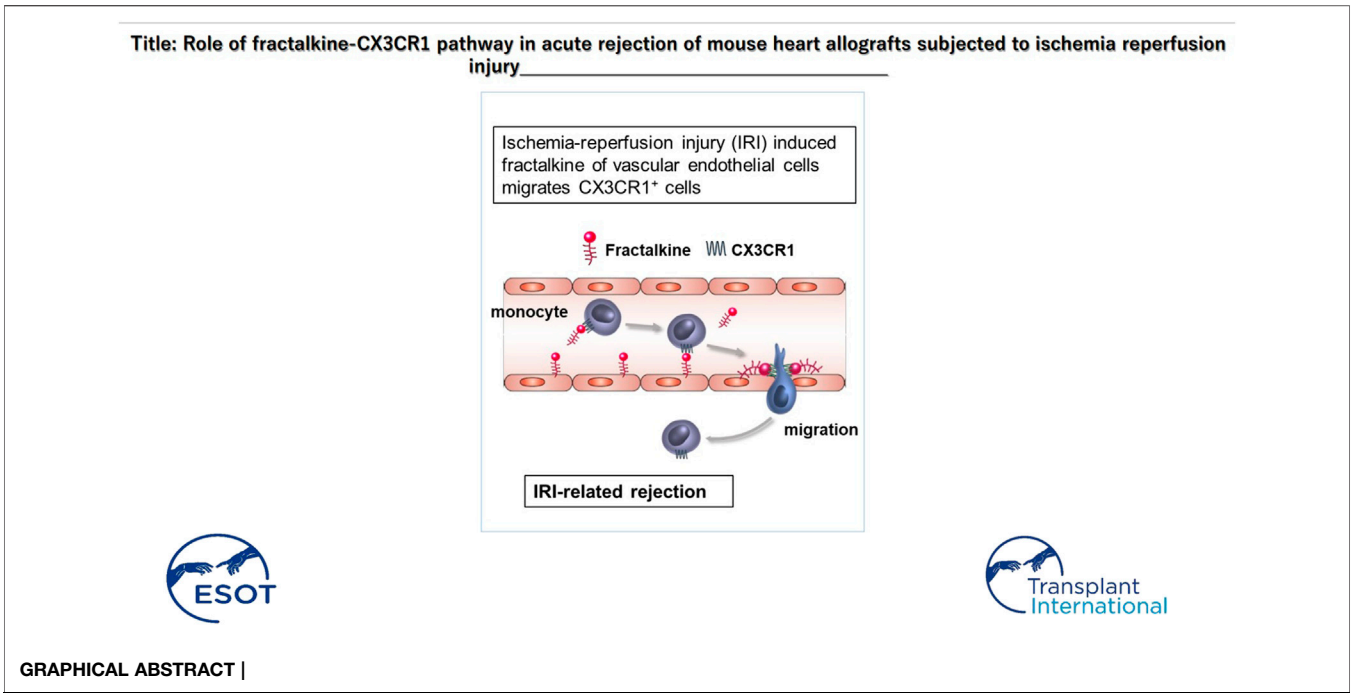
INTRODUCTION

Generally, organs transplanted from living donors have superior function and survival compared with those from deceased donors. The longer the ischemic time imposed on grafts, the lower their function and patient survival rates, which are related to promotion of graft rejection in ischemia reperfusion injury (IRI) (1–8). Length of ischemic time is connected to the severity of primary graft

Abbreviations: CTLA4-Ig, cytotoxic T lymphocyte antigen 4-immunoglobulin; FITC, fluorescein isothiocyanate; FKN, fractalkine; IL, interleukin; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; ICAM-1, intercellular adhesion molecule-1; IFN, interferon; IRI, ischemia reperfusion injury; mAb, monoclonal antibody; MST, median survival time; PE, phycoerythrin; TACE, TNFα cleavage enzyme; TNF, tumor necrosis factor; VCAM-1, vascular cell adhesion molecule-1.



GRAPHICAL ABSTRACT |

dysfunction caused by the generation of oxygen radicals and activation of complement and endothelial cell dysfunction soon after the restoration of blood flow (9–11). During the early inflammatory process after reperfusion, the production of proinflammatory cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-1, and IL-6, is greatly increased, and these cytokines induce and enhance alloimmune responses with the expression of adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), on vascular endothelium, leukocyte infiltration, and tissue injury in grafts (11–14). Increased graft-infiltrating neutrophils, monocytes, and memory $CD8^+$ T cells have been observed in donor organs subjected to prolonged cold ischemia, and these immune cells are implicated in graft dysfunction and transplant rejection (2, 15). Therefore, control of inflammatory cell infiltration in the initial process could be an effective approach to improve graft survival in allogeneic transplantation with donor organs subjected to prolonged ischemic conditions.

Fractalkine (FKN) is the only CX3C chemokine reported to date (16, 17). FKN expression is induced by stimulation of proinflammatory cytokines, such as TNF-α, IL-1, and interferon (IFN)-γ, and the translation product is expressed as a membrane-bound form on vascular endothelial cells (16). Membrane-bound FKN is cleaved by metalloproteinases, including TNF-α cleavage enzyme (TACE) and ADAM10, and released into the blood (18, 19). Soluble FKN acts as a chemokine that migrates immune cells expressing the FKN receptor, CX3CR1 (20), through integrin-independent and -dependent mechanisms (21). CX3CR1-expressing cells include a variety of leukocytes, such as monocytes, macrophages, cytotoxic effector lymphocytes, and natural killer (NK) cells, which migrate along a gradient of soluble FKN and enter through membrane-bound FKN expressed on the endothelium at inflamed sites (16, 22). The FKN-CX3CR1 axis is thought to be involved in the initiation of the innate immune system as well as the continuation of acquired immune response, and is known to play a role in immune defense against infections and tumors (23–27).

In contrast, FKN reportedly contributes to the pathological process of vascular and tissue injury in inflammation-mediated diseases and pathological conditions, including atherosclerosis, glomerulonephritis, rheumatoid arthritis, and transplant rejection, by enhancing migration and adhesion of CX3CR1-expressing leukocytes and promoting their transmigration to inflammatory sites (24, 27, 28). In a mouse heart transplantation model, FKN expression was increased in rejecting grafts, and anti-CX3CR1 neutralizing antibody treatment substantially prolonged graft survival (29). Prophylactic or therapeutic administration of anti-FKN monoclonal antibodies to a mouse collagen-induced arthritis model suppressed the migration of osteoclast progenitor cells derived from a monocyte/macrophage lineage of bone marrow cells into the joint while markedly improving synovitis and joint destruction (30). Furthermore, antibody clone 5H8 reduced skin fibrosis in a systemic sclerosis model (31).

Fractalkine has been reported to exert an effect on monocytes. $CD14^+$ monocytes express CX3CR1 (20), and FKN induces migration (16) and enhances integrin-dependent cell adhesion in monocytes (21,32,33). It has also been documented that CX3CR1 regulates the retention of inflammatory monocytes in blood vessels during inflammation (34). In addition, migration of inflammatory monocytes via a mechanism dependent on the FKN-CX3CR1 axis has been reported to play an important role in renal injury after ischemia reperfusion by cross-clamping kidney pedicles (35). The FKN-CX3CR1 axis is also associated with the

patrolling behavior of $CD115^{+}Gr\text{-}1^{low/-}$ monocytes crawling over the venous endothelium of the inflamed colon in a colitis model, locally producing proinflammatory cytokines and chemokines that promote subsequent leukocyte activation and infiltration. Anti-FKN antibody rapidly eliminated these crawling monocytes and inhibited their patrolling behavior (36).

To date, there have been many reports concerning the FKN-CX3CR1 axis and monocytes in inflammation-mediated pathogenesis, but their relevance to IRI-induced enhancement of rejection that occurs from an early stage after transplantation (IRI-related rejection) has not yet been fully clarified. We here assumed that FKN expression would be induced in grafts under long-term ischemia conditions and that monocytes infiltrated via the FKN-CX3CR1 axis have a significant impact on promotion of transplant rejection and graft failure. In the present study, we investigated the role of the FKN/CX3CR1 axis in a mouse model of IRI-related rejection using CX3CR1-deficient mice as recipients or an intervention with anti-mouse FKN neutralizing antibody (anti-FKN mAb), the emphasis being on the contribution of CX3CR1-positive monocytes.

MATERIALS AND METHODS

Animals

Male BALB/c and C57BL/6 mice were purchased from the Japan SLC Corporation (Hamamatsu, Japan). CX3CR1 homogenous knockout in C57BL/6 background mice was performed by KAN Research Institute (Kobe, Japan). All mice were bred and maintained under specific pathogen-free conditions at the Institute of Laboratory at Tokyo Women's Medical University (Tokyo, Japan). The pathogen-free conditions implemented were based on the criteria of the Central Institute for Experimental Animals (Kawasaki, Japan). The Tokyo Women's Medical University internal committee on the use and care of laboratory animals approved all experiments (Reference ID: AE19-081).

Ectopic Heart Transplantation

All transplant procedures were performed under general anesthesia using sevoflurane. Fully vascularized ectopic heart grafts from BALB/c donors were transplanted into C57BL/6 or CX3CR1-deficient recipients using microsurgical techniques (37). To investigate the influence of IRI on allograft rejection, ectopic heart transplantation was performed after donor hearts were preserved at 4°C for 8 h (prolonged cold ischemia: IRI) or 0.5 h (minimal cold ischemia: non-IRI control) (15). Cytotoxic T lymphocyte antigen 4-immunoglobulin (CTLA4-Ig) (ORENCIA®, Bristol-Myers Squibb, Lawrenceville, NJ, United States) was administered intraperitoneally at a dose of 0.25 mg/day on the day of transplantation (day 0) and day 1. Graft engraftment was assessed by palpation with the presence of contraction as an indicator. Rejection was defined as complete cessation of contraction.

Immunofluorescence Analysis

After perfusion fixation with 1% paraformaldehyde, heart grafts were collected and embedded with O.C.T. compound (Sakura Finetek Japan Co., Ltd, Tokyo, Japan). The tissues were cut into 6 μm-thick sections, blocked with normal donkey serum, and stained with 10 μg/ml of goat anti-rat FKN antibody (R&D Systems, Inc., Minneapolis, MN, United States) and 50-fold diluted rabbit anti-mouse CD31 antibody (Abcam Plc, Cambridge, UK). The combination of 500-fold diluted Alexa fluor 555-conjugated donkey anti-goat IgG (Abcam) and Alexa fluor 488-conjugated goat anti-rabbit IgG (Abcam) was employed as the set of secondary antibodies. Nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI).

Pathological Analysis

Grafts were procured at day 7 and hematoxylin-eosin staining was performed after fixation with 10% neutral phosphate-buffered formalin.

Administration of anti-FKN mAb

Anti-FKN mAb (clone 5H8) and control IgG (anti-dinitrophenol mAb) (30, 31, 36) were provided by KAN Research Institute. Anti-FKN mAb or control IgG was administered at 500 μg/head on days−1, 3, 7, 10, and 14.

Isolation and Adoptive Transfusion of CX3CR1-Positive Monocytes

Bone marrows recovered from 10 week-old wild-type C57BL/6 mice were treated with ammonium chloride buffer for hemolysis, and cultured for 3 days at 37°C in RPMI 1640 medium supplemented with 50 ng/ml of recombinant mouse macrophage colony-stimulating factor (R and D Systems), 10% fetal bovine serum, 0.1 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (pH 7.2–7.5), 1× MEM non-essential amino acid solution, 1 mM sodium pyruvate, 1× penicillin-streptomycin, and 100 μM 2-mercaptoethanol. Monocytes were isolated from post-culture cells using the CD115 MicroBead Kit (mouse; Miltenyi Biotec B.V. & Co. KG, Bergisch Gladbach, Germany). The isolated monocytes were transferred into CX3CR1-deficient recipients at 3×10^{6}/head on day−1.

To confirm the purity of CX3CR1-positive monocytes in the isolated cells, Fc blocking with anti-CD16/CD32 antibody (BD Bioscience, San Jose, CA, United States) was followed by staining with phycoerythrin (PE)-conjugated mouse CX3CR1 antibody and fluorescein isothiocyanate (FITC)-conjugated CD115 (AFS98; Tonbo Biosciences, San Diego, CA, United States). Anti-mouse CX3CR1 antibody (clone L2D11) was provided by KAN Research Institute. Flow cytometry analysis was carried out with a FACSCanto™ II (BD Biosciences) and FlowJo software (Tree Star, Ashland, OR, United States).

Statistical Analysis

Comparisons of graft survival were analyzed by the log-rank test using Prism seven software (GraphPad Software, La Jolla, CA, United States); differences with p values <.05 were considered significant.

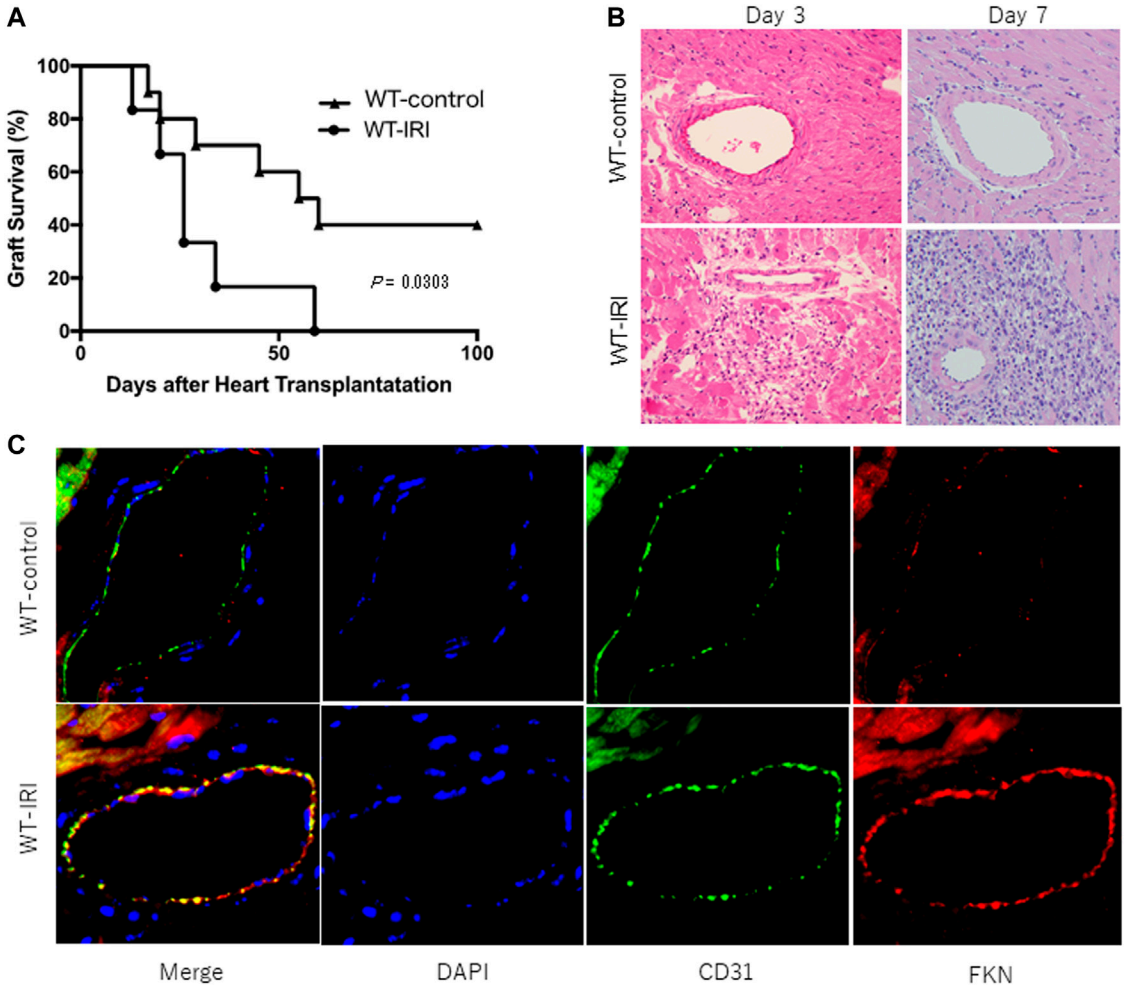


FIGURE 1 | Reduced survival of mouse heart allografts owing to ischemia-reperfusion injury caused by prolonged cold ischemia. BALB/c hearts were transplanted into wild-type C57BL/6 recipients after 8 h of preservation at 4°C (WT-IRI: $n = 6$) or 0.5 h (WT-control: $n = 10$). CTLA4-Ig was administered intraperitoneally at a dose of 0.25 mg/day on the day of transplantation (day 0) and on day 1. **(A)** Graft survival. The comparison between groups was analyzed via the log-rank test. **(B)** Hematoxylin-eosin-stained images of graft tissues at day 3 and 7. **(C)** Expression of fractalkine (FKN) in graft tissues at day 3. Immunofluorescence staining was performed using anti-FKN (red) and anti-CD31 antibodies (green). Nuclei were stained with DAPI (blue).

RESULTS

Enhanced Acute Rejection and Increased Expression of FKN on Vascular Endothelial Cells in Allografts Subjected to Longer Cold Ischemia.

To investigate the influence of IRI on allograft rejection, ectopic heart transplantation was performed on wild-type C57BL/6 recipients after donor hearts were preserved at 4°C for 8 h (WT-IRI) or 0.5 h (WT-control) and graft survival was compared between the two groups. As shown in **Figure 1A**, graft survival in the WT-IRI group was shorter than that in the WT-control group (median survival time (MST): WT-IRI = 26.0 days vs WT-control = 57.5 days; $p = .0303$, log-rank test). Histopathological findings at day 3 and 7 showed more severe cell infiltration in the WT-IRI group than in the WT-control group (**Figure 1B**). These results suggest that IRI was associated with enhanced graft rejection from the initial process (IRI-related rejection) and shortened graft survival in the WT-IRI group. To compare the expression of FKN in graft tissues between both groups, an immunofluorescence assay using anti-FKN antibody was performed on heart allografts at day 3. As shown in **Figure 1C**, FKN was strongly expressed on vascular endothelial cells in the WT-IRI group, which showed enhanced rejection and shortened graft survival compared to the WT control group, whereas less FKN signal was detected in the WT control group. In conclusion, the induction of FKN expression in vascular endothelial cells in graft tissues was

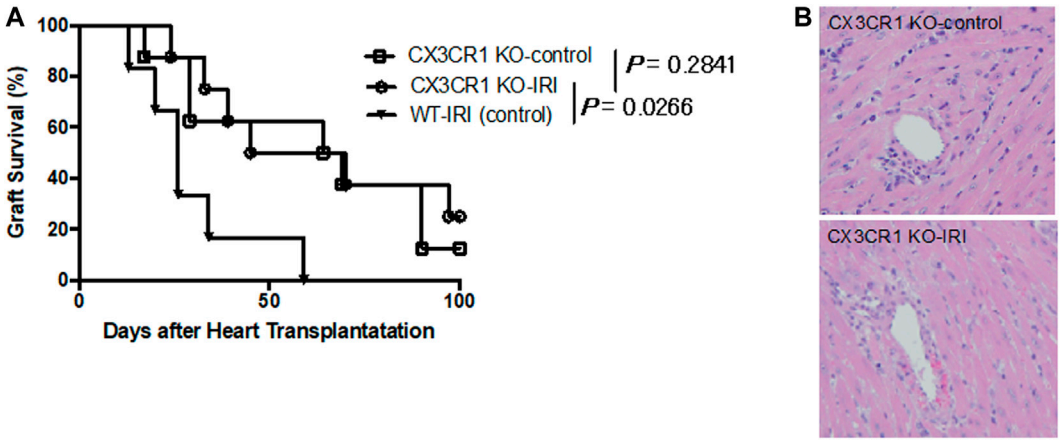


FIGURE 2 | Loss of influence of ischemia-reperfusion injury on graft survival in CX3CR1-deficient recipients. CX3CR1-deficient mice (C57BL/6 background) were transplanted with BALB/c donor hearts preserved at 4°C for 8 h (CX3CR1 KO-IRI: n = 8) or 0.5 h (CX3CR1 KO-control: n = 8). CTLA4-Ig was administered intraperitoneally at a dose of 0.25 mg/day on the day of transplantation (day 0) and day 1. **(A)** Graft survival. The CX3CR1 KO-IRI group was compared with the CX3CR1 KO-control group or the WT-IRI group (see **Figure 1**). The comparisons were analyzed *via* the log-rank test.**(B)** Hematoxylin-eosin-stained images of graft tissues at day 7.

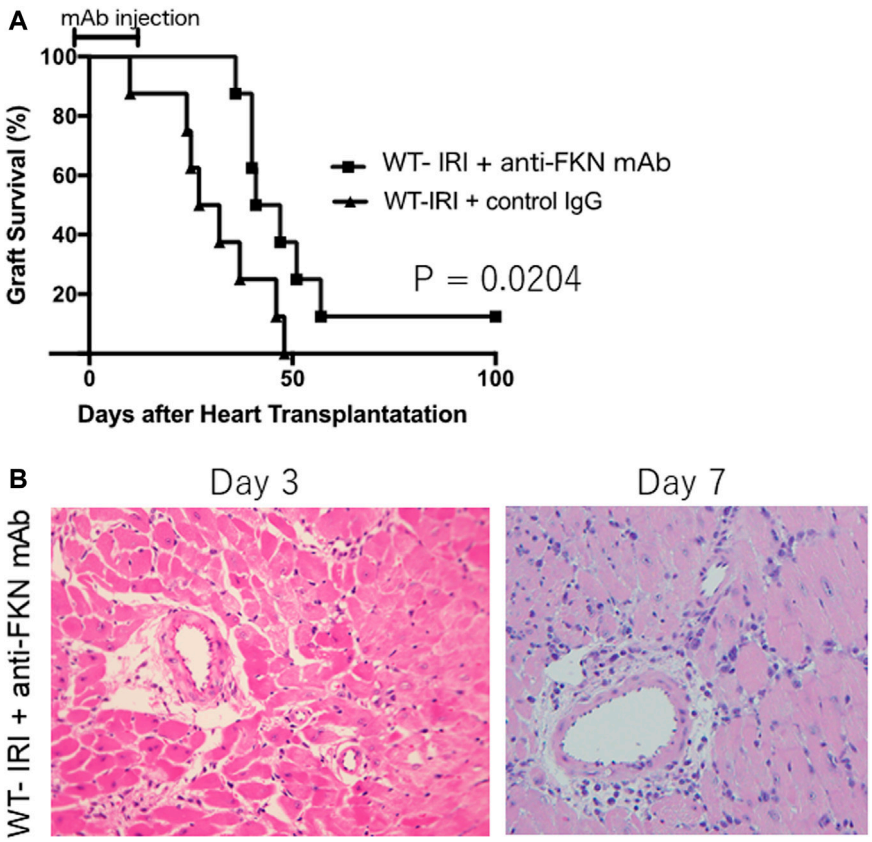


FIGURE 3 | Preventive effect of anti-fractalkine monoclonal antibody (anti-FKN mAb) treatment on reduced allograft survival caused by cold ischemia. We transplanted BALB/c hearts preserved for 8 h at 4°C into wild-type C57BL/6 mice and treated with anti-FKN mAb (WT-IRI + anti-FKN mAb: n = 8) or control IgG (WT-IRI + control IgG: n = 8). Anti-FKN mAb clone 5 H8 was administered at 500 μg on the day before transplantation (day−1) and on days 3, 7, 10, and 14. CTLA4-Ig was administered intraperitoneally at a dose of 0.25 mg/day on days 0 and 1. **(A)** Graft survival. The comparison between groups was analyzed via the log-rank test. **(B)** A hematoxylin-eosin-stained image of graft tissues at day 3 and 7 in the WT-IRI + anti-FKN mAb group.

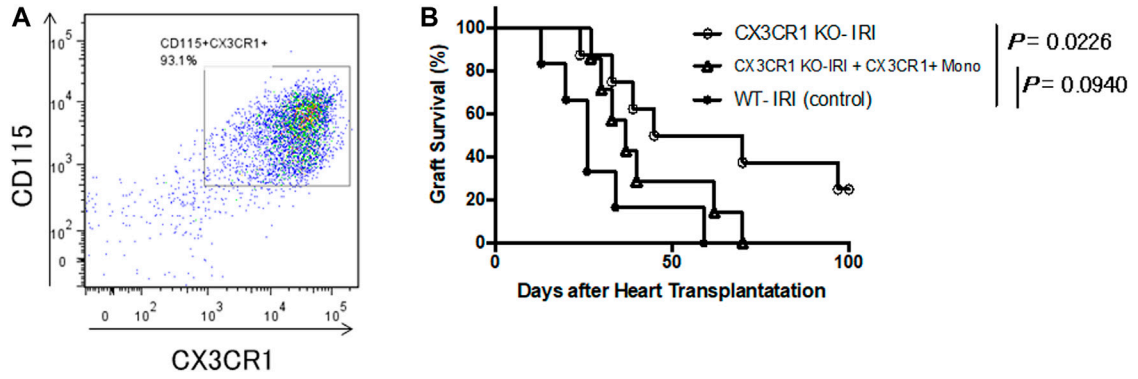


FIGURE 4 | Restoration of the negative influence of cold ischemia on graft survival in CX3CR1-deficient recipients with adoptive transfer of CX3CR1-positive monocytes. Monocytes were purified from wild-type C57BL/6 bone marrow cells stimulated with macrophage colony-stimulating factor using magnetic beads conjugated with anti-CD115 monoclonal antibody. Approximately 3×10^6 of the isolated monocytes were transferred to CX3CR1-deficient mice and the next day, BALB/c hearts preserved at 4°C for 8 h were transplanted (CX3CR1 KO-IRI + CX3CR1$^+$ Mono: $n = 7$). CTLA4-Ig was administered intraperitoneally at a dose of 0.25 mg/day on the day of transplantation (day 0) and day 1. **(A)** Flow cytometric analysis of the isolated monocytes using anti-CD115 and anti-CX3CR1 antibodies. **(B)** The comparison of graft survival between the CX3CR1 KO-IRI + CX3CR1$^+$ Mono group and the CX3CR1 KO-IRI (see **Figure 3**) or the WT-IRI group (see **Figure 1**) was analyzed via the log-rank test.

dependent on IRI, which might affect IRI-related rejection and shortening graft survival.

Lack of Influence of IRI on Allograft Survival in CX3CR1-Deficient Recipients

To investigate the relevance of the FKN receptor, CX3CR1, with respect to augmentation of acute rejection associated with cold ischemia, we transplanted BALB/c donor hearts preserved at 4°C for 8 h (CX3CR1 KO-IRI) or 0.5 h (CX3CR1 KO-control) into CX3CR1-deficient recipients. As shown in **Figure 2A**, there is no significant difference in graft survival between the CX3CR1 KO-IRI group (MST: 57.5 days) and the CX3CR1 KO-control group (MST: 66.5 days). Inflammatory cell infiltration in grafts at day 7 in both groups was similarly mild (**Figure 2B**). Furthermore, graft survival of the CX3CR1 KO-IRI group was significantly longer than that of the WT-IRI group ($p = .0226$, log-rank test) (**Figure 2A**). These results suggest that the presence of CX3CR1 molecules in recipients would be essential for IRI-related rejection.

Improved Survival of Allografts Subjected to IRI in Wild-Type Recipients Receiving Anti-FKN mAb

To further investigate the association between the FKN-CX3CR1 axis and IRI-related augmentation of rejection, we compared the survival of heart grafts subjected to cold ischemia in wild-type recipients with anti-FKN mAb treatment (WT-IRI + anti-FKN mAb) to those with control IgG treatment (WT-IRI + control IgG). As shown in **Figure 3A**, the WT-IRI + anti-FKN mAb group had a longer survival than the WT-IRI + control IgG group (MST: WT-IRI + anti-FKN mAb = 40.5 days vs WT-IRI + control IgG = 29.5 days; $p = .0204$, log-rank test). This result indicates that anti-FKN mAb treatment improves graft survival shortened by cold-ischemia conditions, confirming the importance of the FKN-CX3CR1 axis in IRI-related rejection. We compared the graft tissues at day 3 and 7 between the WT-IRI + anti-FKN mAb group (**Figure 3B**) and the WT-IRI group (**Figure 1B**) and found that the administration of anti-FKN mAb resulted in milder cellular infiltration (**Figure 3B**). These results indicate that blockade of the FKN-CX3CR1 axis inhibits IRI-related rejection.

Importance of CX3CR1-Positive Monocytes in IRI-Related Rejection

We focused on CX3CR1-positive monocytes as effector cells essential for enhancing acute rejection of grafts subjected to prolonged cold ischemia. C57BL/6 bone marrow cells were cultured under M-CSF stimulation for 3 days, and monocytes were purified with anti-CD115 mAb. Flow cytometry analysis showed that more than 93% of the isolated cells were CX3CR1-positive monocytes (**Figure 4A**). Approximately 3×10^6 of the isolated cells were transferred into CX3CR1-deficient mice. The next day, donor hearts preserved for 8 h at 4°C were transplanted into the mice (CX3CR1 KO-IRI + CX3CR1$^+$ Mono). As shown in **Figure 4B**, graft survival in the CX3CR1 KO-IRI group was significantly prolonged as compared to that in the WT-IRI group; adoptive transfer of CX3CR1-positive monocytes attenuated the effect of prolongation of graft survival. As a result of CX3CR1-positive monocyte infusion, there was no significant difference in graft survival between the CX3CR1 KO-IRI + CX3CR1$^+$ Mono group (MST: 37.0 days) and the WT-IRI group (MST: 26.0 days). These results indicate that CX3CR1-positive monocytes are important effector cells in IRI-related rejection.

DISCUSSION

Prevention and mitigation of the impact of IRI is critical for protection against graft dysfunction and improvement of graft survival if the grafts have been ischemic for a long-time following

recovery from donors. For this purpose, regulation of immune cell infiltration in grafts during the initial process would be highly effective. The FKN-CX3CR1 axis plays an important role in immune defense by controlling the migration and adhesion of various types of immune cells involved in immune responses at inflammatory sites or infected areas. Furthermore, the FKN-CX3CR1 axis has been reported to be involved in the development of inflammation-associated diseases and pathological conditions. Using a murine ectopic transplantation model with hearts subjected to cold ischemia, the present study clarified the importance of the FKN-CX3CR1 axis and CX3CR1-positive monocytes in IRI-related rejection, and then demonstrated the potential of the FKN-CX3CR1 axis as a target for prevention of post-transplant graft dysfunction and rejection of long-term preserved grafts, such as those recovered from deceased donors.

In the present study, we first analyzed the influences of cold ischemic time on grafts in a mouse heart transplantation model. The comparison between the grafts preserved for 8 h at 4 °C (IRI) and 0.5 h (non IRI; control) showed that graft survival in the WT-IRI group was significantly shorter than that in the WT-control group (**Figure 1A**), and more severe cell infiltration was observed in the graft pathology of the WT-IRI group as early as day 3 (**Figure 1B**). FKN expression on vascular endothelial cells was detected at day 3 in the WT-IRI group, but minimally in the WT-control group (**Figure 1C**). These results suggest that FKN expression on graft vascular endothelial cells increases infiltrating cells from an early stage and correlates with the severity of IRI-related rejection. It has been previously reported that the expression of FKN on endothelial cells was induced by proinflammatory cytokines, such as IL-1, IFN-γ, and TNF-α (16). Cold ischemia induced activation of the transcription factor, NF-κB, and consequently elevated expression of TNF-α in rat liver allografts (38). The increased levels of IL-1β, IL-6, and TNF-α in human renal graft vein plasma were observed during reperfusion after cold ischemia (39). From the previous findings and our own data here, we infer that in the WT-IRI group, the expression of FKN on endothelial cells would be facilitated by proinflammatory cytokines greatly induced in grafts subjected to IRI. Proinflammatory cytokines also regulate expression of various cell adhesion molecules (40, 41). Cold ischemia leads to expression of P-selectin and ICAM-1 on the endothelium, and augments allogeneic-mediated cell infiltration in rat kidney allografts (42). ICAM-1 antisense oligodesoxynucleotides prevent reperfusion injury and enhance immediate graft function during renal transplantation (13). The engagement of the FKN-CX3CR1 and integrin-ICAM-1 axes enhanced cell adhesion compared to each axis alone (24, 32, 43). Within the allografts subjected to cold ischemia in the WT-IRI group, cooperation of FKN with other molecules associated with cell adhesion would result in increasing infiltrating cells that correlates with the severity of IRI-related rejection.

Graft rejection in the present model is further complicated by influences of allogeneic immunity and IRI caused by cold ischemia. In this study, all experimental groups received intraperitoneal administration of CTLA4-Ig at a dose of 0.25 mg on the day of heart transplantation and the next day. CTLA4-Ig suppresses priming of alloimmune responses by inhibiting T-cell activation mediated by the $CD28^{-}CD80/CD86$ co-stimulatory signals in antigen presentation (44–46) but reportedly has little in the way of suppressive effects on transplant rejection of allografts subjected to cold ischemia (15). In our preliminary study of recipients not receiving CTLA4-Ig, both grafts preserved for 8 h at 4°C and 0.5 h were rejected within 7 days and there was no difference in graft survival between them (data not shown). Acute rejection in these groups is thought to be predominantly caused by alloimmune responses owing to T-cell activation, which was independent of the influences of cold ischemia. The difference in graft survival between the WT-IRI and WT-control groups detected in the present study would be mainly based on IRI as they all survived over 7 days, the maximum survival when not receiving CTLA4-Ig that mitigates the effect of allogeneic immunity.

Next, to examine the importance of the FKN-CX3CR1 axis for graft rejection in cold-ischemic heart transplantation, we analyzed changes in graft survival employing CX3CR1-deficient mice as recipients or treatment with anti-FKN monoclonal antibodies. As shown in **Figure 2A**, in cases of transplantation with hearts subjected to cold ischemia for 8 h, CX3CR1-deficient recipients (CX3CR1 KO-IRI) exhibited prolonged graft survival compared to wild-type recipients (WT-IRI). As previously demonstrated, there is a significant difference in graft survival between donor hearts subjected to cold ischemia or not in wild-type recipients (**Figure 1A**: WT-IRI vs WT-control), but not in CX3CR1-deficient recipients (**Figure 2A**: CX3CR1 KO-IRI vs CX3CR1 KO-control). These results indicate that the loss of the FKN receptor, CX3CR1, almost completely abolishes the negative influence of cold ischemia on graft survival. Furthermore, when anti-FKN mAb was administered to the wild-type recipients transplanted with hearts subjected to prolonged cold ischemia (WT-IRI + anti-FKN mAb), a significant improvement in graft survival was observed (**Figure 3**). Taken together with these findings, the FKN-CX3CR1 axis plays a major role in IRI-related rejection.

Although we showed evidence herein for the relationships involving the FKN-CX3CR1 axis-mediated promotion of graft rejection owing to IRI, the importance of the FKN-CX3CR1 axis in allogeneic acute rejection has already been reported. During mouse heart transplantation, FKN expression was increased in the rejecting allografts and was prominent on vascular tissues and endothelium at early time points. Anti-FKN or anti-CX3CR1 antibodies inhibited the adhesion of peripheral blood mononuclear cells to the vascular endothelium, and treatment with anti-CX3CR1 antibody significantly prolonged survival of mouse cardiac allografts (29). Moreover, when CX3CR1 knockout mice were used as recipients, graft survival was prolonged in the presence of subtherapeutic levels of cyclosporin A with a concomitant reduction in infiltrating macrophages, NK cells, and other leukocytes observed in the grafts (28). These studies highlighted the importance of the FKN-CX3CR1 axis during the pathogenesis of

acute transplant rejection. Based on these findings along with the present data, the FKN-CX3CR1 axis appears to be an effective target for not only preventing acute rejection but also controlling IRI-dependent promotion of rejection responses, suggesting that neutralizing antibodies or other blockers targeting the axis could contribute to protect the long-term preserved donor organs from graft failure.

Next, to evaluate the potential of CX3CR1-positive monocytes as immune cells that exert effector function during IRI-related rejection, CX3CR1-deficient recipients adoptively transferred with monocytes isolated from wild-type mice were transplanted with donor hearts subjected to prolonged cold ischemia (CX3CR1 KO-IRI + CX3CR1$^+$ Mono). As shown in **Figure 4B**, no significant difference was found in graft survival between the CX3CR1 KO-IRI + CX3CR1$^+$ Mono and WT-IRI groups. Moreover, CX3CR1-deficient recipients without transferring monocytes (CX3CR1 KO-IRI) exhibited prolonged survival compared to the WT-IRI group (**Figure 1A**). These results demonstrate that CX3CR1-positive monocytes play an important role in IRI-related rejection in the present model, suggesting that inhibition of monocyte migration through the FKN-CX3CR1 axis may have contributed to the improvement in graft survival shown using CX3CR1-deficient mice as recipients or by treatment with anti-FKN mAb. It has been reported that acute allograft dysfunction is closely related to monocyte infiltration and the monocyte/macrophage lineage cells function as effectors of allograft damage and activate allogeneic responses during acute allograft rejection (47–50). Monocytes that infiltrated into allografts through the FKN-CX3CR1 axis may have contributed to enhancement of graft rejection through similar mechanisms. Conversely, survival rates of the CX3CR1 KO-IRI + CX3CR1$^+$ Mono group were slightly higher than those of the WT-IRI group (**Figure 4B**), suggesting that a transfer of CX3CR1-positive monocytes alone may not fully restore the influence of IRI on grafts exerted in the WT-IRI group. In addition to monocytes, CX3CR1 is known to be expressed in effector memory CD8$^+$ T cells (51). Reportedly, there was a direct association between increased durations of cold ischemic allograft preservation and numbers/enhanced functions of early graft-infiltrating endogenous memory CD8$^+$ T cells, which directly mediate rejection of allografts subjected to prolonged ischemia (15). Although the present data have demonstrated the crucial role of CX3CR1-positive monocytes in graft damage correlated with reduced survival of cold-preserved allografts, analysis of the involvement of other CX3CR1-positive cells is an important topic for future research.

Thus, although there is still room for analysis of immune cells correlated with enhancement of rejection owing to cold ischemia, the importance of the FKN-CX3CR1 axis was clearly demonstrated in the present study. These results strongly suggest that the FKN-CX3CR1 axis may be useful as an interventional target for prophylaxis and therapy to improve survival of allografts affected by IRI. As an initial attempt at clinical application of FKN-CX3CR1 blockades, a humanized mAb against FKN, E6011, has been evaluated in a clinical trial for rheumatoid arthritis. No serious adverse events or deaths were reported in this study, indicating that the FKN-CX3CR1 blockade intervention is safe and well-tolerated, and may have a positive clinical effect in patients with highly active rheumatoid arthritis (52, 53). Based on the present study, clinical applications of FKN-CX3CR1 blockades even in the field of transplantation would be expected, and anti-FKN antibodies hold great promise as an interventional approach to protect grafts that have been left in an ischemic state for a long-time during preservation, such as organs from deceased donors, and to improve the outcomes of organ transplantation.

There are potential limitations to the present study. The following test have not been performed: 1) to compare the early-stage histopathology of the CX3CR1 KO-control, CX3CR1 KO-IRI and WT-IRI groups, 2) to compare the donor-reactive memory CD8$^+$ T cells of wild-type and CX3CR1-KO mice, and 3) to confirm the infiltration of transplanted monocytes into graft tissues and early-stage histopathology. These rigorous analyses are necessary to further validate the role of the FKN-CX3CR1 axis in IRI-related rejection in the future.

In conclusion, ischemia in donor organs over time with respect to transplantation leads to exacerbation of graft rejection via impaired reperfusion after transplantation. CX3CR1-positive monocytes and the FKN-CX3CR1 axis play important roles in this series of tissue disorders that significantly affect allograft survival. Blockade of the FKN-CX3CR1 axis by anti-FKN antibodies or other means reduces the impact of IRI-related rejection and could be an effective intervention to improve the outcomes of deceased donor transplantation.

CAPSULE SENTENCE SUMMARY

This study evaluated the significance of the FKN-CX3CR1 pathway during ischemia-reperfusion injury (IRI)-related graft rejections using a mouse heterotopic heart transplantation model. We believe that our study makes a significant contribution to understanding the mechanism because the roles of the FKN-CX3CR1 axis in IRI-related rejection were directly investigated using the transplant model with CX3CR1-deficient recipients (CX3CR1-KO IRI) or treatment with anti-mouse FKN monoclonal antibodies. Our findings indicated that the FKN-CX3CR1 axis plays a major part during IRI-related rejection and its blockade has the potential to improve the outcomes of deceased donor transplantation. Further, we believe that this paper will be of interest to the readership of your journal because it is known the outcome of transplantation is affected by the promotion of rejection associated with IRI. Our findings suggest there is a way to potentially mitigate this phenomenon and enhance the acceptance of graft transplantations from deceased donors.

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 authors.

ETHICS STATEMENT

The animal study was reviewed and approved by The Tokyo Women's Medical University internal committee on the use and care of laboratory animals (Reference ID: AE19-081).

AUTHOR CONTRIBUTIONS

TK, DT, KS, TY, HI, HF, HK, SM, RI, TH, TI, MO, and KT designed the study and performed data interpretation. TK, TY, HI, HF, HK, SM, and RI performed study and data analysis. TK, DT, KS, and TI wrote the manuscript. All authors critically revised the report, commented on drafts of the manuscript, and approved final report.

CONFLICT OF INTEREST

Author TI is employed by the company KAN Research Institute, Inc.

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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ORIGINAL RESEARCH
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Matching Donor and Recipient Size in Pediatric Heart Transplantation

Tajinder P. Singh[1,2]*, *Steven D. Colan*[1,2] *and Kimberlee Gauvreau*[1,3]

[1]Department of Cardiology, Boston Children's Hospital, Boston, MA, United States, [2]Department of Pediatrics, Harvard Medical School, Boston, MA, United States, [3]Department of Biostatistics, Harvard School of Public Health, Boston, MA, United States

Previous analyses in pediatric heart transplant (HT) recipients using weight or height have not found donor-recipient size-mismatch to be associated with post-transplant mortality. A recent study in 3,215 normal US children developed an equation for left ventricular (LV) mass using body surface area (BSA). We assessed whether donor-recipient size match using predicted LV mass (PLM) is associated with post-transplant in-hospital mortality or 1-year graft survival. We identified 4,717 children <18 yrs old who received primary HT in the US during 01/2000 to 03/2015 and divided them into five groups [10%, 10%, 60% (reference group), 10% and 10%, respectively] with increasing donor-recipient PLM ratio. In adjusted analysis, group 1 children (PLM ratio ≤.90) were at higher risk of post-transplant in-hospital mortality [Odds Ratio (OR) 1.55, 95% CI 1.04, 2.31]. This association of the most undersized donors with recipient in-hospital mortality was similar when donor-recipient weight ratio<.88 or BSA ratio<.92 (lowest decile) were used instead. There was no difference in 1-year graft survival among groups. Utilizing donors with donor-recipient PLM ratio ≤.90 is associated with higher risk of early post-transplant mortality in pediatric HT recipients. However, this metric is not superior to donor-recipient weight ratio or BSA ratio for assessing size match.

Keywords: heart transplant, children, pediatric, survival, donor selection, outcomes

INTRODUCTION

Transplant centers routinely provide a weight-range for an acceptable donor when listing a candidate for heart transplant (HT). This range is often 80%–200% of the recipient weight in children. Donor-recipient (DR) height match may also be considered when reviewing a donor offer. Size match using body measurements is essentially an attempt to match the donor and the recipient for their "normal" or "predicted" heart size to allow adequate mediastinal space for the donor heart and a donor heart that is able to support the recipient circulation after removal of the diseased heart. Previous analyses in pediatric HT recipients using weight or height to assess the effect of DR size match have shown either absent or only a marginal association of DR size-mismatch with recipient survival (1–3). This may be explained by a cautious selection of donors by pediatric HT community over the years such that a large enough sample of size-mismatched DR pairs to demonstrate an effect on graft outcomes does not exist. It may also be that body measurements such as weight or height may not be the best metrics to assess the association of DR size mismatch with outcomes.

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***Correspondence:**
Tajinder P. Singh
tp.singh@cardio.chboston.org

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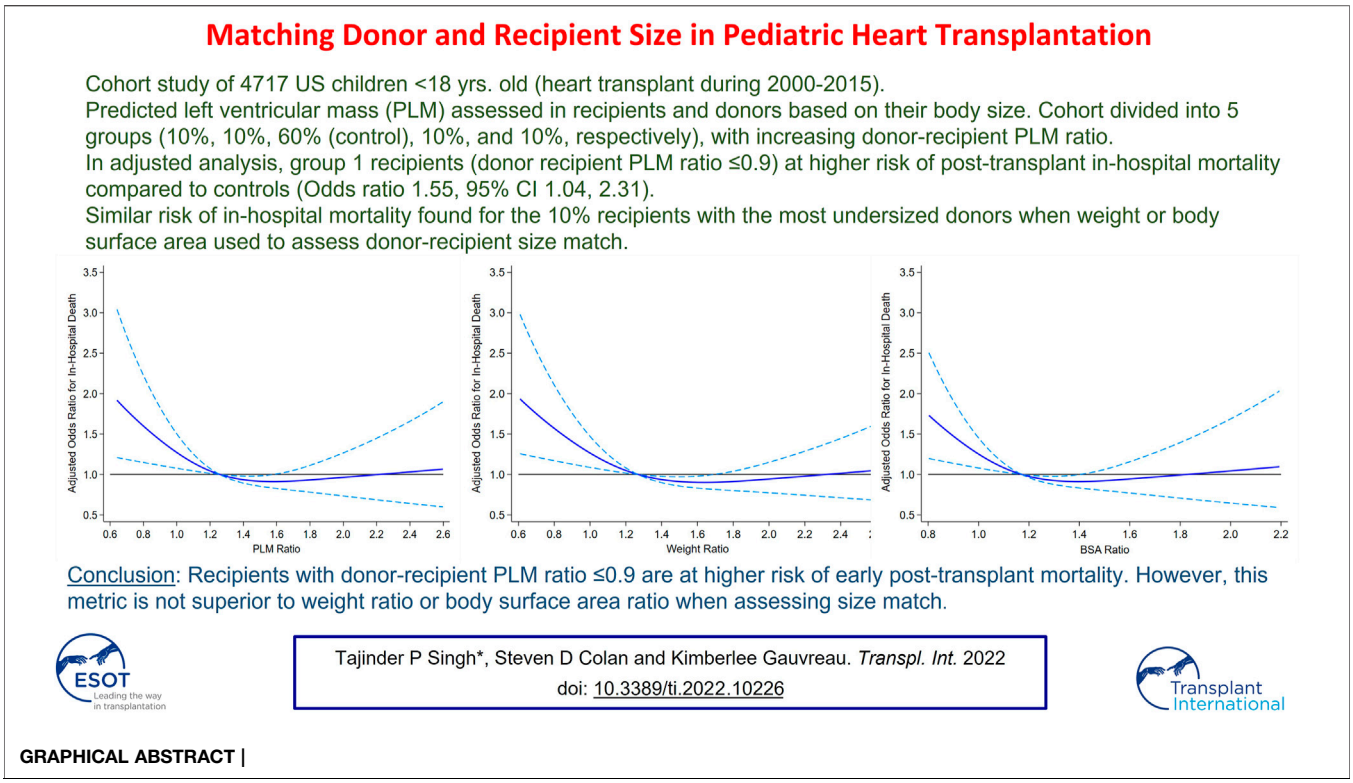
Abbreviations: BSA, body surface area; CI, confidence interval; DR, donor-recipient; GFR, glomerular filtration rate; HR, hazard ratio; HT, heart transplant; LV, left ventricle; MRI, magnetic resonance imaging; OR, odds ratio; OPTN, Organ Procurement and Transplantation Network; PLM, predicted LV mass ratio; RV, right ventricle; US, United States.



GRAPHICAL ABSTRACT |

Until recently, the practice of selecting donor size in adult HT candidates has been similar to the pediatric practice. After investigators of a population-based study (Multi Ethnic Study of Atherosclerosis) in the United States (US) performed cardiac magnetic resonance imaging (MRI) in healthy adults to develop normative equations for left ventricular (LV) and right ventricular (RV) mass using age, gender, height and weight (4, 5), several HT investigators have evaluated the role of predicted heart mass (predicted LV mass + predicted RV mass) as a potential metric for DR size match in adult HT. These analyses have found that recipients with hearts from undersized donors using this metric had significantly worse 1-year HT survival whereas size match assessed using weight, height, BSA or body mass index in the same patient population was not related (6). The superiority of assessing DR size match using predicted heart mass in adult HT recipients was also described in the 2019 annual report of the International Thoracic Registry (7). However, similar analyses were not performed in pediatric HT recipients because MRI-based values of RV or LV mass in normal children are limited to small studies and are not generalizable (3).

A recent Pediatric Heart Network study in 3,215 healthy, racially-diverse US children with adequate representation across the pediatric age range published an equation for left ventricular (LV) mass using body surface area (BSA) which can be used to estimate/predict LV mass for normal children with BSA of the HT recipient and the donor (8). Because LV mass is the dominant contributor to the heart mass after the neonatal period and potentially a surrogate for predicted heart mass (if value for RV mass is not available), we hypothesized that predicted LV mass (PLM) is a better metric for assessing DR size match compared to DR body measurements used in clinical practice and that DR size mismatch using PLM will be associated with short-term pediatric HT outcomes.

The specific aims of this study were 1) to assess the association of DR size match using predicted LV mass with post-transplant in-hospital mortality and 1-year graft survival in pediatric HT recipients and, 2), to compare its performance to the association of DR size-match using weight, height and BSA ratio with these outcomes in the same cohort.

MATERIALS AND METHODS

Study Subjects

We identified all children <18 years old in the Organ Procurement and Transplant Network (OPTN) database who received first HT in the US between January 1st, 2000 and March 31st, 2015. Children who received heart re-transplant or multi-organ transplant were excluded. We also excluded recipients with missing weight or height for the recipient or the donor. The OPTN database includes baseline information at transplant and follow-up data in all recipients in the US submitted by transplant centers. These data are supplemented with death data from the social security master death file and are provided as de-identified data by the United Network for Organ Sharing to investigators. Post-transplant follow-up was available until March 31st, 2016, allowing 1 year of follow-up in all study children. The institutional review board determined that the study was not human subjects research as defined by US federal regulations.

TABLE 1 | Baseline characteristics of study children with increasing donor-recipient PLM ratio.

Variable	PLM Ratio0.55–0.90 (Group 1)	PLM Ratio0.91–1.00 (Group 2)	PLM Ratio1.01–1.60 (Group 3)	PLM Ratio1.61–1.83 (Group 4)	PLM Ratio1.84–3.40 (Group 5)	*p* value
	(*n* = 472)	(*n* = 472)	(*n* = 2,829)	(*n* = 472)	(*n* = 472)	
Age at transplant (years)						<.001
<1	149 (32%)	99 (21%)	759 (27%)	176 (37%)	214 (45%)	
1–10	202 (43%)	188 (40%)	1,023 (36%)	178 (38%)	192 (41%)	
11–17	121 (25%)	185 (39%)	1,047 (37%)	118 (25%)	66 (14%)	
Sex Male	255 (54%)	245 (52%)	1,527 (54%)	264 (56%)	289 (61%)	.032
Race/Ethnicity						.008
White	247 (52%)	266 (56%)	1,543 (55%)	257 (54%)	301 (64%)	
Black	104 (22%)	104 (22%)	595 (21%)	84 (18%)	73 (15%)	
Hispanic	92 (20%)	65 (14%)	494 (17%)	95 (20%)	68 (14%)	
Other	29 (6%)	37 (8%)	197 (7%)	36 (8%)	30 (6%)	
Blood type						.26
O	235 (50%)	207 (44%)	1,252 (44%)	217 (46%)	226 (48%)	
A	166 (35%)	188 (40%)	1,088 (38%)	179 (38%)	161 (34%)	
B	51 (11%)	54 (11%)	385 (14%)	56 (12%)	60 (13%)	
AB	20 (4%)	23 (5%)	104 (4%)	20 (4%)	25 (5%)	
Diagnosis						<.001
Dilated CMP	217 (46%)	214 (45%)	1,282 (45%)	179 (38%)	181 (38%)	
Non-dilated CMP	40 (8%)	55 (12%)	251 (9%)	31 (7%)	26 (6%)	
CHD repaired	161 (34%)	156 (33%)	979 (35%)	201 (43%)	183 (39%)	
CHD unrepaired	35 (7%)	31 (7%)	200 (7%)	42 (9%)	66 (14%)	
Other	19 (4%)	16 (3%)	117 (4%)	19 (4%)	16 (3%)	
Status at transplant						<.001
1A	379 (80%)	356 (75%)	2,251 (80%)	401 (85%)	409 (86%)	
1B	42 (9%)	56 (12%)	315 (11%)	47 (10%)	36 (8%)	
2	51 (11%)	60 (13%)	263 (9%)	24 (5%)	27 (6%)	
Ventilator	100 (21%)	66 (14%)	471 (17%)	107 (23%)	139 (29%)	<.001
Mechanical support						<.001
ECMO	30 (6%)	26 (6%)	153 (5%)	42 (9%)	46 (10%)	
BIVAD	25 (5%)	19 (4%)	164 (6%)	19 (4%)	19 (4%)	
LVAD	50 (11%)	51 (11%)	283 (10%)	29 (6%)	37 (8%)	
Inotropes	233 (49%)	240 (51%)	1,407 (50%)	268 (57%)	268 (57%)	.005
Bilirubin (mg/dl)	0.6 [0.3, 1.0]	0.7 [0.4, 1.1]	0.7 [0.4, 1.2]	0.6 [0.4, 1.3]	0.7 [0.4, 1.6]	<.001
Renal dysfunction						.042
Normal	409 (87%)	419 (89%)	2,439 (86%)	398 (84%)	383 (81%)	
Moderate	44 (9%)	38 (8%)	270 (10%)	50 (11%)	68 (14%)	
Severe	19 (4%)	15 (3%)	120 (4%)	24 (5%)	21 (4%)	
PRA (%)						.009
≤10	389 (82%)	375 (79%)	2,220 (78%)	387 (82%)	394 (83%)	
11–25	20 (4%)	38 (8%)	173 (6%)	20 (4%)	15 (3%)	
>25	63 (13%)	59 (13%)	436 (15%)	65 (14%)	63 (13%)	
Medicaid insurance	214 (45%)	194 (41%)	1,173 (41%)	204 (43%)	201 (43%)	.56
Year of transplant						.008
2000–2002	72 (15%)	77 (16%)	423 (15%)	65 (14%)	95 (20%)	
2003–2005	76 (16%)	63 (13%)	489 (17%)	98 (21%)	86 (18%)	
2006–2008	84 (18%)	91 (19%)	549 (19%)	102 (22%)	105 (22%)	
2009–2011	100 (21%)	103 (22%)	632 (22%)	94 (20%)	85 (18%)	
2012–2015	140 (30%)	138 (29%)	736 (26%)	113 (24%)	101 (21%)	
Donor age (years)						<.001
<1	197 (42%)	122 (26%)	648 (23%)	86 (18%)	52 (11%)	
1–10	179 (38%)	194 (41%)	1,046 (37%)	211 (45%)	267 (57%)	
11–17	79 (17%)	130 (28%)	656 (23%)	68 (14%)	57 (12%)	
≥18	17 (4%)	26 (6%)	479 (17%)	107 (23%)	96 (20%)	
Donor ischemic time (hours)						.039
<4	300 (64%)	295 (62%)	1826 (65%)	294 (62%)	260 (55%)	
≥4	152 (32%)	159 (34%)	893 (32%)	157 (33%)	187 (40%)	
Not reported	20 (4%)	18 (4%)	110 (4%)	21 (5%)	25 (5%)	
Donor-recipient weight ratio	0.83 [0.77, 0.88]	0.96 [0.92, 1.00]	1.27 [1.12, 1.46]	1.82 [1.69, 1.92]	2.24 [2.04, 2.50]	<.001
Donor-recipient height ratio	0.91 [0.85, 0.97]	0.97 [0.92, 1.02]	1.08 [1.01, 1.16]	1.23 [1.15, 1.32]	1.38 [1.27, 1.51]	<.001
Donor-recipient BSA ratio	0.87 [0.84, 0.90]	0.97 [0.95, 0.99]	1.17 [1.08, 1.29]	1.51 [1.47, 1.56]	1.75 [1.66, 1.89]	<.001
Male recipient/Female donor	103 (22%)	95 (20%)	595 (21%)	108 (23%)	136 (29%)	.004

Data are expressed as number (%) or median (interquartile range), PLM, predicted left ventricular mass; CMP, cardiomyopathy; CHD, congenital heart disease; ECMO, extracorporeal membrane oxygenation; BIVAD, biventricular assist device; LVAD, left ventricular assist device; PRA, panel reactive antibody; BSA, body surface area.

Study Design and Variables

This was a retrospective cohort study. Data were analyzed during March-December 2020. Two primary endpoints, post-transplant in-hospital mortality and graft loss during the first post-transplant year (time to death or re-transplant) were evaluated. The primary predictor was donor-recipient PLM ratio (=donor PLM divided by recipient PLM). This report follows the Strengthening the Reporting of Observational Studies in Epidemiology reporting guideline (9).

For all study subjects, BSA was calculated using the Haycock formula [BSA = 0.024265 x height (cm)$^{0.3964}$ x weight (kg)$^{0.5378}$] for both the recipient and the donor. This was used to generate PLM for all recipients and for donors up to 18 years old as follows (8):

Predicted LV mass = 53.02 × BSA$^{1.25}$

For donors >18 years old (because pediatric equation is not validated), we calculated donor PLM using the MRI-derived adult equation (4):

PLM (>18 years) = a × Height$^{0.54}$ (m) × Weight$^{0.61}$ (kg)), where a = 6.82 for women, 8.25 for men.

Demographic and clinical variables were defined at transplant. Race/ethnicity was recorded as reported by center and analyzed as White (non-Hispanic White), Black (non-Hispanic Black), Hispanic or Other. Renal function was analyzed as estimated glomerular filtration rate (GFR, in ml/min/1.73 m^2) using serum creatinine and the modified Schwartz equation (10). For children ≥1 year old, normal renal function was defined as GFR >60, moderate dysfunction as GFR 30–60, and severe dysfunction as GFR <30 or dialysis support. For infants <1 year old, normal renal function was defined as GFR >40, moderate dysfunction as GFR 20–40, and severe dysfunction as GFR <20 or dialysis support (11).

No subject had missing data for the variables age, gender, race/ethnicity, cardiac diagnosis, blood type, hemodynamic support (inotrope support, ventilator, type of mechanical support), health insurance (i.e., Medicaid), dialysis and the dates of transplant, death or re-transplant. For children with missing values of serum creatinine (2%) or bilirubin (7%), we used a multiple imputation technique to impute their GFR and serum bilirubin respectively using clinical variables at transplant and 10 imputations for each missing value (12).

Statistical Analysis

Baseline characteristics are presented as median (Interquartile range, IQR) or number (percent). Study subjects were divided into five groups with increasing donor-recipient PLM ratio consisting of 10%, 10%, 60% (reference group), 10% and 10%, respectively of study subjects. This distribution was chosen to evaluate both ends of the size match spectrum (undersized and oversized donors including possible U-shaped relationship), with a reasonable sample size in exposure groups to detect the association of DR size mismatch with outcomes if present and to detect any trends with outcomes on either end, assuming the middle 60% would be the best matched group by size. The groups were compared for the distribution of baseline demographic and clinical (recipient and donor) variables as well as the distribution of DR weight-, height- and BSA ratio using chi-square tests or Kruskal-Wallis tests, as appropriate.

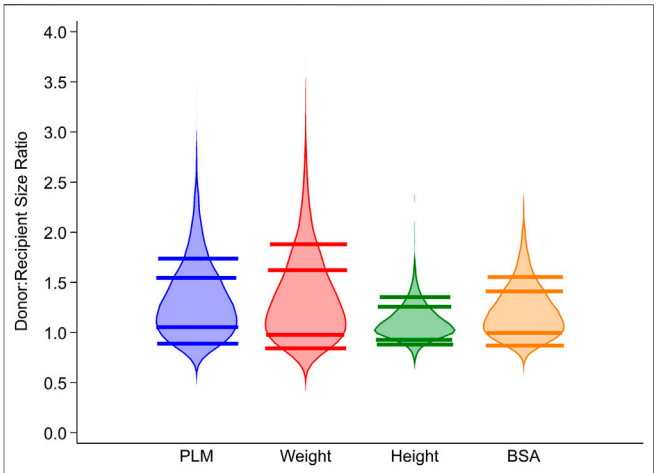


FIGURE 1 | Distribution of donor-recipient PLM ratio, weight ratio, height ratio and BSA ratio in the study cohort. PLM ratio, weight ratio, height ratio and BSA ratio were used to divide the study cohort into five groups representing 10%, 10%, 60% (reference group), 10% and 10% of subjects with increasing value of the ratio. PLM, predicted left ventricular mass; BSA, body surface area.

A multivariable logistic regression model using variables at HT and forward selection was developed for post-transplant in-hospital mortality retaining variables significant at the 0.10 level based on a likelihood ratio test; all variables in **Table 1**, other than DR weight-, height- and BSA ratio were considered. We decided a priori to adjust the model for the calendar year of HT irrespective of significance due to potential changes in clinical practices and recipients and improvement in early post-transplant survival over time (3). We then assessed the association of size match using donor-recipient PLM ratio adjusted for all factors in the model. We assessed the interaction of size match with model variables to determine a disproportionate effect, if any, on early post-transplant mortality. To compare the performance of PLM ratio with currently used metrics, we performed analyses using DR weight-, height- or BSA ratio (instead of PLM ratio) with post-transplant in-hospital mortality adjusted for all variables in the multivariable model. For each model, we used the middle 60% subjects for the corresponding variable as the reference group. We also evaluated adjusted risk of post-transplant in-hospital mortality with size match variables (PLM ratio, weight ratio and BSA ratio) assessed as continuous variables.

Kaplan Meier curves and log rank test were used to compare cumulative 1st year post-HT graft loss (death or re-HT) among the five groups. Multivariable Cox models were built to assess the association of size match using different metrics for 1 year graft survival, adjusted for baseline characteristics and year of transplant. For each model, the middle 60% subjects for the specific metric were used as the reference group.

We performed a sensitivity analysis by repeating/limiting all multivariable analyses only in recipients who received a heart

TABLE 2 | Multivariable model for post-transplant in-hospital mortality.

	Odds ratio	95% confidence interval	*p* value
Age at transplant <1 Year	1.99	1.48, 2.67	<.001
Diagnosis (vs. Dilated CMP)			<.001
Non-dilated CMP	1.86	0.99, 3.50	
CHD repaired	3.89	2.75, 5.50	
CHD unrepaired	1.56	0.90, 2.69	
Other	1.91	0.94, 3.87	
Ventilator	1.84	1.36, 2.50	<.001
Mechanical support (vs. none)			<.001
ECMO	3.30	2.30, 4.74	
BIVAD	2.23	1.25, 3.98	
LVAD	1.08	0.57, 2.04	
Bilirubin (vs. < 0.6 mg/dl)			<.001
0.6–1.9	1.55	1.12, 2.14	
≥2.0	2.26	1.55, 3.30	
Renal dysfunction (vs. normal)			<.001
Mild-moderate	2.06	1.45, 2.94	
Severe	3.88	2.59, 5.80	
Donor ischemic time (vs. < 4 h)			.002
≥4	1.59	1.21, 2.09	
Not reported	1.67	0.91, 3.07	
Male recipient/Female donor	0.74	0.54, 1.02	.068
Year of transplant (vs. 2000–2002)			
2003–2005	1.04	0.68, 1.58	
2006–2008	0.82	0.53, 1.27	
2009–2011	0.81	0.52, 1.27	
2012–2015	0.86	0.56, 1.31	

CMP, cardiomyopathy; CHD, congenital heart disease; ECMO, extracorporeal membrane oxygenation; BIVAD, biventricular assist device; LVAD, left ventricular assist device.

from a donor up to 18 years old so that both the recipient and the donor PLM were derived using the PHN equation.

Data were analyzed using SAS version 9.4 (SAS Institute, Inc., Cary, NC) and Stata version 15 (StataCorp, College Station, TX). All statistical tests were two-sided and a $p < 0.05$ defined statistical significance. The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

RESULTS

During the 15 year study period, 4,797 children <18 years old underwent primary HT in the US. Of these, 23 received a multi-organ transplant and 57 had missing weight or height for the recipient or the donor and were excluded. The remaining 4,717 children in whom PLM could be estimated for both the recipient and the donor formed the study cohort. Of these, 30% were infants <1 year old, 55% were male, 52% had cardiomyopathy, 44% had congenital heart disease and 21% were on a mechanical support (6% on extracorporeal membrane oxygenation, 5% on biventricular assist device and 10% on left ventricular assist device) at transplant. Overall, 85% of these recipients received a heart from a pediatric donor <18 years old, the remaining being adult donors.

Donor-recipient PLM ratio ranged from 0.55 to 3.40 in the study cohort and was 0.55–0.90 in group 1 (most undersized donors), 0.91–1.00 in group 2, 1.01–1.60 in group 3 (reference group), 1.61–1.83 in group 4 and 1.84–3.40 in group 5 (most oversized donors), respectively. The distribution of baseline recipient and donor characteristics among the five groups with increasing donor-recipient PLM ratio is illustrated in **Table 1**. As expected, recipients with higher donor-recipient PLM ratio had higher DR weight ratio, higher DR height ratio and higher BSA ratio (p for trend<.001 for all, **Table 1**). **Figure 1** illustrates violin plots with the distribution of study cohort into five groups (10%, 10%, 60%, 10% and 10%) using donor-recipient PLM, weight, height and BSA ratio, respectively.

Post-Transplant In-Hospital Mortality

Overall, 283 (6%) children died prior to hospital discharge. In-hospital mortality was 8.3%, 4.9%, 5.5%, 7.0% and 6.8%, respectively in PLM Groups 1–5 (p = .10). In multivariable analysis, recipient age, cardiac diagnosis, ventilator or mechanical support, renal dysfunction, hepatic dysfunction and donor ischemic time were all significantly associated with in-hospital mortality (**Table 2**). In adjusted analysis (adjusted for factors in **Table 2**), HT recipients with the lowest donor-recipient PLM ratio (group 1, PLM ratio ≤.9) were at a significantly higher risk of in-hospital mortality [Odds Ratio (OR) 1.55, 95% CI 1.04, 2.32, p = .03] compared to the reference group (PLM group 3) whereas HT recipients in PLM group 2 (OR 1.01, 95% CI 0.62, 1.64), group 4 (OR 0.95, 95% CI 0.62, 1.47) or group 5 (OR 0.78, 95% CI 0.50, 1.20) were not at higher risk of in-hospital mortality. There was no significant interaction of PLM group 1 with any risk factor in the multivariable model.

There was no difference in the distribution of causes of in-hospital mortality among PLM groups 1–5. There was also no difference among groups in the proportion of children who

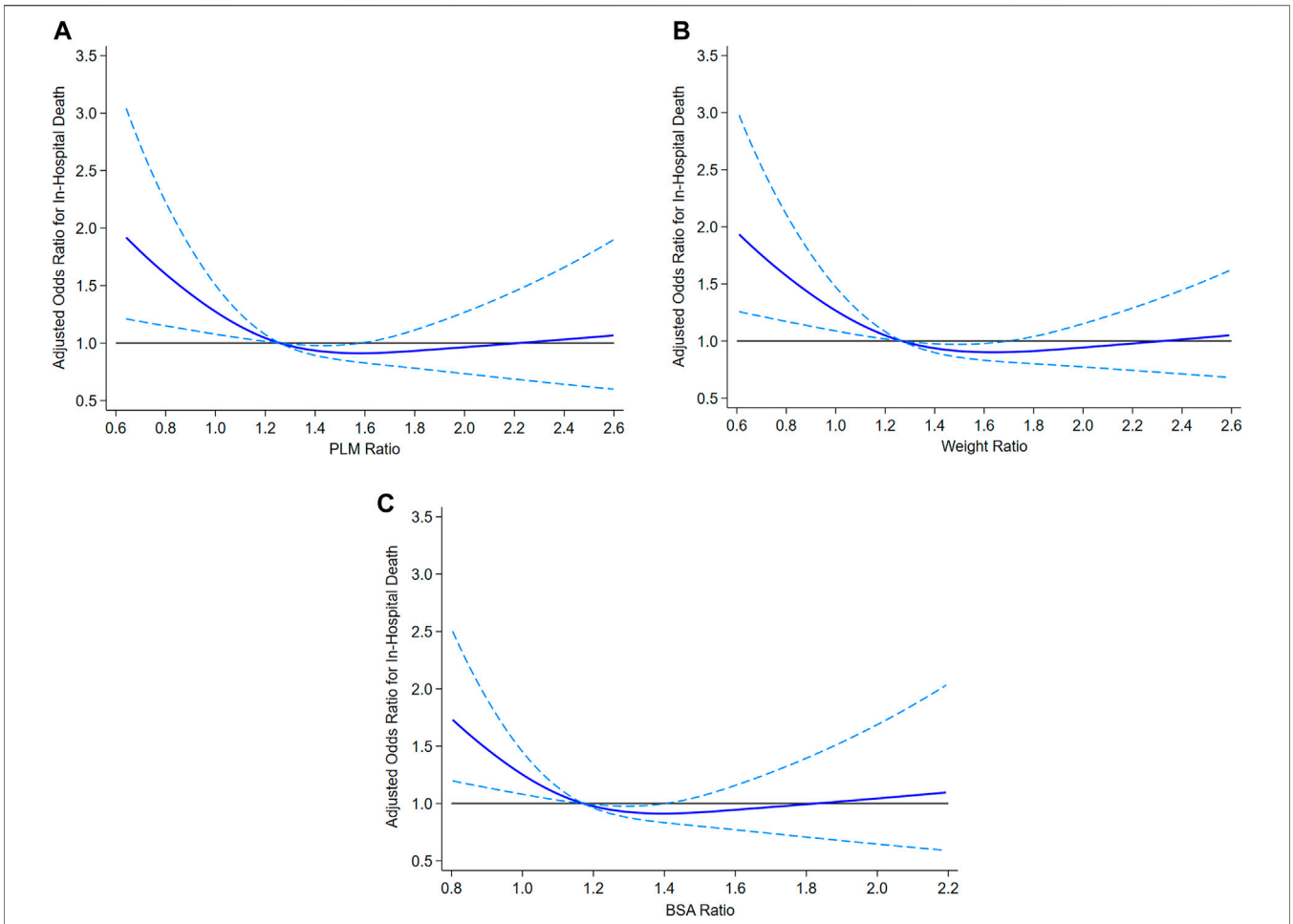


FIGURE 2 | Association of post-transplant in-hospital mortality with donor-recipient size match assessed as PLM ratio (2A), weight ratio (2B) and BSA ratio (2C). PLM, predicted left ventricular mass; BSA, body surface area.

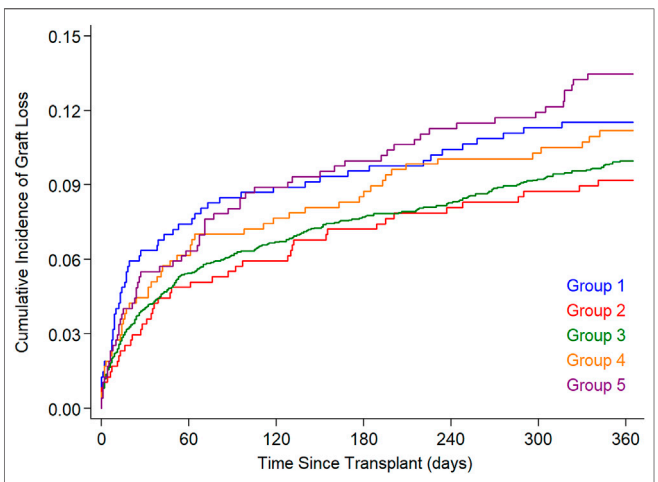


FIGURE 3 | Cumulative graft loss (death or re-transplant) in the 5 PLM groups. The PLM groups 1–5 represent 10%, 10%, 60% (reference group), 10% and 10% of study subjects with increasing PLM ratio. PLM, predicted left ventricular mass.

developed severe primary graft dysfunction (6%, 4%, 4%, 7%, 6%, respectively, p = .11), defined as initiation of extra-corporeal membrane oxygenation support within 2 days following transplant (13, 14). However, the association of PLM group 1 with in-hospital mortality was weaker (adjusted OR 1.45, 95% CI 0.95, 2.22) when primary graft dysfunction (yes/no) variable was added to the multivariable model.

There was a borderline increased risk of in-hospital mortality in adjusted analysis in recipients in the lowest decile of DR weight ratio defined as <0.88 (OR 1.49, 95% CI 0.99, 2.25, p = .05, **Supplementary Table S1**) whereas recipients in the lowest decile of DR height ratio were not at increased risk (OR 1.15, 95% CI 0.74, 1.77, p = .54, **Supplementary Table S1**). Using BSA ratio for DR size match demonstrated a significantly increased risk of in-hospital mortality among recipients in the lowest decile, defined as <0.92 (OR 1.53, 95% CI 1.02, 2.30, p = .04). The area under the receiver operating characteristic curve for the multivariable models for in-hospital mortality was identical (c statistic = 0.81) whether donor-recipient PLM ratio, weight ratio or BSA ratio was used in the multivariable model.

TABLE 3 | Multivariable cox model for graft loss within 1 Year of heart transplant.

	Hazard ratio	95% confidence interval	*p* value
Age at transplant <1 year	1.58	1.29, 1.94	<.001
Race/Ethnicity (vs. White)			.003
Black	1.33	1.06, 1.67	
Hispanic	0.77	0.58, 1.02	
Other	1.33	0.94, 1.90	
Diagnosis (vs. Dilated CMP)			<.001
Non-dilated CMP	1.85	1.24, 2.76	
CHD repaired	2.92	2.31, 3.68	
CHD unrepaired	1.57	1.08, 2.27	
Other	1.40	0.82, 2.36	
Ventilator	1.56	1.26, 1.94	<.001
Mechanical support (vs. none)			<.001
ECMO	2.58	2.00, 3.32	
BIVAD	1.82	1.23, 2.71	
LVAD	0.98	0.63, 1.52	
Bilirubin (mg/dl) (vs. < 0.6)			<0.001
0.6–1.9	1.23	0.99, 1.53	
≥2.0	1.59	1.23, 2.05	
Renal dysfunction (vs. none)			<.001
Mild-moderate	1.59	1.23, 2.04	
Severe	2.72	2.07, 3.59	
Donor ischemic time (hours) (vs. < 4)			.001
≥4	1.29	1.07, 1.55	
Not reported	1.24	0.81, 1.90	
Male recipient/Female donor	0.72	0.58, 0.90	.003
Year of transplant (vs. 2000–2002)			<.001
2003–2005	1.00	0.76, 1.33	
2006–2008	0.90	0.67, 1.19	
2009–2011	0.75	0.55, 1.01	
2012–2015	0.64	0.47, 0.86	

CMP, cardiomyopathy; CHD, congenital heart disease; ECMO, extracorporeal membrane oxygenation; BIVAD, biventricular assist device; LVAD, left ventricular assist device.

Figure 2 illustrates the association of post-transplant in-hospital mortality with DR size match when donor-recipient PLM ratio (2A), weight ratio (2B), and BSA ratio (2C) were assessed as continuous variables.

The donor age was 18 years or younger for 3992 HT recipients in the study and therefore the PHN equation for PLM was applicable for both the donor and the recipient. Among these recipients, those with the lowest donor-recipient size ratio were at higher risk of post-transplant in-hospital mortality in adjusted analysis whether donor-recipient PLM ratio (OR 1.55, 95% CI 1.03, 2.35), weight ratio (OR 1.59, 95% CI 1.04, 2.45) or BSA ratio (OR 1.62, 95% CI 1.06, 2.48) were used to define the most undersized decile of donors.

Post-Transplant 1-Year Graft Survival

Figure 3 illustrates cumulative 1 year graft loss among HT recipients stratified by donor-recipient PLM ratio. Graft loss during the first post-transplant year occurred in 11.4%, 9.1%, 9.9%, 11.0%, and 13.4% in Groups 1–5. The difference among groups was not statistically significant (p = .14, log rank test).

In a multivariable Cox model, risk factors associated with graft loss during the first post-transplant year included recipient age, cardiac diagnosis, black race, hemodynamic support at transplant, renal or hepatic dysfunction and donor ischemic time (**Table 3**). PRA was not associated with survival. In analysis adjusted for factors in **Table 3**, HT with either undersized donors (PLM group 1, hazard ratio [HR] 1.20, 95% CI 0.89, 1.61; PLM group 2, HR 1.03, 95% CI 0.74, 1.42) or with oversized donors (PLM group 4, HR 0.91, 95% CI 0.67, 1.22; PLM group 5, HR 1.00, 95% CI 0.75, 1.32) was not associated with 1 year graft loss. Similarly, there was no association of donor-recipient size mismatch with graft loss during the first year when the DR size match groups were based on the distribution of DR weight-, height or BSA ratio (**Supplementary Table S2**). There was no association of size mismatch with 1 year graft loss when the analysis was limited to donors up to 18 years old.

DISCUSSION

A longstanding wisdom when evaluating a donor for HT is to avoid undersized donors due to the risk of primary graft failure in the recipient. In this study of US children who received primary HT in the US during a 15 year period, we calculated donor and recipient PLM using a recently described equation in normal US children. We found that 10% of HT recipients received a heart with donor-recipient PLM ratio of ≤.90. These children were at 55% higher risk of post-transplant in-hospital mortality compared to the reference group in adjusted analysis. When size match was assessed with PLM ratio as a continuous variable, the adjusted risk of in-hospital mortality was higher the more undersized the donor heart. Recipients who received oversized hearts were not at increased risk. There was no association of DR

size mismatch with 1 year graft survival suggesting that the risk associated with using hearts from undersized donors is short-term. The association of undersized donors with in-hospital mortality was also demonstrable to a comparable degree when size match was assessed using DR weight ratio or donor-recipient BSA ratio. These findings are different from analyses in adult HT recipients where use of predicted heart mass formula to assess DR size match is superior to using body measurements. Considering the lack of superiority of PLM ratio and the simplicity in using DR weight ratio or BSA ratio when evaluating size match, it is difficult to justify a routine use of donor-recipient PLM ratio when evaluating donors. DR height ratio was not associated with post-transplant in-hospital mortality or 1 year graft loss.

We were inspired to ask the study question after several studies in adult heart transplantation (6, 15) and the 2019 annual report of the International Thoracic Registry in adult HT recipients showed that DR predicted heart mass ratio was the optimal metric for assessing DR size match by being associated with 1 year post-transplant survival whereas DR weight-, height- or BSA ratio were not (7). Prior to these reports, DR weight ratio was the most common metric for assessing size match in adult HT candidates (16). The ability to estimate predicted heart mass in adults followed publications of normative equations for LV and RV mass using gender, height and weight based on cardiac MRI data in a multi-ethnic population-based study in the US (4, 5). These equations have not been validated in children and MRI-based values of RV or LV mass in normal children are limited to small studies (17). Echocardiography is limited in its ability to image RV due to its proximity to sternum, its geometry and a thin RV free wall. LV mass measurements have however been routinely performed in clinical practice using echocardiography (18). LV mass is the dominant contributor to the heart mass after the first 4–6 weeks of life. This is supported by an MRI study in 50 healthy children where the BSA-based regression equations showed the mean LV mass to be > 3 times the RV mass during childhood (17). This is similar to adults where applying the MRI-derived equations to a few real life examples shows that LV mass contributes 75%–80% to the predicted heart mass (4, 5). Lacking an equation for predicted RV mass in children, we reasoned that LV mass would contribute about the same proportion to total heart mass in most children making predicted LV mass a reasonable surrogate for predicted heart mass and designed the current study as we did.

Previous analyses in children using weight or height have shown absent or marginal association of DR size-mismatch with recipient survival. Tang et al analyzed 3048 US pediatric HT recipients during 1994–2008 for DR size match using weight (1). There were 204 (6.7%) recipients with donor weight <80% of the recipient weight. They found no effect on post-transplant survival when the donor weight was 60%–80% of the recipient weight but reported lower 30 day survival in infant recipients with donor weight <60% of the recipient weight. In another report, Patel et al analyzed 2133 US children who underwent HT for dilated cardiomyopathy during 1989–2012 (2). DR size mismatch using either weight or height was not associated with post-transplant survival in multivariable analysis. The 2019 annual ISHLT pediatric analysis did not find association of DR weight mismatch with 1 year post-transplant mortality in adjusted analysis (p = .09) (3). The association of using hearts from undersized donors with post-transplant in-hospital mortality in the current analysis illustrates that the major consideration in DR size match is limited to the immediate post-transplant period. The loss of this association with longer follow-up may be explained by echocardiographic studies in pediatric HT recipients with DR size mismatch that have shown that LV mass regresses or grows to become near-normal for the recipient size within the first few weeks and months post-transplant (19, 20). Furthermore, the number of recipients exposed to this risk factor was small (one 10th of the cohort). Therefore, when analyzed for the full cohort, the risk was short-lived, and with time, other factors that were important in the full cohort became more important.

Study Implications

Our analysis shows a significant association of HT from undersized donors with early post-transplant mortality. Because cardiac mass in normal children increases as the body size increases (8), the association when expressed as donor-recipient PLM ratio - while performing similar to the DR body size ratios—provides a physiologic correlate for the risk associated with undersized donors. If the ultimate goal is to match the donor and the recipient for their predicted heart mass, the weight ratio and BSA ratio appear to be reasonable surrogates in children unlike in adults. The difference between adults and children in this regard may be best explained by the gender difference in calculation of predicted heart mass. It is notable that the pediatric LV mass equation is the same in boys and girls with similar BSA. In contrast, there is a significant difference in values for the predicted LV (and RV) mass by gender such that with the same body weight and height as that of a man, the LV mass in a woman calculates to 82.7% of that man, thus explaining the increased risk of mortality in adult male recipients when receiving HT from a female donor. This is the likely explanation for a much superior performance of heart mass calculation in adult HT recipients over body measurements whereas they appear to perform no differently in children.

The size match categories in our analysis were chosen to understand if either undersized or oversized donors were associated with worse outcomes compared to the reference group and were guided in part by an adult study where seven equal-size groups were analyzed for size match with just the middle group being the reference group (6). With a much smaller study population in children, we needed the reference group to be larger than the exposed groups. Because PLM ratio is a continuous variable, we also analyzed it as such and as expected, the risk of graft loss was higher the more undersized the donor. Our primary study finding does indeed support the current clinical practice of caution with undersized donors and defines the threshold to be donor-recipient PLM ratio of ≤.9 or weight ratio <.88 or BSA ratio of <.92, each seen in pediatric HT in 10% of all recipients. It is important to note however, that despite the higher relative risk, the observed outcomes seen with such undersized donor hearts may be considered quite reasonable in many HT candidates who may not otherwise receive another donor call. The decision when evaluating such donors would require one to balance the consequences of accepting an undersized heart vs the risk of wait-list mortality.

Limitations

This study has several limitations. First, this was a retrospective study using registry data with inherent limitations of such data. However, submission of these data to UNOS by centers is required, the data are used on an ongoing basis for organ allocation and are periodically audited by UNOS, thus allowing safeguards to data quality. Second, although we describe increased mortality risk in 10% of HT recipients with the most undersized donors, the category as defined is somewhat arbitrary and the risk is continuous with a higher risk the more undersized the donor rather than present at a specific PLM ratio. Third, donor-recipient size mismatch may be clinically reasonable in a cachectic or an overweight recipient in whom ideal body weight, such as the 50th percentile weight for current height, instead of the current weight, may be considered. We did not analyze such examples in this study for statistical reasons.

CONCLUSION

Pediatric HT recipients who receive hearts from donors with donor-recipient PLM ratio ≤.9 are at significantly increased risk of early post-transplant mortality. However, this metric is not superior to donor-recipient weight ratio or BSA ratio when assessing size match as this association is also seen when evaluating donors and recipients using weight ratio or BSA ratio. These findings should be considered during decision making when assessing potential donors for HT candidates.

CAPSULE SENTENCE SUMMARY

A longstanding wisdom when evaluating a donor heart for a heart transplant candidate is to avoid undersized donors due to the risk of primary graft failure. However, previous analyses in pediatric heart transplant recipients using weight or height have not found donor-recipient size-mismatch to be associated with post-transplant mortality. A recent study in healthy US children using echocardiography described an equation for LV mass using body surface area. We assessed if donor-recipient size mismatch assessed using predicted LV mass ratio is associated with post-transplant mortality. In a study of 4,717 pediatric heart transplants in the US over 15 years study duration, we found that children with donor-recipient predicted LV mass ratio <.9 (10% with most undersized donor hearts) were at higher risk of post-transplant in-hospital mortality adjusted for other risk factors. The metric was not superior to donor-recipient weight ratio or BSA ratio for assessing size match however because recipients in the lowest decile of donor-recipient weight ratio or body surface area ratio were also at increased risk of in-hospital mortality.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: The data are available upon request to participating centers in the US. Requests to access these datasets should be directed to https://optn.transplant.hrsa.gov › data › request-data.

ETHICS STATEMENT

The institutional review board (Boston Children's Hospital) determined that the study was not human subjects research as defined by US federal regulations.

AUTHOR CONTRIBUTIONS

TS participated in all aspects of the research including study design, performance, analysis, interpretation, writing and editing of the manuscript. SC participated in study design, interpretation and in editing of the manuscript text. KG participated in study design, data analysis and in editing of the manuscript text.

FUNDING

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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.

ACKNOWLEDGMENTS

KG had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of data analysis. The data were supplied by the UNOS as the contractor for the OPTN. The interpretation and reporting of these data are the responsibility of the authors and in no way should be seen as an official policy of or interpretation by the OPTN or the US Government.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10226/full#supplementary-material

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Metagenomic Next-Generation Sequencing for Diagnosing Infections in Lung Transplant Recipients: A Retrospective Study

Chun-Rong Ju[1†], Qiao-Yan Lian[1†], Wei-Jie Guan[1†], Ao Chen[1], Jian-Heng Zhang[1], Xin Xu[1,2], Rong-Chang Chen[3], Shi-Yue Li[1] and Jian-Xing He[1,2*]*

[1]State Key Laboratory of Respiratory Disease, National Clinical Research Center for Respiratory Disease, Guangzhou Institute of Respiratory Health, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China, [2]Department of Thoracic Surgery, Guangzhou Institute of Respiratory Disease, First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China, [3]Department of Respiratory and Critical Care Medicine, First Affiliated Hospital of Southern University of Science and Technology, Second Clinical Medical College of Jinan University, Shenzhen People's Hospital, Shenzhen Institute of Respiratory Diseases, Shenzhen, China

Background: Accurate identification of pathogens is essential for the diagnosis and control of infections. We aimed to compare the diagnostic performance of metagenomic next-generation sequencing (mNGS) and conventional detection methods (CDM) in lung transplant recipients (LTRs).

Methods: We retrospectively analyzed 107 LTRs with suspected infection of pulmonary, blood, central nervous system or chest wall between March 2018 and November 2020. Bronchoalveolar lavage fluid and other body fluids were subject to pathogen detection by both mNGS and CDM.

Results: Of the 163 specimens, 84 (51.5%) tested positive for both mNGS and culture, 19 (11.7%) of which were completely consistent, 44 (27.0%) were partially congruent, and 21 (12.9%) were discordant (kappa = .215; $p = .001$). Compared with CDM, mNGS detected a higher diversity of pathogens. Moreover, the turn-around time was significantly shorter for mNGS compared with culture (2.7 ± .4 vs. 5.5 ± 1.6 days, $p < .001$). As an auxiliary method, treatment strategies were adjusted according to mNGS findings in 31 cases (29.0%), including eight patients with non-infectious diseases, who were finally cured.

Conclusion: mNGS can identify pathogens with a shorter turn-around time and therefore provide a more accurate and timely diagnostic information to ascertaining pulmonary infections. mNGS might have a role in differentiating infectious from non-infectious lung diseases in LTRs.

Keywords: infection, metagenomic next-generation sequencing, lung transplant recipients, pathogen, conventional detection methods

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***Correspondence:**
Shi-Yue Li
lishiyue@188.com
Jian-Xing He
drjianxing.he@gmail.com

[†]These authors have contributed equally to this work

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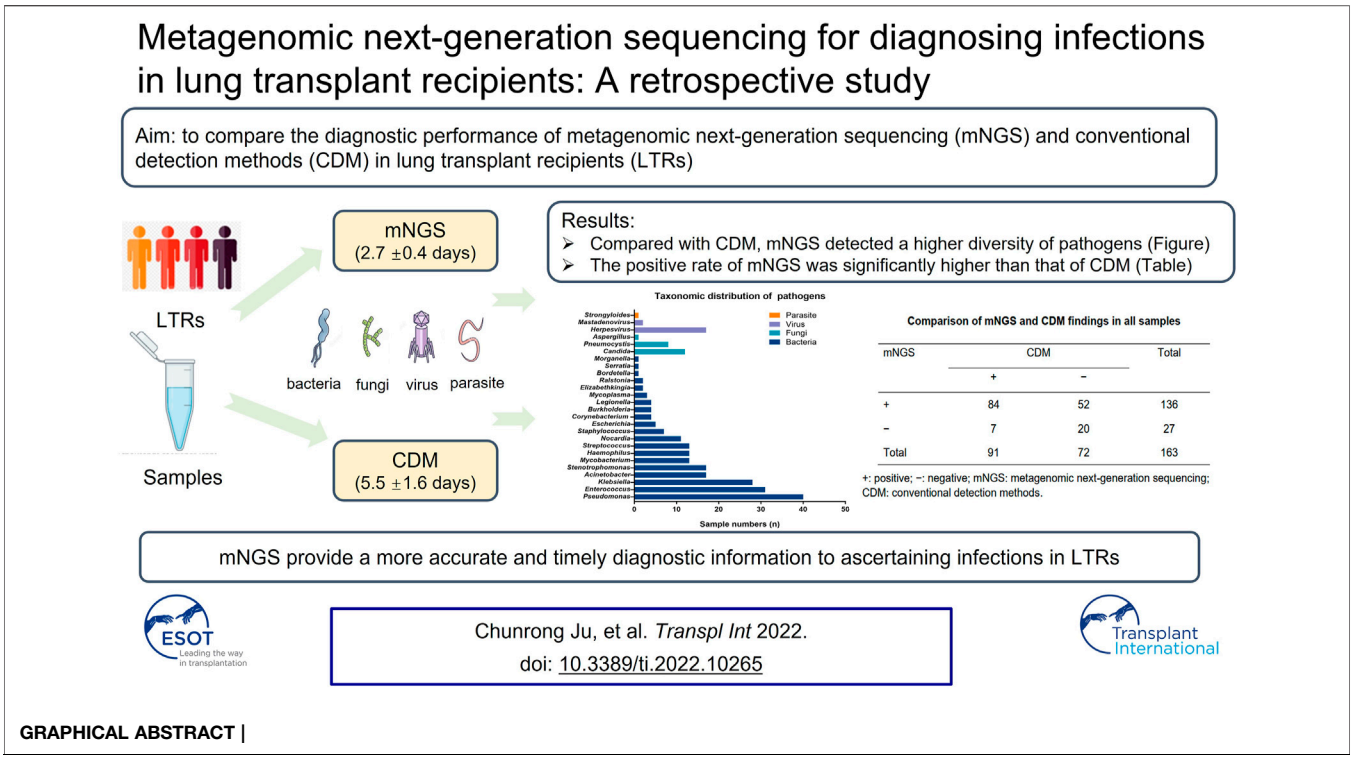
Abbreviations: BALF, bronchoalveolar lavage fluid; BDG, (1/3)-β-D-glucan; BOS, bronchiolitis obliterans syndrome; CDM, conventional detection methods; CMV, cytomegaloviruses; COPD, chronic obstructive pulmonary disease; CSF, cerebrospinal fluid; CT, computed tomography; EBV, Epstein-Barr virus; GM, galactomannan; LAM, Lymphangioleiomyomatosis; LTRs, lung transplant recipients; mNGS, metagenomic next-generation sequencing; NTM, non-tuberculosis mycobacterium; PCR, polymerase chain reaction; SOT, solid organ transplant; TBLB, trans-bronchoscopic lung biopsy.



GRAPHICAL ABSTRACT |

INTRODUCTION

Infection is the main cause of death in lung transplant recipients (LTRs), especially at the early postoperative stages (1, 2). Compared with other solid organ transplant (SOT) recipients, LTRs are at significantly higher risk of acquiring infections because the lungs are constantly exposed to the atmospheric environment. This increased risk is further aggravated by the maintenance treatment with high-dose immunosuppressants, the impaired cough reflex, and the decreased mucociliary clearance especially at the early stage after lung transplantation (3, 4). The timely and accurate initiation of anti-infective treatment is vital to the clinical outcomes, which depends on the rapid and accurate pathogen identification. In real-world clinical practice, conventional detection methods (CDM) have a lower sensitivity and a relatively long turn-around time for detecting opportunistic pathogens such as *Pneumocystis jirovecii*, mycobacteria, *Nocardia* spp., fungi, and other atypical pathogens (5–7). Moreover, it is difficult to distinguish non-infectious diseases from infections because the clinical manifestations and radiologic characteristics are non-specific in LTRs (8–10). Therefore, accurate diagnosis of infection based on the exact identification of the pathogens are crucial to inform the decisions of therapeutic interventions.

Metagenomic next-generation sequencing (mNGS) is an emerging culture-independent assay that facilitates rapid and sensitive detection of various pathogens (5, 11). mNGS has recently been adopted for detecting pathogens in the respiratory, neurologic, urinary, pediatric, cardiovascular and orthopedic diseases (12–17). However, data among the LTRs have been scarce. The only existing study regarding mNGS mainly focused on the identification of viral species and explored the usefulness in LTRs with a previously undetectable source of infection (18). The value of mNGS for detecting other pathogens in LTRs has not been well elucidated. However, given the complexity of pathogens and the difficulty in differentiating the clinical diagnosis in LTRs, a thorough evaluation with mNGS is urgently needed.

In this study, we retrospectively analyzed the diagnostic performance of mNGS in diagnosing infectious diseases through the comparison with CDM. Our findings might help explore the role of mNGS in differentiating infectious from non-infectious pulmonary complication in LTRs.

MATERIALS AND METHODS

Study Population

In this retrospective study, LTRs hospitalized in the First Affiliated Hospital of Guangzhou Medical University between March 2018 and November 2020 underwent screening. Inclusion criteria consisted of the following: 1) Aged 18 years or greater; 2) LTRs with new-onset pulmonary complication; and 3) BALF sample was available for pathogen detection by both mNGS and CDM. Patients with the undetermined diagnoses were excluded from our study.

Data of the LTRs that were collected retrospectively consisted of the demographics, primary underlying diseases before lung

transplantation, the type of surgery (unilateral, bilateral, or heart-lung transplantation), clinical symptoms, signs, chest imaging findings, time from transplantation to sampling, laboratory routine tests, biochemical tests, treatment schemes (immunosuppressive and antimicrobial regimens) and clinical outcomes. All lungs were derived from the deceased cardiovascular or brain donors.

This study was carried out in accordance with the principles of the Declaration of Helsinki and was approved by the Ethics Review Committee of the First Affiliated Hospital of Guangzhou Medical University (No. 128, 2020). Patient approval and informed consent were waived because of the retrospective review of patient's records.

Criteria of Defining Pulmonary Infections

Pulmonary infection was diagnosed comprehensively according to the overall condition of the LTRs, which included the clinical manifestations (including symptoms), thoracic imaging, and laboratory findings, etc. We mainly took into account the thoracic imaging findings for diagnosing pulmonary infection. A new patchy or progressive infiltrate, consolidation, or ground-glass opacity should be shown on chest X-ray or computed tomography (CT). Meanwhile, patients would have to satisfy at least one of the following five items: 1) New-onset cough or expectoration, or aggravation of the existing respiratory tract symptoms with or without purulent sputum production, chest discomfort, dyspnea, or hemoptysis; 2) Fever; 3) Pulmonary consolidation and/or moist rales; 4) Peripheral blood white blood cell count $>10 \times 10^9$/L or $<4 \times 10^9$/L; 5) An evidence of pathogen infection. The differential diagnosis of infections and non-infectious diseases was established by combining the comprehensive clinical information and a review of the therapeutic outcomes.

Sample Collection Schemes

Bronchoalveolar lavage fluid (BALF) samples were collected from patients with a new-onset pulmonary complication who were suspected as having infectious disease based on the overall clinical conditions. In addition, blood samples and cerebrospinal fluid (CSF) were collected from the patients who were suspected as having infection of the blood stream and the central nervous system, respectively. The exudate from the chest wall soft tissue mass was collected from patients suspected as having chest infections. The lung lobes with the most prominent lesions according to chest CT were selected for performing lavage with fiberoptic bronchoscopy according to the standardized operating procedures. 50–60 ml normal saline was instilled into the affected bronchial segment, with the target recovery rate of 40%–60%. Samples were immediately stored in sterilized containers and subjected to pathogen detection with CDM and mNGS.

The CDM included a minimal bundle of the bacterial and fungal smear and culture with the Grocott's methenamine staining and acid-fast staining, real-time polymerase chain reaction (PCR) for cytomegaloviruses (CMV), Epstein-Barr virus (EBV), and *Mycobacterium tuberculosis* (TB), serum antibody assays (with indirect immunofluorescence assay) for respiratory syncytial virus, influenza A/B virus, parainfluenza virus, adenovirus, *Legionella pneumophila*, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae*. In addition, galactomannan (GM) antigen and (1/3)-β-D-glucan (BDG) assays were adopted for detecting fungi. GeneXpert MTB/RIF, enzyme-linked immunospot assay (T-SPOT) and tuberculin skin test were performed among patients highly suspected as having TB. Meanwhile, an aliquot was stored at 4°C before immediately (within 4 h) being transferred to a designated central laboratory for performing mNGS. Trans-bronchoscopic lung biopsy (TBLB) was also performed among patients who could tolerate the procedure when non-infectious lung diseases (e.g., allograft rejection) were suspected.

Sample Processing and DNA Extraction

The clinical samples mainly included BALF, peripheral blood, CSF, and exudate from the chest wall soft tissue mass. To prepare for the BALF samples, a 600 μl aliquot was aspired into a sterile container for breaking the cellular wall (esp. fungi), and another aliquot of 300 μl was subject to DNA extraction by using a TIANamp Micro DNA Kit (DP316; Tiangen Biotech, Beijing, China), according to the manufacturer's instructions. For processing other samples such as the blood, CSF, and exudate from the chest wall soft tissue mass, 300–600 μl of samples was adopted.

The extracted DNA was subject to the comparison with the sequences in the genomic libraries through transposase indexing of each sample. After purification, amplification, and re-purification of the library, the fragment sizes and library concentrations were assessed by using Qsep1 (BiOptic, Hubei, China) and Qubit (Thermo Fisher Scientific, Waltham, MA, United States) devices, respectively. DNA nanoballs were prepared by using single-stranded DNA. Finally, each DNA nanoball was loaded into a single lane for sequencing. The sequencing was performed on the Illumina NextSeq 550Dx platform (Illumina, San Diego, CA, United States).

Metagenomic Next-Generation Sequencing Data Analysis

Quality control was performed on the raw sequencing data by using the BWA platform (http://bio-bwa.sourceforge.net/). Low-quality reads and reads shorter than 35 bp were removed. The remaining reads were further filtered by using a human host DNA subtraction database. The sequences were then annotated by using a dedicated pathogen database after removing the low-complexity reads, and subsequently classified according to their taxonomic groups, such as viruses, bacteria, fungi, parasites, and other pathogens. The non-human sequence reads from each sample were deposited at the NCBI BioProject database (https://www.ncbi.nlm.nih.gov/bioproject) under the accession number PRJNA737316.

TABLE 1 | Patient and sample characteristics.

Characteristics	Value
Lung transplant recipients (*n* = 107)	
Age (years), mean ± SD	56.1 ± 13.3
Sex (male, %)	90 (84.1%)
BMI (kg/m^2), mean ± SD	20.2 ± 3.6
Primary indications for lung transplantation, *n* (%)	
COPD	36 (33.6%)
Interstitial lung disease	46 (43.0%)
Bronchiectasis	10 (9.4%)
Pneumosilicosis	4 (3.7%)
Eisenmenger syndrome	4 (3.7%)
Pulmonary arterial hypertension	2 (1.9%)
BOS	2 (1.9%)
PLAM	1 (0.9%)
Re-transplantation	2 (1.9%)
Type of lung transplantation	
Unilateral lung transplantation	60 (56.1%)
Bilateral lung transplantation	41 (38.3%)
Heart–lung transplantation	6 (5.6%)
Total number of samples (*n* = 163)	
Sample type, *n* (%)	
BALF	159 (97.5%)
Blood	2 (1.2%)
CSF	1 (0.6%)
Exudate from the chest wall mass	1 (0.6%)
Time from transplant to sampling (days), median (IQR)	108 (18–419)
Clinical symptoms at sampling, *n* (%)	
Fever	21 (12.9%)
Cough/purulent sputum	134 (82.2%)
Dyspnea	74 (45.4%)
Chest tightness/pain	27 (16.6%)
Hemoptysis	6 (3.7%)
Headache	1 (0.6%)
Antimicrobial prophylaxis at sampling, *n* (%)	
[a]β-Lactams	134 (82.2%)
[b]Quinolones	21 (12.9%)
[c]Glycopeptides	52 (31.9%)
[d]Triazoles	123 (75.5%)
Ganciclovir	79 (48.5%)
[e]Other antibiotics	18 (11.0%)
None	9 (5.5%)

COPD, chronic obstructive pulmonary disease; CSF, cerebrospinal fluid; PLAM, pulmonary lymphangioleiomyomatosis; BOS, bronchiolitis obliterans syndrome.
[a]β-Lactam: including meropenem, imipenem, piperacillin and cefoperazone.
[b]Quinolones including moxifloxacin and levofloxacin.
[c]Glycopeptides including vancomycin and teicoplanin.
[d]Triazoles including voriconazole and posaconazole.
[e]Other antibiotics including trimethoprim-sulfamethoxazole, minocycline and linezolid.

Criteria for Defining Positive Findings of Metagenomic Next-Generation Sequencing

For mNGS assay, microorganism detection (bacteria, viruses and fungi) was considered positive if satisfying any of the following thresholds: 1) The relative abundance of bacteria (excluding *Mycobacterium tuberculosis* complex) and fungi was greater than 30% at the genera level; 2) Virus detection was considered when the stringent map read number (SMRN) was 3 or greater. 3) For *Mycobacterium tuberculosis* complex, at least one read should be aligned to the reference genome at species or the genera level (19) due to the technical challenges of DNA extraction and the low probability of contamination (20). However, positive mNGS finding did not invariably indicate the presence of causative pathogen, which required immediate treatment in clinical settings. It would be the clinician's responsibility to determine the treatment strategy through comprehensive clinical assessments.

Microorganisms detected with mNGS were categorized into colonized microorganism, putative pathogen, and pathogenic microorganism. Torque teno virus, parvovirus, *Ureaplasma*, *Staphylococcus epidermidis*, intestinal colonized flora and anaerobic bacteria were deemed colonized microorganism should the patients remained clinically stable. Putative pathogens and pathogenic microorganisms were ascertained by two specialist clinicians according to the comprehensive assessments which consisted of the number of reads for mNGS, the clinical presentations, radiologic manifestations, conventional detection findings, and the clinical epidemiology. The putative pathogens or pathogenic microorganisms could be ascertained if consensus was achieved by the two clinicians. A third senior clinician and a fourth clinical microbiologist were further involved in the discussion in case of a major disagreement between the first two clinicians.

Pathogens Identified by Conventional Detection Methods

Culture positive was considered if the microbial (bacterial and fungal) load exceeded 10^4 CFU/ml. Positive BALF smear was defined as a Gram-positive and/or -negative bacterium or fungal spore/hyphae being detected by microscopic investigation. For fungi, both the positive results for BGD and GM antigen in the serum and the positive results for GM antigen in BALF were applied as the adjunct diagnostic criteria, except that pneumocystis was confirmed by PCR assay for the BALF

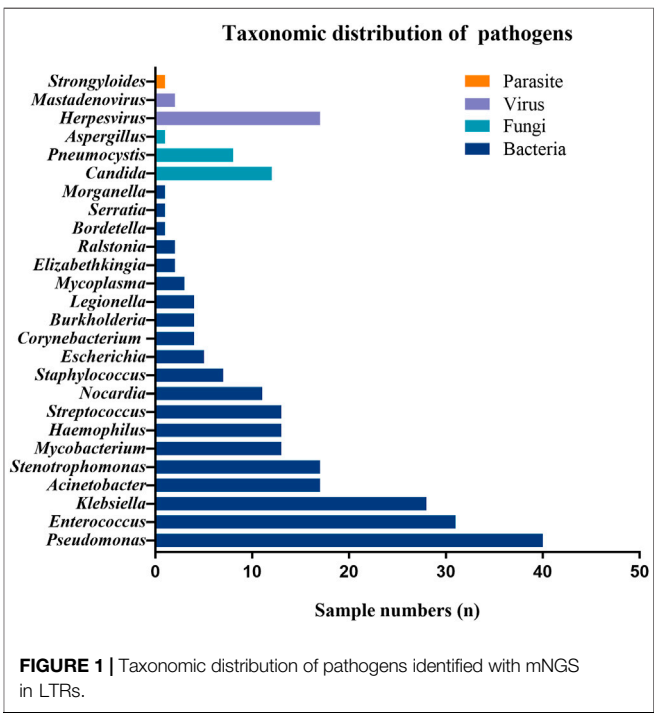


FIGURE 1 | Taxonomic distribution of pathogens identified with mNGS in LTRs.

TABLE 2 | Comparison of mNGS and CDM findings in all samples.

mNGS	CDM		Total
	+	–	
+	84	52	136
–	7	20	27
Total	91	72	163

+, positive; –, negative; mNGS, metagenomic next-generation sequencing; CDM, conventional detection methods.

samples. The targeted viruses, such as CMV or EBV, were detected with PCR assays of the BALF samples. The diagnosis of Mycobacterium infection was based on sputum smear for acid-fast bacilli, and the definitive diagnosis of TB or non-tuberculosis mycobacterium (NTM) was based on both culture and PCR, respectively. Moreover, the diagnosis of pulmonary TB was established according to the TB-related clinical symptoms, along with CT imaging findings and the results of the TB-spot and/or GeneXpert MTB/RIF.

Statistical Analyses

Continuous variables were expressed as means ± standard deviation or median (IQR), and categorical variables as count (percentage). Paired McNemar chi-square tests and Cohens' kappa were used to compare the difference and the concordance of mNGS with that of CDM. Statistical significance was defined at $p < .05$. Statistical analyses and plots were processed by using SPSS statistical software (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY, United States) and GraphPad Prism software (GraphPad Prism version 6.0.0 for Windows, GraphPad Software, San Diego, CA, United States).

RESULTS

General Information of Study Participants

After screening for 266 LTRs, 107 eligible patients were included in our final analysis. The reasons for exclusion consisted of the following: mNGS not available for pathogen detection (n = 138) and unclear final diagnoses (n = 21). There were 90 males, and the mean age was 56.1 years. The mean body-mass index was 20.2 kg/m^2. Of all LTRs, 60 underwent unilateral transplantation, 41 bilateral transplantation, and six combined heart-lung transplantation. The most common primary disease was interstitial lung disease (43.0%), followed by chronic obstructive pulmonary disease (33.6%). All LTRs received standard triple immunosuppressive regimens consisting of calcineurin inhibitors (tacrolimus/cyclosporin A), mycophenolate mofetil, and prednisolone. **Table 1** demonstrates the characteristics of the LTRs.

Sample Types

BALF samples were collected from 106 LTRs (159 samples) at each episode of clinical exacerbation. Blood samples were collected from two patients who were suspected as having bloodstream infection, the exudate was sampled from one patient with a soft tissue mass on the chest wall, and CSF sample was collected from a patient suspected as having intracranial infection. Therefore, 163 specimens of different types were included in our analysis (**Table 1**).

Spectrum of Pathogens Detected by Metagenomic Next-Generation Sequencing

For the detection of pathogens in 163 specimens, 136 (83.4%) tested positive for mNGS with a significantly higher positive rate compared with CDM (83.4% vs. 55.8%, $p = .027$). Of these, 59 (36.2%) tested positive for a single pathogen and 77 (47.2%) for two or more pathogens. Herpesvirus was the most prevalent virus in BALF, whereas *Candida* was the most common fungi detected with mNGS. The three most common bacteria consisted of *Pseudomonas aeruginosa*, *Enterococcus* and *Klebsiella pneumoniae*. The detailed compositions of the putative pathogens detected with mNGS are demonstrated in **Figure 1**. Further details are shown in **Supplementary Table S1**.

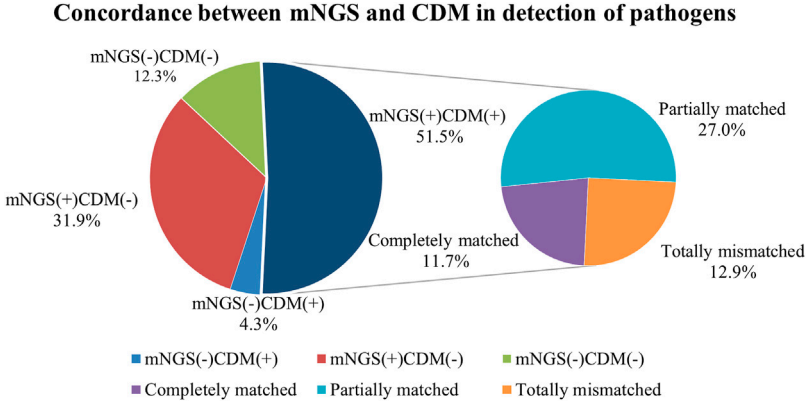


FIGURE 2 | Pathogen detection congruence of mNGS and CDM.

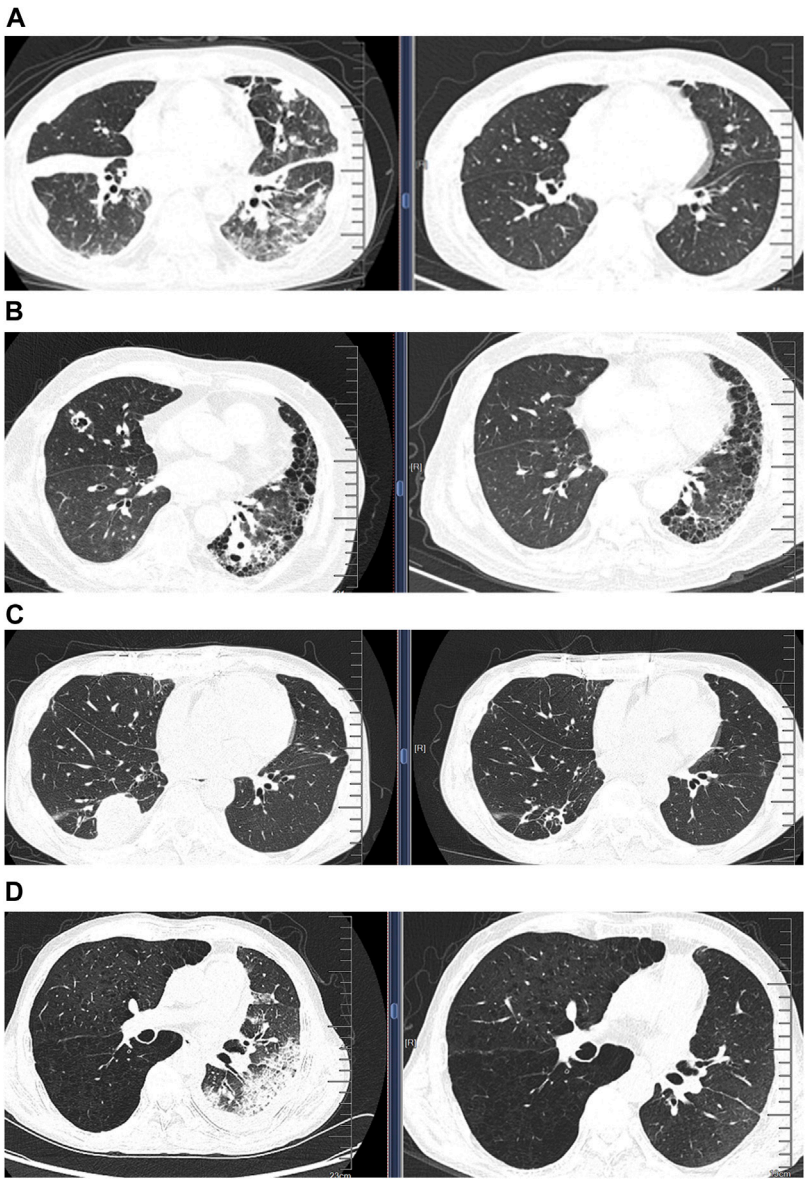


FIGURE 3 | Comparison of chest computed tomographic (CT) images before and after treatment in four patients whose treatment regimens were switched thoroughly according to the mNGS findings. **(A)** CT images from a patient diagnosed as having *Pneumocystis jirovecii* pneumonia according to mNGS; CT images showing significant improvement of infiltration after treatment (right) compared with that before treatment (left); **(B)** CT images of disseminated nocardiosis before and after treatment; **(C)** CT images of NTM pulmonary disease before and after treatment; **(D)** CT images of acute rejection before and after treatment.

Of the 163 specimens, 91 (55.8%) tested positive and 72 (44.2%) tested negative for CDM. Both CDM and mNGS tested positive among 84 samples (51.5%), and negative among 20 samples (12.3%). We also noted inconsistent findings among the two methods [negative CDM but positive mNGS findings in 52 (31.9%) samples, and positive CDM but negative mNGS findings in seven (4.3%) samples] (**Table 2**). The concordance of findings was moderate between mNGS and CDM findings (Cohen's Kappa = .215; p = .001). The positive rate of mNGS was significantly higher than that of CDM (McNemar test p < .001; **Table 2**).

The pathogens identified by CDM and mNGS were completely matched in 19 samples (11.7%). Of these, the three most common bacteria were *Pseudomonas aeruginosa* (n = 7), *Acinetobacter baumannii* (n = 5) and *Klebsiella pneumoniae* (n = 4). CDM findings were partially concordant with those of mNGS in 44 samples (27.0%). For instance, mNGS has revealed other pathogens (i.e., *Acinetobacter baumannii*) aside from the

pathogens that were identified with culture alone (i.e. *Pseudomonas aeruginosa*). However, inconsistent findings were identified between mNGS and CDM in 21 samples (12.9%; **Figure 2**). In addition, mNGS was associated with a significantly shorter turn-around time as compared with CDM (2.7 ± .4 vs. 5.5 ± 1.6 days, $p < .001$).

Of the two blood samples, one was considered positive according to both mNGS and CDM which were consistently positive. These results were consistent with the clinical manifestations. For the other blood sample, the detection yielded inconsistent findings, with positive mNGS findings and negative blood culture findings. The pathogens detected by mNGS were *Klebsiella pneumoniae* and *Nocardia*, and the patient presented with the clinical manifestations of severe infection and sepsis. The pleural exudate sample tested negative for both mNGS and CDM. The single CSF sample tested positive for *Nocardia* with mNGS but not CDM (which did not reveal any pathogen). This occurred in a single patient who suffered repetitively from fever and headache for more than 1 month during which the pathogen had not been detected, with the clinical conditions worsening despite the use of broad-spectrum antibiotics.

Treatment Adjustments According to the Positive Metagenomic Next-Generation Sequencing Findings

The treatment strategies were amended among 23 patients (21.5%) at an early stage based on the mNGS findings. Seven patients were diagnosed as having *Pneumocystis jirovecii* pneumonia, seven patients as having mycobacterial disease (including five patients with NTM pulmonary disease and two patients with pulmonary TB), four patients as having pulmonary nocardiosis, one patient as having legionellosis, one patient as having *Strongyloidiasis stercoralis* pneumonia, and one patient as having invasive pulmonary aspergillosis.

Moreover, one patient was treated immediately according to the blood mNGS findings who had been confirmed to have suffered from *Klebsiella pneumoniae* bloodstream infection according to the clinical manifestations and the delayed culture findings. For the patient whose CSF tested positive for *Nocardia* by mNGS, cotrimoxazole and linezolid were administered immediately, after which the clinical condition improved significantly within 1 week until clinical cure. **Figures 3A–C** shows the comparison of chest CT images before and after treatment in the three LTRs whose treatment strategy switched from the initial anti-infectious regimens into a different anti-infectious regimen according to the mNGS findings (**Supplementary Table S2**).

Negative Metagenomic Next-Generation Sequencing Fingdings as an Auxiliary Diagnosis of Non-Infectious Pulmonary Disease

We finally analyzed the negative mNGS findings as an auxiliary diagnosis of non-infectious diseases. Among the eight cases (7.5%) who yielded negative mNGS findings, one was eventually diagnosed as having pulmonary mucinous adenocarcinoma based on TBLB histopathology. Six patients were suspected as having acute rejection according to the comprehensive assessment of clinical characteristics; however, biopsy was not possible due to the poor clinical conditions. Because of the absence of pathological evidence, the patients were deemed to have acute rejection according to the negative mNGS findings along with the clinical manifestations. Therefore, the treatment strategies switched from antibiotics to an escalation of the dose of immunosuppressants. This led to the progressively improved clinical conditions and significantly diminished pulmonary infiltration, which collectively indicated resolved acute rejection (**Figure 3D**; **Supplementary Table S2**). The remaining one patient who had a soft tissue mass on the chest wall had initially been prescribed with antibiotics which was subsequently withheld because of the negative mNGS findings. The soft tissue mass was diagnosed to be local lymphatic fistula, and the exudate finally dissipated.

DISCUSSION

We have for the first time delineated the strengths of mNGS for ascertaining the infection status and pathogen identification in LTRs. We have also explored the diagnostic performance of mNGS as an auxiliary diagnostic approach of non-infectious complications, revealing how the treatment strategies could be amended by taking into account the findings from mNGS.

In this study, mNGS was employed to identify the pathogens in various body fluid samples, revealing a significantly higher positive rate and diversity compared with CDM. Our findings were in line with those of other recent mNGS studies (19–22), suggesting that mNGS could result in a higher positive rate and a greater accuracy of diagnosing pulmonary infection in LTRs. There may be two explanations for these outcomes: 1) mNGS can detect a wide range of pathogenic and non-pathogenic microorganisms which reside within the lungs; 2) mNGS might be capable of detecting dead pathogens whereas culture could only identify live microorganisms. Thus, whether the microorganisms detected by mNGS are causative or colonized pathogens should be determined by clinicians based on the comprehensive assessment of the clinical information.

Our results supported the assumption that mNGS has considerable advantages over CDM (6). While some shortcomings of mNGS such as higher cost need to be resolved before the extensive application as a reliable routine diagnostic method in LTRs. However, mNGS is characterized by the rapid turn-around time which takes from less than 3 days to, until recently, within 24 h only. Accurate administration of antibiotics is important for improving the prognosis among LRTs with infections. However, this depends heavily on the early identification of pathogens (23). Our results were consistent with those of the recent studies which showed that mNGS could be used for diagnosing clinical infectious diseases with the advantages of a high throughput, rapid turn-around, and high sensitivity (19, 21, 22). Taken together, mNGS confers considerable advantages over CDM for diagnosing pulmonary infections in LTRs.

In addition, compared with CDM, mNGS yielded a significantly higher sensitivity for the sample types other than respiratory specimens, such as blood and CSF (13, 24). For bloodstream infections, mNGS was less affected by the previously administered antibiotics compared with culture (24–26), which might help interpret why mNGS also yielded a higher sensitivity. In fact, most LTRs were treated with antibiotics at the time of specimen collection.

For diagnosing pathogen which was responsible for pulmonary infection, mNGS assays showed that the most prevalent pathogens mainly consisted of bacteria, particularly in LTRs at the early post-lung transplantation stages, which was in line with the results of several studies (27–29). The three most prevalent bacteria were *Pseudomonas aeruginosa*, *Enterococcus* and *Klebsiella pneumoniae*. Although mNGS can help detect clinically common multi-drug resistant bacteria with a higher sensitivity compared with conventional culture, the CDM could determine antibiotic sensitivity which cannot be achieved by mNGS. Therefore, the selection of antibiotics in our study was mainly based on culture, and our results concurred with the opinion that culture methods might be more informative than mNGS for detecting bacterial drug-resistance (30).

Pneumocystis jirovecii is one of the most common opportunistic pathogens in LTRs. However, the low rate of confirmed diagnoses as revealed with CDM could readily result in a high mortality rate. In our study, seven LTRs were diagnosed as having pneumocystis jirovecii pneumonia based on mNGS results, which were verified by PCR subsequently. The LTRs were cured after a timely adjustment of treatment with sulfamethoxazole-trimethoprim. Our results were in line with previous studies, suggesting that mNGS would be a promising method for rapid and accurate detection of *Pneumocystis jirovecii* (31, 32). Our findings supported the conclusions of the previous studies which posited that patients would benefit from mNGS assay due to the high sensitivity of pathogen detection (33, 34).

Due to the non-specific clinical manifestations and the low positive rates, nocardiosis cannot be readily diagnosed or is prone to be misdiagnosed in clinical settings (35, 36). In our study, a patient with cerebral nocardiosis suffered from refractory fever and headaches for more than 1 month, mNGS finally unraveled the culprit pathogen within the CSF. Furthermore, another patient with disseminated nocardiosis, the diagnosis was entirely based on mNGS findings. In addition, other pathogens such as *Legionella pneumoniae*, *Mycoplasma pneumoniae*, and *Strongyloidiasis stercoralis* were detected in BALF by mNGS but not CDM. Our findings suggested a considerable clinical value of mNGS for diagnosing the infections with rare pathogens and the atypical pathogens which were associated with the low detection rates according to the CDM. Other studies have also shown a higher positive rate of detection for certain fungal species and some rare pathogens with mNGS (25, 37–39).

It was worth noting that there were seven LTRs who showed lung infiltration in the chest CT, but pathogen detection in BALF using mNGS was negative. It was challenging to obtain lung tissue biopsy samples, and the six patients were diagnosed as having probable acute rejection based on their overall clinical manifestations. After initiating the immunosuppressive therapy, the pulmonary infiltration was well absorbed, and the clinical condition improved considerably. In another patient, the negative mNGS finding from BALF samples have informed physicians to perform invasive biopsy although the patient might not tolerate the procedures. The final diagnosis was confirmed to be lung adenocarcinoma according to the pathology findings. Therefore, our study results suggested that negative mNGS results might also be useful for the differential diagnosis of infectious and non-infectious pulmonary complications after lung transplantation.

Limitations

First, in this retrospective study, most patients had already received antibiotic treatment prior to collecting the samples which might have resulted in a decreased positivity rate compared with CDM. Second, samples were collected only at the initial stage of the disease for comparison with CDM. Due to the high cost of mNGS, we did not perform mNGS to test RNA virus and no longitudinal comparison was performed after the condition had improved. Finally, ascertaining the putative pathogen of infection should be made in conjunction with the clinical manifestations, the findings of both mNGS and CDM, while the interpretation of mNGS findings depends on the clinician's expertise, therefore some bias may still remain.

CONCLUSION

Compared with CDM, mNGS is associated with a higher diagnostic yield of identifying infection and could help differentiate infectious from non-infectious diseases in LTRs. Because of the advantages such as the short turn-around time and the high sensitivity, mNGS might be further pursued as a routine approach for the management of LTRs.

CAPSULE SENTENCE SUMMARY

Infection is the predominant cause of death in lung transplant recipients, timely and accurate anti-infection schemes are vital to ensure the best possible treatment outcomes. However, it is difficult to detect some pathogens using conventional detection methods in clinical practice for various reasons, and conventional culture suffers from the limitations such as being time-consuming. Thus, the diagnosis of lung infection and identification of pathogens is crucial for determining the treatment options in this population. As far as we know, this is first investigation on the clinical application of metagenomic next-generation sequencing (mNGS) in lung transplant recipients. In this study, we collected 159 bronchoalveolar lavage fluid (BALF) and four samples of other body fluid in lung transplant recipients. We found that mNGS detection sensitivity of plumonary infections in lung transplant recipients was significantly higher than that of conventional detection methods. In particular, mNGS revealed the infection of some pathogens that were difficult to detect using conventional detection methods, including *Pneumocystis jirovecii*, mycobacteria, and *Nocardia*. mNGS offers not only a substantially higher

diagnostic sensitivity with a more rapid diagnosis of infectious diseases, but can also help differentiate infectious from non-infectious lung diseases in lung transplant recipients.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, PRJNA737316.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Review Committee of the First Affiliated Hospital of Guangzhou Medical University (No. 128, 2020). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

J-XH and S-YL supervised the study; C-RJ designed the study; C-RJ, Q-YL, W-JG performed the experiments, analyzed the data, interpreted the data, and drafted the manuscript; AC, J-HZ, and XX performed the experiments and collected the study samples; R-CC, S-YL, and J-XH revised the manuscript. All authors reviewed the results and approved the final version of the manuscript.

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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.

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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.2022.10265/full#supplementary-material

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ORIGINAL RESEARCH
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Hepatolithiasis After Living Donor Liver Transplantation in Pediatric Patients: Mechanism, Diagnosis, Treatment, and Prognosis

Yukihiro Sanada, Yasunaru Sakuma, Yasuharu Onishi, Noriki Okada, Yuta Hirata, Toshio Horiuchi, Takahiko Omameuda, Alan Kawarai Lefor and Naohiro Sata*

Division of Gastroenterological, General and Transplant Surgery, Department of Surgery, Jichi Medical University, Shimotsuke, Japan

There is little information about the outcomes of pediatric patients with hepatolithiasis after living donor liver transplantation (LDLT). We retrospectively reviewed hepatolithiasis after pediatric LDLT. Between May 2001 and December 2020, 310 pediatric patients underwent LDLT with hepaticojejunostomy. Treatment for 57 patients (18%) with post-transplant biliary strictures included interventions through double-balloon enteroscopy (DBE) in 100 times, percutaneous transhepatic biliary drainage (PTBD) in 43, surgical re-anastomosis in 4, and repeat liver transplantation in 3. The median age and interval at treatment were 12.3 years old and 2.4 years after LDLT, respectively. At the time of treatments, 23 patients (7%) had developed hepatolithiasis of whom 12 (52%) were diagnosed by computed tomography before treatment. Treatment for hepatolithiasis included intervention through DBE performed 34 times and PTBD 6, including lithotripsy by catheter 23 times, removal of plastic stent in 8, natural exclusion after balloon dilatation in 7, and impossibility of removal in 2. The incidence of recurrent hepatolithiasis was 30%. The 15-years graft survival rates in patients with and without hepatolithiasis were 91% and 89%, respectively (p = 0.860). Although hepatolithiasis after pediatric LDLT can be treated using interventions through DBE or PTBD and its long-term prognosis is good, the recurrence rate is somewhat high.

Keywords: hepatolithiasis, pediatric living donor liver transplantation, percutaneous transhepatic biliary drainage, double-balloon enteroscopy, computed tomography scan

Abbreviations: LT, liver transplantation; LDLT, living donor liver transplantation; PTBD, percutaneous transhepatic biliary drainage; DBE, double-balloon enteroscopy; CT, computed tomography.

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Correspondence:
Yukihiro Sanada
yuki371@jichi.ac.jp

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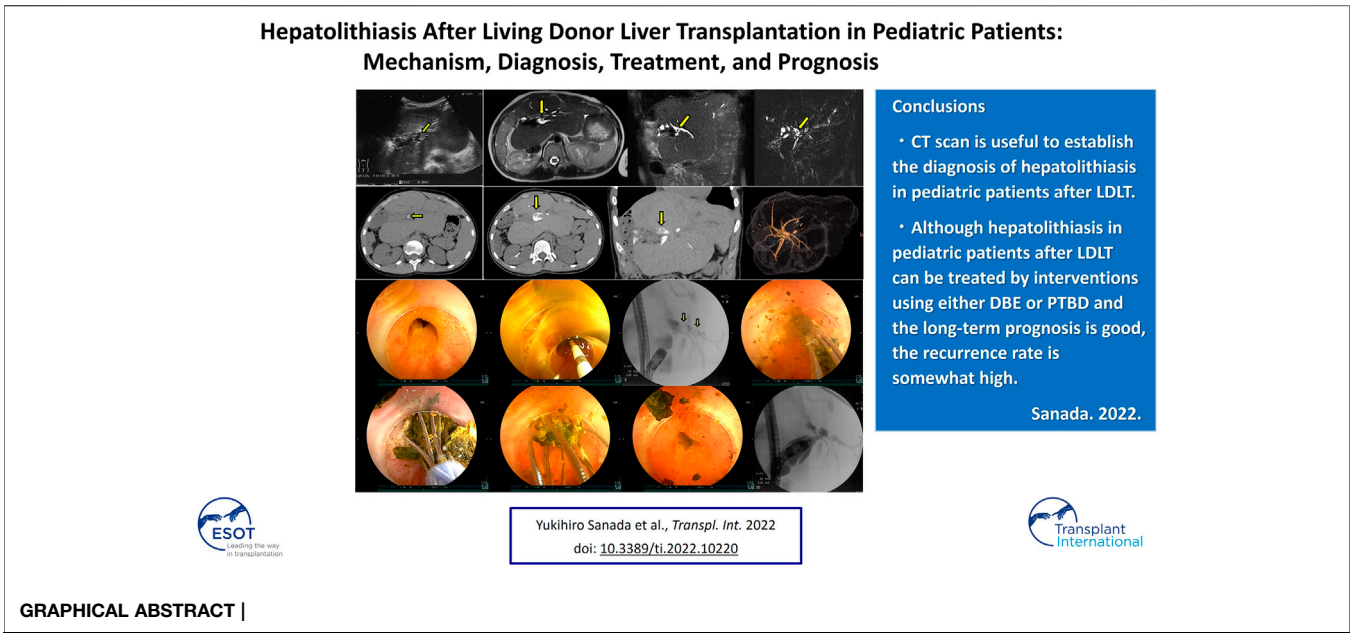

GRAPHICAL ABSTRACT |

INTRODUCTION

Liver transplantation (LT) is an established curative treatment for pediatric patients with end-stage liver disease or acute liver failure. However, post-transplant biliary complications are still frequent despite improvements and innovations in surgical techniques, and these complications occasionally lead to graft failure or even death. The reported incidence of biliary complications after living donor liver transplantation (LDLT) is 10–35% in pediatric recipients (1–6). However, hepatolithiasis after LT has been rarely reported. The reported incidence of hepatolithiasis or biliary cast syndrome after LT is 2.1–9.1% in adult recipients (7–10). The suggested risk factors for hepatolithiasis or biliary cast syndrome after LT include acute cellular rejection, prolonged warm ischemic time, and others (7–10). Few studies have analyzed the risk factors for hepatolithiasis after LT in pediatric recipients.

There are currently two major therapeutic options for biliary complications: surgical and non-surgical interventions. Non-surgical interventions, including percutaneous transhepatic biliary drainage (PTBD) and endoscopic interventions, have emerged as an attractive and less invasive alternatives to surgical intervention in recent years (2, 3). Endoscopic interventions remain controversial in pediatric recipients with a Roux-en-Y hepaticojejunostomy due to the presence of abdominal adhesions, the pediatric physique, and uncertain long-term patency. We reported that endoscopic interventions through double-balloon enteroscopy (DBE) for biliary strictures in pediatric recipients with Roux-en-Y hepaticojejunostomy after LDLT is safer and less invasive than surgical interventions (6). Few studies have analyzed the treatment options for hepatolithiasis after LT in pediatric recipients, and no consensus regarding the optimal approach has yet been reached.

We retrospectively reviewed the mechanism, diagnosis, treatment options and prognosis for pediatric patients with hepatolithiasis after LDLT.

MATERIALS AND METHODS

Patients

Between May 2001 and December 2020, 314 LDLTs were performed for pediatric patients with end-stage liver disease or acute liver failure at the Department of Surgery, Division of Gastroenterological, General and Transplant Surgery, Jichi Medical University, Japan. Of these, four patients underwent LDLT with a choledochocholedochostomy; these patients were excluded from this study. Therefore, a total of 310 LDLTs with a hepaticojejunostomy were reviewed in the present study. Demographic data for recipients and graft information are shown in **Table 1**. Approval to conduct this study was obtained from the Ethics Committees of Jichi Medical University (Ethics Committee Approval Case Number 20-001).

Surgical Procedure of LDLT

The type of donor hepatectomy was selected based on the recipient's standard liver volume, weight and graft volume determined by preoperative computed tomographic volumetry. The donor's biliary anatomy was evaluated using intraoperative real-time cholangiography performed three times to determine the biliary anatomy, decide on the biliary transection line, and confirm absence of biliary leakage. A routine donor hepatectomy was performed using intraoperative ultrasonic guidance. The donor's left hilar plate was transected using a scalpel.

For the recipient's operation, inverted T-shaped or transverse incisions was made, and total hepatectomy was performed. In many infants, after total hepatectomy, the recipient's right, middle and left hepatic veins were formed into a single orifice, and the recipient's hepatic vein was anastomosed to the graft's hepatic vein. The recipient's portal vein was anastomosed to the graft's left portal vein. Hepatic artery reconstruction was performed using microsurgical techniques. Biliary

TABLE 1 | Demographic data for recipients and graft information.

Patient	Recipients with hepatolithiasis	Recipients without hepatolithiasis	*p*-value
Period	May 2001–December 2020		
Number	23	287	
Gender	Male: 11, Female: 12	Male: 107, Female: 180	0.374
Age (years old)	1.8 (0.6–16.0) years old	1.4 (0.0–16.5) years old	0.191
Weight	11.1 (5.8–64.9) kg	9.7 (2.6–62.9) kg	0.108
Original disease	Biliary atresia: 19, OTCD: 2, Wilson's disease: 1, Primary sclerosing cholangitis: 1	Biliary atresia: 202, OTCD: 17, Graft failure: 12, Alagille syndrome: 11, Acute liver failure: 7 Hepatoblastoma: 5, Neonatal hemochromatosis: 5, Others: 28	
ABO-compatibility	Identical/Compatible: 20, Incompatible: 3	Identical/Compatible: 235, Incompatible: 52	0.777
PELD/MELD score	12 (0–26)	9 (0–37)	0.304
Type of graft	Left lateral segment: 13, Left lobe: 6, Left lobe + caudate lobe: 3, Reduced left lateral segment: 1	Left lateral segment: 193, Left lobe: 57, Reduced left lateral segment: 14, Segment 2 monosegment: 13, Left lobe + caudate lobe: 8, Segment 3 monosegment: 1, Posterior segment: 1	
GV/SLV	67.1 ± 25.4%	72.1 ± 20.0%	0.239
Operation time	15 hr38 min ± 5 hr03 min	14 hr30 min ± 4 hr34 min	0.244
Cold ischemic time	2 hr27 min ± 1 hr 29min	2 hr12 min ± 1 hr44 min	0.193
Warm ischemic time	54 min ± 24 min	52 min ± 19 min	0.959
Bleeding volume	78.7 ± 56.1 ml/kg	106.8 ± 124.3 ml/kg	0.837
Transfusion volume	102.7 ± 90.9 ml/kg	135.0 ± 143.3 ml/kg	0.297
Observation period	10.3 ± 5.6 years		

OTCD; ornithine transcarbamylase deficiency, PELD; pediatric end-stage liver disease, MELD; model for end-stage liver disease, GV/SLV; graft volume/standard liver volume ratio.

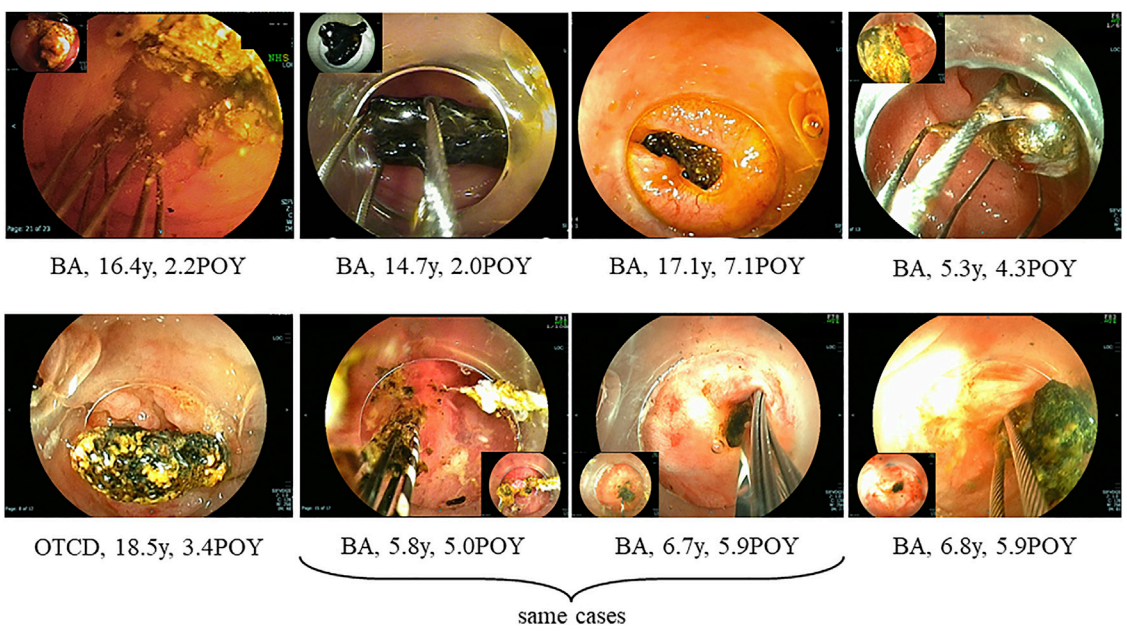

FIGURE 1 | Types of hepatolithiasis diagnosed by computed tomography scan before treatment for post-transplant anastomotic biliary strictures. BA; biliary atresia, POY; post-operative year, OTCD; ornithine transcarbamylase deficiency.

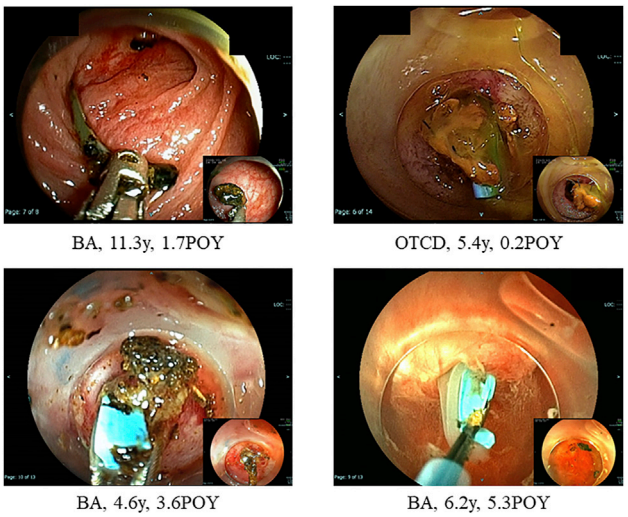

FIGURE 2 | Hepatolith adhering to an internal stent at the time of living donor liver transplantation. BA; biliary atresia, POY; post-operative year, OTCD; ornithine transcarbamylase deficiency.

reconstruction was performed using a Roux-en-Y hepaticojejunostomy which was performed using intraluminal continuous 6-0 absorbable monofilament sutures on the posterior wall and extraluminal interrupted 6-0 absorbable monofilament sutures under surgical loupe vision. Recipients who underwent LDLT between May 2001 and March 2004, and between July 2008 and May 2011 underwent hepaticojejunostomy using an internal stent (4 or 5 Fr pancreatic duct tube, Sumitomo Bakelite co., Ltd., Tokyo, JAPAN, or 10 Fr Blake silicone drains, Johnson & Johnson, Tokyo, JAPAN), while recipients who underwent LDLT between April 2004 and July 2008 underwent biliary reconstruction without a stent. Recipients who underwent LDLT from June 2011 onwards underwent hepaticojejunostomy using an external stent (4 Fr pancreatic duct tube, Sumitomo Bakelite co., Ltd., Tokyo, JAPAN). If drained bile volume decreased to less than 50 ml/day without liver dysfunction, clamping of the external stent without cholangiography was considered. The external stent was removed 3 months after LDLT without cholangiography.

Diagnosis of Post-Transplant Biliary Complications

We diagnosed post-transplant biliary complications including hepatolithiasis when radiologic, endoscopic, or surgical interventions were performed for patients with liver dysfunction or cholangitis due to biliary complications detected by ultrasonography or computed tomography (CT) scan. Obstruction at the biliary stricture site was diagnosed when contrast medium delivered via PTBD did not flow into the Roux-en-Y limb, when no real-time moving images were obtained under fluoroscopy, or when the hepaticojejunal anastomotic site could not be confirmed with DBE.

Therapeutic Strategy for Biliary Complications

We present a summary of the therapeutic strategy for biliary complications including hepatolithiasis based on a previous

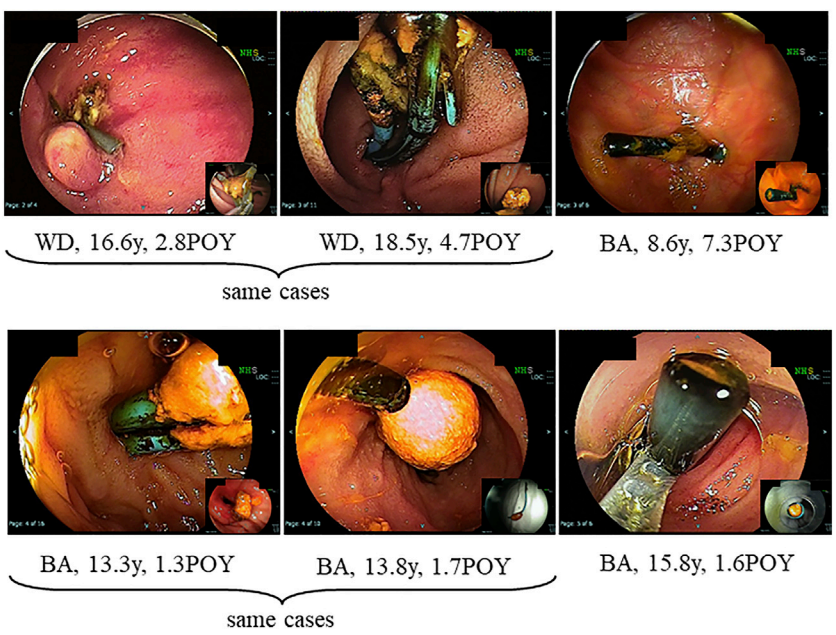

FIGURE 3 | Hepatolith adhering to a plastic stent after treatment for post-transplant anastomotic biliary stricture. WD; Wilson's disease, POY; post-operative year, BA; biliary atresia.

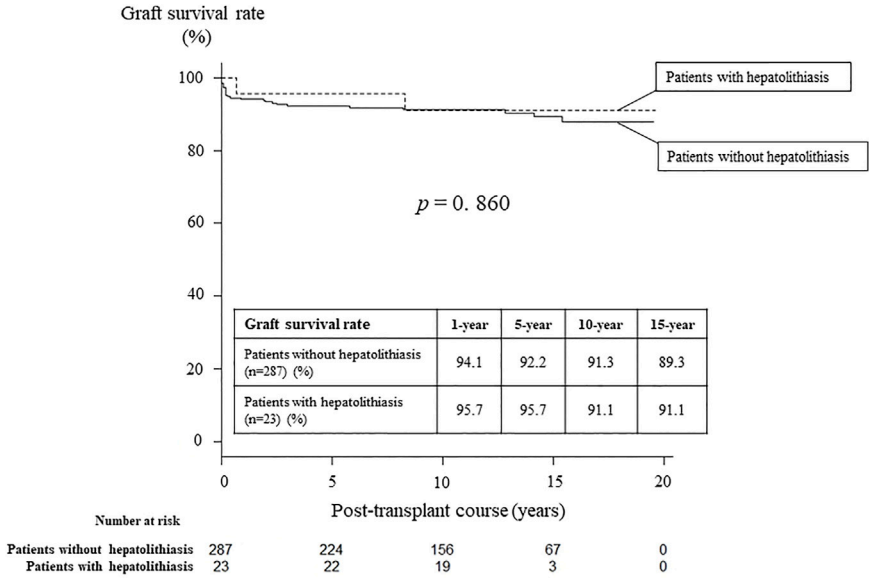

FIGURE 4 | Graft survival rates in patients with and without hepatolithiasis after living donor liver transplantation.

report (6). When patients with suspected biliary complications experience persistent liver dysfunction or recurrent cholangitis, we assessed the hepaticojejunal anastomotic site using PTBD or DBE (EN-450P5/20 or EC-450BI5; Fujifilm Corp., Tokyo, Japan). The indication for DBE is weight greater than 15 kg because of instrument and technical limitations. When patients are diagnosed with biliary strictures by PTBD or DBE, balloon dilatation is performed. When obstruction of the hepaticojejunal anastomotic site or intrahepatic bile duct is diagnosed, balloon dilatation using the Rendezvous penetration method with DBE and PTBD is performed. If non-surgical interventions by balloon dilatation or the Rendezvous technique are unsuccessful, surgical re-anastomosis or repeat LT is performed.

Statistical Analysis

Graft survival rate was calculated by the Kaplan-Meier product-limited method, and differences in survival between two groups then compared using the log-rank test. Statistical analysis was performed using the EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria), and differences were considered to be significant with values of $p < 0.05$.

RESULTS

The overall incidence of post-transplant biliary strictures was 18% (57/310). Treatment for patients with post-transplant biliary strictures included interventions through DBE in 100 patients, interventions after PTBD in 43, surgical re-anastomosis in 4, and repeat LT in 3. The median age and post-transplant interval at treatment were 12.3 years old (range 0.7–25.8) and 2.3 years after LDLT (range 0.1–19.3), respectively.

At treatments, 23 patients (7%) had hepatolithiasis of whom 12 patients (52%) were diagnosed by CT scan before

TABLE 2 | Univariate analysis of risk factors for post-transplant complications in recipients with and without hepatolithiasis.

Variable	Recipients with hepatolithiasis N = 23	Recipients without hepatolithiasis N = 287	*p*-value
Hepatic vein complications	0 (0%)	24 (8.4%)	0.236
Portal vein complications	5 (21.7%)	41 (14.3%)	0.358
Hepatic artery complications	2 (8.7%)	15 (5.2%)	0.365
Re-laparotomy after LDLT	4 (17.4%)	33 (11.5%)	0.498
Acute cellular rejection	11 (47.8%)	115 (40.1%)	0.512
Steroid-resistant acute rejection	2 (8.7%)	32 (11.1%)	0.999
Cytomegalovirus viremia	9 (39.1%)	106 (36.9%)	0.826
Post-transplant lymphoproliferative disorder	1 (4.3%)	5 (1.7%)	0.373
Hospital length of stay	43 ± 28 days	47 ± 46 days	0.501

LDLT; living donor liver transplantation.

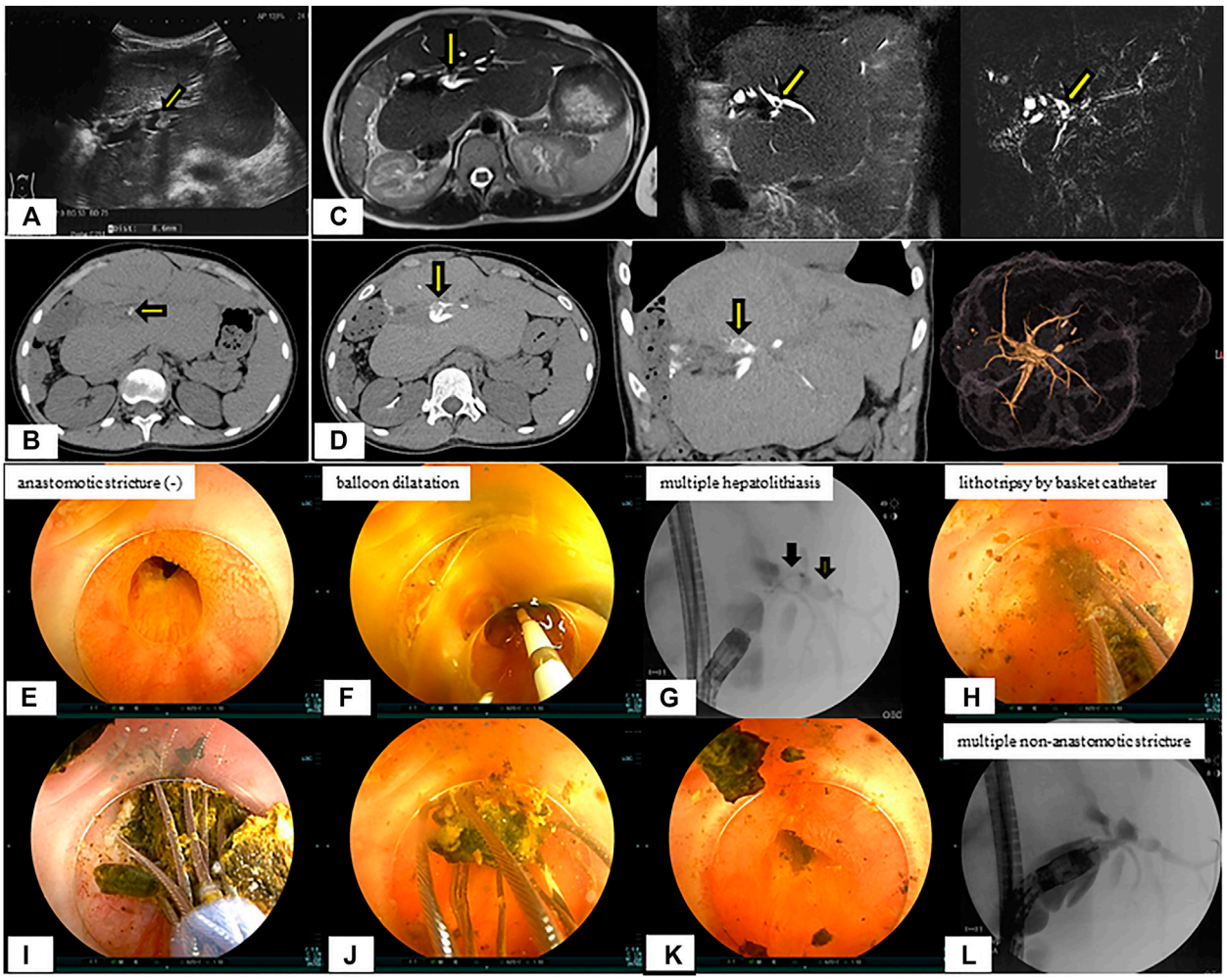

FIGURE 5 | Radiological findings and endoscopic lithotripsy for hepatolithiasis in a patient with multiple non-anastomotic biliary strictures. **(A)** Hepatolith (diameter 8.6 mm) with acoustic shadowing on ultrasonography was observed at the confluence of the biliary tracts of Segments 2 and 3. **(B)** Hepatolithiasis with a high density lesion on computed tomography scan was observed at the confluence of the biliary tracts of Segments 2 and 3. **(C)** Magnetic resonance imaging. **(D)** Drip infusion cholangiographic-computed tomography. **(E)** Anastomotic biliary stricture was not observed by double-balloon enteroscopy. **(F)** Balloon dilatation for intrahepatic bile duct stricture was performed. **(G)** Multiple hepatoliths were demonstrared on cholangiography at the confluence of the biliary tracts of Segments 2 and 3. **(H–K)** Lithotripsy by basket catheter was performed. **(L)** Multiple intrahepatic bile duct strictures on cholangiography were observed and hepatoliths disappeared.

treatment (**Figure 1**). The hepatoliths were all calcium bilirubinate calculi. Treatment for hepatolithiasis included interventions through DBE in 34 times and interventions through PTBD in 6 times, including lithotripsy by catheter 23 times, removal of the plastic stent in 8 (**Figures 2**, **3**), natural exclusion after balloon dilatation in 8, and impossibility of removal in 2. The incidence of recurrent hepatolithiasis was 30%, and repeat treatment was performed multiple times (range 2–6). The 15-years graft survival rates in patients with and without hepatolithiasis after LDLT were 91% and 89%, respectively (p = 0.860) (**Figure 4**), and the causes of graft failure included antibody-mediated rejection due to ABO-incompatible LDLT and chronic rejection. There was no significant difference in the rate of post-transplant complications between patients with and without hepatolithiasis (**Table 2**).

Presentation of a Patient With Hepatolithiasis

A female with biliary atresia underwent LDLT using a left lateral segment graft from her mother at age 0.8 years. Hepatic arterial thrombosis developed on post-operative days 4, 7, and 17. She underwent percutaneous transfemoral artery balloon dilatation on each occasion. Non-anastomotic biliary strictures developed on post-operative days 36 and 50. She underwent PTBD on each occasion. Thereafter, she has suffered from repeat episodes of cholangitis and mild liver dysfunction.

After developing acute cholangitis at age 15.3 years, she was diagnosed with hepatolithiasis by imaging studies (**Figures 5A–D**). She was diagnosed with hepatolithiasis with a non-anastomotic biliary stricture by direct vision and cholangiography using DBE. Lithotripsy by basket catheter was performed (**Figures 5E–L**). Although she had a small hepatolith a year after treatment, she is doing well without further episodes of cholangitis.

DISCUSSION

Hepatolithiasis after LT has been rarely reported. The reported incidence of hepatolithiasis or biliary cast syndrome after LT is 2.1–9.1% in adult recipients (7–10). However, few studies have analyzed the incidence of hepatolithiasis after LT in pediatric recipients. Therefore, no consensus on the optimal diagnostic or treatment strategies has been reached, and the prognosis of hepatolithiasis after LT in pediatric recipients is not defined. The suggested risk factors for hepatolithiasis or biliary cast syndrome after LT include acute cellular rejection, prolonged warm ischemic time, and others in adult recipients (7–10). In this study, the suggested risk factors for hepatolithiasis after LDLT in pediatric recipients made clear hepaticojejunostomy, internal stent placed during LDLT, plastic stent placed after treatment for post-transplant anastomotic biliary stricture, and non-anastomotic biliary stricture. The hepatoliths were all calcium bilirubinate calculi. Therefore, reflux of intestinal juice via hepaticojejunostomy, adhesion to an internal stent placed during LDLT, a plastic stent placed after treatment for post-transplant anastomotic biliary stricture (**Figures 2**, **3**), and biliary stasis due to non-anastomotic biliary strictures were associated with the development of hepatolithiasis. In our institution, recipients who underwent LDLT after June 2011 underwent hepaticojejunostomy using an external stent. Thereafter, the incidence of post-transplant anastomotic biliary strictures was significantly decreased. After this, patients with adhesion to an internal stent placed at LDLT or a plastic stent placed after treatment for post-transplant anastomotic biliary strictures should be decreasing, and therefore, the incidence of hepatolithiasis is also expected to decrease. On the other hands, the causes and prognosis of post-transplant non-anastomotic biliary strictures are unclear, but in adult recipients, it has been reported that the incidence of repeat LT and mortality was high because it is difficult to treat non-anastomotic biliary strictures and to resolve the cause of non-anastomotic biliary strictures (11, 12). In this study, one patient after lithotripsy for hepatolithiasis developed intractable cholangitis with repeat hepatolithiasis. The long-term prognosis of patients with post-transplant non-anastomotic biliary strictures is not defined, and patients with post-transplant non-anastomotic biliary strictures may eventually need repeat LT.

We diagnosed post-transplant biliary complications including hepatolithiasis when radiologic, endoscopic, or surgical interventions were performed for the patients with liver dysfunction or cholangitis due to biliary complications detected by ultrasonography or CT scan (**Figure 5B**). Hepatolithiasis was diagnosed in 12 patients (52%) by CT scan before treatment was performed (**Figure 1**). Indwelling internal stents and long-term indwelling plastic stents should be noted, and CT scan is useful to establish the diagnosis of hepatolithiasis in these patients after LDLT in pediatric patients.

There are currently two major therapeutic options for patients with biliary complications that can be classified as surgical and non-surgical interventions. Non-surgical interventions, including PTBD or endoscopic interventions, have emerged as an attractive and less invasive alternative to surgical interventions in recent years (2, 3). We have reported that endoscopic interventions through DBE to evaluate and treat biliary strictures in pediatric patients with Roux-en-Y hepaticojejunostomies after LDLT is safer and less invasive than surgical interventions (6). Although few studies have analyzed treatment options for pediatric patients with hepatolithiasis after LT, with advances in endoscopic instrumentation and techniques in recent years, endoscopic treatment of hepatolithiasis using DBE has become possible. Therefore, in our institution, the first-line treatment for post-transplant biliary complications including hepatolithiasis in pediatric patients with a Roux-en-Y hepaticojejunostomy is endoscopic intervention using DBE. However, in this study, the incidence of recurrent hepatolithiasis was 30%, and treatment was repeated multiple times (range 2–6). This recurrence rate of hepatolithiasis is thought to be associated with the use of endoscopic interventions using DBE.

In conclusion, mechanisms causing hepatholitiasis following pediatric LDLT and also preventive measures were made clear in this study. In addition, diagnostic methods and treatment options for hepatolithiasis following pediatric LDLT were showed for the first time. CT scan is useful to establish the diagnosis of hepatolithiasis in pediatric patients after LDLT. Although hepatolithiasis in pediatric patients after LDLT can be treated by interventions using either DBE or PTBD and the long-term prognosis is good, the recurrence rate is somewhat high. Further studies of our policy for the diagnosis and treatment of hepatolithiasis after LDLT and the accumulation more experience are necessary.

CAPSULE SENTENCE SUMMARY

The overall incidence of hepatolithiasis after pediatric living donor liver transplnatation was 7% (23/310). Although hepatolithiasis in pediatric patients can be treated by interventions using either double-balloon enteroscopy or percutaneous transhepatic biliary drainage and the long-term prognosis is good, the recurrence rate is somewhat high.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committees of Jichi Medical University.

Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

Study design: YuS. Acquisition of data: YuS, YO, NO, YH, TH, and TO. Analysis and interpretation: YuS. Revision: YaS, AL, and NS.

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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ORIGINAL RESEARCH
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The Influence of Diabetes Mellitus on the Risks of End-Stage Kidney Disease and Mortality After Liver Transplantation

*Chung-Ying Lee[1,2†], Mei-Yi Wu[3,4,5,6†], Hsiu-Chen Chan[7], Tzu-Ting Chen[5,8], Le-Yin Hsu[5], Mai-Szu Wu[3,4,6,9] and Yih-Giun Cherng[10,11]**

[1]Division of Gastroenterology, Department of Internal Medicine, Shuang Ho Hospital, Taipei Medical University, New Taipei City, Taiwan, [2]Division of Gastroenterology, Department of Internal Medicine, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan, [3]Division of Nephrology, Department of Internal Medicine, Shuang Ho Hospital, Taipei Medical University, New Taipei City, Taiwan, [4]Division of Nephrology, Department of Internal Medicine, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan, [5]Institute of Epidemiology and Preventive Medicine, College of Public Health, National Taiwan University, Taipei, Taiwan, [6]TMU Research Center of Urology and Kidney, Taipei Medical University, Taipei, Taiwan, [7]Department of Pharmacy, Shuang Ho Hospital, New Taipei City, Taiwan, [8]Center for Neuropsychiatric Research, National Health Research Institutes, Miaoli, Taiwan, [9]Graduate Institute of Clinical Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan, [10]Department of Anesthesiology, Shuang Ho Hospital, Taipei Medical University, New Taipei City, Taiwan, [11]Department of Anesthesiology, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan

This retrospective study aimed to investigate the effect of diabetes mellitus (DM) on the risks of end-stage kidney disease (ESKD) and post-liver transplantation (post-LT) mortality. Using data from the National Health Insurance Research Database, Taiwan, 3,489 patients who received a LT between 1 January 2005, and 31 December 2015, were enrolled in this study and divided into the pre-existing DM, post-LT DM (PLTDM), and without DM groups. All subjects were followed up from 1 year after LT to the index date for ESKD, and the occurrence of death, or until 31 December 2016. Of the 3,489 patients with LT, 1,016 had pre-existing DM, 215 had PLTDM, and 2,258 had no DM pre- or post-LT. The adjusted HRs of ESKD were 1.77 (95% Confidence Interval [CI], .78–3.99) and 2.61 (95% CI, 1.63–4.18) for PLTDM group and pre-existing DM group compared to without DM group, respectively. For the risk of death, the adjusted HRs were 1.05 (95% CI, .72–1.55) and 1.28 (95% CI, 1.04–1.59) for PLTDM group and pre-existing DM group compared to those without DM group, respectively. The sensitivity analysis for the risk of ESKD and death also revealed the consistent result. Pre-existing DM has significant increase the risk of post-LT ESKD and mortality. The role of PLTDM should be explored to explain postoperative morbidity and mortality.

Keywords: liver transplantation, diabetes mellitus, risk, end-stage kidney disease, mortality

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***Correspondence:**
Yih-Giun Cherng
stainless@s.tmu.edu.tw

†These authors have contributed equally to this work

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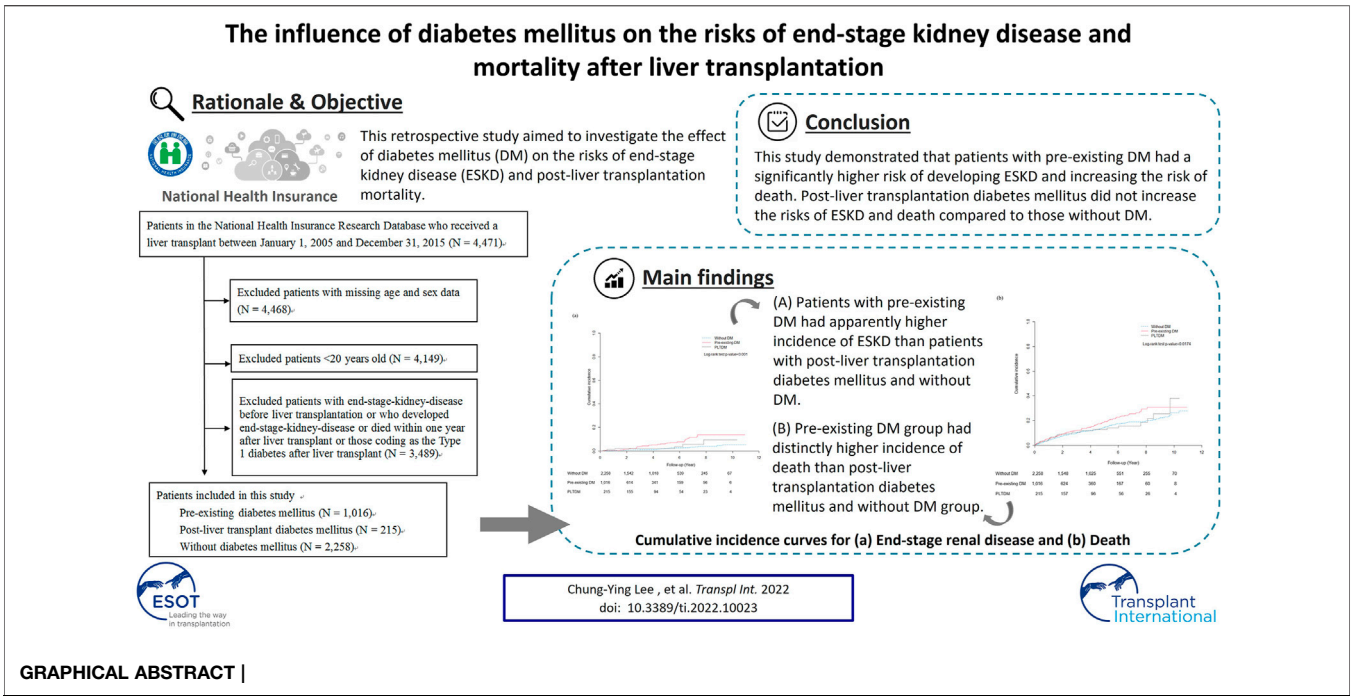
Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CCB, calcium channel blocker; CI, confidence interval; CKD, chronic kidney disease; DM, diabetes mellitus; ESKD, end-stage kidney disease; HRs, hazard ratios; ICD-9-CM, International Classification of Disease, Revision 9, Clinical Modification; LT, liver transplantation; mTOR, mammalian target of rapamycin; NHI, National Health Insurance; NHIRD, National Health Insurance Research Database; PLTDM, post-liver transplantation diabetes mellitus; Post-LT, post-liver transplantation.



GRAPHICAL ABSTRACT |

INTRODUCTION

Liver transplantation (LT) is an effective strategy for treating patients with end-stage liver disease and some types of hepatocellular carcinomas (1). With the advancements in surgical techniques and the use of immunosuppressants, patient survival rates have improved globally, reaching nearly 85% at 1 year and 73% at 5 years in Europe and 88% at 1 year and 70% at 5 years in the United States (2). A recent population-based study in Taiwan revealed that the overall 1-year and 5-year survival rate post-LT was 85.1% and 79.6%, respectively (3). An improvement in the early post-LT survival rate underscores the importance of understanding the causes and risk factors for late post-LT mortality.

Renal dysfunction is common in recipients of liver transplant and is a known risk factor for mortality in patients who have undergone LT (4, 5). Cohen et al. reported that 27.5% of LT patients had severe renal dysfunction (measured glomerular filtration rate <40 ml/min/1.73 m^2) at 5 years with a cumulative incidence of end-stage kidney disease (ESKD) of 6.25% at 7 years and 10% at 10 years (6). Moreover, studies have shown that pre-existing diabetes mellitus (DM), associated with microvascular and macrovascular complications, may influence post-LT morbidity and mortality (7, 8). Post-LT DM (PLTDM) develops in up to 30% of liver transplant recipients, negatively affecting long-term survival (9). However, conflicting results on the effect of PLTDM on post-LT mortality rates exist (10). The relatively limited number of studies examining the impact of pre-existing DM and PLTDM on long-term renal outcomes and mortality, especially the risks of ESKD, prompted us to conduct this retrospective study to investigate the influence of DM on the risks of ESKD and all-cause mortality post-LT by using patient data from the National Health Insurance Research Database (NHIRD) in Taiwan.

METHODS

Data Collection

We conducted a Nationwide population-based retrospective cohort study using data from the NHIRD, Taiwan. Taiwan initiated its National Health Insurance (NHI) program in 1995. The system covered almost 99% of the entire population in 2007. Taiwan's population in 2015 was approximately 23 million and the more than 99% of the population is covered by the NHI program. De-identified and computerized data were provided by the National Health Insurance Administration, which organizes claims data for NHI and established the NHIRD. The NHIRD contains basic patient information and medical data from medical claims, including clinical diagnostic codes based on the International Classification of Disease, Revision 9, Clinical Modification (ICD-9-CM). According to the guidelines of the NHI program, the diagnosis code for LT would have been entered by a qualified gastroenterologist or transplant surgeon. The study adhered to the ethical standards of the 2000 Declaration of Helsinki and the Declaration of Istanbul 2008. No executed prisoners were used as donors.

Study Population and Study Design

The recipients were identified from the NHIRD database using the LT surgery code (codes 75020A or 75020B) from 1 January 2005, to 31 December 2015. We excluded patients with missing age and sex data, who were <20 years old at the time of surgery,

who had been diagnosed with ESKD before LT, or who had been coding as the Type 1 diabetes after LT. We also excluded patients who had developed ESKD or died within 1 year after LT to reduce the immortal time bias. The recipients were divided into three groups: pre-existing DM, PLTDM, and without DM. DM (ICD-9-CM code: 250) was identified from medical notes recorded either three or more times in the outpatient department or one or more times in the inpatient department within 1 year before the index date of LT. PLTDM group was defined as those diagnosed as having DM after LT within 1 year. After these three groups were defined, all subjects were start followed from 1 year after LT to the index date for ESKD, the occurrence of death, or until 31 December 2016 to evaluate the risk of ESKD. To estimate the risk of death, all subjects were followed from 1 year after LT to the occurrence of death or until 31 December 2016. We showed our detailed main study design for ESKD and death in **Supplementary Figures S1, S2**, respectively.

Outcomes

The primary outcomes in this study are ESKD and death. Patients who had been diagnosed with ESKD were identified when the use of hemodialysis codes (58001C, 58014C, 58019C, 58020C, 58021C, 58022C, 58023C, 58024C, 58025C, 58027C, 58029C, 58030B, 69006C) was more than 24 times in three consecutive months and peritoneal dialysis codes (58002C, 58009B, 58010A, 58010B, 58011A, 58011AB, 58011B, 58011C, 58012A, 58012B, 58017B, 58017C, 58028C) was more than three consecutive months or renal transplantation surgery (76020A, 76020B) was performed. Mortality data were obtained from the Taiwanese Ministry of Internal Affairs, cause of death database and included information on the date and cause of death.

Covariates

Comorbidities were identified from medical notes recorded either three or more times in the outpatient department or one or more times in the inpatient department within 1 year before the index date for LT. The following comorbidities were identified among patients in our study cohort with ICD-9-CM codes: hypertension (ICD-9-CM codes: 401–405), hyperlipidemia (ICD-9-CM codes: 272.0–272.4), chronic kidney disease (CKD) (ICD-9-CM codes: 2504, 2741, 28311, 403, 404, 4401, 4421, 4473, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 6421, and 6462), myocardial infarction (ICD-9-CM codes: 410 and 412), and congestive heart failure (ICD-9-CM codes: 398.91, 402.01, 402.11, 402.91, 404.01, 404.03, 404.11, 404.13, 404.91, 404.93, and 428). Immunosuppressant-use was defined as the use of calcineurin inhibitors, antimetabolic agents (purine antagonist), mammalian target of rapamycin (mTOR) inhibitors, and corticosteroids during hospitalization. The usage of antihypertensive agent for more than 90 days within 1 year before the date for LT was also recorded, including angiotensin-converting enzyme inhibitor (ACEI), angiotensin receptor blocker (ARB), calcium channel blocker (CCB), diuretics, β-blockers and α-blockers.

Statistical Analysis

For baseline covariates, we used the analysis of variance and chi-square test to test continuous variables and category variables among three groups, respectively. To evaluate the risk of ESKD and death, we used the Cox proportional hazard models. In Cox proportional hazard models, we adjusted for potential confounders, such as age, sex, hypertension, hyperlipidemia, CKD, myocardial infarction, congestive heart failure, calcineurin inhibitors, antimetabolic agent (purine antagonist), mTOR inhibitors, corticosteroids, and antihypertensive agents to minimize confounding bias. We also assessed the assumption of proportional hazards for Cox proportional hazard models.

Sensitivity Analyses

To deal with the immortal time bias, we did sensitivity analyses by using different study design (**Supplementary Figures S3, S4**). In the sensitivity analyses, all subjects were followed from the hospital discharge date for LT to the index date for ESKD, the occurrence of death, or until 31 December 2016 to evaluate the risk of ESKD. To estimate the risk of death, all subjects were followed from the hospital discharge date for LT to the occurrence of death or until 31 December 2016. We excluded patients who had developed ESKD before the index date for LT, but we did not exclude patients who had developed ESKD or died within 1 year after LT. Moreover, the time from the hospital discharge date for LT to the index date for PLTDM was accounted as the time in non-DM.

RESULTS

A flowchart of the patient selection process is shown in **Figure 1**. After excluding patients with missing age and sex data, age <20 years old at the time of surgery, with ESKD before LT, who had been coding as the Type 1 diabetes after LT and who had developed ESKD or died within 1 year after LT, a total of 3,489 patients were included, of which, 1016 (29.1%) had pre-existing DM, 215 (6.2%) had PLTDM, and 2,258 (64.7%) did not have DM before or after LT. The distribution of demographic characteristics, comorbid medical disorders, and the use of immunosuppressant and antihypertensive agents for pre-existing DM group, PLTDM group, and without DM group are shown in **Table 1**. The mean age was higher in the pre-existing DM group (54.90 ± 7.28 years) than in the PLTDM (53.52 ± 7.99 years) and without DM groups (51.53 ± 9.34 years). Patients in the pre-existing DM group had a higher rate of comorbid medical disorders, including hypertension, hyperlipidemia, and CKD, than the PLTDM and without DM groups. For the use of immunosuppressants, including calcineurin inhibitor, antimetabolic agent (purine antagonist), mTOR inhibitors, corticosteroids and antihypertensive agents, including ACEI, ARB, CCB, diuretics, β-blockers and α-blockers, were reported.

Table 2 shows the incidence rate, crude and the adjusted hazard ratios (HRs) for ESKD among three groups during the 12-year follow-up. The incidence rates were 4.2, 8.1 and 13.1 per

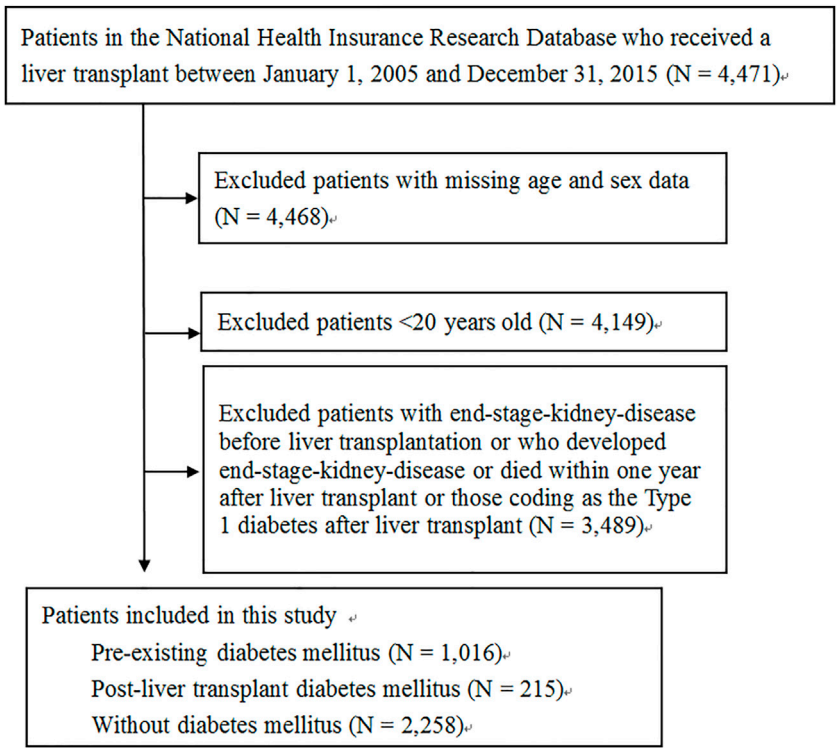


FIGURE 1 | Flowchart of the selection criteria and process of selecting eligible patients.

1,000 person-years for without DM group, PLTDM group and pre-existing DM group, respectively. The crude HRs of ESKD for PLTDM group and pre-existing DM group were 1.92 (95% confidence interval [CI], .86–4.31, p = .1117) and 3.29 (95% confidence interval [CI], 2.12–5.10, p < .001) compared to without DM group, respectively. After adjustment for age, sex, hypertension, hyperlipidemia, CKD, myocardial infarction, congestive heart failure, calcineurin inhibitors, antimetabolic agent (purine antagonist), mTOR inhibitors, corticosteroids, and antihypertensive agents, the adjusted HRs of ESKD were 1.77 (95% CI, .78–3.99, p = .1694) and 2.61 (95% CI, 1.63–4.18, p< .001) for PLTDM group and pre-existing DM group compared to without DM group, respectively. We showed the models for ESKD and death in **Supplementary Tables S1, S2**, respectively.

For the risk of death, we show the incidence rate, the crude and the adjusted HRs among three groups during the 12-year follow-up in **Table 3**. The incidence rates were 31.6, 33.0 and 43.1 per 1,000 person-years for without DM group, PLTDM group and pre-existing DM group, respectively. The crude HRs were 1.05 (95% CI, .72–1.54, p = .7979) and 1.34 (95% CI, 1.10–1.64, p = .0045) for PLTDM group and pre-existing DM group compared to those without DM group, respectively. After adjustment for age, sex, hypertension, hyperlipidemia, CKD, myocardial infarction, congestive heart failure, calcineurin inhibitors, antimetabolic agent (purine antagonist), mTOR inhibitors, corticosteroids, and antihypertensive agents, the adjusted HRs were 1.05 (95% CI, .72–1.55, p = .7915) and 1.28 (95% CI, 1.04–1.59, p = .0204) for PLTDM group and pre-existing DM group compared to those without DM group, respectively.

Otherwise, we also performed the sensitivity analysis for the risk of ESKD and death, which is disclosed in **Table 4**. When compared to those without DM group, the adjusted HRs for the risk of ESKD and death were 1.70 (95% CI, .93–3.09, p = .0847) and 0.89 (95% CI, .69–1.14, p = .3383) for PLTDM group, and were 2.28 (95% CI, 1.51–3.43, p < .001) and 1.18 (95% CI, 1.01–1.39, p = .0373) for pre-existing DM group, respectively.

The cumulative incidence curves for ESKD and death during the follow-up period are shown in **Figure 2**. The cumulative incidence of ESKD for pre-existing DM, PLTDM, and without DM groups were significantly different (Log-rank test p < .001). Patients with pre-existing DM had apparently higher incidence of ESKD than patients with PLTDM and without DM. For mortality, the cumulative incidence for pre-existing DM, PLTDM, and without DM groups were significantly different (Log-rank test p = .0174). Overall, pre-existing DM group had distinctly higher incidence of death than PLTDM and without DM group.

TABLE 1 | Characteristics of liver transplant patients.

Characteristic	DM	PLTDM	Non-DM	*p*-value
Number of patients	1,016	215	2,258	
Age, mean (SD), years	54.90 ± 7.28	53.52 ± 7.99	51.53 ± 9.34	<.0001
Age group, years, *N* (%)				<.0001
20–39	28 (2.8)	14 (6.5)	249 (11.0)	
40–59	699 (68.8)	150 (69.8)	1,568 (69.4)	
60–79	289 (28.4)	51 (23.7)	441 (19.5)	
Sex, male, *N* (%)	736 (72.4)	153 (71.2)	1,656 (73.3)	.7209
Comorbidities before the index date, *N* (%)				
Hypertension	362 (35.6)	38 (17.7)	345 (15.3)	<.0001
Hyperlipidemia	128 (12.6)	4 (1.9)	78 (3.5)	<.0001
Chronic kidney disease	179 (17.6)	20 (9.3)	190 (8.4)	<.0001
Myocardial infarction	5 (.5)	0 (.0)	2 (.1)	.0460
Congestive heart failure	15 (1.5)	3 (1.4)	19 (.8)	.2303
Treatment with drugs after liver transplant, *N* (%)				
Calcineurin inhibitors	1,004 (98.8)	214 (99.5)	2,229 (98.7)	.5729
Antimetabolic agent (Purine antagonist)	759 (74.7)	145 (67.4)	1,614 (71.5)	.0455
MTORIs	204 (20.1)	19 (8.8)	393 (17.4)	.0004
Corticosteroids	1,014 (99.8)	215 (100.0)	2,253 (99.8)	.7859
Treatment with drugs within 1 year prior to liver transplant, *N* (%)				
Antihypertensive agents	524 (51.6)	81 (37.7)	647 (28.7)	<.0001
ACEI	43 (4.2)	4 (1.9)	20 (0.1)	<.0001
ARB	132 (13.0)	8 (3.7)	83 (3.7)	<.0001
CCB	132 (13.0)	13 (6.1)	111 (4.9)	<.0001
Diuretic	582 (57.3)	135 (62.8)	1,033 (45.8)	<.0001
β-blockers	379 (37.3)	64 (29.8)	513 (22.7)	<.0001
α-blockers	15 (1.5)	2 (0.9)	10 (.4)	.0074
Hypoglycemic agent	731 (72.0)	—	—	—
Type I DM	74 (7.3)	0 (0.00)	—	—

TABLE 2 | Incidence rate for end-stage renal disease.

	No. of event	Person-years	Incidence rate	Crude		Adjusted[a]	
				Hazard ratio (95% CI)	*p*-value	Hazard ratio (95% CI)	*p*-value
Non-DM	38	8,947	4.2	Ref.		Ref.	
PLTDM	7	860	8.1	1.92 (0.86–4.31)	.1117	1.77 (.78–3.99)	.1694
DM	43	3,279	13.1	3.29 (2.12–5.10)	<.0001	2.61 (1.63–4.18)	<.0001

[a]*Adjustment: age, sex, hypertension, hyperlipidemia, chronic kidney disease, myocardial infarction, congestive heart failure, calcineurin inhibitors, antimetabolic agent (Purine antagonist), mTORIs and corticosteroids, antihypertensive agents.*

DISCUSSION

We performed a Nationwide population-based retrospective cohort study of patients who received an LT between 2005 and 2015 to evaluate the influence of DM on the risk of ESKD and mortality after LT. During the 12-year follow-up period, patients with pre-existing DM had a significantly higher risk of ESKD and mortality after LT. Patients with PLTDM did not increase the risk of ESKD and death after LT compared to those without DM. To our knowledge, this is the first study to examine the risk of ESKD and mortality after LT among patients with pre-existing DM, PLTDM and without DM.

DM is a group of metabolic diseases characterized by hyperglycemia, which is associated with microvascular and macrovascular complications resulting in long-term damage and failure of various organ systems (11). A case–control study that compared mortality rates after LT, including 57 patients with pre-existing DM (3, type I; 54, type II) and 114 age-, sex-, and race-matched patients without DM showed that 5-year survival was significantly lower in the DM group (34.4% vs. 67.7%, $p = .002$) (7). Here, the study population was large, and the results consistently showed that pre-existing DM reduces 12-year post-operation long-term survival for patients who have received LT.

In addition to pre-existing DM, PLTDM has emerged as a problem, which is diagnosed according to the 2003 International Consensus Guidelines. LT recipients who had no DM before transplantation but developed symptoms of DM with an elevated random plasma glucose (≥200 mg/dl) or an elevated fasting plasma glucose (≥126 mg/dl) or an elevated 2-h plasma

TABLE 3 | Incidence rate for death.

	No. of event	Person-years	Incidence rate	Crude		Adjusted[a]	
				Hazard ratio (95% CI)	*p*-value	Hazard ratio (95% CI)	*p*-value
Non-DM	285	9,032	31.6	Ref.		Ref.	
PLTDM	29	878	33.0	1.05 (.72–1.54)	.7979	1.05 (.72–1.55)	.7915
DM	145	3,362	43.1	1.34 (1.10–1.64)	.0045	1.28 (1.04–1.59)	.0204

[a]*Adjustment: age, sex, hypertension, hyperlipidemia, chronic kidney disease, myocardial infarction, congestive heart failure, calcineurin inhibitors, antimetabolic agent (Purine antagonist), mTORIs and corticosteroids, antihypertensive agents.*

TABLE 4 | Sensitivity analysis of risk of end-stage renal disease and death.

	No. of event	Person-years	Incidence rate	Crude		Adjusted[a]	
				Hazard ratio (95% CI)	*p*-value	Hazard ratio (95% CI)	*p*-value
End-stage renal disease							
Non-DM	47	10,021	4.7	Ref.		Ref.	
PLTDM	14	1,740	8.0	1.74 (0.96–3.16)	.0692	1.70 (.93–3.09)	.0847
DM	63	4,345	14.5	3.15 (2.15–4.60)	<.0001	2.28 (1.51–3.43)	<.0001
Death							
Non-DM	456	10,146	44.9	Ref.		Ref.	
PLTDM	73	1,770	41.2	.90 (.70–1.15)	.4104	.89 (.69–1.14)	.3383
DM	279	4,471	62.4	1.32 (1.14–1.53)	.0003	1.18 (1.01–1.39)	.0373

[a]*Adjustment: age, sex, hypertension, hyperlipidemia, chronic kidney disease, myocardial infarction, congestive heart failure, calcineurin inhibitors, antimetabolic agent (Purine antagonist), mTORIs and corticosteroids, antihypertensive agents.*

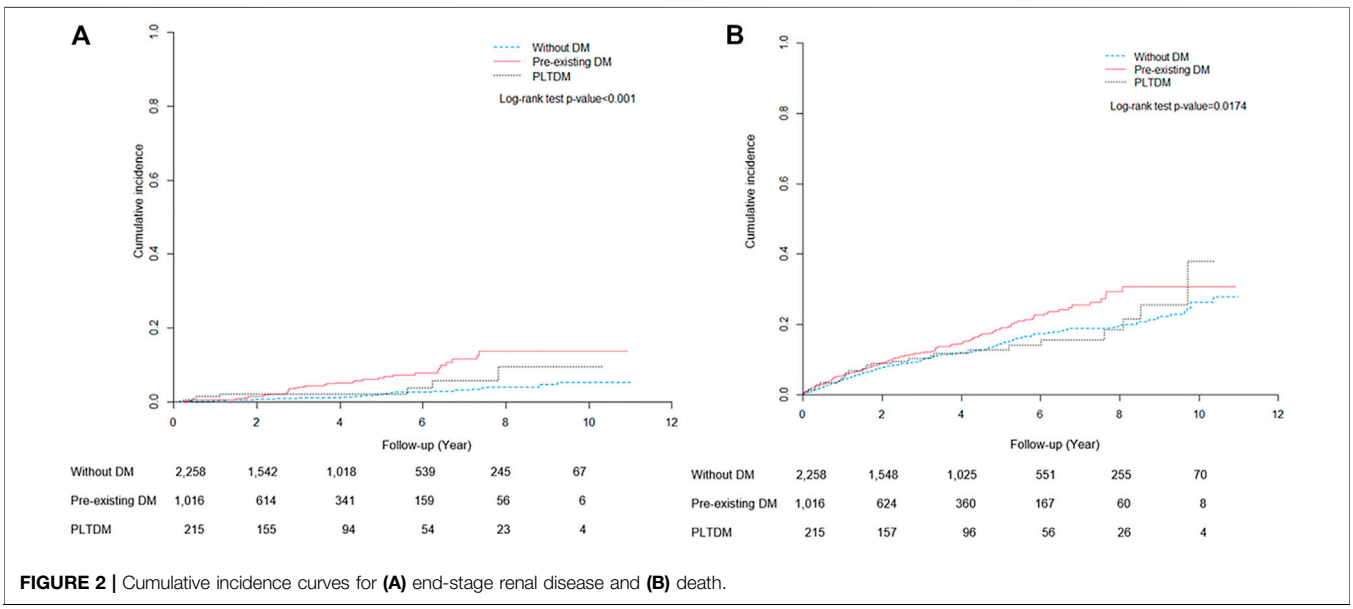


FIGURE 2 | Cumulative incidence curves for **(A)** end-stage renal disease and **(B)** death.

glucose (≥200 mg/dl) during an oral glucose tolerance test were diagnosed to have PLTDM (12). PLTDM is a disorder with many risk factors, such as sex, use of immunosuppressants of the CNI family (tacrolimus and cyclosporine) or corticosteroid, pretransplant overweight, nonalcoholic steatohepatitis or hepatitis C infection, and type of liver donor (9). In this study, we found that 8.7% (215/2473) of patients who received LT developed PLTDM. The incidence of PLTDM was reported to range between 7.2% and 38% and may increase the risk of mortality and multiple morbidities (9). Studies have shown

that PLTDM contributes to an increased risk of cardiovascular disease, infection, and transplant rejection, which negatively affects graft survival and survival (13, 14). In DM, elevated blood sugar levels are expected to increase cardiovascular risk and therefore mortality (15). Our results showed patients with PLTDM did not significantly reduce the long-term survival after LT during 12-years follow-up period when compared to those without DM. However, this relationship has not been clearly established due to the relatively few studies with small sample sizes. Two studies have reported conflicting results and shown that PLTDM is associated with an improved 5-year survival after LT, probably due to uncontrolled confounding factors (10, 16). Patients often present with cachexia and sarcopenia prior to LT (17). Studies have suggested that patients who recover from preoperative cachexia, sarcopenia, and malnutrition show better survival post LT (18). However, because the patients are recovering from malnutrition and gain weight, they may be at a greater risk of developing PLTDM (19), which may have better survival than those without DM only with good glycemic control after LT.

LT is associated with a deterioration of renal function in both early and late postoperative periods (20, 21). Kang et al. reported that renal function significantly decreased in the first year after LT (22). Kamei et al. showed that CKD developed in 26% of the patients with a median follow-up of 9.2 years after LT (23). Cohen et al. reported that 27.5% of the patients had severe renal dysfunction (measured GFR <40 ml/min/1.73 m^2) at 5 years and the cumulative incidence of ESKD was 6.25% at 7 years and 10% at 10 years (6). Immunosuppressive treatment, essential for patients with LT to prevent graft rejection, is associated with nephrotoxicity, especially when calcineurin inhibitors are used. A study showed that a higher trough blood tacrolimus concentration correlated with reduced eGFR (24). However, we observed that no significant differences correlated with the use of immunosuppressive agents between the three groups. Previous studies have found that the etiologies of LT, such as hepatitis C infection and ethanol abuse, and donor type (circulatory death) may worsen renal function, increasing the prevalence of CKD after LT (24, 25). Our study shows that pre-existing DM is a significant risk factor for developing ESKD post-LT during a 12-year follow-up period, after adjustments for age, sex, co-morbidity, the usage of immunosuppressant, corticosteroid and antihypertensive agents.

As follow-up is started from date of transplant and PLTDM is defined at any time point during the first post-transplant year, this group will inevitably have survived until diagnosis of PLTDM and as such patients in this group will not be able to experience mortality until their diagnosis of PLTDM. This creates a biased low mortality rate in this group. In order to deal with the immortal time bias in the PLTDM group, we defined PLTDM as those who had been diagnosed of DM within 1-year after LT. We then excluded the patients who had developed ESKD or died within 1 year after LT and started follow-up 1 year after liver transplantation (all groups are defined at this time point as pre-existing DM, PLTDM, or non-DM). Furthermore, we also performed the sensitivity analysis for the risk of ESKD and death, which disclosed the consistent result that patients with pre-existing DM had a significantly higher risk of ESKD and mortality after LT during 12-year follow-up period.

Since the administrative health database have become more accessible, the validity of *ICD-9-CM* is crucial for the accuracy of the study. The k statistic, which assesses how well the administrative data set extracted from the electronic health record of *ICD-9-CM* agrees with actual chart review, confirmed substantial agreement in DM (k range from .7 to .8) and CKD patients (k > .8) (26, 27).

A major strength of this retrospective study is the relatively large number of patients with long follow-up periods. We have established the risk of developing ESKD and long-term survival in patients with pre-existing DM and PLTDM and without DM after LT. However, this study has some limitations. First, our results were based on a retrospective cohort study. The NHIRD is a secondary database and information on medical examination data, laboratory data, detailed rejection condition, transient hyperglycemia condition post-LT and the etiology of DM, CKD, death, and LT was not provided by the administrative database. PLTDM can be defined as a degree of hyperglycemia after LT. A reliable diagnosis of PLTDM must be made after the doses of immunosuppressive agents or steroid have been tapered and are stable. In our study, we defined PLTDM as those patients who had been diagnosed of DM within 1-year post-LT and it may create bias of inevitably included post-LT transient hyperglycemia. Second, our cohort study included patients from a 12-year period, and variations in the type of liver donor and the selection criteria for LT may influence long-term outcome. Thirds, ESKD takes times to develop, therefore, if PLTDM affect the outcome of ESKD, it may need longer follow-up period to elucidate the difference. We recommend extending the follow-up period and conducting further prospective studies to clarify long-term outcomes due to the conflicting survival rates reported between patients with PLTDM and without DM. Last, the power of the model for ESKD may not be large enough when we included many covariates in the model. We recommend to included more patients in the LT cohort in the future study.

In conclusion, this study demonstrated that patients with pre-existing DM had a significantly higher risk of developing ESKD and increasing the risk of death. PLTDM did not increase the risks of ESKD and death compared to those without DM. We emphasize the need for adequately powered studies and extending the follow-up period to explore the long-term outcome of PLTDM.

CAPSULE SUMMARY SENTENCE

Improvements in early post-liver transplantation (LT) survival rates have increased the importance of understanding the risks factors for late post-LT morbidity and mortality. This retrospective study aimed to investigate the effect of diabetes mellitus (DM) on the risks of end-stage kidney disease (ESKD) and post-LT mortality. This study demonstrated that patients with pre-existing DM had a significantly higher risk of developing

ESKD and increasing the risk of death compared to without DM group. We emphasize the need for adequately powered studies to explore the role of Post-LT DM (PLTDM) to explain post-LT morbidity and mortality.

DATA AVAILABILITY STATEMENT

The data underlying this article were provided by National Health Insurance Research Database in Taiwan under license/by permission. The datasets presented in this article are not readily available because it is a restricted database only accessible by formal application to the Health and Welfare Data Science Center of Taiwan. Requests to access the datasets should be directed to the Health and Welfare Data Science Center of Taiwan (http://dep.mohw.gov.tw/DOS/cp-5119-59201-113.html).

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

C-YL participated in research design, writing, and performance of research. M-YW participated in research design, writing, and performance of research. H-CC participated in research design, writing, and performance of research. T-TC participated in writing and data analysis. L-YH participated in writing and data analysis. M-SW participated in research design, performance of research, and critically revised the manuscript. Y-GC participated in research design, critically revised the manuscript, and performance of research. All authors provided approval for publication of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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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.2022.10023/full#supplementary-material

Supplementary Figure 1 | Main study design for ESKD.

Supplementary Figure 2 | Main study design for death.

Supplementary Figure 3 | Study design in sensitivity analysis for ESKD.

Supplementary Figure 4 | Study design in sensitivity analysis for death.

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Prolonged Organ Extraction Time Negatively Impacts Kidney Transplantation Outcome

Hanno Maassen[1,2*†], *Henri G. D. Leuvenink*[1†], *Harry van Goor*[2†], *Jan-Stephan F. Sanders*[3†], *Robert A. Pol*[1†], *Cyril Moers*[1†] *and H. Sijbrand Hofker*[1†]

[1]*Department of Surgery, University Medical Center Groningen, University of Groningen, Groningen, Netherlands,* [2]*Department of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands,* [3]*Department of Nephrology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands*

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***Correspondence:**
Hanno Maassen
H.maassen@umcg.nl

†ORCID:
Hanno Maassen
orcid.org/0000-0003-1447-7427
Henri G. D. Leuvenink
orcid.org/0000-0001-5036-2999
Harry van Goor
orcid.org/0000-0002-6670-1577
Jan-Stephan F. Sanders
orcid.org/0000-0002-0904-3969
Robert A. Pol
orcid.org/0000-0002-2236-5233
Cyril Moers
orcid.org/0000-0001-8014-8387
H. Sijbrand Hofker
orcid.org/0000-0002-0112-4430

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Main Problem: Following cold aortic flush in a deceased organ donation procedure, kidneys never reach the intended 0–4°C and stay ischemic at around 20°C in the donor's body until actual surgical retrieval. Therefore, organ extraction time could have a detrimental influence on kidney transplant outcome.

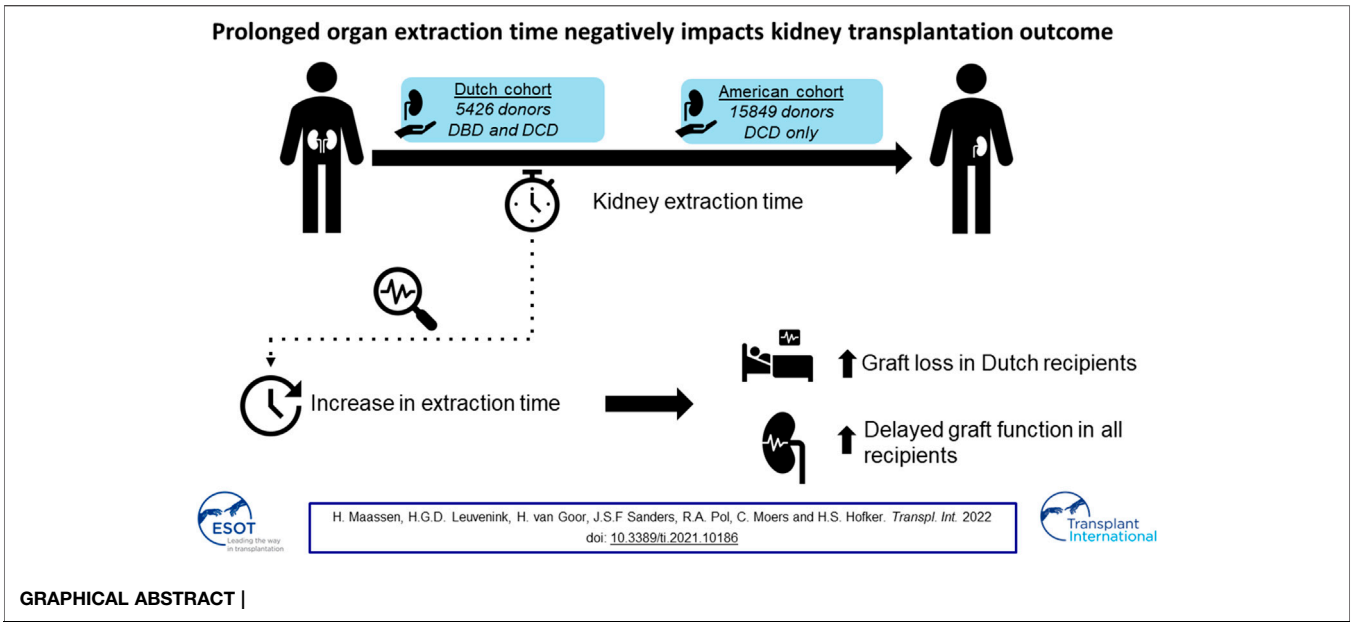
Materials and Methods: We analyzed the association between extraction time and kidney transplant outcome in multicenter data of 5,426 transplant procedures from the Dutch Organ Transplantation Registry (NOTR) and 15,849 transplant procedures from the United Network for Organ Sharing (UNOS).

Results: Extraction time was grouped per 10-min increment. In the NOTR database, extraction time was independently associated with graft loss [HR 1.027 (1.004–1.050); $p = 0.022$] and with DGF [OR 1.043 (1.021–1.066); $p < 0.005$]. An extraction time >80 min was associated with a 27.4% higher hazard rate of graft failure [HR 1.274 (1.080–1.502); $p = 0.004$] and such kidneys had 43.8% higher odds of developing DGF [OR 1.438, (1.236–1.673); $p < 0.005$]. In the UNOS database, increasing extraction times in DCD donors were associated with DGF [OR 1.036 (1.016–1.055); $p < 0.005$]. An extraction time >30 min was associated with 14.5% higher odds of developing DGF [OR 1.145 (1.063–1.233); $p < 0.005$].

Discussion: Prolonged kidney extraction time negatively influenced graft survival in Dutch donors and increased DGF risk in all deceased donor recipients.

Keywords: extraction, time, nephrectomy, kidney, transplantation outcome

Abbreviations: BMI, Body mass index; DBD, Donation after brain death; DCD, Donation after circulatory death; DGF, Delayed graft function; eGFR, Estimated glomerular filtration rate; HCV, Hepatitis C virus; KDRI, Kidney donor risk index; NOTR, Dutch Organ Transplant Registry; UNOS, United Network for Organ Sharing.



GRAPHICAL ABSTRACT |

INTRODUCTION

Kidney transplantation is the preferred treatment for end-stage chronic kidney disease (1). Although kidney transplant outcomes have improved over time and new preservation techniques show promising results, (2) further improvements may be possible. In deceased donor organ procurement, the extraction time is the time interval between the start of the cold flush with preservation solution through an aortic cannula and the actual extraction of the organ from the body. The aim is to reduce temperature and metabolism, and thus protect organs against ischemic injury. Unfortunately, despite the cold flush and topical cooling of the abdominal cavity with slushed ice, the temperature of the kidneys does not reach the intended 4°C required to fully minimize metabolism (3) and remains up to around 20°C right before the actual extraction (4). This is in line with liver procurement, where the organ does not reach the preferred 4°C during procurement surgery either (5, 6). In deceased liver donation, prolonged liver extraction time has been shown to impair liver transplant outcome (7). Prolonged kidney extraction time could also be detrimental to organ quality and kidney transplantation outcome. The effect of the extraction time on kidney graft function is a subject of debate. Data from a single organ procurement organization showed a higher risk of delayed graft function (DGF) with increasing extraction time (8). Another study found no association between extraction time and early graft failure, DGF or graft survival, but there was an association between extraction time and rate of recovery from DGF (9). Unfortunately, the relatively small number of kidney transplantations analyzed in these studies restricts generalization of findings. Heylen et al. analyzed the Eurotransplant database in a multicentre cohort study and found that prolonged extraction time was associated with graft loss after donation after circulatory death (DCD), but not after brain death donation (DBD) (10). The current study analyzed multicenter data of transplant procedures in the Netherlands and the United States, aiming to determine an association between kidney extraction time and post-transplantation kidney function, DGF, graft failure and possibly patient mortality.

METHODS AND MATERIALS

Study Population

Data on all kidney transplantations performed between January 2002 and December 2016 were obtained from the Dutch Organ Transplant Registry (NOTR). Consent for the conduct of this retrospective database study was obtained from the Netherlands Transplantation Foundation data governance board, representing all Dutch transplant centers. Deceased donor, DBD and DCD, and recipient data were analyzed. Follow-up data up to May 2018 were available.

Data from the United Network for Organ Sharing (UNOS) were also used. Data submitted to the registry between February 2005 and March 2019 were analyzed. In the UNOS database extraction time was only available for DCD donors, hence no analyses could be performed on DBD donor kidneys from this database. Follow-up data up to June 2019 were available. Studies using the UNOS dataset are exempt from review by the Institutional Review Board.

Inclusion criteria were all deceased kidney transplantations with available extraction times. Exclusion criteria were extraction times under 5 min or over 5 h and missing outcome data (i.e., patient survival, graft failure and DGF, and in the NOTR database unknown renal function at 3 months post-transplantation) (**Figure 1**).

Extraction time of the kidney was calculated and defined as start of the cold aortic flush until end of nephrectomy, and times

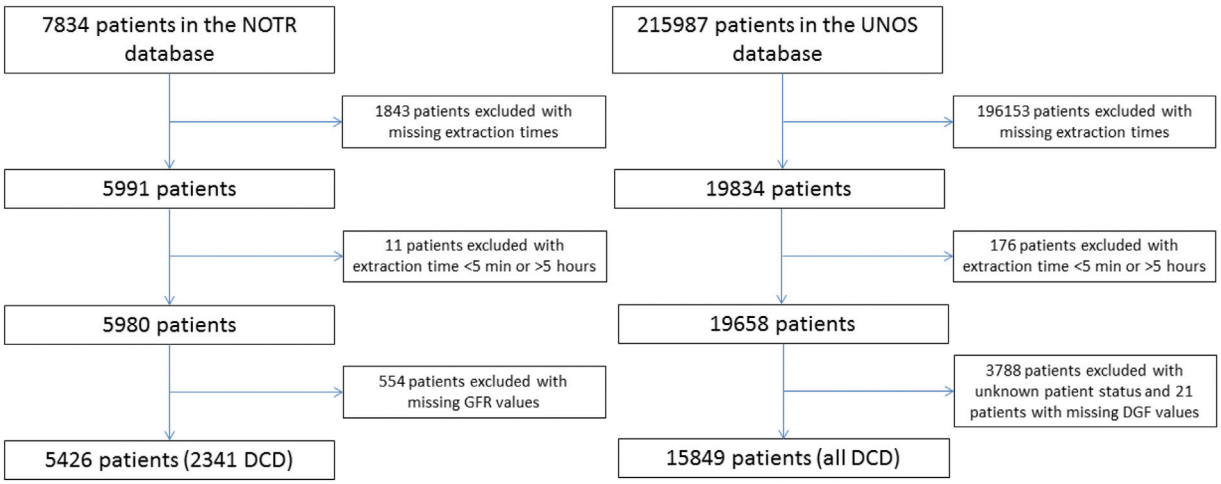


FIGURE 1 | Flowchart exclusion of patients in both the NOTR database and the UNOS database.

were grouped in 10-min increments. In addition to extraction time, warm ischemic time (DCD only), cold ischemic time, and anastomosis time were defined accordingly to the Eurotransplant manual (11). Post-transplantation estimated Glomerular Filtration Rate (eGFR) was calculated in the NOTR according to the MDRD formula: $186 \times (\text{creatinin}/88.4)^{-1.154} \times (\text{age})^{-0.203} \times$ (0.742 with female). In the UNOS database, necessary data was missing for calculation of the eGFR post-transplantation. Patient survival was defined as the time from transplantation until death. Graft survival was defined as the time from transplantation until failure of the graft, death-censored and it includes all causes of graft failure. DGF was defined as any dialysis requirement in the first week post transplantation.

Kidney Donor Risk Index

The Kidney Donor Risk Index (KDRI) was calculated in the Dutch database using a standardized formula including age, height, weight, history of hypertension, history of diabetes, cause of death, serum creatinine and DCD status (12). Hepatitis C virus (HCV) status and ethnicity were not available in the database, therefore we assumed all patients were Caucasian and were not infected with HCV. HCV infection was stated to be 0.2% in other research conducted with Dutch transplant donors and recipients (13). First, KDRI_{rao} was calculated with the previously mentioned variables. Next, $\text{KDRI}_{\text{median}}$ was calculated using the same scaling factor used in the UNOS database (1.250695754). The KDRI was already available in UNOS, so no further calculations were performed on those data.

Statistical Analysis

Statistical analysis was performed on cases for which extraction time and outcome was available using multivariable Cox regression for patient survival and graft failure, logistic binary regression for DGF and graft rejection or multivariable linear regression for exploring factors influencing extraction time. Missing values for the variables history of hypertension and history of diabetes were defined as "not present." Median values were imputed in the NOTR database for missing values in the variables "reported number of organs" (1,014 cases), warm ischemic time (631 cases), cold ischemic time (576 cases), second ischemic time (642 cases), HLA mismatches (29 cases), body mass index (BMI) (2 cases) and KDRI median (8 cases). Baseline characteristics are presented as median with range or number with percentage. Univariable variables were tested for normal distribution and comparisons between groups were performed accordingly. Pearson's chi-square test was used for donor sex, donor hypertension, donor diabetes, recipient sex, DGF occurrence and previous kidney transplantations, Mann-Whitney U-test for donor age, donor BMI, cold ischemic time, extraction time, extraction time kidney-only donation, $\text{KDRI}_{\text{median}}$, recipient age and HLA mismatches and log-rank test for 5 years graft survival. Performance of univariable analysis and determination of potential confounders were followed by a stepwise multivariable analysis. A p-value of ≤ 0.05 was assumed to be statistically significant. An interaction analysis was performed for DBD/DCD and extraction time in the NOTR database based on model 5 of **Table 2**, with the addition of DBD/DCD*extraction time for outcomes with a significant association with extraction time. Extraction time was dichotomized to perform a cut-off value analysis. After dividing the data binary, multiple analysis were performed to find the cut-off value. Multivariable cox regression was used for patient survival and graft failure, binary logistic regression was used for DGF. An increase in familywise error rate was controlled by Bonferroni correction. Leading to a p-value of 0.00625 to be regarded statistically significant at the cut-off analysis part of the manuscript. Statistical analysis was performed using SPSS Statistics version 23.

RESULTS

Donor and Recipient Characteristics

Donor and recipient characteristics are shown in **Table 1**, displaying NOTR data with DBD and DCD donors, NOTR

TABLE 1 | Donor and Recipient Characteristics of NOTR (Jan 2002–Dec 2016) and UNOS (Feb 2005–Mar 2019) databases.

Characteristics	NOTR all (5426)	NOTR DCD only (2341)	UNOS DCD only (15849)	DCD NOTR vs UNOS
Donor				
Age, years	52 (1–86)	52 (1–78)	39 (1–69)	$p < 0.005$
Sex				$p < 0.005$
Male	2814 (51.9%)	1374 (58.7%)	10542 (66.5%)	
Female	2612 (48.1%)	967 (41.3%)	5307 (33.5%)	
BMI	24.7 (9.8–55.6)	24.7 (12.5–55.6)	26.9 (8.91–69.2)	$p < 0.005$
Donor Type				
DBD	3085 (56.9%)			
DCD	2341 (43.1%)	2341 (100%)	15849 (100%)	
Cause of Death				
CVA	1406 (25.9%)	538 (23%)	2455 (15.5%)	
Trauma	1153 (21.2%)	609 (26%)		
Head trauma			5089 (32.1%)	
Anoxia			7543 (47.6%)	
Other	2867 (52.8%)	1194 (51%)	762 (4.8%)	
Hypertension				$p < 0.005$
Yes	1243 (22.9%)	448 (19.1%)	3778 (23.8%)	
No	4183 (77.1%)	1893 (80.9%)	12071 (76.2%)	
Diabetes				$p < 0.005$
Yes	269 (5%)	115 (4.9%)	903 (5.7%)	
No	5157 (95%)	2226 (95.1%)	14946 (94.3%)	
Reported number of organs (NOTR)*			Extracted no. organs (UNOS)*	
1	5 (0.1%)	5 (0.2%)	56 (0.4%)	
2	669 (12.3%)	600 (25.6%)	8657 (54.6%)	
3	705 (13%)	531 (22.7%	5672 (35.8%)	
4	1851 (34.1%)	528 (22.6%)	866 (5.5%)	
5	544 (10%)	197 (8.4%)	527 (3.3%)	
6	846 (15.6%)	458 (19.6%)	71 (0.4%)	
7	806 (14.9%)	22 (0.9%)		
Warm ischemic time (DCD only), min		17 (6–54)	17 (0–180)**	
Cold ischemic time, min	961 (60–2880)	961 (119–2797)	1080 (0.6–5940)	$p < 0.005$
Anastomosis time, min	33 (10–180)	33 (11–180)		
Extraction time, min	58 (5–300)	59 (5–293)	38 (5–259)	$p < 0.005$
Extraction time kidney-only donation, min	52 (5–293)	53 (5–293)	33 (6–165)	$p < 0.005$
$KDRI_{median}$	1.0395 (0.51–2.85)	1.099 (0.57–2.35)	0.9515 (0.56–2.49)	$p < 0.005$
Recipient				
Age	54 (2–85)	56 (8–81)	53 (1–86)	$p = 0.002$
Sex				$p = 0.755$
Male	3254 (60%)	1457 (62.2%)	9811 (61.9%)	
Female	2172 (40%)	884 (37.8%)	6038 (38.1%)	
HLA mismatches	3 (0–6)	3 (0–6)	5 (0–6)	$p < 0.005$
Delayed graft function				$p < 0.005$
No	3592 (66.2%)	1107 (47.3%)	9476 (59.8%)	
Yes	1834 (33.8%)	1234 (52.7%)	6373 (40.2%)	
Death -censored graft survival rate after 5 years	90.3%	90.6%	92.2%	$p = 0.273$
eGFR 3 Months, ml/min*173^2	43.7 (1.4–340.7)	41.5 (1.4–279.2)	***	
eGFR 12 Months, ml/min*173^2	46.1 (2.6–376.3)	45.2 (2.6–232.7)	***	
Number of Rejections			Rejection 1 year post-transpl.	
0	4830 (89%)	2069 (88.4%)	14911 (94.1%)	
1 or more	596 (11%)	272 (11.6%)	938 (5.9%)	
Previous kidney transplantation				
No	4578 (84.8%)	2040 (87.1%)	14102 (89%)	$p = 0.009$
Yes	848 (15.2%)	301 (12.9%)	1747 (11%)	

**Both lungs are counted as an individual organ.*

***Value not reliable due to high number of missing values.*

****Value not available.*

Showing median + range or number + percentage.

UNOS database only contain DCD donors.

BMI, body mass index; CVA, cerebrovascular accident; GFR, glomerular filtration rate; KDRI, kidney donor risk index.

data with only DCD donors, and UNOS data (the extraction time was only available in DCD donors in the UNOS data). Notable differences between DBD and DCD donors from the Dutch database are a higher rate of males among DCD donors (DCD: 58.7% vs. DBC: 46.7%) and the occurrence of more DGF in DCD versus DBD kidney recipients (DCD: 52.7% vs. DBD: 19.4%). Median donor age was much higher in DCD donors from the NOTR database compared to UNOS [52, (1–78) vs. 39 (1–69), $p < 0.005$]. Of the 2341 DCD donors, 58.6% were male in NOTR compared to 66.5% in 15849 in the UNOS database ($p < 0.005$). **Table 1** shows the number of reported organs in NOTR and the number of extracted organs in UNOS. The number of extracted organs per donor was not available in NOTR. Cold ischemic time was significantly shorter in NOTR [NOTR: 961 min (119–2797) vs. UNOS: 1080 min (0.6–5940), $p < 0.005$], with a significantly longer median kidney extraction time (NOTR: 59 min vs. UNOS: 38 min, $p < 0.005$). In the kidney-only donation, the median extraction time was also significantly longer in NOTR (NOTR: 53 min vs. UNOS: 33 min, $p < 0.005$). $KDRI_{median}$ was significantly higher in NOTR, showing on average a better quality of donors from UNOS [NOTR: 1.099 (0.57–2.35) vs. UNOS: 0.9515 (0.56–2.49), $p < 0.005$].

Extraction time was not available in a large percentage of the UNOS patients, this lead to a large exclusion in patients (215.987). The difference between the whole cohort and the selection we used is presented in **Supplementary Table S1**. There seems to be an overall similarity in donor characteristics such as age, sex, diabetes and hypertension. Striking is the difference seen in the number of extracted organs. In the data available for our analysis, fewer organs were procured for each donor than what is reported in the complete data. This might account for the difference seen between the Dutch NOTR database and the UNOS database with regard to extraction time, were UNOS had a shorter extraction time than the NOTR database (58 vs. 38 min). The NOTR had more comparable data on the number of extracted organs compared to the UNOS complete dataset, due to more complete registration of extraction times. More organs extracted per donor leads of course to an increase in average extraction time.

Median recipient age was similar in both datasets [NOTR: 54 (2–85) vs. UNOS: 53 (1–86), $p = 0.058$]. The number of HLA mismatches was significantly higher in the UNOS database ($p < 0.005$). There was also a significantly higher rate of delayed graft function in the recipients of DCD donor kidneys in the NOTR database compared to UNOS (NOTR: 52.7% vs. UNOS: 40.2%, $p < 0.005$). Overall graft survival did not differ between the DCD donors and all donors of the two cohorts though (5 years graft survival DCD only NOTR: 90.6% vs. UNOS 92.2%, $p = 0.273$ and NOTR: 90.3% vs. UNOS 92.2%, $p = 0.151$).

NOTR Extraction Time

The impact of extraction time in the NOTR data, grouped per 10 min, on patient survival, graft survival and DGF is shown in **Table 2**. Increasing extraction times were significantly associated with a higher hazard rate of graft failure [HR 1.027 (1.004–1.050) $p = 0.022$] and odds for the development of DGF [OR 1.043 (1.021–1.066) $p < 0.005$]. These associations remained unchanged when adjusted for potential confounders (**Table 2**, models 1–5). Increasing extraction times were not significantly associated with a higher hazard rate of recipient death [HR 0.999 (0.981–1.0167) $p = 0.916$]. Interaction analysis on model 5 of **Table 2** showed that the relationship between extraction time and the outcomes graft survival and DGF was not different for DBD and DCD ($p = 0.111$ and $p = 0.080$ respectively). Prolonged extraction times were associated with significantly lower eGFR values at both 3 months [B −0.305 (−0.519 to −0.092) $p = 0.005$] and 1 year [B −0.334 (−0.542 to −0.126) $p = 0.002$] post-transplantation in fully adjusted models (**Supplementary Table S3**). Analysis of eGFR values at 1 year is conducted with 432 missing cases, longer follow up with eGFR was not conducted because of too much missing cases. Increasing extraction times were not significantly associated with rejection post-transplantation. Next, multivariable analysis using model 4 of **Table 2** was performed to examine the influence of prolonged extraction times on specific deceased donor-subgroups DBD and DCD (**Supplementary Table S4**). Increasing extraction times were not associated with a higher hazard rate of graft failure when the DBD and DCD groups were analyzed separately. A higher odds of developing DGF with increasing extraction time was only seen in the DCD group [OR 1.058 (1.030–1.087) $p < 0.005$].

UNOS Extraction Time

Impact of extraction time in the UNOS data, grouped per 10 min, on patient survival, graft survival and DGF is shown in **Table 3**. Similarly to the Dutch database, UNOS data showed a significant association of prolonged extraction times with DGF [OR 1.036 (1.016–1.055) $p < 0.005$]. Prolonged extraction times were, however, not associated with a higher hazard rate of graft failure [HR 0.997 (0.970–1.025) $p = 0.829$] or a higher hazard rate of patient death in the UNOS data [HR 0.995 (0.971–1.019) $p = 0.667$]. Increasing extraction times were not significantly associated with acute rejection ($p = 0.448$) and rejection 1 year post-transplantation ($p = 0.158$).

NOTR Cut-off Value

Multivariable Cox regression or binary logistic regression was used to find a cut-off value for the extraction time upper limit in the NOTR data. Extraction time was dichotomized divided into different time intervals between 40 and 110 min (**Table 4**) and analyses were performed using model 5 of **Table 2**, including all potential confounders. An extraction time over 80 min was associated with a 27.4% higher hazard rate of graft failure (8.0–50.2%; $p = 0.004$) (**Figure 2**); kidneys with an extraction time over 70 min had 23.7% higher odds of developing DGF (7.9–41.7%; $p = 0.002$), and those over 80 min as much as 43.8% higher odds (23.6–67.3%; $p < 0.005$).

UNOS Cut-off Value

A similar analysis was performed on the UNOS database to find a cut-off value for extraction time, using model 4 of **Table 3** (**Table 5**). An extraction time over 30 min was associated with 14.5% higher odds of developing DGF (1.063–1.233; $p < 0.005$).

TABLE 2 | Multivariable Cox regression/binary logistic regression on extraction time (10 min) and patient survival, graft failure and DGF NOTR (DBD and DCD). Coefficients of full models are listed in **Supplementary Table S1**.

	Patient death HR [95% CI]	*p*	Graft failure HR [95% CI]	*p*	DGF OR [95% CI]	*p*
Univariable	0.977 [0.960–0.994]	0.010	1.011 [0.989–1.034]	0.312	1.055 [1.036–1.075]	<0.005
Model 1	1.000 [0.982–1.018]	0.982	1.033 [1.011–1.056]	0.004	1.067 [1.047–1.088]	<0.005
Model 2	1.001 [0.983–1.019]	0.926	1.036 [1.016–1.059]	0.002	1.068 [1.048–1.089]	<0.005
Model 3	0.998 [0.980–1.016]	0.834	1.028 [1.006–1.051]	0.014	1.029 [1.008–1.051]	0.007
Model 4	1.000 [0.982–1.017]	0.956	1.028 [1.005–1.051]	0.016	1.030 [1.009–1.052]	0.006
Model 5	0.999 [0.981–1.017]	0.916	1.027 [1.004–1.050]	0.022	1.043 [1.021–1.066]	<0.005

Model 1: extraction time + donor age, BMI and gender.
Model 2: model 1 + cause of death, donor diabetes, hypertension and last serum creatinine.*
*Model 3: model 2 + cold ischemic time, warm ischemic time, anastomosis time and number of reported organs**.*
Model 4: model 3 + number of previous transplants, HLA mismatches, age recipient, gender recipient.
Model 5: model 4 + DBD/DCD.
**CVA, Trauma or other.*
***Divided as <=2 or >2 organs.*
BMI, body mass index.

TABLE 3 | Multivariable Cox regression/binary logistic regression on extraction time (10 min) and patient survival, graft failure and DGF UNOS (DCD only). Coefficients of full models are listed in **Supplementary Table S4**.

	Patient death HR [95% CI]	*p*	Graft failure HR [95% CI]	*p*	DGF OR [95% CI]	*p*
Univariable	0.967 [0.945–0.990]	0.004	0.981 [0.956–1.007]	0.156	1.004 [0.987–1.020]	0.663
Model 1	0.987 [0.964–1.010]	0.272	0.992 [0.967–1.018]	0.557	1.018 [1.000–1.035]	0.044
Model 2	0.987 [0.964–1.010]	0.256	0.992 [0.967–1.018]	0.559	1.022 [1.005–1.040]	0.013
Model 3	0.988 [0.965–1.013]	0.350	1.001 [0.974–1.029]	0.943	1.036 [1.017–1.055]	<0.005
Model 4	0.995 [0.971–1.019]	0.667	0.997 [0.970–1.025]	0.829	1.036 [1.016–1.055]	<0.005

Model 1: extraction time + donor age, BMI, ethnicity and gender.*
*Model 2: model 1 + cause of death**, donor diabetes, hypertension and last serum creatinine.*
*Model 3: model 2 + cold ischemic time and number of recovered organs***.*
Model 4: model 3 + previous transplants, HLA mismatches, recipient age and gender.
**African American or other.*
***CVA, head trauma, anoxia or other.*
****Divided as <=2 or >2 organs.*
BMI, body mass index.

Factors Influencing Extraction Time

Multivariable linear regression was performed to explore factors that significantly influenced extraction time. The analysis was performed in a merged dataset, which combined data of all kidney transplant donors and recipients of both the NOTR and UNOS databases. The variables donor gender, BMI, history of hypertension and diabetes and NOTR vs. UNOS database (DCD only) were used in the regression analysis. In this combined dataset, all the aforementioned factors together accounted for 172% of the variability in extraction time [R^2 = 0.172, adjusted R^2 = 0.1172, F (5, 21235) = 880.5, $p < 0.005$]. Nonstandardized (B) and standardized (β) regression coefficients for each predictor in the regression model are reported in **Supplementary Table S6**. The largest contributor to a longer extraction time was country of the donation, NOTR (the Netherlands) vs. UNOS (United States) [B −23.557 (−24.274, −22.841) $p < 0.005$].

DISCUSSION

Prolonged postmortem kidney extraction times in the Dutch NOTR database, increasing per 10 min, were associated with a higher hazard rate of graft loss, delayed graft function and lower eGFR values 3 months and 1 year after transplantation. Extraction times over 80 min in DBD and DCD donors combined, significantly increased the hazard rate of graft loss and the odds of developing DGF compared to extraction times lower than 80 min. A large difference in the median extraction time was seen between the Dutch donors and donors from America (58 vs. 38 min). In the UNOS database, increasing extraction time in DCD donors was not associated with patient survival and graft survival but increased the odds of developing DGF. In addition, extraction times over 30 min showed increased odds of developing DGF compared to extraction times under 30 min.

TABLE 4 | Multivariable Cox regression/binary logistic regression on binary extraction times and patient survival, graft failure and DGF NOTR (DBD and DCD).

Cut-off value (min)	% Patients per time interval (n = 5426)	Patient survival HR [95% CI]	*p*	Graft failure HR [95% CI]	*p*	DGF OR [95% CI]	*p*
40	<40: 1016 (18.7%) >40: 4410 (81.3%)	1.072 [0.940–1.222]	0.298	1.140 [0.957–1.370]	0.140	1.090 [0.929–1.279]	0.291
50	<50: 1953 (36%) >50: 3473 (64%)	1.040 [0.932–1.160]	0.482	1.013 [0.877–1.171]	0.856	1.013 [0.877–1.171]	0.856
60	<60: 2928 (54%) >60: 2498 (46%)	1.013 [0.911–1.128]	0.807	1.061 [0.921–1.222]	0.410	1.110 [0.976–1.261]	0.111
70	<70: 3708 (68.3%) >70: 1718 (31.7%)	0.898 [0.883–1.115]	0.898	1.126 [0.969–1.308]	0.122	1.237 [1.079–1.417]	0.002
80	<80: 4246 (78.3%) >80: 1180 (21.7%)	1.050 [0.920–1.199]	0.470	1.274 [1.080–1.502]	0.004	1.438 [1.236–1.673]	<0.005
90	<90: 4617 (85.1%) >90: 809 (14.9%)	1.039 [0.890–1.212]	0.630	1.258 [1.038–1.523]	0.019	1.428 [1.199–1.700]	<0.005
100	<100: 4833 (89%) >100: 593 (11%)	0.974 [0.813–1.165]	0.771	1.239 [0.995–1.543]	0.055	1.337 [1.095–1.631]	0.004
110	<110: 4996 (92%) >110: 430 (8%)	0.912 [0.738–1.128]	0.395	1.292 [1.009–1.654]	0.042	1.501 [1.194–1.887]	<0.005

Model: extraction time + donor age, BMI, gender, cause of death, diabetes, hypertension and last serum creatinine, cold ischemic time, warm ischemic time, anastomosis time, number of reported organs, number of previous transplants, HLA mismatches; recipient age, gender and DBD/DCD.
BMI, body mass index.

Life table - Numbers at risk						
Time point	0 years	2 years	4 years	6 years	8 years	10 years
<80 min	3976	3063	2180	1498	985	396
>80 min	1094	837	594	402	277	133

Log-rank test P=0.067

FIGURE 2 | Kaplan-Meier curve displaying 10-years death-censored graft survival in the NOTR database, extraction time divided into over and under 80 min.

TABLE 5 | Multivariable Cox regression/binary logistic regression on binary extraction times and patient survival, graft failure and DGF UNOS (DCD only).

Cut-off value (min)	% Patients per time interval (n = 15849)	Patient survival HR [95% CI]	*p*	Graft failure HR [95% CI]	*p*	DGF OR [95% CI]	*p*
20	<20: 1189 (7.5%) >20: 14660 (92.5%)	0.906 [0.773–1.061]	0.220	1.043 [0.861–1.264]	0.667	1.056 [0.931–1.197]	0.396
30	<30: 4823 (30.4%) >30: 11026 (69.6%)	0.999 [0.905–1.103]	0.988	1.008 [0.900–1.129]	0.890	1.145 [1.063–1.233]	<0.005
40	<40: 8871 (56%) >40: 6978 (44%)	1.033 [0.940–1.135]	0.501	0.995 [0.894–1.108]	0.928	1.182 [1.102–1.268]	<0.005
50	<50: 11812 (74.5%) >50: 4037 (25.5%)	0.932 [0.835–1.041]	0.215	0.941 [0.831–1.067]	0.344	1.116 [1.031–1.208]	0.007
60	<60: 13588 (85.7%) >60: 2261 (14.3%)	1.021 [0.892–1.168]	0.768	1.024 [0.882–1.188]	0.755	1.146 [1.040–1.263]	0.006
70	<70: 14691 (92.7%) >70: 1158 (7.3%)	0.934 [0.780–1.119]	0.461	0.953 [0.783–1.160]	0.630	1.061 [0.933–1.207]	0.369
80	<80: 15214 (96%) >80: 635 (4%)	0.884 [0.697–1.121]	0.310	0.913 [0.706–1.180]	0.486	1.138 [0.961–1.347]	0.134
90	<90: 15533 (98%) >90: 316 (2%)	0.865 [0.621–1.206]	0.393	0.719 [0.487–1.062]	0.098	1.133 [0.895–1.434]	0.298

Model: extraction time + donor age, BMI, ethnicity, gender, cause of death, diabetes, hypertension, last serum creatinine, cold ischemic time, number of recovered organs, previous transplants, HLA mismatches; recipient age and gender.
BMI, body mass index.

Our data suggest that, in addition to other factors, extended kidney extraction time is an important variable that determines deceased donors' kidney transplantation outcome. It was not possible to state a universal extraction time cut-off value for all kidney donors. It nonetheless seems that extraction times higher than 80 min lead to a greater odds of developing DGF and a higher hazard of developing graft failure in Dutch transplant recipients. This means that postmortem donor operation times should be kept as short as possible in similar cases. In addition, preservation during this time-period could be improved, especially in prolonged extraction times. When analyzing the data of the cut-off value, we need to take in consideration that there might be a loss of impact at the higher and lower extraction times in the UNOS database (e.g., <20 min and >70 min), where just over 7% of the patients are in one group and the rest are in the other. This could explain why the association between extraction time and DGF is lost >70 min in the UNOS database.

The different effect of prolonged extraction time on outcomes in both databases is likely caused by the inequality between the two organ donation and transplantation systems (**Table 1**). A factor that influences transplantation outcome adversely, i.e., increased donor age, (14) was higher in the NOTR database, while cold ischemic time (15–17) was longer in the UNOS database. KDRI was calculated for a better understanding of the differences in kidney donor quality resulting from different baseline characteristics. KDRI combines ten donor factors and gives a validated estimate of the relative risk of post-transplantation kidney graft failure (12, 13). NOTR donors had a significantly higher $KDRI_{median}$ value than UNOS donors, indicating a higher relative risk of post-transplantation kidney graft failure and suggesting an average inferior quality of transplanted kidneys in the Dutch NOTR database. The difference in KDRI value is a plausible explanation for the different influence of prolonged extraction time on transplantation outcome between the two groups, where increased extraction time could have a detrimental effect on transplantation outcome if the donor kidney was already more susceptible to graft failure.

Besides the difference in KDRI value, extraction times too are different between the databases, with a median value of 58 min for NOTR compared to 38 min for UNOS. A prolonged extraction time could be the result of more organs being procured from each individual donor in the Dutch cohort compared to the American cohort, or might be explained by differences in donation procedures. Since NOTR shows reported number of organs and UNOS extracted number of organs, a comparison between the two databases may not be entirely correct. The lower number of extracted donor organs in the UNOS data compared to the number of reported organs in the NOTR data could be explained by the fact that not all reported organs are always procured, due to some degree of organ discard prior to retrieval. There is no clear explanation as to why extraction times differed, the experience of surgeons was not measured, and the specific surgical procedure was not part of our analysis. Given that, when looking at kidney-only procurement, the median time of kidney extraction was still longer in the NOTR database (52 vs. 33 min) while possible operating time-increasing variables (such as male gender and BMI) (18, 19) were less favorable among US donors, a relevant difference in expertise and/or surgical technique cannot be ruled out. In addition, by only using a selection of the full UNOS database due to limited available extraction times, selection bias might be introduced. There is a difference in number of extracted organs between the full UNOS database and the cases we used for our calculation. The more organs extracted, the longer the extraction time, so we might underreported the actual extraction time for the full UNOS database and thereby the possible effect of extraction time on transplantation outcome. This might also explain the difference seen in cut-off point between the NOTR and UNOS (80 vs. 30 min).

To the best of our knowledge, only a few other studies have focused on the effect of extraction time on kidney transplantation outcome. A previous study, conducted on smaller patient cohort (n = 576), emphasized the influence of extraction times higher than 60 min on the occurrence of DGF (8). Another study by Heylen et al., found that prolonged extraction time was associated with graft loss after donation after circulatory death (DCD), but not after brain death donation (DBD) (10). This analysis was performed on the Eurotransplant region which includes the Netherlands, between 2004 and 2013. Although it is performed in an overlapping time interval, Heylen et al. does not show an association between extraction time and graft loss in DBD and DCD donors combined as we do. In the NOTR database, prolonged extraction time lost its association when the database was split into DBD or DCD donors only. The association between prolonged extraction times and the occurrence of DGF remained only in the DCD group. When analyzing the whole NOTR database, with DBD/DCD as a covariate in the multivariable analysis, the association of kidney extraction time with both graft failure and DGF remained significant. This could mean that by dividing the NOTR database into two groups the number of donors became too small to maintain enough power for the graft failure analysis. Interaction analyses showed that the relationship between extraction time and graft survival and DGF was not different for DBD and DCD. Although, by performing statistical analysis on a combined group we cannot rule out that we have measured an artificial effect of extraction time on transplantation outcome, even though we corrected for the donor type in our analysis. Apart from a slightly different outcome in graft survival between the study by Heylen et al. and ours, we were able to perform additional analyses on the outcome DGF, patient survival and eGFR, giving more insight on the impact of extraction time on kidney transplantation outcome.

Besides kidney extraction time, hepatectomy time has also been associated with impaired transplantation outcome (7). Donor risk index was used by Jochmans et al. as a marker for organ quality, showing that livers from DCD and higher-risk donors are most affected by prolonged extraction time (7). This is in line with the results from Heylen on nephrectomy time and our obtained data in the NOTR database, where the KDRI was higher than in the UNOS database and the effect of prolonged extraction times on transplantation outcome was stronger.

More research needs to be conducted on how to improve or at least maintain organ quality during the period of extraction. Flushing the organ via the aorta in a fairly warm body results in sub-normothermic conditions which are most likely suboptimal for organ preservation. Higher organ temperatures result in higher metabolism, (3) and in current organ retrieval practice the kidneys receive no oxygen or nutrients, which causes a discrepancy between cellular demand and supply. Better temperature control during the extraction or otherwise reducing kidney metabolism, for example with the use of hydrogen sulphide, (20) could improve transplantation outcome even with longer extraction times. Since shortening of organ extraction time may not always be feasible, future research should focus on alternative improvements that protect the kidneys during organ procurement.

After an analysis among Dutch donors, the hepatectomy time proved to be a significant independent risk factor for the development of non-anastomotic biliary strictures after DCD liver transplantation (21). This led to the implementation of a new protocol, combined with extra training of surgeons and creating awareness on this important and potentially modifiable risk factor. By creating awareness that extraction time is an important factor that could influence transplantation outcome, extraction times themselves could be reduced.

A limitation of this study is the nature of its design. The large cohort size ensures a good power to find significant associations, but does not establish causality. We show different results in the different cohorts, therefore the results should be interpreted with care since generalization is not possible. In addition, not all data were fully available in the two databases, and UNOS only had data on extraction times of DCD donors. The large number of extraction times that were not available in the UNOS database could have induced bias regarding this analysis. Also, several other subtle differences existed in how data were stored in the databases—in some cases, data values were missing and data had to be imputed. This could have introduced bias, although in our opinion not all differences between the databases can be explained by these dissimilarities. Also, there could be an immeasurable bias in the prolonged extraction time of donors itself. Factors that predispose the fact that they needed a longer extraction time could explain the generally worse transplantation outcome instead of the prolonged extraction time itself. Even so, extraction time is an easily measured variable that a transplantation professional can take into account in the decision to accept a donor kidney or not. If these unmeasured factors contribute to a worse transplantation outcome but also to a prolonged extraction time, extraction time itself is still a variable to contemplate and should be taken into account.

In conclusion, extraction time during deceased donor procedures was associated with graft loss, delayed graft function and lower eGFR values in Dutch kidney transplant recipients, and with delayed graft function in American transplant recipients. Prolonged extraction time seems a potentially important determinant of kidney transplantation outcome, especially in kidneys recovered from high-risk donors.

CAPSULE SUMMARY SENTENCE

The aim of the present manuscript was to investigate the impact of kidney extraction time on eGFR, delayed graft function (DGF), graft failure, and patient survival after renal transplantation. We analyzed this in two large cohorts of both Netherlands (5,426 transplant procedures) and United States (15,849 transplant procedures). Our results show that prolonged extraction time increases the risk of DGF in both Dutch and American recipients and even leads to an elevated graft failure rate in Dutch recipients. In addition, longer extraction times were associated with lower eGFR values after transplantation. We believe that our manuscript demonstrates the detrimental influence of a potentially modifiable surgical factor during deceased donor organ donation. Shortening kidney extraction times could improve renal transplantation outcome.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: The data that support the findings of this study are available on request from the UNOS and/or NOTR. Requests to access these datasets should be directed to info@transplantatiestichting.nl and UNOS.org.

ETHICS STATEMENT

Consent for the conduct of the retrospective database study of the NOTR database was obtained from the Netherlands Transplantation Foundation data governance board, representing all Dutch transplant centers. Studies using the UNOS dataset are exempt from review by the Review Board of the University Medical Center Groningen.

AUTHOR CONTRIBUTIONS

HM participated in research design, writing of the paper, performance of the research and data analysis. HL participated in research design, writing of the paper and data analysis. HV participated in research design, writing of the paper and data analysis. J-SS participated in research design, writing of the paper and data analysis. RP participated in research design, writing of the paper and data analysis. CM participated in research design, writing of the paper, performance of the research and data analysis. HH participated in research design, writing of the paper, performance of the research and data analysis.

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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.

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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.2021.10186/full#supplementary-material

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ORIGINAL RESEARCH
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Illness Perceptions and Medication Nonadherence to Immunosuppressants After Successful Kidney Transplantation: A Cross-Sectional Study

Yiman Wang[1], Denise M. J. Veltkamp[2], Paul J. M. van der Boog[2,3], Marc H. Hemmelder[4,5], Friedo W. Dekker[1], Aiko P. J. de Vries[2,3] and Yvette Meuleman[1]*

[1]Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, Netherlands, [2]Department of Internal Medicine, Division of Nephrology, Leiden University Medical Center, Leiden, Netherlands, [3]Transplant Center, Leiden University Medical Center, Leiden, Netherlands, [4]Department of Internal Medicine, Division of Nephrology, Maastricht University Medical Center, Maastricht, Netherlands, [5]CARIM School for Cardiovascular Research, University Maastricht, Maastricht, Netherlands

Background: Medication nonadherence to immunosuppressants is a well-known risk factor for suboptimal health outcomes in kidney transplant recipients (KTRs). This study examined the relationship between illness perceptions and medication nonadherence in prevalent Dutch KTRs and whether this relationship depended on post-transplant time.

Methods: Eligible KTRs transplanted in Leiden University Medical Center were invited for this cross-sectional study. The illness perceptions and medication nonadherence were measured *via* validated questionnaires. Associations between illness perceptions and medication nonadherence were investigated using multivariable logistic regression models.

Results: For the study, 627 participating KTRs were analyzed. 203 (32.4%) KTRs were considered nonadherent to their immunosuppressants with "taking medication more than 2 h from the prescribed dosing time" as the most prevalent nonadherent behaviour (n = 171; 27.3%). Three illness perceptions were significantly associated with medication nonadherence: *illness identity* (adjusted odds ratio [OR_{adj}] = 1.07; 95% confidence interval [CI], 1.00–1.14), *concern* (OR_{adj} = 1.07; 95%CI,1.00–1.14), and *illness coherence* (OR_{adj} = 1.11; 95% CI,1.01–1.22). The relationships between illness perceptions and medication nonadherence did not differ depending on post-transplant time (p-values ranged from 0.48 to 0.96).

Conclusion: Stronger negative illness perceptions are associated with medication nonadherence to immunosuppressants. Targeting negative illness perceptions by means of psychoeducational interventions could optimize medication adherence and consequently improve health outcomes in KTRs.

Keywords: kidney transplantation, adult, illness perceptions, immunosuppressants, medication nonadherence

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Correspondence:
Yiman Wang
y.wang.epid@lumc.nl

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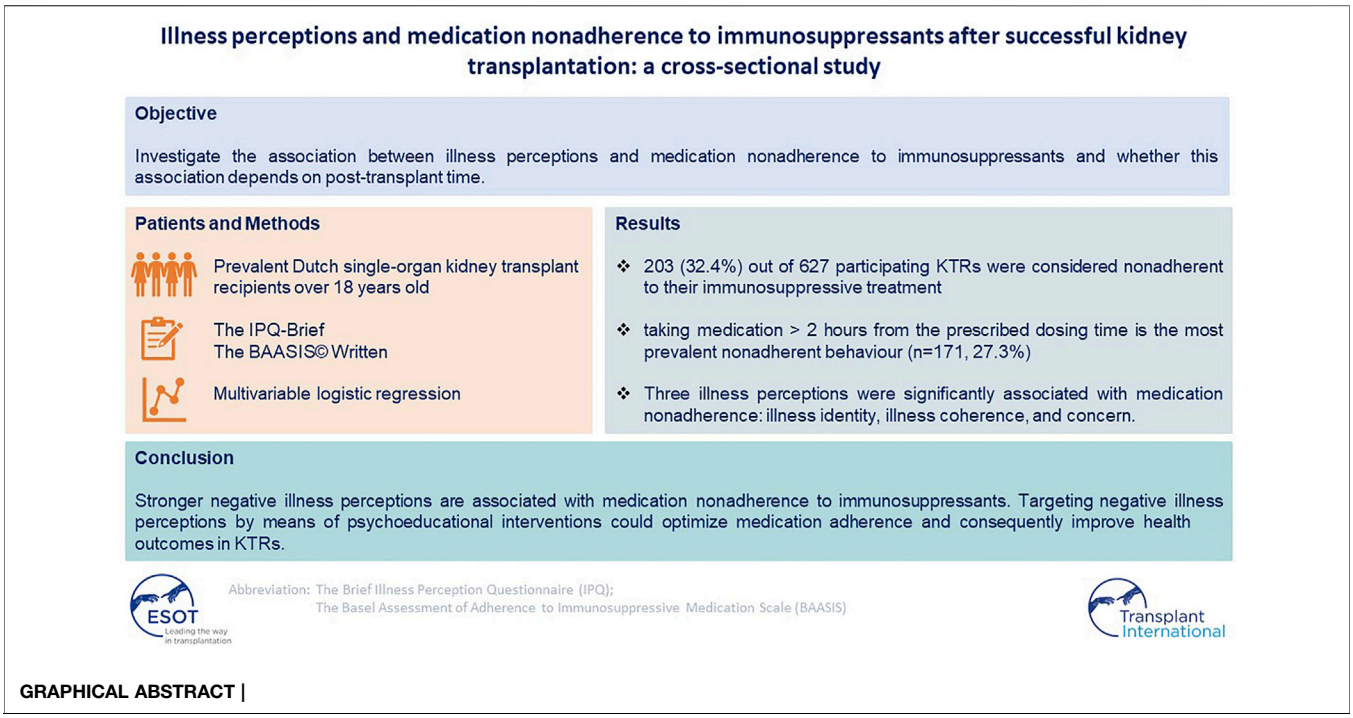
Abbreviation: BAASIS, the basel assessment of adherence to immunosuppressive medication scale; BMI, body mass index; CI, confidence interval; CSM, common sense model of self-regulation; EMERGE, the ESPACOMP medication adherence reporting guidelines checklist; IPQ, illness perception questionnaire; IQR, interquatile range; KT, kidney transplantation; KTRs, kidney transplant recipients; LUMC, Leiden university medical centre; OR, odds ratio; ORadj, adjusted odds ratio; PKD, primary kidney disease; SD, standard deviation; SES, socioeconomic status; STROBE, the strengthening the reporting of observational studies in epidemiology guideline; WMO, the medical research involving human subjects act.



GRAPHICAL ABSTRACT |

INTRODUCTION

Successful kidney transplantation requires strict adherence to chronic immunosuppressive regimens (1). Failure to take immunosuppressants as prescribed has been identified as a risk factor for adverse clinical outcomes among kidney transplant recipients (KTRs), including graft loss and reduced patient survival (2, 3). Butler et al. reported a seven-fold higher odds of graft failure in nonadherent KTRs than in adherent KTRs (2). Furthermore, persistent medication nonadherence can lead to increased individual medical costs (4). Despite the obvious negative impact, medication nonadherence in KTRs remains substantial, with a broadly consistent prevalence of 20% or higher (1, 5).

Leventhal's widely-used Common Sense Model (CSM) of Self-regulation provides us with explanations for patients' behaviour when facing health threats and may aid our understanding of the behavioural mechanism explaining medication nonadherence (6). According to the CSM, patients' illness perceptions directly influence their coping behaviour (e.g., medication adherence) with the medical condition; thereafter, they appraise the effect of such behavioural adaptions and the result of the appraisal therof can shape their illness perceptions (6). Consequently, illness perceptions—referring to patients' appraisal and understanding of their medical condition—are considered a potential intervention target to improve coping behaviours and subsequent health outcomes.

Previous studies have shown that illness perceptions are associated with various outcomes in patients with chronic conditions, including chronic kidney disease (7–10). In non-KTRs (e.g., patients with hypertension), stronger positive illness perceptions have also been found associated with better medication adherence (11). However, very few studies have shed light on illness perceptions and their associations with medication nonadherence in patients after kidney transplantation, and the existing studies found inconsistent results: Cossart et al. (12) found stronger positive perceptions (i.e., illness coherence) in adherent KTRs, while Massey et al. (13) described a downward trend in medication adherence with improved illness perceptions over time. Therefore, further studies are necessary to understand the influence of illness perceptions on medication nonadherence and to develop effective patient-centered interventions to improve medication adherence in this KTR population.

Finally, the dynamic nature of the self-regulation process is an important feature of the CSM, which suggests that illness perceptions can change throughout the course of a disease (14, 15). A previous study has detected changes in certain illness perceptions in KTRs within 1.5 years after transplantation (13). It is reasonable to speculate that the relief after successful kidney transplantation may positively impact illness perceptions in the short term; however, in the long term, illness perceptions may change due to change in the experience of immunosuppressant-related side effects. Until now, little is known about whether such dynamic feature of KTRs' illness perceptions also plays a role in medication adherence. Therefore, in this study, we will investigate the influence of illness perceptions on medication nonadherence to immunosuppressants among prevalent Dutch KTRs and explore whether such associations differ depending on the time since their kidney transplant.

PATIENTS AND METHODS

For the reporting of this study, we followed the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) guideline (16).

Study Design and Study Population

This study was conducted in Leiden University Medical Center (LUMC) from 1 October 2020 to 30 October 2020. KTRs who met the following criteria were invited to participate in this study: 1) adult KTRs transplanted before 1 April 2019 in LUMC with a functioning graft; 2) the last visit in LUMC took place after 31 December 2010; and 3) patients with a sufficient understanding of the Dutch language. To avoid overburdening of patients, we did not invite patients transplanted after April 2019 as they were already involved in a longitudinal study to measure patient-reported outcomes after kidney transplantation routinely. We excluded patients whose last visit in LUMC was before 31 December 2010 to have more easily accessible administrative and clinical data.The questionnaires used in our study were sent to patients *via* postal service or email along with an informed consent form to use the collected data for research purposes. The questionnaires measured medication adherence and illness perceptions, and collected data about patients' education level, marital status, and employment status at the time of the study. A reminder email was sent to patients with a known email address if they did not respond within 7 days after the first invitation. The institutional review board of LUMC for non-WMO research (i.e., research not subjected to the Medical Research Involving Human Subjects Act [WMO]) approved this study. The study was conducted following the national guidelines for medical scientific research (17).

Medication Nonadherence

Self-reported medication adherence to immunosuppressants was measured using a commonly used and validated questionnaire, the Basel Assessment of Adherence to Immunosuppressive Medication Scale (BAASIS© Written) (18). The questionnaire contains four questions to measure medication adherence in the implementation phase (i.e., issue with taking, changed timing, drug holidays, and dose reduction). Each question asks the occurrence of the medication-taking behaviour (yes or no) and the frequency of corresponding nonadherent behaviour (i.e., once a month, once every 2 weeks, every week, more than once a week, and every day) in the past 4 weeks prior to the measurement. Regardless of the frequency, any "yes" to the above four questions implied medication nonadherence to immunosuppressants. The reporting of medication adherence followed the ESPACOMP Medication Adherence Reporting Guidelines (EMERGE) checklist (19).

Illness Perceptions

The following eight illness perceptions were measured on a 0-to-10 response scale using the commonly used and validated questionnaire, the Brief Illness Perception Questionnaire (Brief-IPQ) (20): *consequences*, *timeline*, *personal control*, *treatment control*, *illness identity*, *concern*, *illness coherence,* and *emotional response*. In this study, we omitted illness perception domain *cause* from our analysis as the cause of kidney disease is very heterogeneous (7). To facilitate interpretation, we recoded the scores of three perceptions (i.e., *personal control*, *treatment control,* and *illness coherence*) in such a way that for all perceptions, a higher score indicated more negative illness perceptions (e.g., a higher score of treatment control now implies a lower belief of patients in that the treatment they receive can relieve or cure their illness).

Sociodemographic and Clinical Characteristics

Data on sociodemographic and clinical characteristics were collected *via* questionnaires or from patients' medical records, including age at transplantation, age at study participation, sex, socioeconomic status (SES), education level, marital status, number of transplantation, primary kidney disease, donor type (living donor and deceased donor), pre-emptive kidney transplantation, time since kidney transplantation (i.e., post-transplant time), body mass index (BMI), comorbidities, and type of immunosuppressants at study. The SES of study participants was obtained by linking the four digits of their postcode with the latest SES-score per postcode area reported by the Netherlands Institute for Social Research; the SES was divided into three groups: low, medium, and high (21). Primary kidney disease (PKD) was classified into eight categories: congenital and hereditary kidney disease, cystic kidney disease, diabetes mellitus, glomerulonephritis, renal vascular disease, interstitial nephritis/pyelonephritis, other diseases, and unknown ontology (22). Data about comorbidities at transplantation were collected. Comorbidities were indicated by a history of diabetes mellitus, cardiac event, vascular event, and cerebrovascular event before the study. Post-transplant time was ategorized into three groups: ≤5 years, 5–15 years, and >15 years. The most recent BMI was also collected, with the average time between BMI measurement and study participating being approximiatly 1 year (mean = 12.5 months; SD = 13.7 months).

Statistical Analysis

Continuous variables were presented as mean with standard deviation (SD) if normally distributed and as median with interquartile range (IQR) if not normally distributed. Count (percentage) was used for categorical variables. Medication adherence and illness perceptions were described in the total study population and in subgroups stratified by post-transplant time. Multivariable logistic regression models were employed to analyse the impact of each separate illness perception on medication adherence while adjusting for potential confounders, including age at study participation, sex, SES, marital status, education level, employment status, donor type, number of transplantation, PKD, comorbidities, and post-transplant time. The interaction term "post-transplant time

TABLE 1 | Patient characteristics of the total study population and stratified by categories of post-transplant time.

Characteristic	Total (*n* = 627)	Post-transplant time		
		<5 years (*n* = 158)	5–15 years (*n* = 312)	>15 years (*n* = 157)
Mean age (SD) at study, yr	61.4 (11.3)	58.0 (11.9)	61.8 (11.5)	63.9 (9.3)
Age structure at study, n (%)				
18~39	31 (4.9)	14 (8.9)	15 (4.8)	2 (1.3)
40~59	233 (37.2)	68 (43.0)	114 (36.5)	51 (32.5)
60~79	350 (55.8)	76 (48.1)	176 (56.4)	98 (62.5)
80~	13 (2.1)	0 (0)	7 (2.2)	6 (3.8)
Mean (SD) age at KT, yr	50.0 (13.1)	54.9 (11.8)	52.5 (11.8)	40.0 (11.5)
Median (IQR) time after KT, yr	9.0 (10.2)	3.1 (1.8)	9.0 (4.8)	20.7 (11.3)
Female, n(%)	233 (37.2)	53 (33.5)	124 (39.7)	56 (35.7)
SES, n(%)[a]				
Low	64 (10.2)	22 (13.9)	26 (8.3)	16 (10.2)
Middle	397 (63.3)	101 (63.9)	200 (64.1)	96 (61.1)
High	161 (25.7)	34 (21.5)	83 (26.6)	44 (28.0)
Marital status, n(%)				
Single/separated	160 (25.5)	53 (33.5)	71 (22.8)	36 (22.9)
Married/living together	467 (74.5)	105 (66.5)	241 (77.2)	121 (77.1)
Education				
Low	52 (8.3)	12 (7.6)	22 (7.1)	18 (11.5)
Middle	215 (34.3)	52 (32.9)	107 (34.3)	56 (35.6)
High	360 (57.4)	94 (59.5)	183 (58.7)	83 (52.9)
Employment, n(%)				
Employed	291 (46.4)	83 (52.5)	142 (45.5)	66 (42.0)
Unemployed	69 (11.0)	24 (15.2)	32 (10.3)	13 (8.3)
Retired/Student	267 (42.6)	51 (32.3)	138 (44.2)	78 (49.7)
Primary Kidney Disease, n(%)[a]				
Congenital/hereditary kidney disease	15 (2.4)	0 (0)	8 (2.6)	7 (4.5)
Cystic kidney disease	139 (22.2)	38 (24.1)	78 (25.0)	23 (14.6)
Diabetes	33 (5.3)	21 (13.3)	12 (3.8)	0 (0)
Glomerulonephritis	136 (21.7)	34 (21.5)	75 (24.0)	27 (17.2)
Interstitial nephritis/pyelonephritis	51 (8.1)	11 (7.0)	21 (6.7)	19 (12.1)
Renal vascular disease	61 (9.7)	18 (11.4)	31 (9.9)	12 (7.6)
Other diseases	45 (7.2)	11 (7.0)	27 (8.7)	7 (4.5)
Unknown	102 (16.3)	24 (15.2)	51 (16.3)	27 (17.2)
Number of KTs, n(%)[a]				
1	540 (86.1)	133 (84.2)	263 (84.3)	144 (91.7)
>1	77 (12.3)	24 (15.2)	40 (12.8)	13 (8.3)
Donor type, n(%)[a]				
Living donor	376 (60.0)	102 (64.6)	212 (67.9)	62 (39.5)
Deceased donor	241 (38.4)	55 (34.8)	91 (29.2)	95 (60.5)
Mean (SD) BMI, kg/m^2 [a]	26.2 (4.6)	26.6 (4.5)	25.7 (4.3)	27.0 (5.4)
Comorbidities, n(%)[a]				
Diabetes Mellitus	97 (15.5)	31 (19.6)	47 (15.1)	19 (12.1)
Cardiovascular event	169 (27.0)	53 (33.5)	67 (21.5)	49 (31.2)
Cerebrovascular event	42 (6.7)	12 (7.6)	23 (7.4)	7 (4.5)
Immunosuppressants, n(%)[a]				
Prednisone	556 (88.7)	148 (93.7)	281 (90.1)	127 (80.9)
Tacrolimus	348 (55.5)	123 (77.8)	193 (61.9)	32 (20.4)
Mycophenolic acid	361 (57.6)	120 (75.9)	182 (58.3)	59 (37.6)

Data are presented as mean (SD) or median (IQR) for continuous variables and n (%) for categorical variables. Abbreviations: BMI, body mass index; IQR, interquartile range; KT, kidney transplantation; SES, socioeconomic status; SD, standard deviation.

[a]*Variables with missing values: SES (0.8%), primary kidney disease (7.2%), number of KT (1.6%), donor type (1.6%), BMI (22.2%), diabetes (42.6%), cardiovascular event (39.1%), cerebrovascular event (47.8%), immunosuppressants (3.2%).*

(categorical) * illness perception" was added to evaluate whether the influence of individual illness perception on medication nonadherence differed depending on post-transplant time. A variable "IPQ score/n" was used in the logistic regression models to assess the risk of medication nonadherence with n increments in IPQ-score (i.e., one or two increments on a 11-point scale).

Missing values were considered "missing at random" and were imputed with 10-folds multiple imputation (23). In addition to the variables with missing values (see **Table 1**), variables used for multiple imputation included illness perceptions, medication adherence, and other variables adjusted for in the logistical regression model. Abnormally distributed continuous variables were log-transformed for imputation. As sensitivity analyses, we

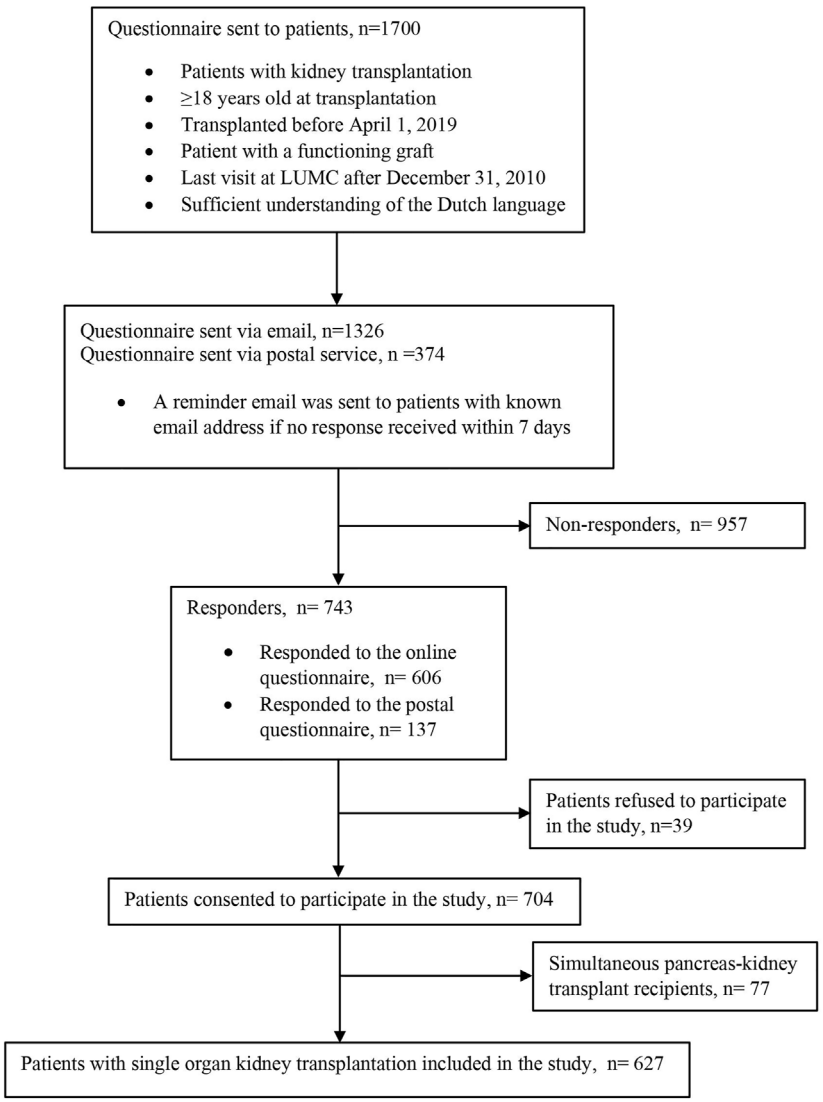


FIGURE 1 | Flow chart of the study population.

repeated all analyses but now excluded comorbidities and BMI from the multivariable model due to a relatively high percentage of missing values. The patient characteristics of responders and nonresponders are presented in **Supplementary Table S1**. *P*-value < 0.05 was considered significant. We used SPSS software version 25.0. (IBM, Armonk, NY, United States) for all analyses.

RESULTS

Of the 1700 adult KTRs who were transplanted before 1 April 2019, at LUMC and met study inclusion criteria, 743 (43.7%) KTRs responded *via* email (n = 606) or *via* postal service (n = 137). 39 responders filled out the questionnaires but did not want to participate in this study. After excluding another 77 patients who received simultaneous pancreas-kidney transplantation, 627 KTRs were left to be included in the main analysis (**Figure 1**). Please see **Supplementary Table S1** for the characteristics of the nonresponders.

Patient Characteristics

Table 1 shows the sociodemographic and clinical characteristics of the responders in the total population and stratified by post-transplant time. The mean (SD) age of all included KTRs at study participation was 61.4 (11.3) years old; 93% of the KTRS were between 40 and 80 years old at the study. The median (IQR) post-transplant time was 9.0 (10.2) years, 74.5% of the KTRs had a partner, 89.8% had a medium or high SES, 57.4% received a high level of education, and 89.0% were employed, retired, or students. After stratification, KTRs with a post-transplant time of more than 15 years had the oldest age at study participation, the

TABLE 2 | Medication nonadherence in the total study population and stratified by categories of post-transplant time.

Medication nonadherence, n (%)	Total (*n* = 627)	Post-transplant time			A "yes" to the question indicates
		<5 years (*n* = 158)	5–15 years (*n* = 312)	>15 years (*n* = 157)	
Medication nonadherence	203 (32.4)	43 (27.2)	105 (33.7)	55 (35.0)	Nonadherence to immunusuppressants in general[a]
Issues with taking	77 (12.3)	14 (8.8)	41 (13.1)	22 (14.0)	Not taken immunosuppressants some times in the past 4 weeks
Once a month	68 (10.8)	13 (8.2)	36 (11.5)	19 (12.1)	
More than once a month	9 (1.5)	1 (0.6)	5 (1.6)	3 (1.9)	
Drug holiday	5 (0.8)	1 (0.6)	2 (0.6)	2 (1.3)	Skipped several consecutive doses of immunosuppressants in the past 4 weeks
Once a month	3 (0.5)	1 (0.6)	2 (0.6)	0 (0)	
More than once a month	2 (0.3)	0 (0)	0 (0)	2 (1.3)	
Timing	171 (27.3)	35 (22.1)	88 (28.1)	48 (30.6)	Taken immunosuppressants with more than 2 h' time difference from the prescribed dosing time in the past 4 weeks
Once a month	101 (16.1)	22 (13.9)	56 (17.9)	23 (14.6)	
More than once a month	70 (11.2)	13 (8.2)	32 (10.2)	25 (16.0)	
Dose reduction	2 (0.4)	0 (0)	1 (0.3)	1 (0.6)	Reduced the prescribed amount of immunosuppressants in the past 4 weeks
Once a month	1 (0.2)	0 (0)	1 (0.3)	0 (0)	
More than once a month	1 (0.2)	0 (0)	0 (0)	1 (0.6)	

[a]*Any "yes" to the four questions of the four adherence-domains indicates medication nonadherence in general.*

TABLE 3 | Illness perceptions of the total study population and stratified by categories of post-transplant time.

Illness perception, mean (SD)[a]	Total (*n* = 627)	Post-transplant time			A higher score indicates patients believe to a greater extent that...
		<5 years (*n* = 158)	5–15 years (*n* = 312)	>15 years (*n* = 157)	
Consequences	5.0 (2.9)	5.2 (2.9)	4.8 (2.9)	5.0 (3.1)	...their kidney disease has more negative consequences upon their life
Timeline	8.6 (2.7)	8.4 (2.9)	8.8 (2.6)	8.6 (2.7)	...their kidney disease lasts for a longer time
Personal control	3.8 (2.6)	3.4 (2.5)	3.8 (2.6)	4.3 (2.8)	...their kidney disease cannot be effectively controlled by themselves
Treatment control	2.2 (2.3)	1.7 (2.0)	2.2 (2.2)	2.7 (2.6)	...their kidney disease cannot be effectively controlled by their treatment
Illness identity	4.2 (2.9)	3.8 (2.8)	4.2 (2.9)	4.7 (2.9)	...their kidney disease causes more symptoms
Concern	4.7 (2.9)	4.7 (2.8)	4.7 (2.8)	4.9 (3.1)	...their kidney disease causes greater worries about their health
Illness coherence	1.6 (1.9)	1.7 (2.0)	1.3 (1.6)	1.9 (2.3)	...they do not understand their kidney disease
Emotional response	3.8 (2.9)	4.1 (3.1)	3.5 (2.9)	4.0 (2.9)	...their kidney disease causes more emotional distress

[a]*Illness perceptions were measured on an 11-point scale ranging from 0 to 10, with higher scores reflecting stronger negative perceptions of their condition.* Personal control, treatment control *and* illness coherence *were recoded so that a higher score on these perceptions also indicate stronger negative illness perceptions.*

youngest age when receiving the transplantation, and the highest percentage of deceased donor kidney transplantation. KTRs with a post-transplant time of less than 5 years had the highest unemployment rate and the lowest percentage of living alone or being separated. Notably, the percentages of patients with diabetes as either PKD or comorbidity reduced as the post-transplant time increased. Difference in immunosuppressants was also observed in KTRs with different post-transplant time: patients with a post-transplant time of more than 15 years were less likely to receive prednisone, tacrolimus, and mycophenolic acid in comparison to the other two groups. Compared to the nonresponders, the study population had higher SES ranks and a lower percentage of diabetes as their PKD (**Supplementary Table S1**).

Medication Nonadherence

Table 2 presents self-reported nonadherence to immunosuppressants in all study participants: 203 (32.4%) KTRs were identified as nonadherent based on the BAASIS-scoring algorithm. When focusing on the specific medication nonadherence domains, the results showed that nonadherence to *timing* (i.e., taking medication with more than 2 h difference from the prescribed time; 27.3%) was the most frequently reported nonadherent behaviour, followed by *issue with taking* (i.e., not take medication sporadically; 12.3%). Very few KTRs reported *drug holiday* (i.e., not take medication consecutively; 0.8%) or *dose reduction* (i.e., reduce the dosage of prescribed medication; 0.4%). Most nonadherent KTRs reported nonadherent behaviour once a month. After stratification by post-transplant time, the results showed that the proportion of nonadherent patients increased as the time after kidney transplantation increased overall and in the separate nonadherent behaviour domains.

Illness Perceptions

Mean (SD) scores of each illness perception are presented in **Table 3**. In general, the included KTRs believed to a relatively

TABLE 4 | Associations between illness perceptions and medication nonadherence (n = 627).

Illness perception	Crude OR (95% CI)[b]	P-value	Adjusted OR (95% CI)[a,b] per one increment in illness perception	Adjusted OR (95% CI)[a,c] per two increments in illness perception	P-value	P-value for interaction[b] (post-transplant time * illness perception)
Consequences	1.02 (0.97, 1.08)	0.44	1.02 (0.95, 1.08)	1.03 (0.91, 1.16)	0.64	0.48
Timeline	1.04 (0.98, 1.11)	0.21	1.02 (0.96, 1.10)	1.05 (0.91, 1.20)	0.51	0.96
Personal control	1.05 (0.99, 1.12)	0.10	1.05 (0.99, 1.13)	1.11 (0.97, 1.27)	0.12	0.52
Treatment control	1.05 (0.98, 1.23)	0.18	1.05 (0.97, 1.14)	1.11 (0.95, 1.29)	0.20	0.57
Illness identity	1.05 (0.99, 1.11)	0.14	1.07 (1.00, 1.14)	1.14 (1.00, 1.29)	0.05[d]	0.62
Concern	1.06 (1.00, 1.13)	0.04	1.07 (1.00. 1.14)	1.14 (1.00, 1.29)	0.05[d]	0.73
Illness coherence	1.08 (0.99, 1.17)	0.10	1.11 (1.01, 1.22)	1.23 (1.03, 1.48)	0.03	0.69
Emotional response	1.04 (0.98, 1.10)	0.22	1.03 (0.97, 1.10)	1.07 (0.94, 1.21)	0.32	0.64

Abbreviation: BMI, body mass index; CI, confidence interval; OR, odds ratio; SES, socioeconomic status.

[a]*The adjusted variables included age at the study, sex, SES, rank, marital status, employment status, education level, primary kidney disease, comorbidities, BMI, donor type, time after kidney transplantation, the number of transplantations received, and immunosuppressants.*

[b]*OR, of one increment in illness perception scores on an 11-point scale.*

[c]*OR, of every two increments in illness perception scores on an 11-point scale.*

[d]*P-value < 0.05, namely: 0.045 for both illness perceptions "illness identity" and "concern".*

high extent that they understand their kidney disease (*illness coherence*) and that their kidney disease is a life-long chronic condition (*timeline*). They also had a strong belief that their treatment can control their disease (*treatment control*). The perceived *personal control* over their disease was lower than the perceived *treatment control* but could still be considered relatively high. The mean scores of the other illness perceptions laid around the midpoint of the scale (range: 3.8–5.0 on a 11-point scale ranging from 0 to 10), indicating that KTRs believed to a moderate extent that their kidney disease is a cause for concern (*concern*), has negative consequences upon their lives (*consequences*), and causes negative feelings (*emotional response*) and a high symptom burden (*illness identity*). After stratification, the results showed that KTRs with a longer post-transplant time believed to a lesser extent that their disease can be controlled by their treatment or by themselves (*treatment control* and *personal control*) and that they experienced a higher symptom burden due to kidney disease (*illness identity*).

Illness Perceptions and Nonadherence to Immunosuppressants in KTRs

After adjusting for potential confounders, three illness perceptions (i.e., *illness identity*, *concern*, and *illness coherence*) were significantly associated with nonadherence to immunosuppressants in KTRs. More specifically, the results showed that with one increment in scores on the illness perceptions *illness identity*, *concern*, and *illness coherence*, the risk of nonadherence increased by 7%, 7%, and 11%, respectively (**Table 4**). For the other five domains (i.e., *consequences*, *timeline*, *personal control*, *treatment control,* and *emotional response*), the point estimates ranged from 1.02 to 1.05, indicating an association between less favourable illness perceptions of these illness perceptions and increased risk of medication nonadherence but with wider confidence intervals. **Table 4** also shows the increased risk of medication nonadherence with every two increments in illness perception scores. None of the interactions between the separate illness perceptions and time after kidney transplantation were statistically significant (p-values ranged from 0.48 to 0.96).

Sensitivity Analyses

When repeating the logistic regression analysis without comorbidities and BMI (**Supplementary Table S2**), the results showed that, although the association between *illness identity* and *concern* and medication nonadherence became statistically insignificant, the ORs (95%CI) supported the results from the main analysis (i.e., *illness identity*: 1.06, 95%CI, 1.00 to 1.13, p = 0.06; *concern*: 1.06, 95%CI, 1.00 to 1.13, p = 0.06; *illness coherence*: 1.11, 95%CI, 1.02 to 1.22, p = 0.02).

DISCUSSION

Despite the improvements in nephrology care, adherence to immunosuppressants remains a challenge in KTRs. Our study detected nonadherence to immunosuppressants in a considerable proportion of prevalent Dutch KTRs and associations between negative illness perceptions and medication nonadherence to immunosuppressants.

The proportion of nonadherent KTRs in our study (32.4%) is similar to the results of a previous literature review, which also reported a high weighted mean prevalence (28%) of medication nonadherence to immunosuppressants in KTRs (5). However, the prevalence of medication nonadherence reported by different studies may not be directly comparable as their definition for medication nonadherence may differ. Regarding the nonadherence behavioural pattern, taking medication 2 h beyond the recommended dosing time was the most prevalent nonadherent behaviour in our study population (27.3%), followed by not taking their medication sporadically (12.3%). These findings are in line with other studies that also reported nonadherence behavioural patterns in KTRs (24, 25).

Furthermore, our results showed that stronger negative illness perceptions are associated with medication nonadherence to immunosuppressants in KTRs. More specifically, less understanding of kidney disease (*illness coherence*), greater worries about the kidney disease (*concern*), and experiencing more symptoms due to the kidney disease (*illness identity*) significantly increased the risk of medication nonadherence by 7%, 7%, and 11% with one unit increment on a 0-to-10 scale in our Dutch KTRs population. Our findings are in line with the results described by Cossart et al. that nonadherent KTRs believed to a lesser extent that they understand their kidney disease (*illness coherence*) (12). Additionally, our results indicated that the more worried patients were about their kidney disease (*concerns*), the more likely it was that they were nonadherent—an association that has also been reported in patients after myocardial infarction (26). A possible explanation for this finding is that highly concerned patients may have a more fatalistic attitude towards their disease (e.g., progression of their disease is inevitable) and are, therefore, less strict with their medication taking. Finally, our results showed that patients who attributed a greater symptom burden to their kidney disease were less adherent. This result is supported by findings reported by Rosenberger et al. (27) suggesting that KTRs with more adverse effect due to their chronic immunosuppressive treatment (e.g., tremor, diarrhoea, and fatigue) were more likely to be nonadherent. Of note, the results also suggested an association between less favourable illness perceptions of the other five domains (i.e., *consequences*, *timeline*, *personal control*, *treatment control*, and *emotional response*) and increased risk of medication nonadherence despite statistical insignificance.

In general, the association between illness perceptions and medication nonadherence is consistent with Leventhals' CSM (6) and the results reported by others in patients with chronic conditions, such as hypertension and diabetes (28, 29). However, we did not observe the discrepancy found in the study conducted by Massey et al. (13), namely that some illness perceptions (*consequence* and *emotional response*) became more favourable over time while medication nonadherence still increased. The different study populations and study design may explain such differences in findings: Massey et al.' population consisted of newly transplanted patients in a longitudinal study, while our study population was prevalent patients in a cross-sectional study. Notably, we did not detect a difference in the relationships between illness perceptions and medication nonadherence in patients with different time after kidney transplantation; however, we cannot rule out the possibility that these insignificant results are due to the participation of healthier KTRs regardless of their post-transplant time. Future studies with a longitudinal design and sufficient length of follow-up are needed to test the association between illness perceptions and medication nonadherence over time.

Our study suggests a need to improve medication adherence to immunosuppressants in KTRs along with previous research (5), and also suggests that negative illness perceptions could be a potential interventional target to achieve this. In our analyses, a perceived lack of understanding of kidney disease (*illness coherence*) was most strongly associated with medication nonadherence among other illness perceptions. However, a lack of illness understanding among patients is not uncommon in clinical practice: two previous studies in a clinical setting found that only 42% and 77% of the patients were able to list their diagnosis and that 14% and 17% of the patients were able to state the common side effects of their medication (30, 31). Such findings have shown adequate room to modify negative illness perceptions, which are indeed modifiable according to existing evidence in other patient groups and the CSM (6, 32–35). Current interventions to improve illness perceptions are mainly derived from the CSM framework and usually involve behaviour change techniques to modify the psychosocial determinants of unwanted (e.g., nonadherent) behaviour, such as patient education, motivational interviewing, goal setting, identifying and solving problems, improving social support, and facilitating support seeking (33, 34). In recent years, attempts have also been made to introduce self-management support programmes into care for patients with chronic conditions on top of the conventional treatment by healthcare professionals (35). Future studies are needed to facilitate translation of such knowledge into practice by identifying the effects of different behaviour change techniques to modify unhelpful illness perceptions, the efficient approaches to deliver such interventions to the patients, and the optimal logistics to implement such interventions into clinical practice. In addition to cognitive behavioural interventions, our results also suggested that patients could benefit from active management of immunosuppressant-related side effects in KTRs. Future studies may also focus on identifying potential risk factors for unhelpful illness perception to tailor intervention (e.g., age, gender, or SES). Finally, efforts are warranted to understand the clinically relevant level of occurrence and frequency of self-reported nonadherent behaviours in terms of the therapeutic effect of prescribed immunosuppressants to facilitate a more clinically relevant understanding of our results.

The strengths of this study include that our study population consists of KTRs covering a broad time span after kidney transplantation and that we are one of the first studies to examine the associations between illness perceptions and medication nonadherence in this specific population. Additionally, our analyses included a relatively large sample size, especially compared to the previous studies investigating similar topics (12, 13). Our study also has several limitations that should be taken into account. First, medication nonadherence was measured using self-report, which is prone to underestimate medication nonadherence (36). This could have potentially introduced outcome misclassification bias, leading to underestimating the association between illness perceptions and medication nonadherence. Second, the responders may not be representative of the general Dutch KTRs; compared to the nonresponders (**Supplementary Table S1**), responders were more likely to be in a better SES, receive living donor kidney transplantation, and were less likely to have diabetes as PKD. A previous survey study also suggests that responders better adhere to their medication regime than nonresponders (37). Such differences between responders and nonresponders could influence the generalizability of our results. Moreover, the majority of our study population was between 40

and 80 years old, which also limits the generalizability of our results. Third, our study was conducted in prevalent Dutch KTRs, and thus, future studies are needed to investigate whether our results can be generalized to different populations. Finally, due to our observational cross-sectional design, residual confounding as a result of unmeasured confounders (e.g., pill burden) exists and causal interpretation is limited, although the theoretical fundaments of CSM are considered quite robust (6, 38).

In conclusion, this study suggests that stronger negative illness perceptions are associated with medication nonadherence to immunosuppressants in KTRs. The high prevalence of medication nonadherence in our study indicates room for improvement and that KTRs need additional support to adhere to this strict medication regime. Targeting negative illness perceptions utilizing psychoeducational interventions could possibly optimize medication adherence and consequently improve health outcomes in KTRs. Future studies are needed to explore such interventions' effects and identify facilitators and barriers for implementing such support strategies to help its uptake in clinical practice.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because the data was collected for specific research purposes. Requests to access the datasets should be directed to AdV, A.P.J.de_Vries@lumc.nl.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the institutional review board of Leiden University Medical Center for non-WMO research. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YW: concept/design, analysis, interpretation, and drafting article; DV: data collection, interpretation, and critical review of the article; PvB: data collection, interpretation, and critical review of the article; MH: interpretation and critical review of the article; FD: concept/design, analysis, interpretation, critical review of the article, and supervision; AdV: concept/design, data collection, interpretation, critical review of the article, and supervision; YM: concept/design, analysis, interpretation, critical review of the article, and supervision.

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CONFLICT OF INTEREST

The authors declare that this study is part of a larger project, namely, the Patient-reported OutcomeS In kidney Transplant recipients: Input of Valuable Endpoints (POSITIVE) study, and the POSITIVE study received funding from Chiesi Pharmaceuticals BV, Netherlands, and Astellas Pharma Inc. The funder was not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication.

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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.2022.10073/full#supplementary-material

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ORIGINAL RESEARCH
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Interactions Between Donor Age and 12-Month Estimated Glomerular Filtration Rate on Allograft and Patient Outcomes After Kidney Transplantation

Wai H. Lim[1,2], Esther Ooi[2,3], Helen L. Pilmore[4,5], David W. Johnson[6,7,8], Stephen P. McDonald[9,10,11,12], Philip Clayton[9,10,11,12], Carmel Hawley[6,7,8], William R. Mulley[13,14], Ross Francis[6,7], Michael G. Collins[3,4], Bryon Jaques[15], Nicholas G. Larkins[2,16], Christopher E. Davies[9,11,12], Kate Wyburn[17,18], Steve J. Chadban[17,18] and Germaine Wong[17,19,20]*

[1]Department of Renal Medicine, Sir Charles Gairdner Hospital, Perth, WA, Australia, [2]Medical School, University of Western Australia, Perth, WA, Australia, [3]School of Biomedical Sciences, University of Western Australia, Perth, WA, Australia, [4]Department of Renal Medicine, Auckland City Hospital, Auckland, New Zealand, [5]Department of Medicine, University of Auckland, Auckland, New Zealand, [6]Metro South Integrated Nephrology and Transplant Services, Princess Alexandra Hospital, Woolloongabba, QLD, Australia, [7]Faculty of Medicine, University of Queensland, St Lucia, QLD, Australia, [8]Translational Research Institute, Brisbane, QLD, Australia, [9]Australia and New Zealand Dialysis and Transplant Registry, Adelaide, SA, Australia, [10]Central and Northern Adelaide Renal and Transplantation Services, Adelaide, SA, Australia, [11]South Australia Health and Medical Research Institute, Adelaide, SA, Australia, [12]Adelaide Medical School, University of Adelaide, Adelaide, SA, Australia, [13]Department of Nephrology, Monash Medical Centre, Melbourne, VIC, Australia, [14]Department of Medicine, Monash University, Melbourne, VIC, Australia, [15]Western Australia Liver and Kidney Transplant Service, Sir Charles Gairdner Hospital, Perth, WA, Australia, [16]Department of Nephrology, Perth Children's Hospital, Perth, WA, Australia, [17]Faculty of Medicine and Health, University of Sydney, Sydney, NSW, Australia, [18]Renal Medicine, Royal Prince Alfred Hospital, Sydney, NSW, Australia, [19]Centre for Kidney Research, The Children's Hospital at Westmead, Sydney, NSW, Australia, [20]Department of Renal Medicine and National Pancreas Transplant Unit, Westmead Hospital, Sydney, NSW, Australia*

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Correspondence:
Wai H. Lim
wai.lim@health.wa.gov.au

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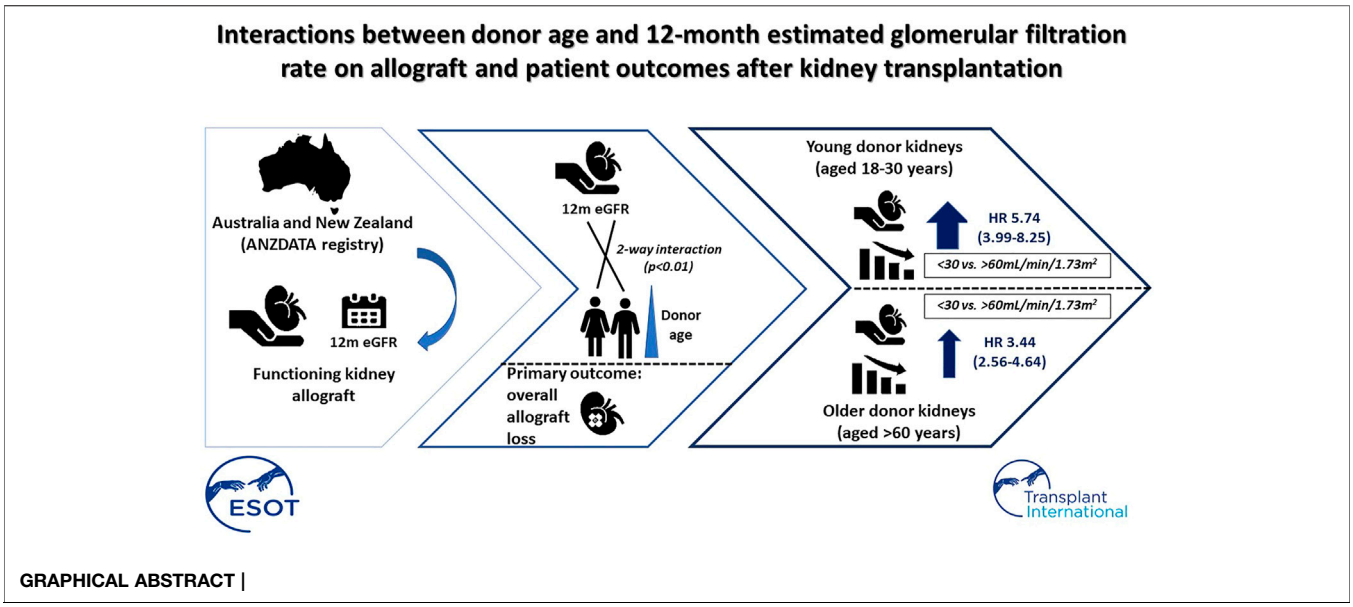
Reduced estimated glomerular filtration rate (eGFR) at 12-months after kidney transplantation is associated with increased risk of allograft loss, but it is uncertain whether donor age and types modify this relationship. Using Australia and New Zealand registry data, multivariable Cox proportional modelling was used to examine the interactive effects between donor age, types and 12-month eGFR on overall allograft loss. We included 11,095 recipients (4,423 received live-donors). Recipients with lowest 12-month eGFR (<30 ml/min/1.73 m^2) experienced the greatest risk of allograft loss, with adjusted HR [95% CI) of 2.65 [2.38–2.95] compared to eGFR of 30–60 ml/min/1.73 m^2; whereas the adjusted HR for highest eGFR (>60 ml/min/1.73 m^2) was 0.67 [0.62–0.74]. The association of 12-month eGFR and allograft loss was modified by donor age (but not donor types) where a higher risk of allograft loss in recipients with lower compared with higher 12-month eGFR being most pronounced in the younger donor age groups ($p < 0.01$). Recipients with eGFR <30 ml/min/1.73 m^2 12-months after transplantation experienced ≥2.5-fold increased risk of overall allograft loss compared to those with eGFR of >60 ml/min/1.73 m^2, and the magnitude of the increased risk is most marked among recipients with younger donors. Careful deliberation of other factors including donor age when considering eGFR as a surrogate for clinical endpoints is warranted.

Keywords: kidney transplantation, registry, allograft failure, patient and graft survival, estimated glomerular filtration rate, donor age, donor type



GRAPHICAL ABSTRACT |

INTRODUCTION

Reduced estimated glomerular filtration rate (eGFR) is associated with an increased risk of all-cause and cardiovascular mortality in the general population and people with chronic kidney disease (1–4). There is an inverse relationship between post-transplant eGFR and the risks of adverse allograft outcomes in kidney transplantation, including death and death-censored allograft loss (5). Post-transplant kidney function, especially allograft function at 12-months post-transplant, is an important outcome measure and is considered one of the most critical outcomes for clinical trials in transplantation by patients and health professionals (5–15). In a systematic review of 169 randomized controlled trials in adult kidney transplant recipients, 60% of trials utilized creatinine-derived eGFR as a study endpoint (28% and 61% as primary and secondary endpoints, respectively), emphasizing the clinical importance of allograft function as a potential surrogate measure of long-term allograft outcome [7].

The growing use of expanded criteria (or higher Kidney Donor Profile Index [KDPI]) donors has prompted clinicians to recognize that specific donor factors, including donor age and comorbidities, may influence short- and long-term outcomes after transplantation (16–18). Many of these confounding factors have been adjusted for in the predictions for allograft loss and mortality. (5, 19, 20) Still, no studies have explicitly examined the potential interaction between donor factors and eGFR for these outcomes. Therefore, the aim of this study was to determine whether donor age and type modify the associations between 12-month allograft function and risk of long-term allograft and patient outcomes in a contemporary cohort of kidney transplant recipients.

MATERIALS AND METHODS

Study Participants

All adult patients with kidney failure (aged 18 years or older) in Australia and New Zealand who had received first kidney transplants from adult living or deceased donors (aged 18 years or older) between 2000 and 2017 were included. Recipients of multiple organ allografts and those who had received prior transplants were excluded. Kidney transplant recipients with failed allografts within 12-months post-transplant and those without a recorded eGFR measurement at 12 months were excluded from the study. This study was approved by the University of Western Australia Human Research Ethics Committee (reference: 2019/RA/4/20/4584) and is reported here according to The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (21).

Demographics and Clinical Characteristics

Baseline characteristics included donor factors (age, donor type [living or deceased], sex, diabetes, hypertension and smoking history); recipient factors (age, sex, ethnicity, body mass index [BMI] at 12-months post-transplant, waiting time pre-transplant [in years], prevalent comorbidities [presence of diabetes, coronary artery disease, cerebrovascular disease or peripheral vascular disease pre-transplantation], smoking history and cause of kidney failure); and transplant-related factors (peak percentage panel reactive antibody [%PRA], number of human leukocyte antigen [HLA] A, B and DR mismatches, transplant era, place of transplantation [Australian states or New Zealand] and initial immunosuppressive agents).

Exposure and Clinical Outcomes

Post-transplant kidney function, especially allograft function at 12-months post-transplant, was chosen as the exposure of interest for three reasons: 1) It is one of the most important clinical outcomes identified by both patients, caregivers and healthcare professionals (14, 15, 22); 2) Previous epidemiological studies have found a strong association between 12-month allograft function and long-term survival (5, 8, 11, 12); 3) There is established evidence to show

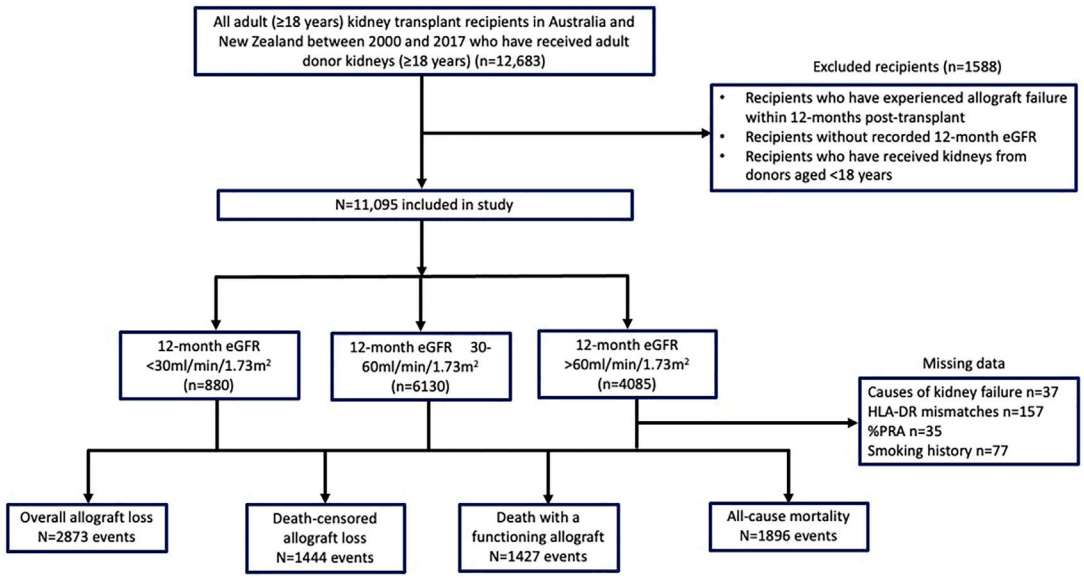


FIGURE 1 | Flow diagram of the study cohort of adult kidney transplant recipients in Australia and New Zealand between 2000 and 2017.

the effect of treatments such as belatacept, on 12-month allograft function has led to improved long-term allograft survival in kidney transplant recipients (9, 23, 24). Recipients' 12-month eGFR values were calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (25), and categorized into prior clinically defined thresholds of >60, 30 to 60 and <30 ml/min/1.73 m^2. The primary outcome of this study was overall allograft loss (includes death-censored allograft loss and death with a functioning graft). The secondary outcomes were death-censored allograft loss, death with a functioning allograft and all-cause mortality (including death after allograft loss).

Statistical Analysis

Data are presented as number (proportion), mean (standard deviation [SD]) and median (interquartile range [IQR]) where appropriate, with comparisons between groups examined by chi-square test, analysis of variance (ANOVA) or Kruskal-Wallis test, respectively. The associations between 12-month eGFR, primary and secondary outcomes were examined using adjusted Cox regression models. Grouped LASSO (least absolute shrinkage and selection operator) regularized logistic regression was used for variables selection (26). The variables of importance were HLA-DR mismatches, prior smoking history, prevalent coronary artery disease, prevalent cerebrovascular disease, prevalent diabetes, primary cause of kidney failure, dialysis duration and peak %PRA in the models that considered overall and death-censored allograft loss, with the addition of recipient age and recipient smoking history in the models for death with a functioning allograft and all-cause mortality. In all Cox regression models, donor age, donor types, transplantation states and transplant era were also included as covariates.

The 12-month eGFR and donor age was considered as the two-way interaction term, and 12-month eGFR, donor age and donor types were considered as the three-way interaction term. We first tested the interaction using continuous measures of 12-month eGFR and donor age, with significant interactions ($p < 0.01$) observed for the outcome of overall allograft loss. We next constructed models evaluating the two-way interaction between categories of 12-month eGFR (according to the prior clinically defined thresholds of >60, 30 to 60 and <30 ml/min/1.73 m^2) and donor age, with donor age thresholds informed by restricted cubic splines (5 knots; **Supplementary Figure S1**). There was a significant interaction ($p < 0.1$) between categories of 12-month eGFR and donor age for overall allograft loss, but not for death-censored allograft loss. However, a three-way interaction between 12-month eGFR, donor age and donor type were not observed for allograft and patient outcomes.

The estimates were expressed as adjusted hazard ratio (HR) and corresponding 95% confidence intervals (95% CI). The proportional hazard assumptions for all Cox regression models were examined graphically by Schoenfeld residuals with no evidence of departures from proportional hazards for allograft loss or mortality. A sensitivity analysis examining the associations between 12-month eGFR and outcomes were undertaken with the inclusion of other donor characteristics of diabetes, hypertension and smoking history in the Cox regression models. All analyses were undertaken using SAS (version 9.4; SAS Institute Inc., Cary, NC) and STATA (Version 15; StataCorp, College Station, TX), with p-values of <0.05 in two-tailed testing considered statistically significant.

RESULTS

Of the 12,683 first kidney transplants performed in 2000–2017, we excluded 1,588 recipients who lost their allografts within

TABLE 1 | Baseline characteristics of kidney transplant recipients transplanted between 2000 and 2017, stratified by 12-month estimated glomerular filtration rate categories.

	eGFR categories			*p-value*
	<30 ml/min/1.73m2 (n = 880)	**30–60 ml/min/1.73m2 (n = 6,130)**	**>60 ml/min/1.73m2 (n = 4,085)**	
Recipient characteristics				
Age (year, mean ± SD)	52.4 ± 13.1	50.1 ± 12.8	46.6 ± 13.7	<0.01
Female (n, %)	351 (39.9)	2,145 (35.0)	1,561 (38.2)	<0.001
BMI (kg/m2, mean ± SD)	28.0 ± 5.9	28.2 ± 5.2	27.2 ± 5.4	<0.001
Ethnicity (n, %)				<0.001
Caucasian	668 (75.9)	4,780 (78.0)	3,000 (73.4)	
Indigenous Australian	38 (4.3)	177 (2.9)	96 (2.4)	
New Zealand Māori	19 (2.2)	171 (2.8)	85 (2.1)	
Others/not recorded	155 (17.6)	1,002 (16.3)	904 (22.1)	
Former/current smokers (n, %)	434 (49.9)	2,749 (45.1)	1,632 (40.3)	<0.001
Coronary artery disease (n, %)	117 (13.3)	642 (10.5)	325 (8.0)	<0.001
Peripheral vascular disease (n, %)	69 (7.8)	386 (6.3)	170 (4.2)	<0.001
Cerebrovascular disease (n, %)	62 (7.1)	312 (5.1)	151 (3.7)	<0.001
Diabetes (n, %)	190 (21.6)	1,031 (16.8)	637 (15.6)	<0.001
Cause of kidney failure (n, %)				0.005
Diabetes	130 (14.8)	703 (11.5)	424 (10.4)	
Glomerulonephritis	370 (42.1)	2,726 (44.6)	1782 (43.8)	
Vascular	56 (6.4)	376 (6.1)	256 (6.3)	
Cystic	131 (14.9)	1,029 (16.8)	677 (16.7)	
Analgesic Nephropathy	7 (0.8)	39 (0.6)	14 (0.3)	
Other or Unknown	185 (21.0)	1,242 (20.4)	911 (22.5)	
Waiting time (years, mean ± SD)	3.6 ± 2.9	2.8 ± 2.7	2.5 ± 2.5	<0.001
eGFR (ml/min/1.73 m2, mean ± SD)[a]	23.0 ± 5.6	46.5 ± 8.1	74.9 ± 12.6	<0.001
12-month eGFR categories (n, %)[a]				<0.001
≥90	0 (0.0)	0 (0.0)	510 (12.5)	
>60–89	0 (0.0)	0 (0.0)	3,575 (87.5)	
45–60	0 (0.0)	3,588 (58.5)	0 (0.0)	
30–44	0 (0.0)	2,542 (41.5)	0 (0.0)	
15–29	791 (89.9)	0 (0.0)	0 (0.0)	
<15	89 (10.1)	0 (0.0)	0 (0.0)	
Donor characteristics				
Age (years, mean ± SD)	57.1 ± 12.5	51.3 ± 12.4	42.4 ± 13.3	<0.001
Female (n, %)	450 (51.8)	3,126 (53.1)	1709 (43.5)	<0.001
Living donor (n, %)	201 (22.8)	2,419 (39.5)	1787 (43.7)	<0.001
Deceased DCD donor (n, %)	123 (14.0)	669 (10.9)	369 (9.0)	0.126
Donor diabetes	66 (7.5)	272 (4.4)	101 (2.5)	<0.001
Donor hypertension	312 (35.5)	1,290 (21.0)	432 (10.6)	<0.001
Donor smoking history	228 (25.9)	502 (24.5)	1,201 (29.4)	<0.001
Transplant characteristics				
HLA-ABDR mismatches (mean ± SD)	3.7 ± 1.7	3.4 ± 1.7	3.3 ± 1.7	<0.001
Ischemic time (hours, mean ± SD)	10.9 ± 6.2	8.7 ± 6.1	8.1 ± 6.0	<0.001
Peak percentage PRA (n, %)				<0.001
0–10	663 (75.5)	5,077 (83.1)	3,378 (83.0)	
11–50	140 (15.9)	667 (10.9)	463 (11.4)	
51–80	41 (4.7)	190 (3.1)	128 (3.1)	
>80	34 (3.9)	176 (2.9)	103 (2.5)	
Transplant year (n, %)				<0.001
2000–2004	221 (25.1)	1,410 (23.0)	729 (17.8)	
2005–2008	154 (17.5)	1,236 (20.2)	788 (19.3)	
2009–2012	210 (23.9)	1,441 (23.5)	1,084 (26.5)	
2013–2017	295 (33.5)	2043 (33.3)	1,484 (36.4)	
Prednisolone at 12 m (n, %)	869 (98.8)	6,055 (98.8)	4,006 (98.1)	0.012
Calcineurin-inhibitor at 12 m (n, %)				0.005
None	12 (1.4)	80 (1.3)	68 (1.7)	
Cyclosporin	161 (18.3)	1,375 (22.4)	35 (0.9)	
Tacrolimus	707 (80.3)	4,675 (76.3)	3,982 (97.4)	
Anti-metabolite at 12 m (n, %)				0.991
Non	15 (1.7)	100 (1.6)	110 (6.5)	
Azathioprine	7 (0.8)	47 (0.8)	117 (7.0)	
Mycophenolic acid	858 (97.5)	5,983 (97.6)	1,456 (86.5)	

[a]*One-year post-transplantation.*

LD, live donor; DD, deceased donor; ESKD, end-stage kidney disease, BMI, body mass index; eGFR, estimated glomerular filtration rate by Chronic Kidney Disease Epidemiology Collaboration equation; DCD, donation after circulatory death; HLA, human leukocyte antigen; PRA, panel reactive antibody; mTOR, mammalian target of rapamycin.

12 months post-transplant or had no recorded 12-month eGFR, leaving a study cohort of 11,095 recipients (**Figure 1**). The mean (SD) age of the study cohort was 49 (13) years, and 37% were females. Eight hundred and eighty recipients (7.9%) had 12-month eGFR <30 ml/min/1.73 m^2 and 4,085 (36.8%) had eGFR <30 and >60 ml/min/1.73 m^2.

Table 1 shows the baseline characteristics of the study cohort, stratified by 12-month eGFR thresholds. Recipients with 12-month eGFR of >60 ml/min/1.73 m^2 were younger, less likely to have prevalent vascular disease or diabetes, and had shorter mean waiting time than recipients with 12-month eGFR ≤60 ml/min/1.73 m^2. Recipients with 12-month eGFR values of >60 ml/min/1.73 m^2 were more likely to have received living donor kidneys and of younger donor age compared to those with 12-month eGFR of ≤60 ml/min/1.73 m^2. The proportion of kidney transplant recipients with 12-month eGFR >60 ml/min/1.73 m^2 increased from 30.9% between 2000 and 2004 to 38.8% between 2013 and 2017. Conversely, the proportion of recipients with 12-month eGFR <30 ml/min/1.73 m^2 reduced from 9.4% between 2000 and 2004 to 7.7% between 2013 and 2017.

Donor Age Categories and 12-Month eGFR

A higher proportion of recipients who received kidneys from younger donors aged 18–30 years had 12-month eGFR >60 ml/min/1.73 m^2 compared to recipients of donor kidneys aged >30–60 and >60 years. Conversely, approximately 17% of recipients with older donor kidneys (aged >60 years) had 12-month eGFR of <30 ml/min/1.73 m^2 compared to 3% of recipients with younger donor kidneys (aged 18–30 years) (**Figure 2** and **Supplementary Table S1**).

Association Between 12-Month eGFR and Overall Allograft Loss

The estimates of the main model for overall allograft loss are shown in **Table 2**. Compared to recipients with 12-month eGFR of 30–60 ml/min/1.73 m^2, recipients with the lowest 12-month eGFR (<30 ml/min/1.73 m^2) experienced the greatest risk of overall allograft loss (adjusted HR [95% CI]: 2.65 [2.38, 2.95]); where those with the highest eGFR at 12-months experienced a lower risk of overall allograft loss (adjusted HR 0.67 [0.62–0.74]). Compared to recipients of older donor kidneys, recipients with younger donor kidneys experienced a reduced risk of overall allograft loss.

Interaction Between Donor Age, 12-Month eGFR and Overall Allograft Loss

Figure 3 shows the adjusted HRs and 95% CI for eGFR categories and overall allograft loss stratified by donor age subgroups of 18–30, >30–60 and >60 years. In recipients of kidneys from younger donors (aged 18–30 years), the adjusted HRs for overall allograft loss were highest in those with the lowest 12-month eGFR values (<30 ml/min/1.73 m^2: HR 5.74 [95% CI 3.99, 8.25]; 30–60 ml/min/1.73 m^2: HR 1.37 [95% CI 1.13, 1.66]; >60 ml/min/1.73 m^2: referent). In recipients of kidneys from older donors aged >60 years, the HRs for overall allograft loss were attenuated at lower 12-month eGFR values (<30 ml/min/1.73 m^2: HR 3.44 [95% CI 2.56, 4.64]; 30–60 ml/min/1.73 m^2: HR 1.45 [95% CI 1.09, 1.92]; >60 ml/min/1.73 m^2: referent) (**Table 2** and **Figure 3**).

Figures 4A–C show the adjusted HR for overall allograft loss across the continuum of 12-month eGFR, stratified by donor age groups. The inflection points of the survival curves corresponding to an increased risk of overall allograft loss occurred at lower eGFR values for recipients of older donor kidneys than younger donor kidneys.

Association Between 12-Month eGFR and Death Censored Allograft Loss, Death With a Functioning Allograft and All-Cause Mortality

The estimates of the main models (without interaction) for death censored allograft loss, death with a functioning allograft and all-cause mortality are shown in **Table 2**. Compared to 12-month eGFR of 30–60 ml/min/1.73 m^2, the adjusted HR for 12-month eGFR of <30 ml/min/1.73 m^2 was 3.94 (3.44, 4.53) for death-censored allograft loss, 1.30 (1.09, 1.54) for death with a functioning allograft and 1.78 (1.56, 2.04) for all-cause mortality. The respective HRs for 12-month eGFR of >60 ml/min/1.73 m^2 were 0.56 (0.49, 0.64), 0.82 (0.73, 0.93) and 0.77 (0.69, 0.86). These relationships were not modified by donor age.

Sensitivity Analysis

A greater proportion of recipients with 12-month eGFR of <30 ml/min/1.73 m^2 received kidneys from donors with a history of diabetes or hypertension compared to recipients with higher 12-month eGFR (**Table 1**). In the sensitivity analysis which included these additional donor characteristics (donor diabetes, donor hypertension and donor smoking history), the two-way interaction between 12-month eGFR and donor age remained statistically significant for overall allograft loss. **Figure 3** shows the adjusted HRs and 95%CI for eGFR categories and overall allograft loss according to the donor age subgroups of 18–30, >30–60 and >60 years.

DISCUSSION

In this contemporary cohort of kidney transplant recipients, recipients with 12-month eGFR less than 30 ml/min/1.73 m^2 experienced at least a 2.5 fold increased risk of overall allograft loss compared to those with higher eGFR at 12 month (>60 ml/min/1.73 m^2). This association was modified by donor age but not donor types. Recipients of younger donor kidneys with a lower 12-month eGFR value of less than 30 ml/min/1.73 m^2 experienced up to 6-times greater risk of overall allograft loss compared to those with higher 12-month eGFR values. This association was attenuated in recipients with older donor kidneys.

Observational data shown a direct association between donor age and kidney function at 12-months and long-term allograft

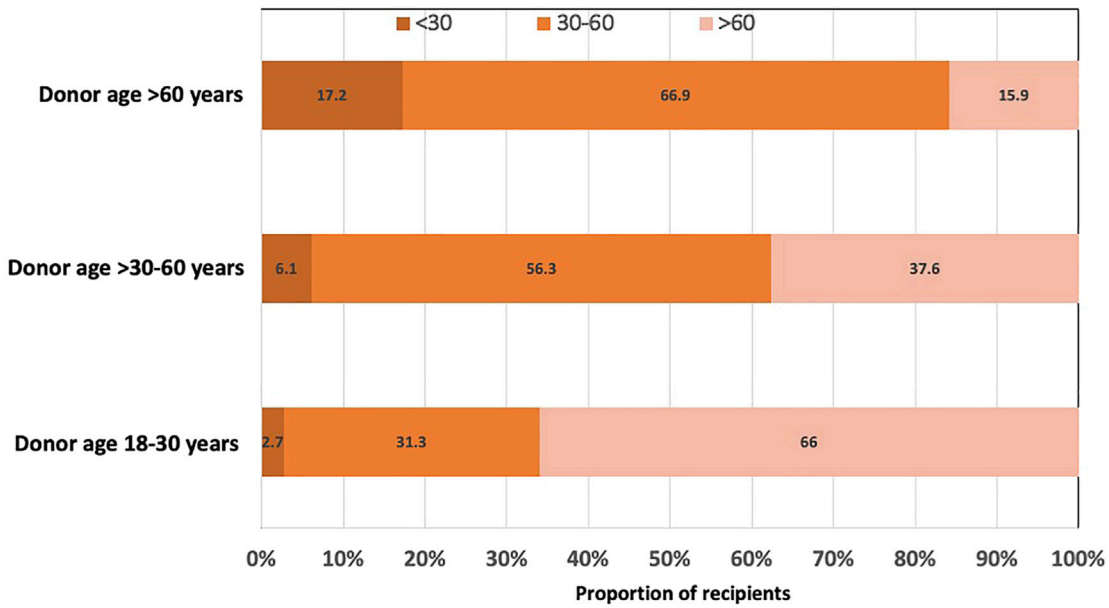


FIGURE 2 | Bar graph showing the proportion of kidney transplant recipients with 12-month estimated glomerular filtration rate (eGFR) of <30, 30–60 and >60 ml/min/1.73 m^2, stratified by donor age groups (18–30, >30–60 and >60 years).

and patient survivals (5, 8, 20, 27–29). but our observed interactive effects between donor age and eGFR at 12 months on allograft loss is novel. Our study findings suggest that the effects of reduced short-term allograft function at 12-month on longer term allograft outcome differs in recipients of younger and older donor kidneys, with the magnitude of the risk for overall allograft loss being higher for recipients of younger donor kidneys with lower 12-month eGFR values than those who received older donor kidneys. The inflection point for the increased risk of allograft loss occurred at a lower eGFR for older donor kidneys than younger donor kidneys. Our current findings may imply that clinical events or disease phenotypes that may have led to a reduced eGFR at 12-months for recipients with younger donor kidneys are different from recipients of older donor kidneys who had reduced eGFR at 12-months. However, these findings also suggest that donor age alone is unlikely the only contributing factor in modifying the association between 12-month eGFR and allograft outcomes. Other mechanisms or influences such as the different etiology of the allograft dysfunction (such as disease recurrence, vascular complications, cellular or antibody-mediated rejection, BK viral nephropathy), the differing susceptibility of the donor kidneys (of varying ages) to clinical insults and the presence of competing events such as death with a functioning allograft may have affected the trajectory for allograft loss for each eGFR threshold according to incremental donor age subgroups.

In a systematic review of 169 randomized controlled trials in kidney transplantation, eGFR was a primary or secondary endpoint in 60% of the trials (7). Clinical trials powered to hard clinical endpoints such as allograft survival are often not feasible in kidney transplantation. Therefore, eGFR is likely to continue to be used as a surrogate measure of allograft survival. In the two largest clinical trials ever conducted in kidney transplantation, the primary endpoint was 12-month eGFR (Efficacy Limiting Toxicity Elimination [ELITE]–Symphony study [n = 1,645]; mean [SD] donor age 45–46 [15–16] years; published 2007) or a composite of acute rejection or eGFR of <50 ml/min/1.73 m^2 at 12-months (TRANSplant eFficacy and Safety Outcomes With an eveRolimus-based regiMen [TRANSFORM] study [n = 2037]; mean [SD] donor age of 48 [15] years, published 2018), indicating that eGFR will likely remain one of the best and practical index measures for longer-term kidney allograft outcome (11, 12). Consequently, a greater understanding of the limitations of the prognostic significance of a single timepoint eGFR is critical when considering clinical trial design and when interpreting the results of clinical trials in kidney transplantation.

Estimated GFR, however, does not necessarily provide accurate quantification of the amount and etiology of the "pathological" acute and chronic changes in the allograft biopsy, which can be influenced by multiple patient- and transplant-related factors, such as the primary cause of kidney failure, body size, age and post-transplant clinical events (e.g. disease recurrence, antibody mediated rejection) and therefore, kidney allograft biopsies are often required to guide clinical management (30). Our study suggests that donor age should be considered when interpreting the clinical applicability and prognostic significance of a single time point eGFR value such that the proportion of recipients attaining different 12-month eGFR thresholds and the association between eGFR and risk of overall allograft loss may be conditional on the effects of donor age. This finding also suggests the need for careful consideration when utilizing a single time point eGFR value as a surrogate measure for overall allograft loss in kidney transplant trials.

TABLE 2 | Association between 12-month eGFR, long-term allograft and patient outcomes (main effects models).

	Overall allograft loss (adjusted HR [95% CI])	Death censored allograft loss (adjusted HR [95% CI])	Death with a functioning allograft (adjusted HR [95% CI])	All-cause mortality (adjusted HR [95% CI])
12-month eGFR (mL/min/1.73m2)				
<30	2.65 (2.38, 2.95)	3.94 (3.44, 4.53)	1.30 (1.09, 1.54)	1.78 (1.56, 2.04)
30–60	1.00	1.00	1.00	1.00
>60	0.67 (0.62, 0.74)	0.56 (0.49, 0.64)	0.82 (0.73, 0.93)	0.77 (0.69, 0.86)
Donor factors				
Live donor (ref: deceased donor)	0.92 (0.84, 1.01)	0.81 (0.72, 0.91)	0.91 (0.79, 1.05)	0.90 (0.80, 1.01)
Donor age (years)				
18–30	0.79 (0.69, 0.90)	0.69 (0.57, 0.85)	0.92 (0.77, 1.11)	0.87 (0.74, 1.02)
>30–60	0.88 (0.80, 0.97)	0.90 (0.79, 1.04)	0.90 (0.79, 1.03)	0.90 (0.80, 1.02)
>60	1.00	1.00	1.00	1.00
Recipient factors				
Recipient age (in years)	—	0.96 (0.95, 0.96)	1.07 (1.06, 1.11)	1.06 (1.05, 1.06)
Prior smoking history (ref: non-smoker)	1.30 (1.20, 1.40)	—	1.35 (1.22, 1.50)	1.37 (1.25, 1.50)
Prior coronary artery disease	1.30 (1.18, 1.44)	1.74 (1.51, 2.01)	1.03 (0.90, 1.18)	1.21 (1.08, 1.36)
Prior cerebrovascular disease	1.42 (1.25, 1.60)	1.78 (1.47, 2.15)	1.10 (0.94, 1.31)	1.20 (1.04, 1.38)
Diabetes	1.53 (1.30, 1.79)	—	1.46 (1.18, 1.80)	1.64 (1.38, 1.96)
Cause of kidney failure		—		
Glomerulonephritis	0.70 (0.60, 0.82)		0.67 (0.55, 0.82)	0.67 (0.56, 0.80)
Diabetes	0.89 (0.72, 1.12)		1.14 (0.85, 1.52)	1.07 (0.84, 1.38)
Hypertension/renovascular disease	1.00		1.00	1.00
Cystic	0.62 (0.52, 0.75)		0.79 (0.64, 0.99)	0.72 (0.59, 0.89)
Analgesic nephropathy	1.68 (1.18, 2.39)		1.53 (1.04, 2.24)	1.48 (1.03, 2.12)
Others	0.77 (0.66, 0.91)		0.85 (0.68, 1.07)	0.89 (0.73, 1.09)
Dialysis duration (in years)	1.06 (1.05, 1.08)	—	1.09 (1.07, 1.11)	1.09 (1.07, 1.11)
Transplant factors				
HLA-DR mismatches				
0	1.00	1.00	1.00	1.00
1	1.26 (1.14, 1.38)	1.59 (1.39, 1.82)	0.95 (0.84, 1.09)	1.04 (0.92, 1.17)
2	1.27 (1.14, 1.41)	1.65 (1.43, 1.92)	1.03 (0.89, 1.19)	1.15 (1.02, 1.30)
Peak PRA (%)	—		-	-
0–10		1.00		
11–50		1.24 (1.06, 1.44)		
51–80		1.31 (1.02, 1.68)		
>80		1.61 (1.26, 2.06)		
Transplant era				
2000–2004	1.00	1.00	1.00	1.00
2005–2008	1.07 (0.97, 1.18)	1.21 (1.06, 1.38)	0.90 (0.78, 1.04)	0.92 (0.82, 1.04)
2009–2012	1.06 (0.94, 1.18)	1.24 (1.06, 1.46)	0.85 (0.72, 0.99)	0.90 (0.78, 1.04)
2013–2017	1.02 (0.87, 1.19)	1.45 (1.16, 1.81)	0.68 (0.54, 0.85)	0.82 (0.67, 1.01)

Data presented as adjusted hazard ratio (HR) and 95% confidence intervals (95% CI) in the multi-variable adjusted Cox regression models, with the estimates of the covariates selected by group least absolute shrinkage and selection operator (LASSO) shown. eGFR, estimated glomerular filtration rate; PRA, panel reactive antibody; HLA-human leukocyte antigen.

There are several strengths and limitations in this study. The prospective nature of a contemporary cohort of kidney transplant recipients and the near completeness of the available data suggest that ascertainment biases of the exposure and outcome measures were minimized and that the study findings reflect current clinical practice. Indication bias remained a possibility because there may have been systematic differences in how clinicians manage kidney transplant recipients with differing eGFR values at 12-months post-transplant. However, the direction of this bias is likely towards the null hypothesis because people with lower eGFR may receive closer monitoring or changes to the management approach due to the lower eGFR. Even though there were multiple confounding factors adjusted for in the analyses, there are likely to be several unmeasured and residual confounders. These include the overall exposure and utilization of immunosuppression (according to clinical risk), the impact of various adverse clinical events/hospitalizations occurring during the time course of the follow-up period, lack of availability of biopsy data and changing nature of immunological risk (such as evidence of transplant glomerulopathy, presence of interstitial fibrosis/tubular atrophy, development of *de novo* donor-specific anti-HLA antibody), presence of and severity of proteinuria and the development (and severity) of *de novo* comorbid conditions such as post-transplant diabetes and hypertension that may have influenced allograft function and allograft survival post-transplant; which were not adequately collected by the ANZDATA registry but may potentially have modified our study findings. It was determined *a priori* that change in eGFR would not be considered in this study given that the majority of landmark clinical studies had utilized a single time point eGFR measurement as the primary or secondary endpoint. However, our other work has shown that change in eGFR

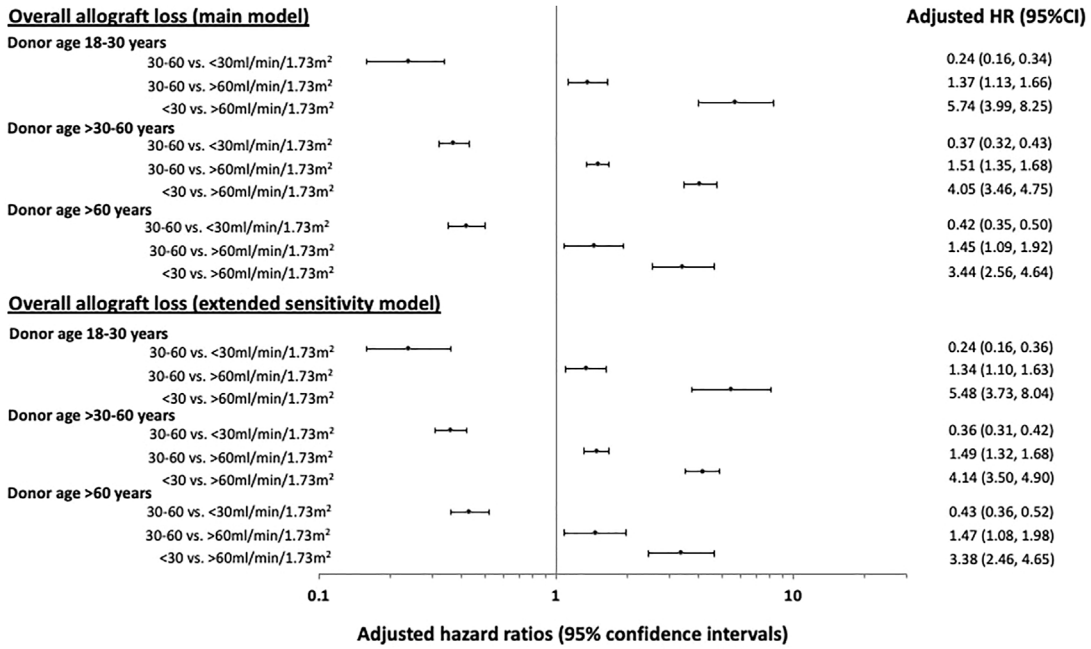


FIGURE 3 | Forest plots showing the adjusted hazard ratio (HR) with 95% confidence intervals (95% CI) of the associations between 12-month estimated glomerular filtration rate (eGFR) of <30, 30–60 and >60 ml/min/1.73 m^2 and overall allograft loss post-kidney transplantation, stratified by donor age groups of 18–30, >30–60 and >60 years. The estimates of the main and extended sensitivity models for overall allograft loss are shown.

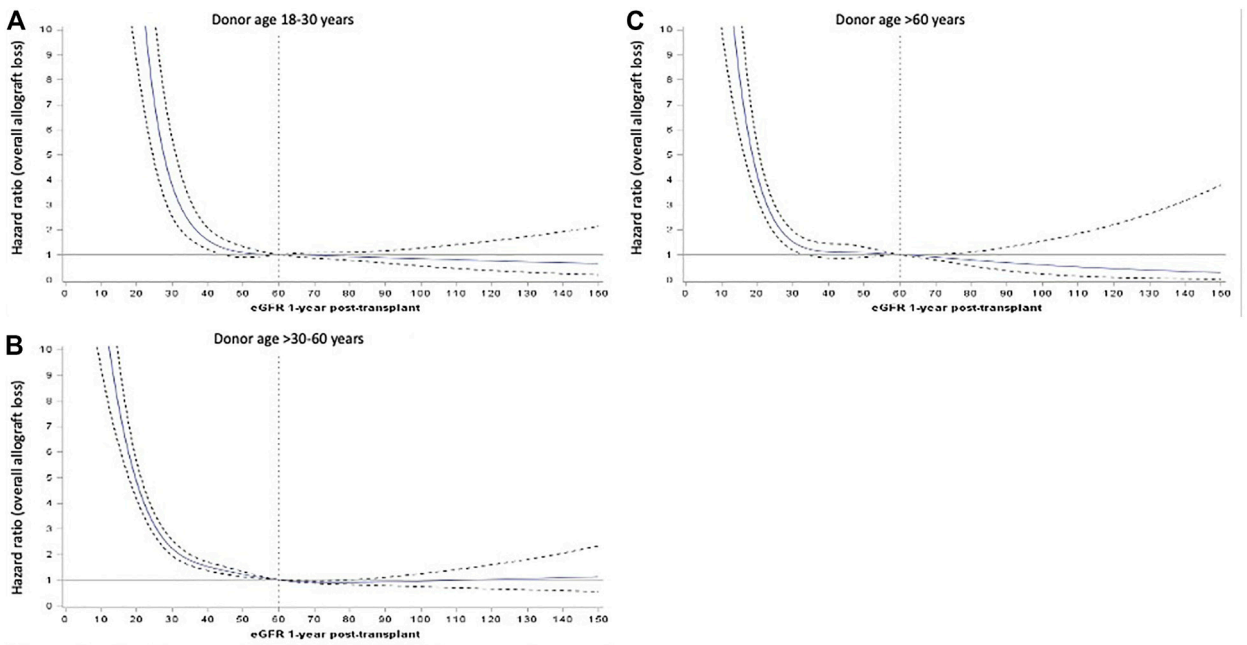


FIGURE 4 | Adjusted hazard ratios (HR, represented by solid blue line) and 95% confidence intervals (95%CI, represented by dotted black lines) of the relationship between 12-month estimated glomerular filtration rate (eGFR, as continuous exposure) and hazards of overall allograft loss for donor age subgroups of 18–30 **(A)**, >30–60 **(B)** and >60 years **(C)**.

is a valuable predictor of long-term outcomes (19). Misclassification bias of actual allograft function may have occurred, however, measured GFR was impractical and costly in the real-world setting. In addition, given the small number of kidney transplant recipients of younger deceased donor kidneys that achieved 12-month eGFR values of <30 ml/min/1.73 m^2, there is likely considerable uncertainty in the estimates to provide an accurate assessment of the true difference between eGFR and allograft outcomes for this group.

In conclusions, our study shows that the association between 12-month eGFR and allograft outcome is modified by donor age. Even though the relationship between eGFR and allograft outcome is similar among different donor age subgroups, an identical single timepoint eGFR as a prognostic indicator of allograft survival and the attainment of a range of eGFR thresholds varies according to these subgroups.

CAPSULE SENTENCE SUMMARY

Reduced estimated glomerular filtration rate (eGFR) 12-month post-transplant is associated with adverse long-term allograft outcomes but whether donor factors such as age, modify this association in unknown. Using data from the Australia and New Zealand Dialysis and Transplant (ANZDATA) registry, we have shown that the relationship between 12-month eGFR and allograft loss was modified by donor age. Even though the trend and nature of the relationships between 12-month eGFR and allograft loss were similar, the magnitudes of the risk were dissimilar among donor age subgroups.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: Use of deidentified data can be requested from ANZDATA registry. Requests to access these datasets should be directed to requests@anzdata.org.au.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University of Western Australia Human Research Ethics Committee. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

WL, EO, and GW conceived the proposal WL, EO, and GW analysed the data. All authors contributed to the writing of 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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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10199/full#supplementary-material

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Relinquishing Anonymity in Living Donor Kidney Transplantation: Lessons Learned From the UK Policy for Anonymous Donors

Mathilde C. Pronk[1]*, *Lisa Burnapp*[2], *Marlies E. J. Reinders*[1] and *Emma K. Massey*[1]

[1]Erasmus MC Transplant Institute, University Medical Center Rotterdam, Rotterdam, Netherlands, [2]Department of Transplantation and Nephrology, Guy's & St Thomas' NHS Foundation Trust, London, United Kingdom

Anonymous living donor kidney transplantation (LDKT) is performed in many countries and policies on anonymity differ. The UK is the only European country with a conditional policy, allowing pairs to break anonymity post-transplant. There is little evidence on how contact after anonymous LDKT is experienced. In this cross-sectional study participants who donated or received a kidney through non-directed altruistic kidney donation or within the UK living kidney sharing scheme completed a questionnaire on their experiences with and attitudes towards anonymity. Non-parametric statistics were used to analyse the data. 207 recipients and 354 donors participated. Anonymity was relinquished among 11% of recipients and 8% of donors. Non-anonymous participants were generally content with non-anonymity. They reported positive experiences with contact/meeting the other party. Participants who remained anonymous were content with anonymity, however, 38% would have liked to meet post-transplant. If the other party would like to meet, this number increased to 64%. Although participants agreed with anonymity before surgery, they believe that, if desired, a meeting should be allowed after surgery. UK donors and recipients were satisfied with conditional anonymity and experiences with breaking anonymity were positive. These results support the expansion of conditional anonymity to other countries that allow anonymous LDKT.

Keywords: kidney transplantation, living donation, Medical Ethics, kidney exchange, anonymity, non-directed altruistic donation

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Correspondence:
Mathilde C. Pronk
m.c.pronk@erasmusmc.nl

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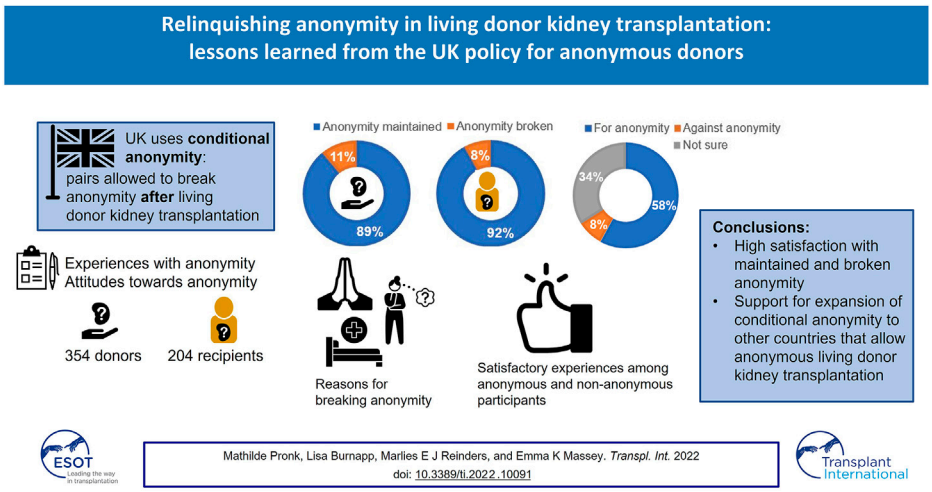
INTRODUCTION

Living donor kidney transplantation (LDKT) is the treatment of choice for patients with end-stage kidney disease. Changes to national legal frameworks and policies have enabled the growth of living donor programmes through both innovative approaches in clinical practice [e.g., kidney exchange programmes (KEPs) and antibody incompatible transplantation] and expansion of the donor pool—from genetically related donors, to inclusion of emotionally related donors (spouses,

Abbreviations: ELPAT, Ethical, Legal and Psychosocial Aspects of Organ Transplantation; KEP, kidney exchange programme; LDKT, living donor kidney transplantation; NDAD, non-directed altruistic donation or donor; UKD, unspecified kidney donation or donor; UKLKSS, UK living kidney sharing scheme.



GRAPHICAL ABSTRACT |

friends), and even strangers. Donation of a kidney from a living person to a stranger (without knowing the identity or any characteristics of the recipient before transplantation) is known as non-directed altruistic donation (NDAD), but it is also described as unspecified kidney donation (UKD), anonymous or "Good Samaritan" donation (1). Non-directed altruistic donors (NDADs) often donate into KEPs to initiate chains of transplants that complete with a recipient on the national transplant list. This is allowed in many countries, such as Australia, Canada, Netherlands, United Kingdom and United States (2). Within Europe, KEPs are especially well established in the Netherlands and in the United Kingdom (3). In these countries, transplants from NDADs make an invaluable contribution to the living donor pool, currently accounting for around 8% (36 NDADs) in the Netherlands (4) to 10% (100 NDADs) in the UK (5) allowing a greater number of transplants to be carried out in these schemes. A detailed description of KEPs in Europe has been provided by Biro et al. (6).

Anonymity of donors and recipients in KEPs is complex and approaches vary between countries based on national policies (6). Anonymity can be absolute (i.e., applicable before and after surgery without permissible exceptions) or conditional (i.e., allowing removal of anonymity under certain circumstances). The advantages and disadvantages of both approaches are well described in an opinion paper by Mamode et al. (7) in which it was concluded that there is compelling evidence for maintaining anonymity of both parties before and after transplantation. However, requiring absolute anonymity when donors and recipients wish to break it, has been perceived as paternalistic by both transplant professionals (7) and donors and recipients who had participated in KEPs in the Netherlands and Sweden (8, 9). In these studies, the experience of anonymity in the Netherlands and Sweden was investigated both retrospectively and prospectively. In general, donors and recipients were satisfied with absolute anonymity, and only a minority of participants would have liked to meet the other party. However, both studies revealed that more than half of all donors and recipients would be open for a meeting if the other party desired that. Moreover, regardless of personal experience or desire for contact, the dominant opinion was that the decision to have contact or meet should be left up to the individuals themselves (8, 9).

These studies were conducted in the Netherlands and Sweden where anonymity is absolute. This differs from the policy in the UK where anonymity is conditional, i.e., anonymity can be revoked by mutual consent of both donor and recipient after surgery. We do not know to what extent donors and recipients in the UK use this option nor their experiences of revoking anonymity. This data is critical in evaluating the effects of maintaining or revoking anonymity after transplantation. The principal aim of this study was to assess the proportion of UK donors and recipients that maintained/broke anonymity after donation or transplantation and to understand their experiences. Secondly, we aimed to assess the attitudes of the UK donors and recipients towards the principle of anonymity and whether these attitudes differed between donors and recipients and between the donors and recipients for whom anonymity was maintained or broken.

MATERIALS AND METHODS

Participants and Procedure

Donors and recipients (≥18 years old) who anonymously donated or received a living donor kidney in the period 2010–2014, with a minimum of 1 year after surgery, were considered for inclusion. These included donor-recipient pairs who participated in paired-pooled donation (part of the UK living kidney sharing scheme, UKLKSS), as well as NDADs and recipients on the UK transplant list. Data collection took place in November and December 2016. 618 Donors and 584 recipients were identified from the electronic UK Transplant Database and invited to participate by a letter from NHS Blood and Transplant. The letter included information on the study and the questionnaire on anonymity. One reminder was sent to non-responders. The participants completed the questionnaire on paper. Informed consent was assumed by

completion and return of the questionnaire. The study protocol received UK Research Ethics Committee approval (NHSBT ID: 16NS0002).

Measures

Socio-Demographic and Medical Characteristics

Self-reported socio-demographic and medical characteristics of participants can be found in **Table 1**.

Experiences With (Revoked) Anonymity and Attitude Towards Anonymity

We used a questionnaire on anonymity, originally developed by a European platform on Ethical, Legal and Psychosocial Aspects of organ Transplantation (ELPAT) and refined by a Dutch research team of transplantation specialists (8). This was adapted for the UK cohort, including adding some country specific questions. In the questionnaire, anonymity was defined as "not knowing from whom the kidney is received or to whom the kidney is donated, except for general characteristics, such as gender or age." The questionnaire consisted of closed and open-ended items that measured experiences with and attitudes towards (revoked) anonymity. The items that were used to measure experiences with anonymity are displayed in **Table 2**. General attitude towards anonymity between donors and unknown recipients was measured (for, against, not sure). A further 12 statements were assessed using 1–7 point Likert scales, as shown in **Table 3**.

Statistical Analyses

Descriptive statistics were used to describe the participants' sociodemographic and medical characteristics, their experiences with anonymity and attitudes towards anonymity. Due to the non-normal distribution of the data, median and ranges were calculated and non-parametric tests conducted. When no significant group differences were found, descriptive statistics for the whole sample are given, referred to as participants. For all analyses we used SPSS 25.0 (IBM) and a *p*-value less than 0.01 was considered statistically significant due to multiple testing.

RESULTS

In total, 354 donors and 204 recipients completed and returned the questionnaire (response rate 57 and 35% respectively). Socio-demographic and medical characteristics are presented in **Table 1**. Half of recipients had received their kidney through the UKLKSS while the other half had received a kidney from a NDAD *via* the UK transplant list. Amongst donors who completed the questionnaire, 63% were NDADs and 37% were paired-pooled donors. Donors were significantly older than recipients and significantly more likely to be male.

Perceived Stress

Participants generally did not find the transplantation or donation stressful (Median = 2, IQR = 1–4). Recipients perceived the transplantation significantly more stressful (Median = 4, IQR = 2–5) than donors perception of donation (Median = 2, IQR = 1–3), $U = 20{,}305$, $p = 0.000$. No significant associations were found between perceived stress experiences with anonymity and attitude towards anonymity.

Knowledge of the Official Policy on Anonymity

Forty-four percent of all participants falsely believed that anonymity was required before and after transplantation. The same proportion of participants (43%) correctly believed that anonymity was required before surgery, but that after surgery donor and recipient can meet if both parties agree. The remaining 13% did not know about an official policy on anonymity. Those who knew about the possibility to rescind anonymity were more likely to do so than those who did not know, $\chi^2 = 17{,}231$, $p = 0.000$.

Experiences With Anonymity

The large majority of participants (among recipients 89%, among donors 92%) reported that anonymity was maintained. Looking back at the donation/transplantation, these donors (median = 7, IQR = 7–7) and recipients (median = 7, IQR = 6–7) were content with anonymity before surgery. The value of the mean ranks indicated that donors (Mean Rank = 256.4) were significantly more content with anonymity before surgery than recipients (Mean Rank = 227.2), $U = 24{,}351$, $p = 0.002$. These donors (median = 7, IQR = 5–7) and recipients (median = 7, IQR = 4–7) were also satisfied with anonymity after surgery. Time since surgery was not related to satisfaction with anonymity before or after surgery.

Of all participants who remained anonymous, only 6% would have liked to meet the other party before surgery and 37% would have liked to meet after surgery. This did not significantly differ between donors and recipients. However, if the other party would like to meet, openness to meeting them rises to 63%. This did not significantly differ between donors and recipients. No relationship was found between preferences for contact and time since surgery.

Half of all recipients who remained anonymous sent an anonymous card to their donor and 24% received an anonymous card from their donor. Amongst donors, 21% sent an anonymous card to their recipient and 34% received an anonymous message from the recipient. Recipients more often sent an anonymous card than donors did, $\chi^2 = 44{,}453$, $p = 0.000$.

Experiences With Broken Anonymity Among Recipients

Amongst recipients, 22 (11%) reported that anonymity was broken, of which 9 only had contact with the donor in writing or on the phone, a median of 7 months after surgery (range 1–48). Twelve recipients actually met their donor, a median of 10 months after surgery (range 3–33). One recipient reported that he accidentally found out about his donor before surgery. It remains unclear how this has happened. These 22 recipients were generally very content with anonymity before surgery (Median = 7, IQR = 6–7) and with the broken anonymity after surgery (Median = 7, IQR = 7–7). All 22 recipients reported positive experiences with the contact/meeting they had with their donor (Median = 7, IQR = 7–7) and did not

TABLE 1 | Socio-demographic and medical characteristics of participants.

	Recipients (n = 204)		Donors (n = 354)		*p* Value
	n	%	n	%	
Age at operation	186		299		0.003
Median (range)	54 (18–76)		58 (21–85)		
Gender	196		349		0.03
Male	78	40	172	50	
Female	118	60	177	50	
Highest education achieved	194		346		n.s.
Secondary school	58	30	97	28	
Further education	136	70	249	72	
Transplant program	196		346		0.008
UK Transplant list/NDAD[1]	98	50	217	63	
Paired pooled recipient/donor	98	50	129	37	
Median months since surgery (range)	188		302		n.s.
	42 (16–93)		40 (23–82)		
Preemptive transplantation	197				
Yes	36	18			
Median months on dialysis before transplantation (range)	156				
	29 (1–240)				
Number of transplants	197				
1	143	73			
2	41	21			
3	13	6			

[1]*NDAD, non-directed altruistic donor.*

TABLE 2 | List of questions measuring experiences with anonymity.

How stressful did you find the donation/transplantation?	1 = not stressful at all; 7 = very stressful
How content are you with your decision to donate your kidney?	1 = completely discontent; 7 = completely content
What do you know about the official policy on anonymity in the UK?	
Anonymity was required both before and after donation	
Anonymity was required before donation, but after donation donor and recipient can meet if both parties agree	
No official policy on anonymity	
Don't know	
When anonymity was maintained:	
How content were you with being anonymous to your donor/recipient before donation?	1 = completely discontent; 7 = completely content
How content were you with being anonymous to your donor/recipient after donation?	1 = completely discontent; 7 = completely content
Would you have liked to have had contact with or meet the donor/recipient of your kidney before donation?	Yes/No/Not sure
Would you have liked to have had contact with or meet the donor/recipient of your kidney after donation?	Yes/No/Not sure
If the donor/recipient would like to make contact with you or meet you, would you be open to such contact/meeting?	Yes/No/Not sure
Did you send an anonymous card, letter or similar item to the donor/recipient?	Yes/No
Did you receive an anonymous card, letter or similar item from the donor/recipient?	Yes/No
When anonymity was broken:	
How was anonymity broken? (multiple answers are possible)	
I had contact with the donor/recipient …..…..…..…..…. month(s) after donation (e.g., by social media, writing e-mails or speaking on the phone)	
I met the donor/recipient in person …..…..…..…..…..… month(s) after donation	
We accidentally found out about each other (e.g. through (social) media)	
We accidentally met each other	
Who initiated this contact or meeting?	
I initiated contact with the donor/recipient	
A member of my family/friend initiated contact with the donor/recipient	
The donor/recipient initiated contact with me	
A member of the donor's/recipient's family/friend initiated contact with me	
Not applicable: we found out about each other or met accidentally	
How content were you with anonymity before donation?	1 = completely discontent; 7 = completely content
How content are you with the fact that your donor/recipient is NOT anonymous to you?	1 = completely discontent; 7 = completely content
How did you experience the contact or meeting with the donor/recipient?	1 = very negatively; 7 = very positively
Do you regret having contact with or meeting the donor/recipient?	1 = not at all; 7 = a great deal

TABLE 3 | Attitude statements for recipients and donors.

Statements[1]	Recipients *n* = 204			Donors *n* = 354			*p*-Value
	Mdn	IQR	*n*	Mdn	IQR	*n*	
There must be anonymity between donor and recipient BEFORE surgery	7	4–7	199	7	5–7	352	n.s.
There must be anonymity between donor and recipient AFTER surgery	4	2–6	197	4	2–6	352	n.s.
If both parties agree, the donor and recipient should be allowed to meet BEFORE surgery	4	2–7	201	3	1–6	351	n.s.
If both parties agree, the donor and recipient should be allowed to meet AFTER surgery	7	4–7	198	7	5–7	349	n.s.
The donor has the right to remain anonymous	7	7–7	202	7	7–7	350	n.s.
The recipient has the right to remain anonymous	7	7–7	202	7	7–7	349	n.s.
The donor has the right to know to whom he/she is donating a kidney	2	1–5	201	1	1–3	349	0.000
The recipient has the right to know from whom he/she is receiving a kidney	1	1–4	201	1	1–4	348	n.s.
Anonymity makes a donation altruistic	6	4–7	189	6	3–7	337	n.s.
The donation should only proceed if the donor agrees to anonymity	4	1–6	200	4	1–7	340	n.s.
If the donation procedure was not anonymous, more people would donate their kidney altruistically to a stranger	3	1–4	198	3	2–4	340	n.s.
In practice, anonymity is difficult to maintain	2	1–4	200	1	1–3	347	n.s.

[1]*All statements are scored on a 7-point Likert scale (1 = completely disagree—7 completely agree).*

regret the contact/meeting (Median = 1, IQR = 1–1). Two recipients who came to know their donor after surgery, were discontent with anonymity before surgery. One of these recipients reported that she found the transplantation extremely stressful.

Major drivers of contact or meetings were the request of the recipient (n = 9) and his or her need to express their gratitude. Some recipients mention that the donor initiated contact (n = 3) or that both they and the donor initiated contact (n = 4). Three recipients accidentally found out who their donor was, due to inadvertent administrative errors or through talking with other patients on the ward. Quotations to illustrate recipients' experiences with the contact or meeting they had with their donor can be found in **Table 4**.

Experiences With Broken Anonymity Among Donors

Amongst donors, 29 (8%) reported that anonymity was broken after surgery, of which 17 only had contact with the recipient in writing or on the phone, a median of 9 months after surgery (range 1–30). Eleven donors actually met their recipient, a median of 12 months after surgery (range 5–36). One donor found out about his recipient through the newspaper (unclear if they had contact at all). In general, these donors were content with anonymity before surgery (Median = 7, IQR = 3–7). Most donors were very content about knowing their recipient afterwards (Median = 7, IQR = 7–7). Most donors experienced the contact/meeting with the recipient as very positive (Median = 7, IQR = 7–7) and did not regret the contact/meeting (Median = 1, IQR = 1–1). One donor felt neutral about the contact she had with her recipient (Median = 4) which was in the form of a formal written thank you card forwarded by the living donor coordinator. Two other donors reported to be only somewhat content with the fact that the recipient is no longer anonymous to them and regret the contact/meeting they had. In both cases, the recipient initiated contact with the donor. Nevertheless, for one of these donors contact with his recipient went beyond his expectations. A major motivation for donors to have contact with the recipient was their curiosity about the outcome. In most cases, the recipient initiated contact (n = 20), however some donors wanted to reassure themselves that the donation was successful and initiated contact with their recipients (n = 7). Quotations to illustrate donors' experiences with the contact or meeting they had with their recipient can be found in **Table 5**.

Attitudes Towards Anonymity

Fifty-eight percent of all participants were for anonymity between donors and unknown recipients, but 34% were not sure. A small group (8%) was against. There was no significant difference between donors and recipients. **Table 6** shows that the distribution of opinions (for, against, not sure) among participants who broke anonymity was significantly different from those who remained anonymous: in the non-anonymous group, a higher number were against anonymity (21%) than in the anonymous group (6%, $\chi^2 = 14{,}229$, $p = 0.001$).

The median attitudes towards anonymity are presented in **Table 3**. Participants agreed strongly with anonymity before the operation and believe that, if desired, a meeting should be allowed after surgery. Participants also agreed with the statement that anonymity makes a donation altruistic. There was less consensus on the statements that there must be anonymity after the surgery and whether surgery should only proceed if the donor agrees to anonymity. In general, participants did not agree that anonymity was difficult to maintain nor that removing anonymity would result in an increase in donors. Participants disagreed with donors and recipients having the right to know the other party.

We found no evidence for differences in attitudes between donors and recipients, except for one statement. Recipients agreed significantly more with the statement that "the donor has the right to know to whom he/she is donating a kidney" (Median = 2, IQR = 1–5) than donors (Median = 1, IQR = 1–3), $U = 27{,}896$, $p = 0.000$.

There were few differences in attitudes between participants who broke anonymity and participants who maintained anonymity. The anonymous group (Median = 4, IQR = 3–6) agreed significantly more with the statement that "there must be anonymity after the operation" than the non-anonymous group (Median = 2, IQR =

TABLE 4 | Quotations to illustrate recipients' experiences with breaking anonymity.

Experiences with written correspondence only	
Male, NTL[1]	"I wanted to thank the donor and explain how they saved my life. We exchanged messages on social media and I sent a letter of thanks. I have chosen not to meet the donor (famous person) as TV would be involved and I would have no control over the TV editing."
Female, PPD[2]	"I was curious to know who has been kind enough to donate a kidney to a stranger. I sent a thank you card, but was told there was a high possibility I wouldn't hear back so when I received a very nice card and letter, I was extremely happy. The contact was only exchanging cards."
Female, NTL	"I needed to express my gratitude and happiness. We exchanged letters. One letter each."
Male, PPD	"My transplant co-ordinator sent me a letter that my donor wished to contact me by letter and email etc. We have never met, but we exchange Christmas cards and email."
Experiences with meeting in person	
Female, PPD	"We were near each other on the ward and we got talking. It was not hard to work out."
Female, NTL	"I wrote to say thank you and received lovely letters back. We met 18 months after and although we have very different lifestyles, we have the same values in life."
Male, NTL	"I wanted to thank my donor for the fantastic gift of one of her kidneys. Because the whole process had been such a major-life event for both couples, we wanted to complete the experience by meeting at least once. I feel that it was beneficial to both parties to form some personal relationship to enhance the experience, I believe the donor would agree."
Male, NTL	"Both myself and the donor wrote a letter to the transplant co-ordinator and after exchanging letters both parties wanted to meet each other. This was strictly connected through the hospital co-ordinator in case either party changed their minds prior to meeting each other."
Female, PPD	"We met one of the couples at clinic, we recognized them as they had appeared on TV promoting transplant donations in the news. The meeting went well and we hugged and thanked each other. It was a three way transplant; have not met the other couple but cards and telephone connections have been exchanged by all three couples. It was a very positive experience. I was very pleased to make contact with both donation couples. Unfortunately, one recipient whose partner donated his kidney to me has since died of cancer and it was extremely upsetting to hear this bad news. It was good to exchange cards with all transplant couples, but to be aware that things don't always go to plan."
Male, NTL	"He changed my life and I wanted to show my gratitude. We met for lunch. My words were "I don't know whether to shake your hand or hug you." We shook hands, later we hugged in private. We have both undertaken skynews interviews (together). We bonded immediately but if meeting prior I would have felt under pressure. Suppose he didn't like me!"
Female, PPD	"I wrote a card to send to my donor *via* the transplant nurse coordinator to say thank you. My donor was really happy I got in touch as she also wanted to find out more about me and eventually meet me."

[1]*NTL, recipient on national transplant list.*
[2]*PPD, recipient registered in paired/pooled donation.*

1–4), $U = 6{,}761$, $p = 0.000$. Non-anonymous participants (Median = 7, IQR = 6–7) also agreed more than anonymous participants (Median = 7, IQR = 4–7) with the statement that "if both parties agree, the donor and recipient should be allowed to meet after surgery," $U = 9596$, $p = 0.005$. Likewise, non-anonymous participants (Median = 3, IQR = 1–5) agreed significantly more than participants who remained anonymous (Median = 1, IQR = 1–3) with the statement that in practice anonymity is difficult to maintain, $U = 9{,}801$, $p = 0.005$.

Associations Between Attitudes and Socio-Demographic and Medical Characteristics

No significant relationships were found between attitudes toward anonymity and gender, education and time since donation. Participants' age was significantly related to three statements. The older the participant, the more they agreed that there must be anonymity before the operation ($r_s = 0.150$, $p = 0.001$) and that the donation should only proceed if the donor agrees to anonymity ($r_s = 0.145$, $p = 0.003$). The younger the participant, the more they agreed that the donor has the right to know to whom he/she is donating a kidney ($r_s = -0.131$, $p = 0.001$).

We found no evidence that attitudes toward anonymity differed according to type of transplant program, except for the following two statements. NDADs (Med = 7, IQR = 6–7) agreed significantly more that there must be anonymity before the operation than paired-pooled donors (Med = 7, IQR = 4–7), $U = 11{,}928$, $p = 0.009$. Likewise, NDADs (Med = 5, IQR = 2–7) agreed significantly more that the donation should only proceed if the donor agrees to anonymity (Med = 3, IQR = 1–5), $U = 10{,}314$, $p = 0.001$.

DISCUSSION

This study shows that, despite the policy on anonymity in the UK, whereby anonymity can be broken after surgery with mutual consent, few donors and recipients make use of this possibility. Only 8% of donors and 11% of recipients reported that anonymity was broken in some way and only 3% of donors and 6% of recipient had met the other party. Experiences with broken anonymity were all positive and all donors and recipients who had contact with, or met the other party, had no regrets. Most participants reported that anonymity was maintained and that they were satisfied with the anonymity of their own procedure. In general, participants agreed strongly with

TABLE 5 | Quotations to illustrate donors' experiences with breaking anonymity.

Experiences with written correspondence only	
Male, PPD[1]	"The recipient contacted me, but I was happy to hear from her to know that all was going well. It was rewarding to know of the benefits the transplant brought to the recipient and her family."
Male, PPD	"The recipient contacted me through the transplant team. We have exchanged letter and e-mails. I don't want to meet the recipient."
Female, NDAD[2]	"I was keen to know the outcome for him or her (hopefully positive but wanted to know even if it's not). We have exchanged emails and have spoken on the phone. I was thrilled to know how the donation has changed not just her life, but also that of her family. We exchange 'anniversary' emails, but may not ever meet."
Male, NDAD	"He sent me a card *via* the hospital and a second card 1 year after donation. He wanted to thank me in person. I wrote to him my name, address and phone number. I told him a bit about myself. I thought that he would be interested. I never received a reply. I wrote another card at Christmas. I never received a reply. Maybe he does not like the Irish. For a little brief moment I felt snubbed, I'm only human."
Female, PPD	"It was lovely to hear from the impact my gift made to the recipient, her immediate family and, particularly, to know she now hopes to see her grandchildren grow up. I've tried to keep in touch with my recipient, but all letters had to go between both coordinators. It felt stalled and eventually I broke contact."
Experiences with meeting in person	
Female, PPD	"As we were part of a pairing scheme, we wanted to see how well they were doing and my husband wanted to thank his donor. We exchanged emails and met up approximately 10 months later."
Male, NDAD	"I wanted to reassure myself that the operation was successful and to confine to myself that what I did was of some purpose. To see that the person was healthy now. They responded to my letter very favourably and wanted to meet me. We then visited each other's families and have become friends."
Female, PPD	"We had several contacts by card/letter *via* the transplant co-ordinators at the hospital. I offered my address to be forwarded to the recipient to make it easier and save NHS money. We had corresponded several times and found each other on Facebook before agreeing to meet. It is amazing to see now she has flourished since receiving the kidney."
Female, NDAD	"I wanted to meet my recipient, because I was curious. She was such a genuine person; her gratitude made me feel good."
Female, PPD	"I was part of a paired donation and my recipient's wife contacted her recipient who gave me their details. I contacted them. My recipient's wife suggested that we all meet up and it seemed like a good idea. We all met for a very emotional day. It was good to see my friend (we were not compatible) and my recipient looking so well."
Male, NDAD	"I thought I preferred not to have contact as I did not wish to establish emotional ties. After exchange of correspondence, I agreed to break anonymity to meet, because it appeared very important to the recipient and his family. When this did happen, it was a very positive experience as the recipient and his family were delighted and clearly the transplant had been successful and their quality of life enhanced immeasurably."
Male, NDAD	"My recipient wished for contact. The whole purpose of donation was to help someone so I wanted to give him the contact he wished for."

[1]*PPD, donor registered in paired/pooled donation.*
[2]*NDAD, non-directed altruistic donor.*

TABLE 6 | General attitude towards anonymity among those who remained anonymous and those who broke anonymity.

Statement	**Anonymity maintained *n* = 507**		**Anonymity broken *n* = 51**	
I am for anonymity between living donors and unknown recipients	284	59%	22	46%
I am against anonymity between living donors and unknown recipients	29	6%	10	21%
I'm not sure	165	35%	16	33%
Missing	29		3	

anonymity before surgery. Opinions about whether there should be anonymity after surgery were mixed. However, all participants believed that a meeting should be allowed after surgery if both parties agree. This seems to indicate that participants are satisfied with the current conditional approach on anonymity.

It is remarkable that the opportunity to revoke anonymity is used so little. Another study among NDADs in the UK also found contact among donors and recipients to be minimal, with only 2% meeting in person (10). Partly, this might be due to participants having mistaken beliefs about the anonymity policy (44% of our participants falsely believed that anonymity was required before and after transplantation). As the possibility of breaking anonymity is discussed with all NDADs and donors and recipients participating in a KEP, it seems that this information is not retained or remembered by participants. It might also indicate that there is little demand for non-anonymous contact among participants in KEPs, because they are content with anonymity and/or because of anxiety about the consequences of breaking it (9).

A similar study has been conducted in the Netherlands and Sweden (where anonymity is perpetual), allowing a comparison of results (9). We found that experiences with anonymity and attitude towards anonymity are similar (9). Some exceptions include greater disagreement among UK participants that a meeting before surgery should be allowed if both parties agree. UK recipients also disagreed more with the statement that donors have the right to know to whom they are donating. However, in both studies, participants strongly believed that a meeting should be allowed after surgery, if both parties agree. This value of autonomous decision-making regarding maintaining or removing anonymity after surgery was also found in another prospective study on attitude towards anonymity among Dutch donors and recipients (8).

Another important finding is that, in all three studies we conducted on anonymity, most participants who remained anonymous did not want to meet their donor/recipient, but were more likely to accept a meeting if the other party would want to meet them (8, 9). This tendency to conform to the needs of the donor/recipient raises the question to what extent recipients and donors might be willing to shift (or even cross) their own boundaries and agree to have contact even when this is not their own personal desire. For recipients, this increased openness could result from a sense of indebtedness towards the donor (11), and for donors, this openness could be explained by their altruistic tendencies (12). However, our finding that written correspondence is more common than meeting directly suggests that donors and recipients do not ultimately all progress to meeting in person and could be interpreted as successful protection of personal boundaries.

One could argue that, since the initiation of KEPs, the discussion on the risks and benefits of anonymity in anonymous donation, has long been more speculative than evidence-based. Several papers described that revoking anonymity after surgery puts donors and recipients at risk for disappointment when the reality differs from an idealized image of recipient/donor or the outcome of the transplantation (7, 13, 14). Also that lifting anonymity could lead to a fall in donation rates, donors attempting to seek reward or to recipients feeling indebted to the donor, and that continued contact might obstruct both parties from achieving closure (7). Although only few studies report on actual experiences with broken anonymity, they provided little evidence for such negative experiences. Rodrigue et al. (15) reported positive experiences of altruistic donors in the US that made contact with their recipient after donation, and Maple et al. (10) found a high satisfaction with non-anonymity in the UK. We are unaware of any empirical studies on the impact of lifting anonymity on donation rates, but as long as maintaining anonymity remains an option, donation rates are unlikely to be influenced by a possible relinquishment of anonymity after donation (5, 7). This is supported by the rising number of unspecified donors in the UK (2, 5) and an increased interest in directed altruistic donation which allows living kidney donors to direct their altruistic donation to a specified recipient they did not know before (often presenting themselves online or in the media). Also, the current study does not support the aforementioned concerns on removal of anonymity in the context of a well-established living donation programme. Rather the findings support the current conditional approach to anonymity that leaves the option for voluntary contact and thereby tailors the donation/transplantation experience to the donors' or recipients' individual wishes to maintain or remove anonymity (if both parties agree). Nevertheless, in clinical practice we should support well-informed decision-making about (removal of) anonymity, by standardized approaches to removal of anonymity, education on risks and benefits of non-anonymous contact between pairs, and pre-operative counselling for donors and recipients.

Despite the strengths of our study, being the first survey study to investigate the issue of anonymity among participants of NDAD and the UKLKSS in the UK, and a large sample size, some limitations should be taken into consideration. Firstly, we did not perform a formal validity assessment of the questionnaire, however using the same questionnaire as previous studies allowed comparison of results. Secondly, the retrospective nature of some questions might have introduced recall bias, as for some participants surgery was almost 8 years ago. However, we found no associations between time since survey and any outcome measures. Thirdly, the response rate was not as high as we hoped for, especially among recipients. This might have introduced a nonresponse bias, for example among those with less positive experiences, and might limit the generalizability of our findings. Another possible limitation of the study is related to the definition of anonymity which may vary between professionals and donors and recipients. Mamode et al. (7) wrote that "anonymity in the context of transplantation could be considered as a situation in which personally identifiable information about the donor is not known by the recipient and vice versa." Based on the responses it appeared that some participants interpreted anonymity differently than we had intended. In this study we chose to follow the perception of the participants and grouped them accordingly in the anonymous or non-anonymous group.

In conclusion, this study demonstrated high satisfaction among UK donors and recipients with the current conditional policy on anonymity, leaving the option for voluntary contact post-transplant. There was little contact between donors and recipients, but the reported experiences with breaking anonymity were positive. These and earlier findings support conditional anonymity over absolute anonymity and are informative for other countries who are assessing current policy on anonymity or considering developing an anonymous living donation programme. Clearly, standardized approaches to removal of anonymity after surgery, education on the advantages and disadvantages of having contact for patients and donors as well as guidelines for healthcare professionals are of utmost importance.

CAPSULE SUMMARY SENTENCE

Currently, almost 5,000 people in the UK are waiting for a kidney transplant. With the national living kidney sharing scheme it is possible to share donated kidneys across the UK. This increases the number of transplantations than can be carried out. The

scheme relies upon anonymity between donor and recipient pairs to avoid disclosure of identity before transplantation. Anonymity has long been seen as a way to prevent commercialization of organs, to prevent disappointment in case of poor recipient outcome, and to protect donor/recipient identity. Unlike in other European countries, in the UK anonymity can be broken with the consent of all parties after the transplantation. As one of few studies, we provide evidence on how often anonymity is broken in the UK and how broken/maintained anonymity is experienced by participants in the kidney sharing scheme. We previously conducted the same study in the Netherlands and Sweden, where it is not allowed to break anonymity after transplantation. Based on these two studies, we make the case that the option of breaking anonymity after transplantation can safely be adopted by other countries in which kidney sharing schemes exist or are to be developed.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

MP: participated in study design, data collection, data analyses, and writing of the paper. LB: participated in study design, data collection, data analyses, and writing and review of the paper. MR: participated in data analyses and review of the paper. EM: participated in study design, data analyses, and writing and review of the paper.

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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.

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ORIGINAL RESEARCH
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Soluble Urokinase Receptor and Mortality in Kidney Transplant Recipients

Christian Morath[1]†, Salim S. Hayek[2]†, Bernd Döhler[3], Christian Nusshag[1], Claudia Sommerer[1], Martin Zeier[1], Jochen Reiser[4] and Caner Süsal[3,5]*

[1]Department of Nephrology, Heidelberg University Hospital, Heidelberg, Germany, [2]Division of Cardiology, Department of Medicine, University of Michigan, Ann Arbor, MI, United States, [3]Institute of Immunology, Heidelberg University Hospital, Heidelberg, Germany, [4]Department of Medicine, Rush Medical College, Rush University, Chicago, IL, United States, [5]Transplant Immunology Research Center of Excellence, Koç Üniversitesi, Istanbul, Turkey

Main problem: Soluble urokinase plasminogen activator receptor (suPAR) is an immunological risk factor for kidney disease and a prognostic marker for cardiovascular events.

Methods: We measured serum suPAR levels in a total of 1,023 kidney transplant recipients either before (cohort 1, n = 474) or at year 1 after transplantation (cohort 2, n = 549). The association of suPAR levels and all-cause and cardiovascular mortality was evaluated by multivariable Cox regression analysis.

Results: The highest suPAR tertile compared to the two lower tertiles had a significantly higher risk of all-cause mortality in both cohorts separately (cohort 1: hazard ratio (HR) 1.92, 95% confidence interval (CI) 1.20–3.08, p = 0.007; cohort 2: HR = 2.78, 95% CI 1.51–5.13, p = 0.001) and combined (n = 1,023, combined HR = 2.14, 95% CI 1.48–3.08, p < 0.001). The association remained significant in the subgroup of patients with normal kidney function (cohort 2: HR = 5.40, 95% CI 1.42–20.5, p = 0.013). The increased mortality risk in patients with high suPAR levels was attributable mainly to an increased rate of cardiovascular death (n = 1,023, HR = 4.24, 95% CI 1.81–9.96, p < 0.001).

Conclusion: A high suPAR level prior to and at 1 year after kidney transplantation was associated with an increased risk of patient death independent of kidney function, predominantly from cardiovascular cause.

Keywords: mortality, kidney, transplantation, suPAR, cardiovascular

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***Correspondence:**
Christian Morath
Christian.Morath@med.uni-heidelberg.de

†These authors have contributed equally to this work and share first authorship

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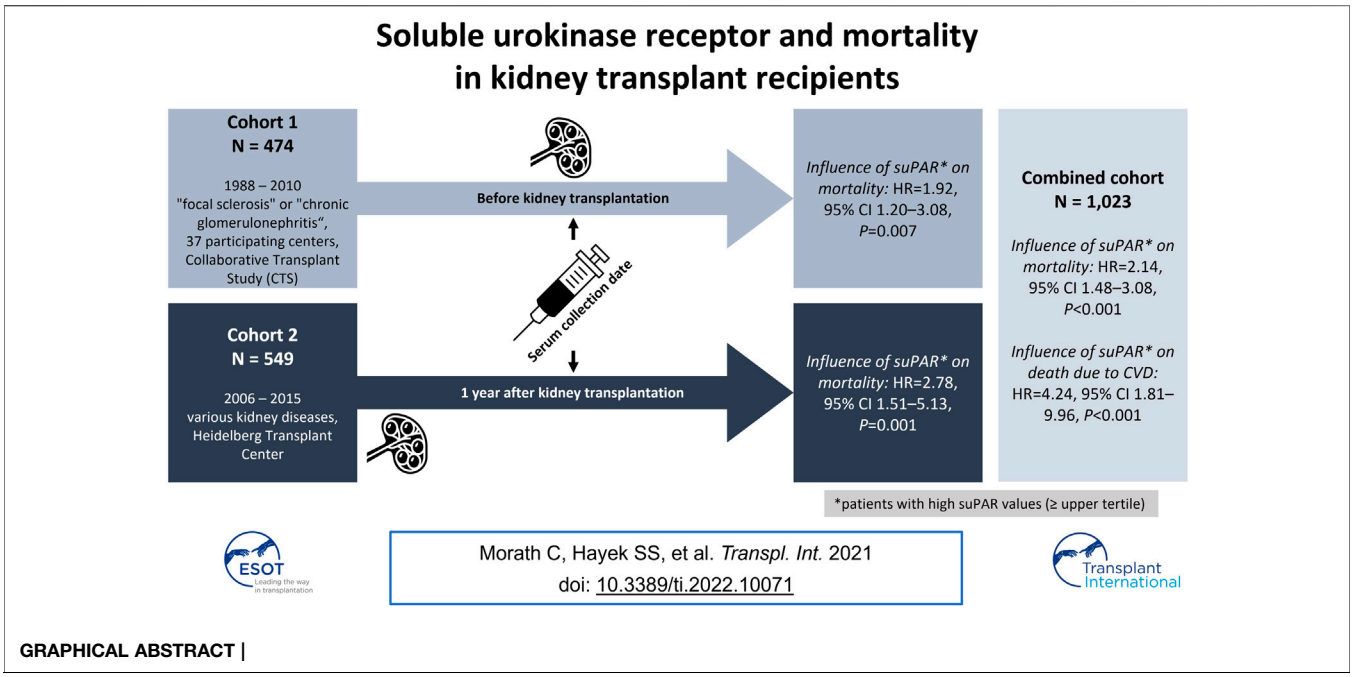
INTRODUCTION

Graft survival after kidney transplantation is limited mainly for two reasons: first, chronic renal allograft dysfunction due to antibody-mediated rejection caused by development of *de novo* donor-specific antibodies and second, death with functioning allograft which in the long term is primarily caused by cardiovascular events (1,2). Early identification of patients at risk for cardiovascular events

Abbreviations: CI, confidence interval; CTS, Collaborative Transplant Study; HR, hazard ratio; HLA, human leukocyte antigen; suPAR, soluble urokinase plasminogen activator receptor; uPA, urokinase.



GRAPHICAL ABSTRACT |

and cardiovascular death may not only reduce the mortality but also the number of graft losses caused by death of the patient with functioning graft.

The soluble urokinase plasminogen activator receptor (suPAR) is the soluble form of uPAR, the membrane-bound receptor for uPA (urokinase). suPAR is a risk factor for kidney disease, both acute and chronic and a biomarker for innate activation of the immune system (3–7). In several studies, serum levels of suPAR were reported to be associated with increased mortality in intensive care unit and septic patients (8,9). In addition, increased suPAR levels were found in patients with a high frequency of cardiovascular events and deaths in populations without chronic kidney disease (10–13). More recently, a high suPAR level was reported to be a predictor of total and cardiovascular mortality in 1,038 hemodialysis patients from 35 dialysis units in Italy (14).

To date, no major study with long-term follow-up has investigated the value of suPAR for the prediction of cardiovascular events and cardiovascular mortality in recipients of kidney transplants. We studied the association between suPAR measured before or at year 1 after transplantation and outcomes in a total of 1,023 kidney transplant recipients.

PATIENTS AND METHODS

Study Population

SuPAR was measured pre-kidney transplant in cohort 1 consisting of 474 patients transplanted between 1988 and 2010 with the primary diagnosis of "focal sclerosis" or "chronic glomerulonephritis" from 37 participating centers that provided a pre-transplant serum from patients reported to the Collaborative Transplant Study (CTS, www.ctstransplant.org). SuPAR was measured 1 year post-kidney transplant in cohort 2, consisting of 549 patients aged 18 years or older who were transplanted at the Heidelberg Transplant Center from 2006 to 2015. The primary diagnosis at the time of transplant was autoimmune disease in 3.1%, disease of blood and blood forming organs in 0.9%, congenital disease in 4.4%, polycystic disease in 16.2%, diabetes in 10.0%, chronic glomerulonephritis in 26.6%, IgA nephropathy in 12.9%, interstitial nephritis in 10.2%, metabolic disease in 2.7%, vascular disease in 4.6%, and other diseases in 1.1% of the patients. In 7.3% of the cases, the original disease could not be specified.

Ethics

The work of the CTS is approved by the Ethics Committee of the Medical Faculty of Heidelberg University (No. 083/2005) and performed in accordance with the World Medical Association Declaration of Helsinki Ethical Principles in the currently valid version.

suPAR Measurements

Serum suPAR was measured in a blinded fashion using either the uPAR Quantikine® ELISA kit (R&D, Minneapolis, MN, United States; cohort 1) or the suPARnostic kit (ViroGates, Birkerød, Denmark; cohort 2) according to the manufacturer's instructions. The lower detection limit is less than 33 and 100 pg/ml, the intra-assay variation less than 5 and 2.75%, and the inter-assay variation less than 5 and 9.17% for the uPAR Quantikine® ELISA and suPARnostic kit, respectively.

Outcomes

The information on date and cause of patient's death was derived from CTS basic follow up forms that are filled out by participating centers at post-transplant months 3, 6, 12 and yearly thereafter.

TABLE 1 | Demographics of study patients, *n* (%).

Characteristic	Unknown (%)	Cohort 1 *n* = 474	Cohort 2 *n* = 549	*p*
Geographical region	–			–
Europe		242 (51%)	549 (100%)	
North America		168 (35%)	–	
Other		64 (14%)	–	
Transplant year	–			–
Range		1988–2010	2006–2015	
Median		2003	2011	
Transplant number	–			0.12
First transplant		402 (85%)	484 (88%)	
Retransplant		72 (15%)	65 (12%)	
Donor relationship	–			<0.001
Living		120 (25%)	217 (40%)	
Deceased		354 (75%)	332 (60%)	
Recipient sex	–			0.001
Female		155 (33%)	233 (42%)	
Male		319 (67%)	316 (58%)	
Recipient age (years)	–			<0.001
<18		52 (11%)	–	
18–59		342 (72%)	403 (73%)	
≥60		80 (17%)	146 (27%)	
Mean ± SD		41.8 ± 17.0	48.4 ± 14.1	<0.001
Donor age (years)	0.3			<0.001
<18		18 (8%)	13 (2%)	
18–59		364 (77%)	360 (66%)	
≥60		69 (15%)	176 (32%)	
Mean ± SD		42.2 ± 16.1	52.2 ± 14.8	<0.001
suPAR (ng/ml)	–			
Range		1.0–26.4	1.1–18.1	
Median (Tertiles)		5.7 (4.9; 6.7)	6.2 (5.2; 7.2)	

SD, standard deviation.

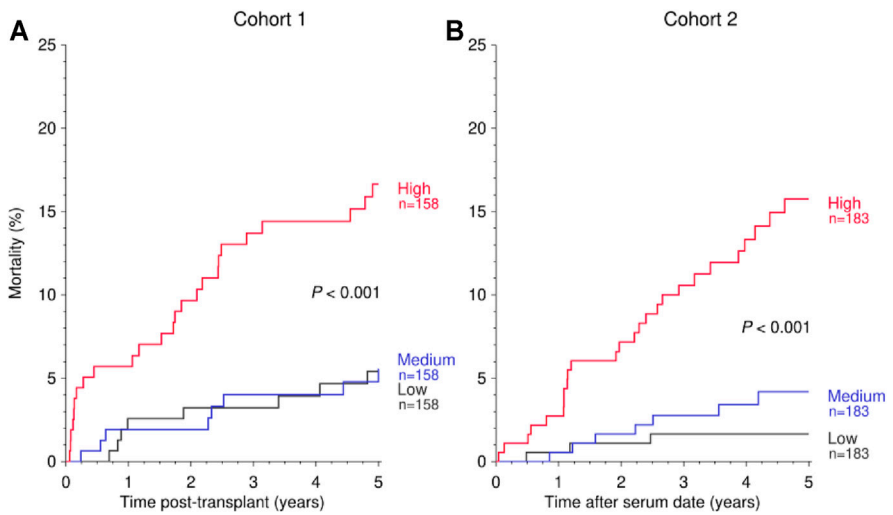


FIGURE 1 | Kaplan-Meier curves demonstrating the impact of suPAR (ng/ml) on 5-year mortality post-transplant in cohort 1 **(A)** and after serum collection date in cohort 2 **(B)**. The categories "Low," "Medium," and "High" are defined by the tertiles of suPAR in each cohort. Log rank *p* values for trend are shown.

Statistics

Time to death was calculated from the serum collection date (cohort 1: serum collection date = transplant date, cohort 2: serum collection date = 1 year after transplantation). Multivariable Cox regression analysis was performed to account for the possible influence of the following confounders separately according to cohort: cohort number, transplant year, transplant number, recipient and donor age, recipient and donor sex, donor relationship, pre-transplant human leukocyte antigen (HLA) antibodies, cold ischemia time (deceased donor), time on

TABLE 2 | Results of the multivariable Cox regression analysis for influence of suPAR on mortality after serum collection date.

Subpopulation	N	HR	95% CI	*p*-value
All study patients	1,023	2.14	1.48–3.08	**<0.001**
Death due to CVD		4.24	1.81–9.96	**<0.001**
Death due to infection		2.20	0.90–5.39	0.083
Death due to cancer		1.61	0.53–4.91	0.40
Cohort 1	474	1.92	1.20–3.08	**0.007**
Cohort 2	549	2.78	1.51–5.13	**0.001**
Good kidney function	255	5.40	1.42–20.5	**0.013**
Female patients	388	1.91	0.97–3.76	0.061
Male patients	635	2.41	1.56–3.73	**<0.001**
Young patients <50 years	584	3.38	1.81–6.34	**<0.001**
Elderly patients ≥50 years	439	1.73	1.10–2.71	**0.017**

Hazard ratios (HR) with 95% confidence interval (CI) of patients with high suPAR values (≥upper tertile) are shown. Significant p-values marked bold.

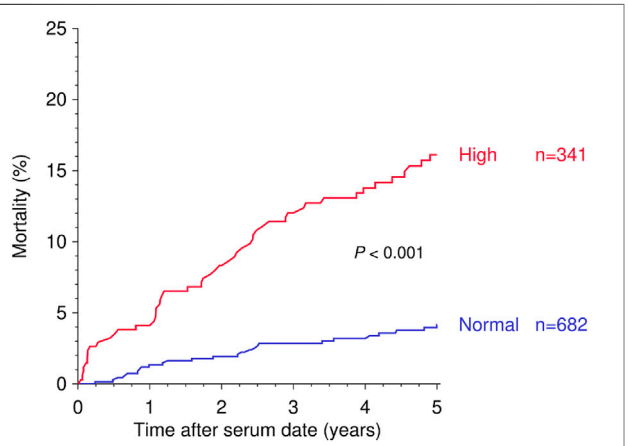


FIGURE 2 | Kaplan-Meier curves demonstrating the impact of suPAR (ng/ml) above the upper tertile ("High") against suPAR values below the upper tertile ("Normal") on 5-year mortality after serum collection date. Log rank *p* value is shown.

dialysis, HLA A + B + DR mismatches, and existence of comorbidities (pretransplant cancer, diabetes mellitus, other reasons of moderate or poor evaluation of the patient as candidate for transplantation). Analysis in cohort 2 included the following additional variables which were not available in cohort 1: rejection treatment during first post-transplant year, 1-year serum creatinine, immunosuppressive therapy at year 1, and presence of an increased cardiovascular risk at year 1 (diabetes mellitus, hypertension, smoking, hypercholesterolemia, obesity). Survival rates were illustrated using the Kaplan-Meier method. The software package IBM SPSS Statistics (SPSS Inc., Chicago, IL, United States) was used.

RESULTS

Patient Demographics

SuPAR levels were measured in a total of 1,023 kidney transplant recipients either before (cohort 1, n = 474) or at year 1 after transplantation (cohort 2, n = 549). In addition to the time of serum sampling, the two cohorts differed with respect to the year of transplantation (cohort 1: 1988–2010, cohort 2: 2006–2015), the geographical region (cohort 1: multicenter, multinational CTS Serum Study, cohort 2: single-center study, Heidelberg, Germany), the donor relationship, the recipient sex, and the donor and recipient age (**Table 1**, **Supplementary Tables 1, 2**). In cohort 1, all patients had marginal renal function at the time of suPAR measurement and suPAR levels did not significantly differ between patients with tissue diagnosis "focal sclerosis" and "chronic glomerulonephritis" (median 5.7 versus 5.8 ng/ml, p = 0.51). In cohort 2, 46.4% of patients had a serum creatinine of <130 µmol/L, 49.0% of patients a serum creatinine of 130–260 µmol/L, and 4.6% of patients a serum creatinine of >260 µmol/L at the time of suPAR measurement at year one.

Serum suPAR Levels and Mortality

The risk of mortality was significantly higher in patients in the high than in the medium or low tertiles ("normal") of suPAR levels (cohort 1: hazard ratio (HR) 1.92, 95% confidence interval (CI) 1.20–3.08, p = 0.007; cohort 2: HR = 2.78, 95% CI 1.51–5.13, p = 0.001; **Figure 1** and **Table 2**). To exclude a decisive influence of kidney function on baseline suPAR levels and subsequent outcomes in cohort 2, we analyzed the impact on mortality of suPAR in patients with good kidney function, e.g. a serum creatinine of <130 µmol/L, separately. Also in this subgroup, the mortality risk was significantly higher in patients with high

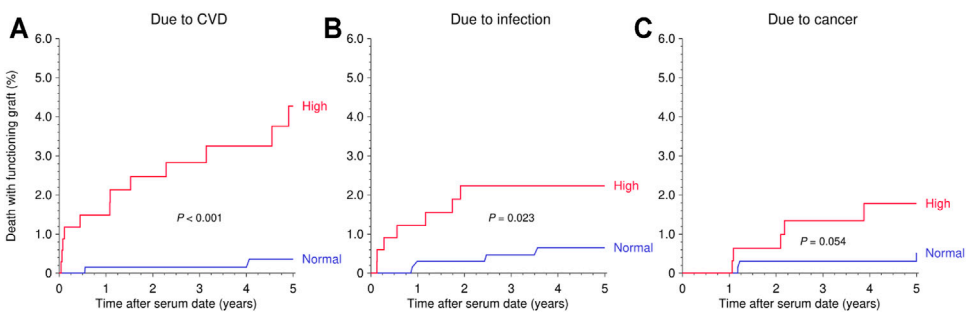


FIGURE 3 | Kaplan-Meier curves demonstrating the impact of suPAR (ng/ml) on death with a functioning graft in the following 5 years after serum collection date as stratified by cause of death. Log rank *p* value is shown. **(A)** Due to CVD. **(B)** Due to infection. **(C)** Due to cancer.

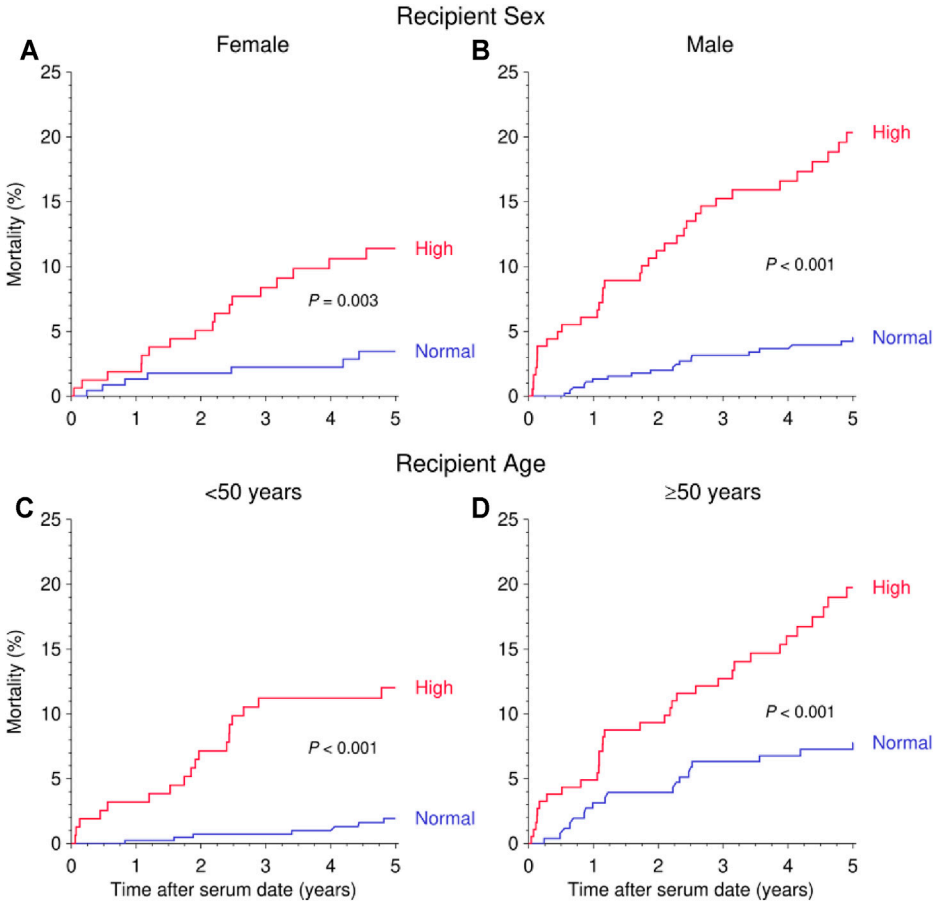


FIGURE 4 | Kaplan-Meier curves demonstrating the impact of suPAR (ng/ml) on 5-years mortality after serum collection date as stratified by recipient sex **(A,B)** and recipient age **(C,D)**. Log rank *p* value is shown.

compared to normal suPAR levels (cohort 2 with good kidney function: HR = 5.40, 95% CI 1.42–20.5, $p = 0.013$; **Table 2**).

Due to the rather comparable risk and a similar distribution of suPAR levels in cohorts 1 and 2 (**Supplementary Figure 1**), both cohorts were combined for further in-depth analysis. The combined risk of mortality during follow-up after the pre- or post-transplant measurement was more than 2-fold higher in patients with a high than normal suPAR level (HR = 2.14, 95% CI 1.48–3.08, $p < 0.001$; **Figure 2** and **Table 2**). As illustrated in **Figure 3** and **Table 2**, the mortality in patients with a high suPAR level was attributable rather to cardiovascular death with a striking HR of 4.24 (95% CI 1.81–9.96, $p < 0.001$) than to death from infection or cancer (infection: HR = 2.20, 95% CI 0.90–5.39, $p = 0.083$; cancer: HR = 1.61, 95% CI 0.53–4.91, $p = 0.40$). The impact of high suPAR level on all-cause mortality was more pronounced in male patients (HR = 2.41, 95% CI 1.56–3.73, $p < 0.001$) than in female patients (HR = 1.91, 95% CI 0.97–3.76, $p = 0.061$; **Table 2** and **Figures 4A,B**) and in younger patients aged <50 years (HR = 3.38, 95% CI 1.81–6.34, $p < 0.001$) than in ≥50-year-old patients (HR = 1.73, 95% CI 1.10–2.71, $p = 0.017$; **Table 2** and **Figures 4C,D**).

DISCUSSION

In this study of 1,023 patients, serum suPAR level was a robust predictor of all-cause mortality after kidney transplantation. A high compared to normal suPAR level was associated with more than doubled risk of mortality during follow-up. This finding was consistent and independent of the time of transplantation (cohort 1: 1988–2010, cohort 2: 2006–2015), the primary kidney disease (cohort 1: glomerulonephritis, cohort 2: various), the time of serum sampling (cohort 1: before transplantation, cohort 2: 1 year after transplantation), or the suPAR assay used (cohort 1: uPAR Quantikine® ELISA kit, cohort 2: suPARnostic kit). The findings were confirmed independently in female versus male, or elderly versus young patients, and most importantly, the influence of suPAR on mortality was constant in patients with different levels of kidney function (cohort 1: marginal kidney function; cohort 2: different levels of kidney function; subgroup of cohort 2 with good kidney function at year 1 and a serum creatinine <130 μmol/L). The main cause of mortality was cardiovascular death with a striking HR of 4.24 in patients with high suPAR level.

SuPAR had been implicated as a biomarker for cardiovascular events and cardiovascular death in the general population as well as in patients with specific diseases, such as type 1 diabetes mellitus and coronary artery disease, or in patients undergoing coronary angiography (11,12,15–17). SuPAR predicted all-cause and cardiovascular mortality independent of classical risk factors or cardiac biomarkers, such as NT-pro BNP, or inflammatory markers, such as CRP. In different studies, suPAR was a strong predictor of cardiovascular death, even after adjustment for cardiovascular risk factors or kidney function (11,12). In kidney disease, suPAR acts as both, a biomarker for future kidney disease as well as being causally implicated through podocyte integrin signaling and tubular cell mitochondrial metabolic adaptation (3,4,18). However, evidence that support the direct involvement of suPAR in cardiovascular mortality is limited and the reported strong associations require to be followed up with translational studies that address the question of suPAR being a cause of cardiovascular disease. Until then, it remains unclear whether there is a causal relationship between elevated suPAR levels and a higher risk of disease or death or whether elevated suPAR levels are merely an unmodifiable marker of disease progression.

Several points are particularly noteworthy in our study. First, regardless of the suPAR assay used, uPAR Quantikine® ELISA kit or suPARnostic kit, the serum suPAR levels in our study were with a median of 5.7 in cohort 1 and 6.2 ng/ml in cohort 2 higher than the median levels reported in other cardiovascular mortality studies with, i.e., a median suPAR level of 3.0 ng/ml in the study by Sommerer et al. (11). The high median level measured in our study can be explained in part by the impaired renal function in kidney transplant recipients (cohort 2) and especially patients awaiting kidney transplantation (cohort 1) due to accumulation of suPAR as result of decreased renal excretion (4). However, the higher suPAR levels in our study may also be an indicator of increased cardiovascular comorbidity of patients with chronic kidney disease. These assumptions are supported by a recently published study from Italy on the predictive value of suPAR on all-cause and cardiovascular mortality in hemodialysis patients (14). In this study, the median suPAR level was with 6.25 ng/ml even slightly higher than that found in the two cohorts studied by us. Second, in patients with high suPAR levels an impressively increased risk of mortality with a HR of 2.14 was observed. In particular the mortality due to cardiovascular death was with a HR of 4.24 above the figures published in other cohorts with, i.e., a median HR for cardiovascular death of 3.43 for the highest suPAR quartile in the study by Sommerer et al. and a HR of 1.48 in Italian dialysis patients when a comparable cut-off as in our study was used (11,14). This in turn could indicate the high susceptibility to cardiovascular death of patients with chronic kidney disease on the waiting list or after transplantation, with high suPAR levels being a strong predictor of increased mortality. Third, high suPAR levels predicted risk of mortality independent of kidney function. The HR for mortality was 1.92 (p = 0.007) in patients with marginal kidney function awaiting transplantation (cohort 1), 2.78 (p = 0.001) in patients with different levels of kidney function 1 year after transplantation (cohort 2), and 5.40 (p = 0.013) when only the subgroup of patients with a serum creatinine <130 µmol/L was analyzed (cohort 2 with good kidney function). This suggests that the link between suPAR and increased mortality is not related to a decreased kidney function, but rather to an unrelated process, i.e., chronic inflammation.

Among the influential factors that were considered in the multivariable analysis, recipient age, suPAR level, and transplant year had the strongest impact on mortality. Pre-transplant cancer, diabetes mellitus, and other reasons of moderate or poor evaluation of the patient as candidate for transplantation (for cohorts 1 and 2), and presence of an increased cardiovascular risk at year 1 (for cohort 2 only) were also considered; however, none of them showed a significant influence. Limitations of the current study include the selection of different cohorts of patients with different underlying diseases ("focal sclerosis" or "chronic glomerulonephritis" in cohort 1 versus various kidney diseases in cohort 2), different timing of serum sample collection (pre-transplant in cohort 1 versus 1 year post-transplant in cohort 2), and the use of different assays for suPAR measurements (uPAR Quantikine® ELISA kit in cohort 1 versus suPARnostic kit in cohort 2). However, as suPAR levels were distributed similarly and not normally in both cohorts and the outcome was similar when cohorts 1 and 2 were analyzed separately, we felt that it was justified to combine both cohorts for further in depth analysis. Moreover, that suPAR levels predicted inferior outcome independent of the primary kidney disease, the time of serum sampling, or the assay, especially in the highest tertile of patients, can also be interpreted as a strength that underlines the robustness of our findings.

In conclusion, a high serum suPAR level was found to be a strong and robust predictor of all-cause and cardiovascular mortality in kidney transplant recipients that may allow for better risk stratification and early intervention in high-risk patients. Most importantly, prediction of risk by suPAR was independent of kidney function at baseline.

CAPSULE SUMMARY SENTENCE

The soluble urokinase plasminogen activator receptor (suPAR) is a risk factor for cardiovascular disease and cardiovascular death in chronic kidney disease and non-chronic kidney disease populations. In hemodialysis patients, the risk of death was almost two times higher in patients with suPAR levels in the highest compared to the lowest tertile with a significantly increased risk of cardiovascular death. So far, no study examined the association of high suPAR levels with overall and cardiovascular mortality in kidney transplant recipients. In two independent cohorts with a total of 1,023 kidney transplant recipients a serum suPAR level in the highest compared to the two lower tertiles was a strong predictor of death, predominantly from cardiovascular cause. These findings were confirmed in different subcohorts. Most importantly, prediction of overall and cardiovascular death was independent of the baseline kidney function indicating that mortality may not be related to a decreased kidney function, but rather to an unrelated process such as chronic inflammation. SuPAR may help to identify kidney transplant recipients at a high risk of cardiovascular death and enable to provide them with the best follow-up and post-transplant care.

DATA AVAILABILITY STATEMENT

The raw data are available upon request to the Collaborative Transplant Study in accordance with the consents of the patients and the participating transplant centers and registries.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics committee of the University of Heidelberg. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CM, BD, CSü created the figures. CM, SH, JR, CSü designed the study. CM, BD, CN, CSo, MZ collected the data, and all authors performed the literature search, data analysis, data interpretation, and writing.

CONFLICT OF INTEREST

CM and MZ, together with the University of Heidelberg, are co-founders of TolerogenixX GmbH, Heidelberg, Germany, a biotechnology company that holds licenses for cell therapies. CM, CSü, and MZ filed a patent application for a cell therapy. JR is cofounder of Trisaq, a biotechnology company developing drugs targeting suPAR. SH and JR are members of the scientific advisory board of Trisaq. JR holds patents and licenses related to suPAR.

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.2021.10071/full#supplementary-material

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ORIGINAL RESEARCH
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Kidney Transplants From Donors on Extracorporeal Membrane Oxygenation Prior to Death Are Associated With Better Long-Term Renal Function Compared to Donors After Circulatory Death

Marilena Gregorini[1,2†], Elena Ticozzelli[3†], Massimo Abelli[4], Maria A. Grignano[2], Eleonora F. Pattonieri[2], Alessandro Giacomoni[5], Luciano De Carlis[6,5], Antonio Dell'Acqua[7], Rossana Caldara[8], Carlo Socci[9], Andrea Bottazzi[10], Carmelo Libetta[1,2], Vincenzo Sepe[2], Stefano Malabarba[3], Federica Manzoni[11], Catherine Klersy[12], Giuseppe Piccolo[13] and Teresa Rampino[2]*

[1]Dipartimento di Medicina Interna e Terapia Medica, Università Degli Studi di Pavia, Pavia, Italy, [2]Unit of Nephrology, Dialysis and Transplant, San Matteo Hospital Foundation (IRCCS), Pavia, Italy, [3]Unit of General Surgery 2, Department of Surgical Sciences, San Matteo Hospital Foundation (IRCCS), Pavia, Italy, [4]Transplant Unit, IRCCS Fondazione Policlinico San Matteo, Pavia, Italy, [5]Transplant Center, Department of General Surgery and Abdominal Transplantation, Niguarda Cà Granda Hospital, Milan, Italy, [6]Department of Medicine and Surgery, University of Milano Bicocca, Milan, Italy, [7]Department of Anesthesia and Critical Care, San Raffaele Scientific Institute (IRCCS), Milan, Italy, [8]Transplant Unit, Department of General Medicine, San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Milan, Italy, [9]Department of Surgery, San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Milan, Italy, [10]ICU1 Department of Intensive Medicine, Foundation IRCCS Policlinico San Matteo, Pavia, Italy, [11]Health Promotion, Environmental Epidemiology Unit, Hygiene and Health Prevention Department, Health Protection Agency, Pavia, Italy, [12]Biometry and Clinical Epidemiology Service, Foundation IRCCS Policlinico San Matteo, Pavia, Italy, [13]Regional Transplant Coordination, Milan, Italy

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***Correspondence:**
Marilena Gregorini
marilena.gregorini@unipv.it

[†]These authors have contributed equally to this work

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Donation after circulatory death (DCD) allows expansion of the donor pool. We report on 11 years of Italian experience by comparing the outcome of grafts from DCD and extracorporeal membrane oxygenation (ECMO) prior to death donation (EPD), a new donor category. We studied 58 kidney recipients from DCD or EPD and collected donor/recipient clinical characteristics. Primary non function (PNF) and delayed graft function (DGF) rates, dialysis need, hospitalization duration, and patient and graft survival rates were compared. The estimated glomerular filtration rate (eGFR) was measured throughout the follow-up. Better clinical outcomes were achieved with EPD than with DCD despite similar graft and patient survival rates The total warm ischemia time (WIT) was longer in the DCD group than in the EPD group. Pure WIT was the highest in the class II group. The DGF rate was higher in the DCD group than in the EPD group. PNF rate was similar in the groups.

Abbreviations: ACLS, advanced cardiovascular life support; BMI, body mass index; CIT, cold ischemia time; CKD-EPI, chronic kidney disease epidemiology collaboration; CPR, cardiopulmonary resuscitation; CS, cold storage; DBD, donation after brain death; DCD, donation after circulatory death; DGF, delayed graft function; ECMO, extracorporeal membrane oxygenation; EEG, Electroencephalography; eGFR, estimated glomerular filtration rate; EKG, electrocardiogram; EPD, ECMO prior to death donation/donor; EPDc, patient's death certified by cardiac criteria; EPDn, patient's death is certified by neurological criteria; ERSD, end stage renal disease; HLA, human leukocyte antigen; HMP, hypothermic perfusion machine; MP, machine perfusion; NRP, normothermic regional perfusion; PNF, primary non function; RR, renal resistances; WIT, warm ischemia time.

Dialysis need was the greatest and hospitalization the longest in the class II DCD group. eGFR was lower in the class II DCD group than in the EPD group. Our results indicate good clinical outcomes of kidney transplants from DCD despite the long "no-touch period" and show that ECMO in the procurement phase improves graft outcome, suggesting EPD as a source for pool expansion.

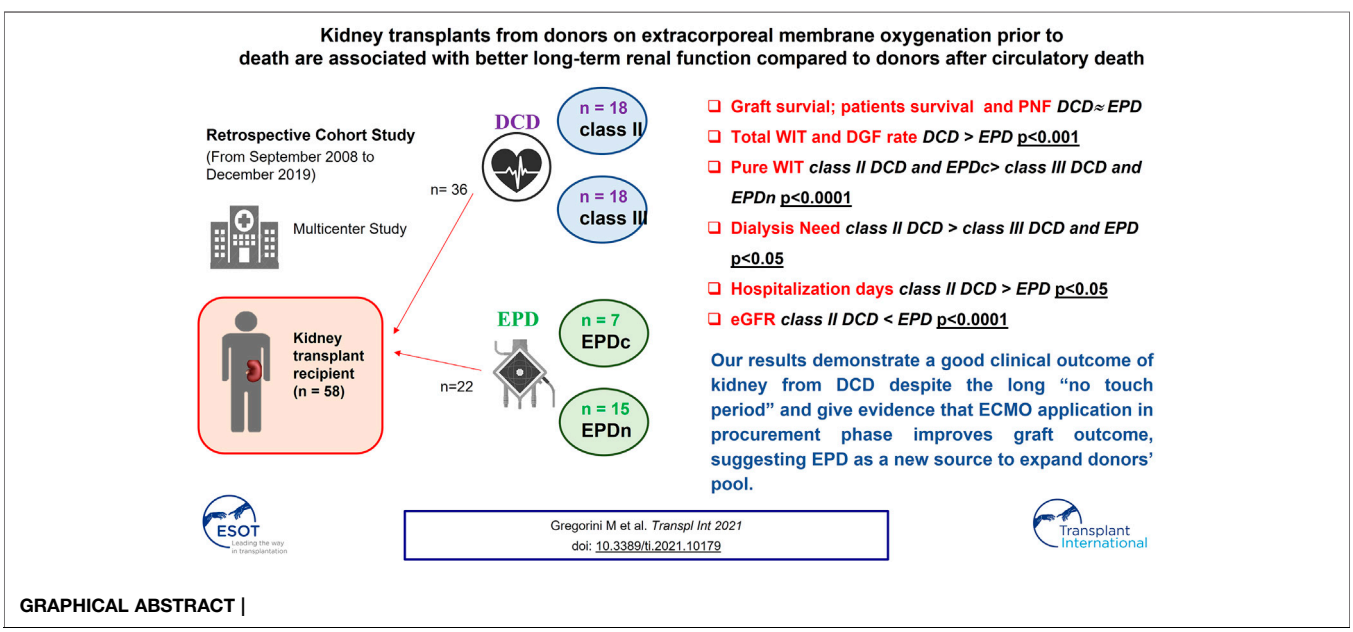
Keywords: donation after circulatory death, extracorporeal membrane oxygenation, renal transplantation, hypothermic perfusion, eGFR

INTRODUCTION

Organ shortage remains the main obstacle in kidney transplantation, thus there is an urgent need for donor pool expansion. Donation after circulatory death (DCD) serves as an additional organ source and has become the current medical practice, despite each country having its own DCD protocol according to its own legislation and healthcare facilities (1). A major difference in the DCD protocols of countries is the "no-touch" period duration, i.e., the time required by law for the circulatory death declaration. Although ethical and practical issues assume the maximal relevance in this type of donation setting, the "no-touch" time ranges from 5 to 30 min in Russia. In Italy, it is 20 min, which is the second longest interval (2, 3). DCD has not been considered in Italy for many years, because of such a prolonged warm ischemia time (WIT); the argument being that it was too long for organ survival. A prolonged WIT is associated with a high rate of organ discard, primary non function (PNF), and delayed graft function (DGF) of kidney transplants from DCD, even if graft and recipient survival is comparable to that linked to donation after brain death (DBD) (4). Due to these complexities, we sought to determine a particular type of donor from among the existing Maastricht categories. It would typically be a patient in whom an advanced resuscitation attempt through extracorporeal life support (ECLS) using extracorporeal membrane oxygenation (ECMO) has failed.

ECLS is an advanced resuscitation technique that ensures blood circulation in asystolic patients. It is a total-body cardiopulmonary by-pass system, applied to allow brain perfusion while cardiac activity restoration is attempted in case of severe heart or lung failure (5). If resuscitation is unsuccessful, these patients become donors with a total-body ECMO already activated. On obtaining family consent, total-body ECMO is switched with normothermic regional perfusion (NRP) by inserting an aortic balloon above the celiac trunk to maintain only abdominal perfusion. These donors do not fit any existing Maastricht criterion because artificial blood circulation starts from cardiac arrest until the patient's death. These patients are always hospitalized in the intensive care unit (ICU) and can be on ECMO for several hours to weeks. Therefore, they are named "donors on ECMO prior to death" (EPD). In this setting, according to Italian legislation, death can be declared by applying cardiac or neurological (EPDc or EPDn, respectively) criteria. When using the latter criteria there is no need to stop circulation while recording the electrocardiogram (EKG) for 20 min, therefore warm ischemia time is shorter.

To our knowledge, there are no studies on EPD as a new potential donor category. Here we report our pragmatic experience over 11 years, comparing graft outcomes achieved with EPD to those achieved with DCD.



GRAPHICAL ABSTRACT |

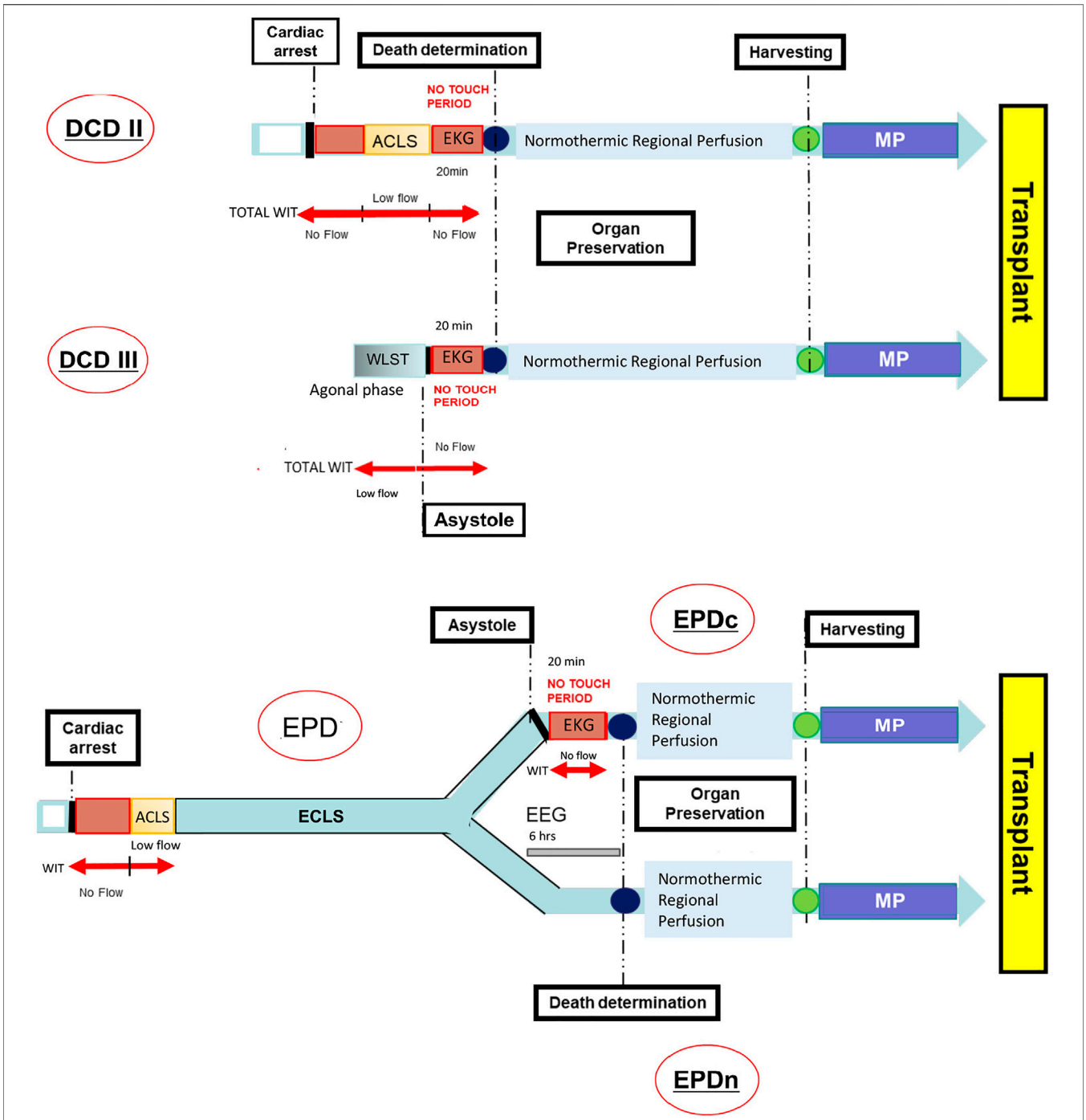


FIGURE 1 | Timeline from the cardiac arrest to the organ transplant in DCD and EPD. ACLS, Advanced Cardiovascular Life Support; DCD, Donation after Circulatory Death; DCDII, Maastricht class II DCD; DCDIII, Maastricht class III DCD; ECLS, Extracorporeal Life Support; EEG, Electroencephalography; EKG, Electrocardiogram; EPD, Extracorporeal Membrane Oxygenation (ECMO) Prior to Death Donation/Donor; EPDc, Patient's death certified by cardiac criteria; EPDn, Patient's death certified by neurological criteria; MP, Machine Perfusion; WIT, Warm Ischemia Time; WLST, Withdrawal Life Sustaining Treatment.

PATIENTS AND METHODS

A total of 58 patients transplanted with kidneys harvested from DCD (36) and donors on EPD (22) between January 2008 and December 2019 were studied. There were 31 donors from DCD (10 recipients received kidneys from five DCD donors), and 21 donors on EPD (2 recipients received kidneys from one donor on EPD). The transplants were performed in three different kidney transplant centers: Foundation IRCCS Policlinico San Matteo Hospital (Pavia), Niguarda Ca' Granda Hospital

(Milan), and San Raffaele Hospital (Milan). The study protocol was approved by the ethics committee (p-20200027199) and fully complied with the 2000 Declaration of Helsinki (6).

DCD categories were defined according to the Maastricht criteria (7). Donors on EPD were recruited from ICU patients, who had undergone an advanced resuscitation protocol, including total-body arterio-venous ECMO as part of ECLS to treat cardiac arrest or severe heart failure. When ECLS therapy failed, the patients became suitable donors.

In this situation, according to Italian legislation, death can be declared using circulatory or neurological criteria. Circulatory death is legally defined as an irreversible cessation of circulatory function, based on definitive proof obtained using an electrocardiogram (EKG). An observation period of 20 min ("no-touch" period), as Italian law imposes, was employed to ensure the irreversibility and permanence of patient's circulatory death. In cases of donors on EPD, death certification was based on neurological criteria; brain death was declared according to international standardized methods (8). Once the consent for donation was obtained, after death declaration, an aortic balloon was inserted through the contralateral iliac artery to ensure selective abdominal circulation and ECMO was restarted to provide NRP (1–4 h) for *in situ* kidney preservation.

Then all kidneys were placed in a hypothermic perfusion machine (HPM RM3-Water Medical System IGL, Lissieu, France and W.A.V.E.S. Water Medical System IGL, Lissieu France) except for the first three procured at the Pavia center, which were preserved in static cold storage because HPM was not yet available.

To avoid PNF, the kidneys were evaluated per donor history, macroscopic appearance, histological criteria and, most importantly, perfusion parameters (9). In particular, the histological pre-transplant examination was performed by obtaining a wedge biopsy specimen from the renal superior pole scoring by using the Remuzzi classification (10). Vascular thrombosis and/or a Remuzzi score of >4 were criteria for organ discard.

The kidneys were also evaluated using perfusion parameters: systolic perfusion pressure was set at 35–40 mmHg. Renal resistance (RR) values were machine-calculated in real-time, as a ratio between the mean perfusion pressure (mmHg) and the flow through the kidney (ml/min); values were recorded every 60 s throughout the perfusion. The perfusion liquid used was a modified Beltzer solution (Perfgen Institut Georges Lopez, Lissieu, France). The perfusion temperature was set at 4–6°C. When the RR value fell below 0.4 mmHg/ml/min, the kidneys were considered suitable for transplantation. In contrast if the RR value remained high after 6 h of perfusion, the kidneys were considered unsuitable for transplantation and discarded, regardless of the biopsy result. The kidneys were detached from the HPM just a few minutes before starting vascular anastomoses.

Figure 1 shows the timeline and event order for the DCD and EPD transplant programs.

CLINICAL VARIABLES

Donors

Donor-related variables included: age, body mass index (BMI), sex, death causes, comorbidities, and Maastricht criteria for DCD.

Donors on EPD were distinguished by EPDn and EPDc depending on the death certification.

Recipients

The recipients were divided into: Maastricht class II DCD, class III DCD, and EPDc and EPDn, according to the donor type. Recipient-related variables included: age, BMI, sex, dialytic age (months), human leukocyte antigen (HLA) match, maximum panel reactive antibody, comorbidities and, primary renal disease.

Transplant-Related Variables

- Total WIT: time elapsed between the moment of cardiac arrest and the beginning of organ preservation by NRP.

The total WIT of the EPD group did not include the total-body ECMO time.

- Pure WIT or no-flow period: asystolic period without any resuscitation maneuvers. This time comprised the first variable period of no flow, the duration of which depends on the celerity in beginning cardiopulmonary resuscitation and a second unchanged period which corresponds to a 20 min recording of EKG.
- The low-flow period: period in which organ blood perfusion was maintained using cardiopulmonary resuscitation, advanced cardiac life support, and chest compression in an attempt to save the patient's life.
- The no-touch period: stand-off time without any intervention to certify the patient's death per circulatory death criteria.
- Cold ischemia time (CIT): time from the beginning of organ perfusion, using cold preservation solutions during the organ retrieval surgery, until the end of graft perfusion through HPM.

Data related to these time intervals, RR, and flow parameters during hypothermic perfusion were collected.

Kidney Suitability for Transplantation

Donor kidneys were discarded according to one of the following criteria:

(1) machine perfusion pump parameters: persistent flow rate of <60 ml/min and/or resistance index of>0.4 mmHg/ml/min;
(2) vascular thrombosis identified upon biopsy analysis performed before hypothermic perfusion; and
(3) Remuzzi score >4.

Immunosuppressive Regimen

Anti-thymocyte globulin or basiliximab was administered as immunosuppression induction therapy. Additionally, methylprednisolone (500 mg/day) was administered intravenously on the first day; its dose was progressively decreased until day 6. Oral methylprednisolone (16 mg) administration was introduced on post-transplant day 7 and reduced every 3 months, to reach a maintenance dose of 4 mg/

TABLE 1 | Clinical and demographic baseline characteristics in DCD and EPD groups.

Variables	Class II DCD group (n = 18)	Class III DCD group (n = 18)	EPD group (n = 22)	p value
Donor age (years) (m ± SD)	49.6 ± 7.94	54.89 ± 8.7	48 ± 11.9	p = 0.08
Recipient age (years) (m ± SD)	55.89 ± 10.9	55.61 ± 8.7	50.86 ± 10.34	p = 0.25
Donor BMI (m ± SD)	27.05 ± 3.4	26.48 ± 3.65	26.51 ± 2.8	p = 0.85
Recipient BMI (m ± SD	25.02 ± 5.1	24.75 ± 3.58	23.33 ± 3.24	p = 0.37
Donor gender (%)				p = 0.7
Male	79.4	75.1	86.3	
Female	22.6	24.9	13.7	
Recipient gender (%)				p = 0.46
Male	66.6	62.5	68.1	
Female	33.4	37.5	31.9	
Donor comorbidity (%)				
Diabetes	5.5	7.3	9	p = 0.6
Hypertension	28.7	29.5	23.6	p = 0.32
Cardiovascular disease	27.5	23.5	31.8	p = 0.07
Dyslipidemia	16.6	17.7	15.6	p = 0.69
Current smoking	21.7	22	20	p = 0.1
Recipient comorbidity (%)				
Diabetes	5	6	3	p = 0.52
Hypertension	77.7	72.3	60.0	p = 0.11
Cardiovascular disease	22.2	18	19.9	p = 0.42
Dyslipidemia	8.3	10	10.1	p = 0.40
Current smoking	19.4	8	10	p = 0.50
Recipient dialytic age (months) (mean ± SD)	39 ± 5.3	37 ± 8	42.8 ± 5.8	p = 0.42
HLA D/R matches (median and IQR)	2 (1–3)	2 (1–3.7)	2 (1–2,25)	p = 0.7
Maximum panel reactive antibody (median; min-max)	0 (0–65)	0 (0–50)	0 (0–65)	p = 0.49
Primary renal disease (%)				
Polycystic kidney disease	19.4	24.5	30.3	p = 0.5
Glomerulonephritis	25.8	18.8	8.6	p = 0.13
Nephroangiosclerosis	29	29	22.2	p = 0.74
Unknown	6.5	10.5	27.8	p = 0.10
Miscellaneous	19.3	17.2	11.1	p = 0.12

day. It was discontinued after 1 year (except in cases of previous acute rejection, re-transplantation, or glomerulonephritis as the primary renal disease). Mycophenolate mofetil was administered at a daily dose of 1–2 g. Tacrolimus or cyclosporine was started on day 1 at 0.1–0.15 mg/kg/day or 6–8 mg/kg/day, respectively. The dose was adjusted to achieve a therapeutic target blood trough level (tacrolimus 8–10 ng/ml, cyclosporine 150–200 ng/ml in the first 3 months). Maintenance immunosuppression regimens consisted of calcineurin and/or mTOR inhibitors, mycophenolate mofetil, and methylprednisolone. Immunosuppressor trough levels were reduced based on transplant age.

Follow-Up

The kidney recipients were followed up for 1–11 years. HLA mismatch between donors and recipients was categorized according to differences at the HLA-A, HLA-B, HLA-DR, and HLA-DQ loci.

Routine blood tests and serum levels of immunosuppressive drugs were regularly assessed. Follow-up ceased due to patient death, PNF, or graft failure.

The following end points were evaluated after transplantation:

- PNF was defined as the immediate failure of the graft function, requiring permanent dialysis or a re-transplantation;
- DGF was defined as a need for dialysis within the first week after transplantation, DGF duration was measured as the number of dialysis sessions
- Dialysis need was defined as the number of dialysis sessions required after transplantation;
- Length of hospital stay in the post-transplant period was determined based on the number of days; and
- Graft survival rate was defined as the time from transplantation to graft nephrectomy, return to dialysis, or re-transplantation. It did not cover patient death with a functioning graft.

Incidences of PNF, DGF, and acute rejection were retrospectively analyzed in all groups of patients. Acute rejection biopsies were classified according to the Banff 2013 classification (11).

In addition, severe post-operative complications such as viral and bacterial infections, severe bleeding, renal vein/artery thrombosis, stenosis of the bladder-ureter anastomosis, allograft rupture, lymphorrhagia, and urine leakage were analyzed retrospectively.

Assessment of Graft Function

Estimated glomerular filtration rate (eGFR), calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI)

TABLE 2 | Transplant-related variables in DCD and EPD groups.

	DCDII	DCDIII	EPD	*p*-value
Total WIT (minutes, m ± SD)	142 ± 40°	60.5 ± 8.1*	25.25 ± 9.3	°$p < 0.0001$ vs. DCDIII, EPD *$p < 0.001$ vs. EPD
Pure WIT (minutes, m ± SD)	28 ± 3.1*	20.8 ± 3.1°	19.8 ± 7.13	*$p < 0.0005$ EPD °$p < 0.0001$ vs. DCDII
CIT (minutes, median, and IQR)	1,065 (540–1,440)	975 (660–1,440)	1,080 (915–1,230)	NS
TB ECMO time (hours, median, and IQR)		36 (19.88–63.38)		
NRP time (minutes, median, and IQR)	210 (190–230)	240 (220–250)	200 (180–230)	NS
HMP time (minutes, median, and IQR)	720 (330–1,260)	660 (375–1,380)	1,080 (915–1,230)	NS
Perfusion parameters	DCD	EPD		
Flow (ml/min, m ± SD)	96.13 ± 27.55	82.69 ± 21.26	NS	
RR (m ± SD mmHg/mlmin^{-1})	0.25 ± 0.09	0.28 ± 0.12	NS	

*The symbols * and ° refer to the statistical significance levels reported in* p *value column. WIT, warm ischemia time; CIT, cold ischemia time, TB ECMO, total body extracorporeal membrane oxygenation; NRP, normothermic regional perfusion; HPM, hypothermic perfusion machine; RR, renal resistance; SD, standard deviation; IQR, interquartile range.*

formula (expressed in milliliters per minute and adjusted for body surface area), was determined on days 7, 1, 3, 6, and 12 months after transplantation and every year throughout the follow up period.

A percutaneous renal graft biopsy was performed 2 or 3 weeks after transplantation when DGF persisted. Furthermore, a biopsy was performed for patients presenting with an abrupt decrease or a lengthy deterioration of renal function, significant proteinuria, or finally, the appearance of specific antibodies against the donor during the follow-up period.

Statistical Analysis

Parametric variables are expressed as mean and standard deviation or standard error values and non-parametric variables as medians and interquartile ranges. The categorical variables are expressed as percentages. Chi-square or Fisher test was used for comparative analysis of categorical variables. Differences in eGFR were evaluated using repeated-measures ANOVA.

Patient and graft survival rates were estimated using the Kaplan-Meyer method, the differences were compared using the log rank test. All tests were two-tailed and considered statistically significant at $p < 0.05$.

RESULTS

From September 2008 to December 2019, 58 kidney transplants were performed; of which 36 kidneys came from DCD and 22 kidneys from EPD. According to the Maastricht DCD criteria, 18 donors each belonged to classes II and III. In the EPD group, death was certified by circulatory criteria in seven donors and by neurological criteria in 15 donors.

Baseline Characteristics

As shown in **Table 1**, baseline characteristics were similar in the groups. All recipients were Caucasian. All donor deaths were caused by cardiac arrest and cardiogenic shock. Basiliximab and/or rabbit ATG were used for induction therapy (basiliximab: 69.4 and 68.8% of DCD and EPD recipients, respectively; rabbit ATG: 30.6 and 31.2% of DCD and EPD recipients, respectively; $p = 0.7$).

Donor Kidney-Related Variables

As shown in **Table 2**, total WIT was the longest in the class II DCD group and the shortest in the EPDn group ($p < 0.0001$).

Similarly, pure WIT was the longest in the class II DCD group and the shortest in the EPDn group ($p < 0.0001$). TB ECMO time was similar in the two EPD subgroups (EPDc and EPDn).

The groups showed no significant differences in CIT, NRP time, and perfusion parameters (flow and RR).

Clinical Outcomes

PNF occurred in two patients, one in the class II DCD group (2.7%) and the other in the EPDc group (4.5%). In both cases, renal biopsy revealed ischemic coagulation necrosis.

Immediate recovery of renal function was noted predominantly in the EPD group (EPD 76.19%, DCD 29.4%, $p < 0.0001$), while the DGF rate was higher in the class II DCD group than in the EPD group (class II DCD: 76.47%, class III DCD: 38.89%, EPD 23.81%) (**Figure 2A**).

The need for dialysis was higher in class II than in class III DCD and EPD recipients (**Figure 2B**). Hospital length of stay was significantly higher in the class II DCD recipients than in EPD recipients ($p < 0.05$) (**Figure 2C**).

Graft Function

During the follow-up period, the highest eGFR was observed in the EPD group, but it was not significantly different from that in the class III DCD group (**Figure 2D**). Interestingly, the eGFR was higher in class III than in class II DCD, but the difference among the two groups was significant only for the first month after transplant. In addition, eGFR was similar in EPDc and EPDn (**Supplementary Figure S1**).

Graft and Patient Survival

Graft survival and patient survival were similar in the DCD and EPD groups, as shown by Kaplan-–Meier curves in **Figures 2E,F**. All deceased patients had functioning grafts.

Causes of death are described in **Supplementary Table S1**.

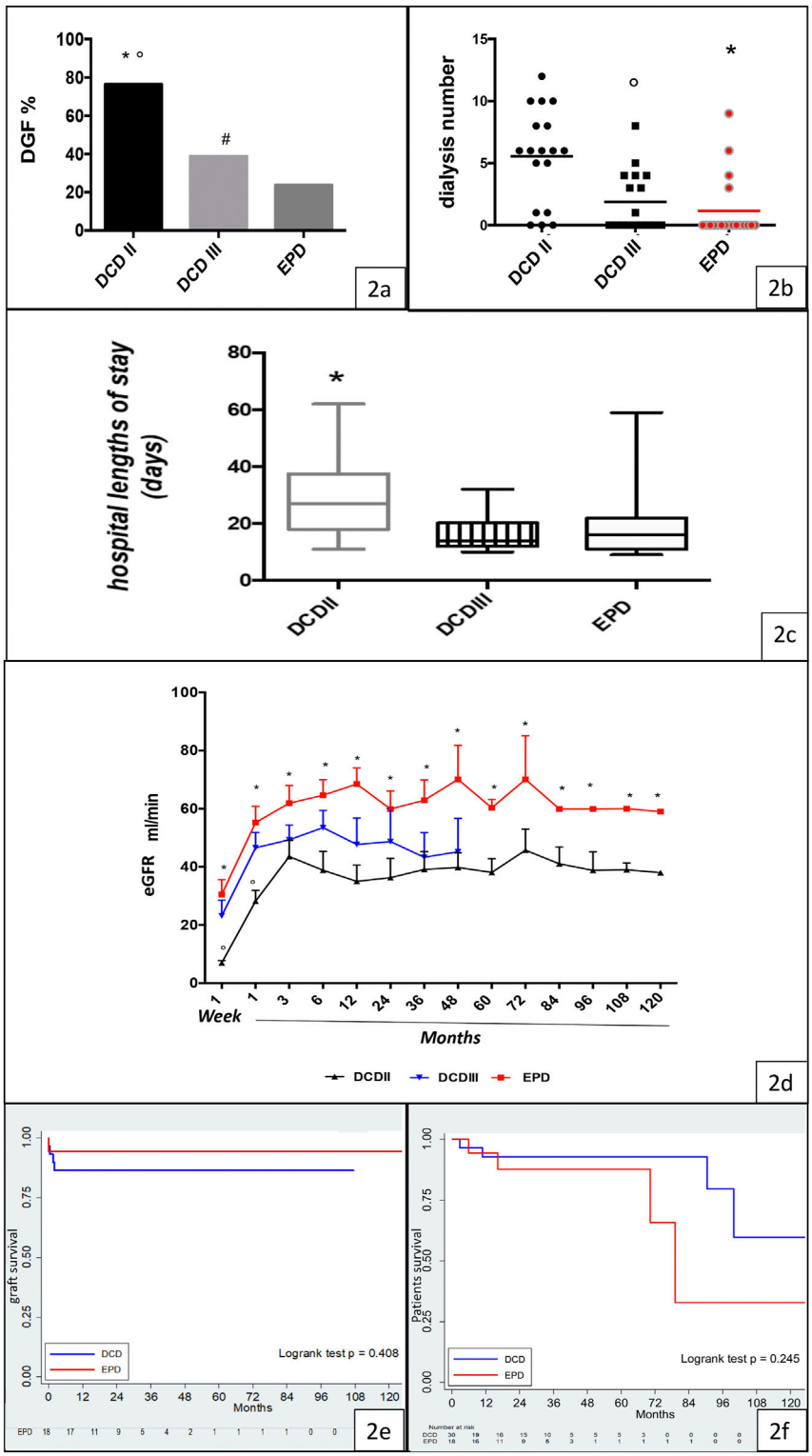


FIGURE 2 | Clinical outcome, graft function, and graft and patient survival in the DCD and EPD groups. **(A)** DGF rate in the Maastricht class II DCD, Maastricht class III DCD, and EPD groups (DCDII vs. DCDIII, *$p < 0.0001$; DCDII vs. EPD, °$p < 0.0001$; DCDIII vs. EPD, #$p < 0.0001$). **(B)** Dialysis requirement in Maastricht class II DCD, Maastricht class III DCD, and EPD recipients (EPD vs. DCDII, *$p < 0.001$; DCDIII vs. DCDII, °$p < 0.05$). **(C)** Hospital length of stay in the studied groups (DCDII vs. EPD, *$p < 0.05$). **(D)** eGFR in Maastricht class II DCD, Maastricht class III DCD, and EPD recipients (DCDII vs DCDIII, °$p < 0.005$; EPD vs. DCDII, *$p < 0.001$). **(E)** Kaplan-Meier curve of graft survival, by group (log rank test $p = 0.408$). **(F)** Kaplan-Meyer curve of patient survival, by group (log rank test $p = 0.245$).

Medical and Surgical Complications Post-Transplant

The rates of post-transplant complications causing graft loss did not differ among the DCD (11%) and EPD (4.5%) groups.

In the DCD group, three grafts were explanted because of renal vein thrombosis, severe hemorrhage secondary to a mycotic aneurysm, or severe life-threatening sepsis requiring immunosuppressive therapy suspension.

The surgical complication rate was higher in the DCD group than in the EPD group: lymphocele was observed in four DCD recipients (11.1%), urinary leakage in two DCD recipients (5.5%), and perirenal hematoma in two EPD (9%) and one DCD recipient (2.7%).

Kidney acute rejection (Banff 2a) occurred in only one patient belonging to the EPD group.

DISCUSSION

We report the renal transplantation results after 11 years of follow-up, by comparing the outcomes for EPD and DCD grafts. Our data showed excellent clinical outcomes in the recipients belonging to all groups. EPD was revealed to be a novel and promising category of donor, that has not been taken into consideration previously.

The EPD recipients achieved better outcomes than the DCD recipients. They showed better renal function, lower DGF rates, reduced dialysis need, shorter post-transplant hospital stays, and lower short- and long-term medical and surgical complication rates. However, the two groups of recipients showed no differences in PNF or graft and patient survival rates. Several factors, both immunological and non-immunological, are known to affect DGF occurrence and influence graft outcomes.

The emergence of new therapies as well as the advancements in mesenchymal stem cell and growth factor therapies and drug monitoring have improved the graft outcomes (12–17), but reduction of risk factors to prevent organ failure remains an important step.

Donor-related risk factors include age, body weight, cause of death, CIT, and WIT (9, 18), while recipient-related factors include the time spent on dialysis, obesity, diabetes, age, and race (18–22). However, WIT remains the most critical determinant of renal tissue injury, which is also related to DGF occurrence (9, 23–24). Despite the limitations of a retrospective study, the donors and recipients of the groups in this study had similar demographic and clinical characteristics; since they showed no differences in the average CIT, NRP, and HPM times. The only significant differences were noted in relation to WIT, which was longer in the DCD group than in the EPD group; notably, the maximum WIT was found in the class II DCD group, and the lowest value was observed in the EPDn group. Pure WIT was similar in class II DCD and EPDc groups; however, it was shorter in the class III DCD group and even shorter in the EPDn group. WIT is known to be an independent risk factor for DGF and acute kidney injury (23, 24). Our findings confirm the harmful influence of WIT on graft outcome. WIT is a hemodynamic impairment that implies a cessation of oxygen and nutrient delivery to the tissues and accumulation of metabolic waste products, which is followed by endothelial and epithelial necrosis, severe inflammation and immune cell activation, and a frequently maladaptive repair process, all of which lead to fibrosis. The pathogenesis of kidney fibrosis induced by ischemia remains an unresolved issue. The nature and the exact moment of the molecular switch between renal tubular repair and progression to atrophy/fibrosis as a response to injury is currently unknown (25). The successful outcomes of grafts from EPD support the hypothesis that early ECMO application could protect renal tissue from severe ischemic injury and predispose the tissue to switch to the correct repair mechanism. The main benefit derived from the immediate application of the ECMO device in the EPD group is the possibility of restoring stable blood circulation (i.e., a mean arterial pressure ranging from 50 to 60 mmHg, an SaO_2 value ranging from 98 to 100%, good gas exchange, and a normothermic body temperature), which ensure good tissue perfusion (26–29).

In fact, the advantage derived from ECMO explains why eGFR was not different between the EPDc and EPDn subgroups, although pure and total WIT were significantly lower in the EPDn subgroup.

In contrast, in DCD, extracorporeal circulation is performed as a method of organ preservation only after the patient's death declaration; therefore the long unstable circulatory period affects the performance of organs. Few reports have investigated the influence of total-body ECMO on donors arising from an unsuccessful extracorporeal life support treatment, and its advantages are unclear. In contrast, several clinical and animal studies have already proven the efficacy of NRP in reversing warm ischemic damage (30–33).

On the other hand, in our protocol, early application of ECMO was aimed at patient resuscitation and was not meant for organ procurement. Interestingly, total-body ECMO, which was applied to assist the circulatory and respiratory functions in DBD, reduced the ischemic damage caused by amines and improved organ quality, leading to a decrease in organ discard rates (33). This effect seems to depend on cellular energy restoration, as supported by studies in animal models (12, 13, 32). Moreover, some authors demonstrated that ECMO in cardiogenic shock was associated with lower levels of systemic inflammation (34). Thus, it could be argued that the comparison between DCD and EPD is improper because EPDn is more similar to DBD than to DCD.

Indeed, the EPDn recipients were subjected to a shorter WIT than the others, because death certification occurs by neurological criteria but these donors cannot be considered the same as DBD donors because they suffer from a refractory cardiac arrest or a cardiogenic shock; thus, the patients can remain on ECMO for hours, days, or weeks before the treatment is declared to be futile and unsuccessful. We would like to emphasize that EPD donors undergo a warm ischemia period during the asystolic phase as the class II DCD. Finally, ECMO provides artificial blood circulation through a roller pump, which cannot induce physiological cardiac systole and diastole as in DBD.

In summary, we have provided preliminary evidence showing that ECMO, applied before the patient's death declaration, protects the kidney against ischemic injury, as demonstrated by the higher eGFR achieved with EPD grafts than with DCD grafts throughout the follow-up period. Furthermore, our results support the use of

DCD despite the higher rate of DGF, but no study has yet reported the excellent long-term outcome of kidney transplants from DCD with a 20 min "no-touch period." Patient and graft survival rates do not significantly differ in kidney transplants from DCD and EPD.

We are aware of the methodological limitations of the study, including the retrospective approach. The lack of controls selected according to a priori criteria precluded definitive conclusions. Nevertheless, we wish to highlight how EPD may be considered a new source of donors with excellent outcomes, at least for kidney transplants. We will test this hypothesis in a sound prospective investigation.

In the Maastricht class II DCD group, the cardiac arrest may occur out of - or in-hospital witnessed by standers. Resuscitation maneuvers are performed to save the patient's life. WIT consists of a no-flow time (circulatory time elapsed from the cardiac arrest to the beginning of ACLS plus a 20 min no-touch period) and a low-flow time (up to a maximum of 120 min), during which a basic level of oxygenated blood circulation is restored by means of a cardiac compressor and mechanical ventilation.

Maastricht class III DCD typically includes an unpredictable agonal phase (maximum 2 h) following the WLST and a no-flow period while the EKG is recorded. Therefore, WIT consists of the time of the agonal phase plus the no-touch period. EPD is a type of donor resulting from an unsuccessful ECMO treatment after an irreversible cardiac arrest. In this setting, the patient's death can be certified by either cardiac (EPDc) or neurological criteria (EPDn). The first choice requires recording a 20 min EKG (no-touchperiod). In contrast, if the patient's death is certified by the neurological criteria the no-touch period can be avoided. After death determination, all types of donors undergo regional normothermic perfusion aimed at preserving the abdominal organs until harvesting. The removed organ is subsequently stored in the mechanical pulsatile perfusion machine until transplantation.

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 ethics committee (p-20200027199) of Fondazione IRCCS Policlinico San Matteo Pavia. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MG participated in research design, statistical analysis, and the writing of the paper. ET participated in research design, data collection, and the writing of the paper. MA participated in research design, data collection, and critical revision of the paper. MAG contributed to data collection and statistical analysis. EP and AG contributed to data collection and manuscript revision. LDC, AD'A, RC, CS, AB, and SM contributed to data collection. CL and VS contributed to manuscript revision. FM and CK performed data analysis. GP contributed to data collection and manuscript revision. TR participated in research design, writing of the paper, and critical revision of the final version of the paper.

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.2021.10179/full#supplementary-material

Supplementary Figure 1 | eGFR in EPDn and EPDc recipients.

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Temporal Reduction in COVID-19-Associated Fatality Among Kidney Transplant Recipients: The Brazilian COVID-19 Registry Cohort Study

Tainá Veras de Sandes-Freitas[1,2,3], Marina Pontello Cristelli[4], Lucio Roberto Requião-Moura[4,5,6], Luís Gustavo Modelli de Andrade[7], Laila Almeida Viana[4], Valter Duro Garcia[8], Claudia Maria Costa de Oliveira[2], Ronaldo de Matos Esmeraldo[3], Paula Roberta de Lima[1], Ida Maria Maximina Fernandes Charpiot[9], Teresa Cristina Alves Ferreira[10], Rodrigo Fontanive Franco[11], Kellen Micheline Alves Henrique Costa[12], Denise Rodrigues Simão[13], Gustavo Fernandes Ferreira[14], Viviane Brandão Bandeira de Mello Santana[15], Ricardo Augusto Monteiro de Barros Almeida[7], Luciane Monica Deboni[16], Anita Leme da Rocha Saldanha[17], Irene de Lourdes Noronha[17,18], Lívia Cláudio de Oliveira[19], Deise De Boni Monteiro de Carvalho[20], Reinaldo Barreto Oriá[1], Jose Osmar Medina-Pestana[4,5] and Helio Tedesco-Silva Junior[4,5] on behalf of the COVID-19 KT Brazil Study Group*

[1]*Programa de Pós-Graduação em Ciências Médicas, Departamento de Medicina Clínica, Faculdade de Medicina, Universidade Federal do Ceará, Fortaleza, Brazil,* [2]*Hospital Universitário Walter Cantídio, Fortaleza, Brazil,* [3]*Hospital Geral de Fortaleza, Fortaleza, Brazil,* [4]*Hospital do Rim, Fundção Oswaldo Ramos, São Paulo, Brazil,* [5]*Departamento de Medicina, Divisão de Nefrologia, Universidade Federal de São Paulo, São Paulo, Brazil,* [6]*Unidade de Transplante Renal, Hospital Israelita Albert Einstein, São Paulo, Brazil,* [7]*Departamento de Medicina Interna, Universidade Estadual Paulista-UNESP, Botucatu, Brazil,* [8]*Santa Casa de Misericórdia de Porto Alegre, Porto Alegre, Brazil,* [9]*Hospital de Base, Faculdade de Medicina de São José do Rio Preto (FAMERP), São José do Rio Preto, Brazil,* [10]*Universidade Federal do Maranhão, São Luiz, Brazil,* [11]*Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil,* [12]*Divisão de Nefrologia e Transplante Renal, Hospital Universitário Onofre Lopes (HOUL), Natal, Brazil,* [13]*Hospital Santa Isabel, Blumenau, Brazil,* [14]*Santa Casa de Misericórdia de Juiz de Fora, Juiz de Fora, Brazil,* [15]*Hospital de Base do Distrito Federal, Brasília, Brazil,* [16]*Hospital Municipal São José (HMSJ), Joinville, Brazil,* [17]*Hospital Beneficência Portuguesa de São Paulo (BP), São Paulo, Brazil,* [18]*Divisão de Nefrologia, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil,* [19]*Unidade de Transplantes, Hospital Universitário de Brasília, Universidade de Brasília (UnB), Brasília, Brazil,* [20]*Hospital São Francisco na Providência de Deus, Rio de Janeiro, Brazil*

Data from the general population suggest that fatality rates declined during the course of the pandemic. This analysis, using data extracted from the Brazilian Kidney Transplant COVID-19 Registry, seeks to determine fatality rates over time since the index case on March 3rd, 2020. Data from hospitalized patients with RT-PCR positive SARS-CoV-2 infection from March to August 2020 (35 sites, 878 patients) were compared using trend tests according to quartiles (Q1: <72 days; Q2: 72–104 days; Q3: 105–140 days; Q4: >140 days after the index case). The 28-day fatality decreased from 29.5% (Q1) to 18.8% (Q4) ($p_{for\text{-}trend} = 0.004$). In multivariable analysis, patients diagnosed in Q4 showed a 35% reduced risk of death. The

Abbreviations: AKI, Acute kidney injury; AUC-ROC, Area Under the Receiver Operating Curve; COVID-19, Coronavirus disease 2019; eGFR, Glomerular filtration rate; ESKD, End-stage kidney disease; GLMM, Generalized Linear Mixed Models; IQR, Interquartile range; IRB, Institutional Review Board; KT, Kidney transplant; rATG, Antithymocyte globulin; RT-PCR, Reverse-transcription polymerase chain reaction; sCr, Serum creatinine; ST, Steroid; Δ sCr, Delta serum creatinine.

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Correspondence:
Tainá Veras de Sandes-Freitas
taina.sandes@gmail.com

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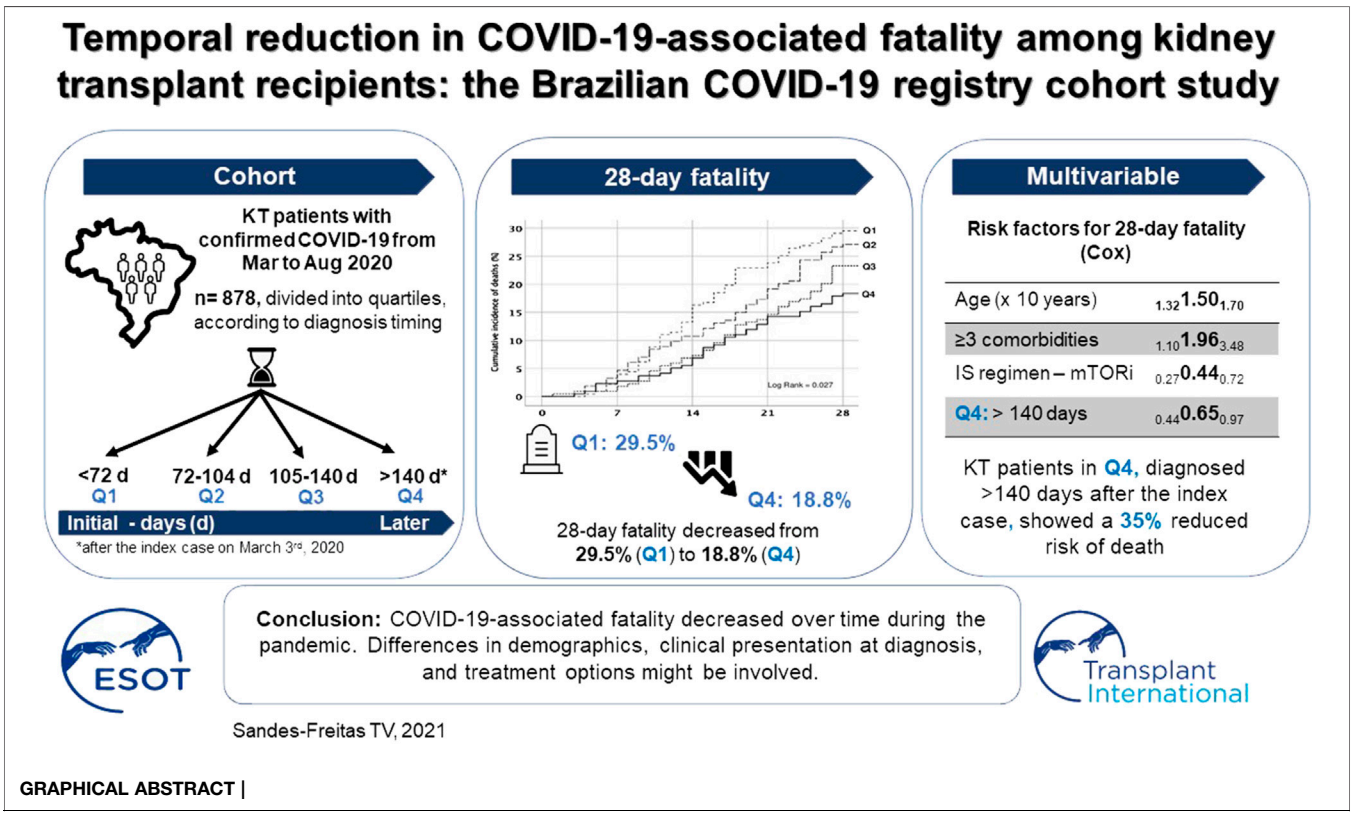
trend of reducing fatality was associated with a lower number of comorbidities (20.7–10.6%, $p_{for\text{-}trend}$ = 0.002), younger age (55–53 years, $p_{for\text{-}trend}$ = 0.062), and better baseline renal function (43.6–47.7 ml/min/1.73 m^2, $p_{for\text{-}trend}$ = 0.060), and were confirmed by multivariable analysis. The proportion of patients presenting dyspnea ($p_{for\text{-}trend}$ = 0.001) and hypoxemia ($p_{for\text{-}trend}$ < 0.001) at diagnosis, and requiring intensive care was also found reduced ($p_{for\text{-}trend}$ = 0.038). Despite possible confounding variables and time-dependent sampling differences, we conclude that COVID-19-associated fatality decreased over time. Differences in demographics, clinical presentation, and treatment options might be involved.

Keywords: Sars-CoV-2, Covid-19, kidney transplant, coronavirus, renal transplantation



GRAPHICAL ABSTRACT |

INTRODUCTION

Over the past year, the coronavirus disease 2019 (COVID-19) global pandemic has been responsible for more than 126 million cases of severe acute respiratory syndrome worldwide and over 2.76 million deaths. With large numbers of COVID cases, Brazil has become an epicenter of the COVID-19 outbreak in the world (1, 2). Among many specific vulnerable groups affected by SARS-COV-2 infection, transplant immunocompromised recipients represent a recognized high-risk group for this infection (3).

Although to date there is still no specific treatment for COVID-19, several pharmacological and non-pharmacological strategies have been explored to improve the clinical outcomes. Among these strategies, the following are noteworthy: 1) the use of prehospital pulse oximetry to early detect silent hypoxemia (4); 2) the important role of non-invasive mechanical ventilation often avoiding unnecessary early intubation (5); 3) prone position to improve oxygenation in intubated and non-intubated patients with COVID-19-related acute respiratory distress syndrome (6, 7); 4) anticoagulant treatment in patients with coagulopathy (8); and 5) corticosteroids in patients with severe disease (9).

Data from the general population suggest an improvement in survival rates during the pandemic, mainly among critically ill patients (10–13). Multicenter national studies have reported COVID-19-related fatality rates varying from 20.5 to 32% among hospitalized kidney transplant (KT) patients (14–18), but no study evaluated the impact of the timing on deaths in this population.

In this analysis of the multicenter national Brazilian registry of SARS-CoV-2 infection study, we aimed to assess fatality rates

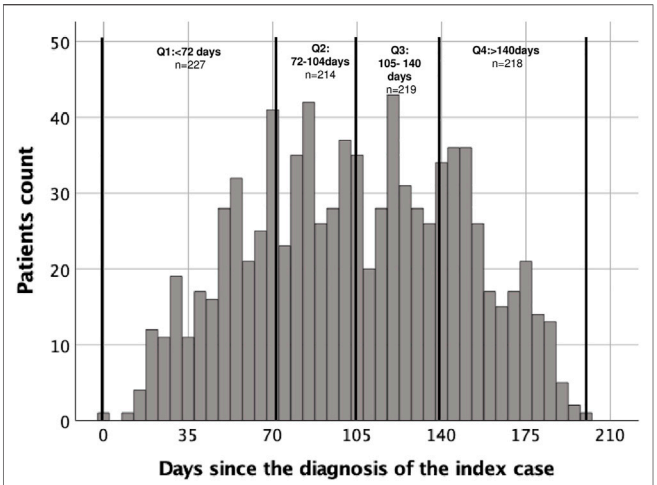


FIGURE 1 | Distribution of COVID-19 diagnosed transplant patients after the index case, on March 3rd, 2020, according to quartiles.

over the first 6 months of pandemic and to explore whether demographics, clinical profile, and in-hospital management of COVID-19 were associated with trends in the outcomes.

MATERIALS AND METHODS

Study Design

This is an ongoing multicenter national Brazilian registry of SARS-CoV-2 infection among kidney transplant recipients (ClinicalTrials.gov: NCT04494776) (19). For this analysis, we extracted data of patients with COVID-19-related signs and symptoms and SARS-CoV-2 detected by reverse-transcription polymerase chain reaction (RT-PCR) of a respiratory sample, between 3rd March and 31st August 2020, who required hospitalization, totalizing 878 patients from 35 transplant centers of four Brazilian Regions (615 from the Southeast, 124 Northeast, 111 South, and 28 from the Midwest). Patients were followed for 3 months after the diagnosis or until death or graft loss, and the end-of-study data was 30th November 2020.

Variables

Patient age, gender, ethnicity, and body mass index were collected and included in the analysis. Comorbidities comprised the following conditions: hypertension, diabetes, cardiovascular, pulmonary, neurological or hepatic diseases, current or previous neoplasia, and autoimmune disease. The following clinical presentation parameters were also included in the analysis: fever and/or chills, cough, dyspnea, myalgia, diarrhea, headache, fatigue and or/asthenia, runny nose, and nausea and/or vomiting. Data related to KT such as donor source, end-stage kidney disease (ESKD) etiology, time after transplantation, baseline renal function, maintenance immunosuppressive (IS) drugs, steroid (ST) pulse therapy <3 months, use of rabbit antithymocyte globulin (rATG) <3 months were analyzed.

The following laboratory exams at admission were recorded: lymphocytes count, hemoglobin, platelets count, C-reactive protein, lactic dehydrogenase, aspartate transaminase; alanine transaminase; creatine phosphokinase, serum sodium, ferritin, serum creatinine. Chest radiography and/or computed tomography at admission were used to classify pulmonary abnormalities.

The following treatments available in the registry were analyzed: antibiotics, particularly azithromycin, high-dose steroids, prophylactic or therapeutic use of anticoagulants, and use of oseltamivir, ivermectin, and chloroquine or hydroxychloroquine.

The analysis of outcomes in COVID-19 transplant recipients across time was carried out considering fatality rates and the following variables: invasive mechanical ventilation, intensive care unit admission, and development of AKI with dialysis requirement.

Definitions

The COVID-19-associated fatality rate was defined as the percentage of deaths that occurred in patients with confirmed SARS-CoV-2 infection. Hospital admission criteria and the use of pharmacological and non-pharmacological treatments were at the discretion of each of the participating centers. The definition of "high-dose steroids" was at the center discretion, according to their local practices.

We considered as the index case the first KT patient diagnosed with COVID-19 and included in the Brazilian Kidney Transplant COVID-19 Registry (March 3rd, 2020). The sample was divided into quartiles, as demonstrated in **Figure 1**: Q1: patients diagnosed <72 days after the index case (n = 227); Q2: 72–104 days (n = 214); Q3: 105–140 days (n = 219); Q4: >140 days (n = 218).

Baseline serum creatinine (sCr) was defined as the last three available sCr measurements before COVID-19 infection. Glomerular filtration rate (eGFR) was estimated by the CKD-EPI formula. Delta sCr (Δ sCr) was the difference between admission and baseline sCr values. Acute kidney injury (AKI) was defined as a rise in sCr of ≥50% from its baseline value (20). Graft loss was defined as the return to long-term dialysis therapy or retransplantation.

Statistical Analysis

Categorical variables were presented as frequency and percentage. All continuous variables were non-normally distributed and were summarized as median and interquartile range (IQR). Trend analyses comparing data across the quartiles were performed using Cochran–Armitage test for categorical variables, and Jonckheere-Terpstra test for numerical variables. Survival curves were obtained using Kaplan-Meier method and compared using the log-rank test. Univariable and multivariable analyses to identify independent risk factors associated with death were performed using Cox regression, with center-based random effects (frailty model). Collinear variables, and those poorly associated with death in univariable analysis (p > 0.15) were excluded from the multivariable model. No variable exceeded 5% of missing

TABLE 1 | Demographic characteristics of kidney transplanted patients at COVID-19 diagnosis across quartiles of time.

	Non-missing cases	Total	Q1	Q2	Q3	Q4	$p_{for\text{-}trend}$
		N = 878	*N* = 227	*N* = 214	*N* = 219	*N* = 218	
Age (years-old)	878	54 (45–62)	55 (46–64)	54 (44–61)	54 (45–61)	53 (44–62)	0.062
Male gender	878	535 (60.9)	146 (64.3)	131 (61.2)	134 (61.2)	124 (56.9)	0.127
Ethnicity	878						0.204
Caucasian		483 (55.0)	111 (48.9)	108 (50.5)	125 (57.1)	139 (63.8)	
Mixed race		255 (29.0)	79 (34.8)	68 (31.8)	63 (28.8)	45 (20.6)	
Afro-Brazilian		112 (12.8)	28 (12.3)	28 (13.1)	24 (11.0)	32 (14.7)	
Asian		14 (1.6)	6 (2.6)	3 (1.4)	4 (1.8)	1 (0.5)	
Indian		1 (0.1)	0 (0)	0 (0)	1 (0.5)	0 (0)	
Not available		13 (1.5)	3 (1.3)	7 (3.3)	2 (0.9)	1 (0.5)	
BMI (kg/m^2)	842	26.5 (23.6–30.0)	26.4 (23.3–29.5)	26.0 (22.9–29.7)	27.3 (24.4–30.9)	26.8 (23.9–29.9)	**0.031**
Donor source	878						0.084
KT - LD		259 (29.5)	79 (34.8)	62 (29.0)	67 (30.6)	51 (23.4)	
KT - DD		601 (68.5)	142 (62.6)	151 (70.6)	146 (66.7)	162 (74.3)	
Combined KT[a]		18 (2.1)	6 (0.7)	1 (0.1)	6 (0.7)	5 (0.6)	
ESKD etiology	878						0.230
Unknown		266 (30.3)	57 (25.1)	80 (37.4)	69 (31.5)	60 (27.5)	
Diabetes		174 (19.8)	53 (23.3)	41 (19.2)	38 (17.4)	42 (19.3)	
Chronic GN		151 (17.2)	33 (14.5)	30 (14.0)	51 (23.3)	37 (17.0)	
Hypertension		103 (11.7)	34 (15.0)	22 (10.3)	20 (9.1)	27 (12.4)	
PKD		73 (8.3)	20 (8.8)	14 (6.5)	19 (8.7)	20 (9.2)	
Urological		14 (1.6)	4 (1.8)	4 (1.9)	3 (1.4)	3 (1.4)	
Other		97 (11.0)	26 (11.5)	23 (10.7)	19 (8.7)	29 (13.3)	
Time after KT (years)	875	6.1 (2.2–11.2)	6.9 (2.5–11.8)	5.6 (2.1–10.3)	6.1 (2.0–11.7)	5.7 (2.5–11.2)	0.541
Comorbidities	878						
Hypertension		689 (78.5)	179 (78.9)	170 (79.4)	175 (79.9)	165 (75.7)	0.471
Diabetes		351 (40.0)	101 (44.5)	84 (39.3)	89 (40.6)	77 (35.2)	0.075
Cardiovascular disease		142 (16.2)	49 (21.6)	33 (23.2)	32 (14.6)	28 (12.8)	**0.014**
Pulmonary disease		30 (3.4)	10 (4.4)	7 (3.3)	7 (3.2)	6 (2.8)	0.353
Neurological disease		10 (1.1)	5 (2.2)	1 (0.5)	1 (0.5)	3 (1.4)	0.416
Hepatic disease		35 (4.0)	8 (3.5)	8 (3.7)	8 (3.7)	11 (5.0)	0.449
Current or previous neoplasia		59 (6.7)	31 (13.7)	14 (6.5)	10 (4.6)	4 (1.8)	**<0.001**
Autoimmune disease		22 (2.5)	11 (4.8)	2 (0.9)	6 (2.7)	3 (1.4)	0.062
No. of comorbidities	878						**0.002**
None		111 (12.6)	23 (10.1)	26 (12.1)	31 (14.2)	31 (14.2)	
1–2		644 (73.3)	157 (69.2)	161 (75.2)	162 (74.0)	164 (75.2)	
3 or more		123 (14.0)	47 (20.7)	27 (12.6)	26 (11.9)	23 (10.6)	
Maintenance IS drugs	872						
CNI		691 (79.2)	170 (74.9)	170 (79.8)	180 (83.3)	171 (79.2)	0.172
MPA or AZA		653 (74.9)	163 (71.8)	152 (71.4)	167 (77.3)	171 (79.2)	**0.033**
mTORi		135 (15.5)	40 (17.9)	42 (19.7)	26 (12.2)	267 (12.7)	**0.038**
ST		826 (94.7)	212 (93.4)	203 (94.9)	202 (92.2)	209 (95.9)	0.496
RAAS blockade	866	294 (33.9)	74 (32.6)	65 (30.4)	76 (34.7)	79 (36.2)	0.787
ST pulse therapy ≤3 months	859	49 (5.7)	11 (4.8)	7 (3.3)	12 (5.5)	19 (8.7)	0.460
rATG ≤3 months	844	30 (3.6)	8 (3.5)	6 (2.8)	7 (3.2)	9 (4.1)	0.222
eGFR (ml/min/1.73 m^2)	846	44.5 (28.7–60.9)	43.6 (25.4–57.9)	46.3 (30.0–61.1)	40.9 (27.3–59.3)	47.7 (31.9–66.7)	0.060

Trend analysis for categorical and continuous data were performed using Cochran–Armitage test and Jonckheere-Terpstra test, respectively. BMI, body mass index; KT, kidney transplant; LD, living donor; DD, deceased donor; CNI, calcineurin inhibitor; AZA, azathioprine; MPA, mycophenolate; mTORi, mammalian target of rapamycin inhibitor; RAAS, renin-angiotensin-aldosterone system; ST, steroids; rATG, rabbit antithymocyte globulin; ESKD, end-stage kidney disease; GN, glomerulonephritis; PKD, polycystic kidney disease; IS, immunosuppressive; eGFR, estimated glomerular filtration rate.

Bold values denote statistical significance at the $p < 0.05$ *level.*

[a]*Simultaneous pancreas-kidney = 8; simultaneous liver-kidney = 6; kidney after liver = 3; simultaneous heart-kidney = 1.*

values and Multiple Imputation by Chained Equation (MICE) was used to replace missing data values, as follows: 1) generating replacement values ("imputations") for missing data and repeating this procedure 10 times, 2) analyzing the 10 imputed data sets, and 3) combining (pooling) the results using Rubin's Rules (21). A significantly statistical difference was assumed when the *p*-value was less than 0.05. Statistical analysis was performed using the IBM SPSS 25 and R 4.0.2.

RESULTS

Demographic Characteristics Across the Quartiles

The baseline demographic characteristics at COVID-19 diagnosis are shown in **Table 1**. Changes in patients' clinical profile occurred over time, with a significant reduction in age, and in the percentage of patients with ≥3 comorbidities.

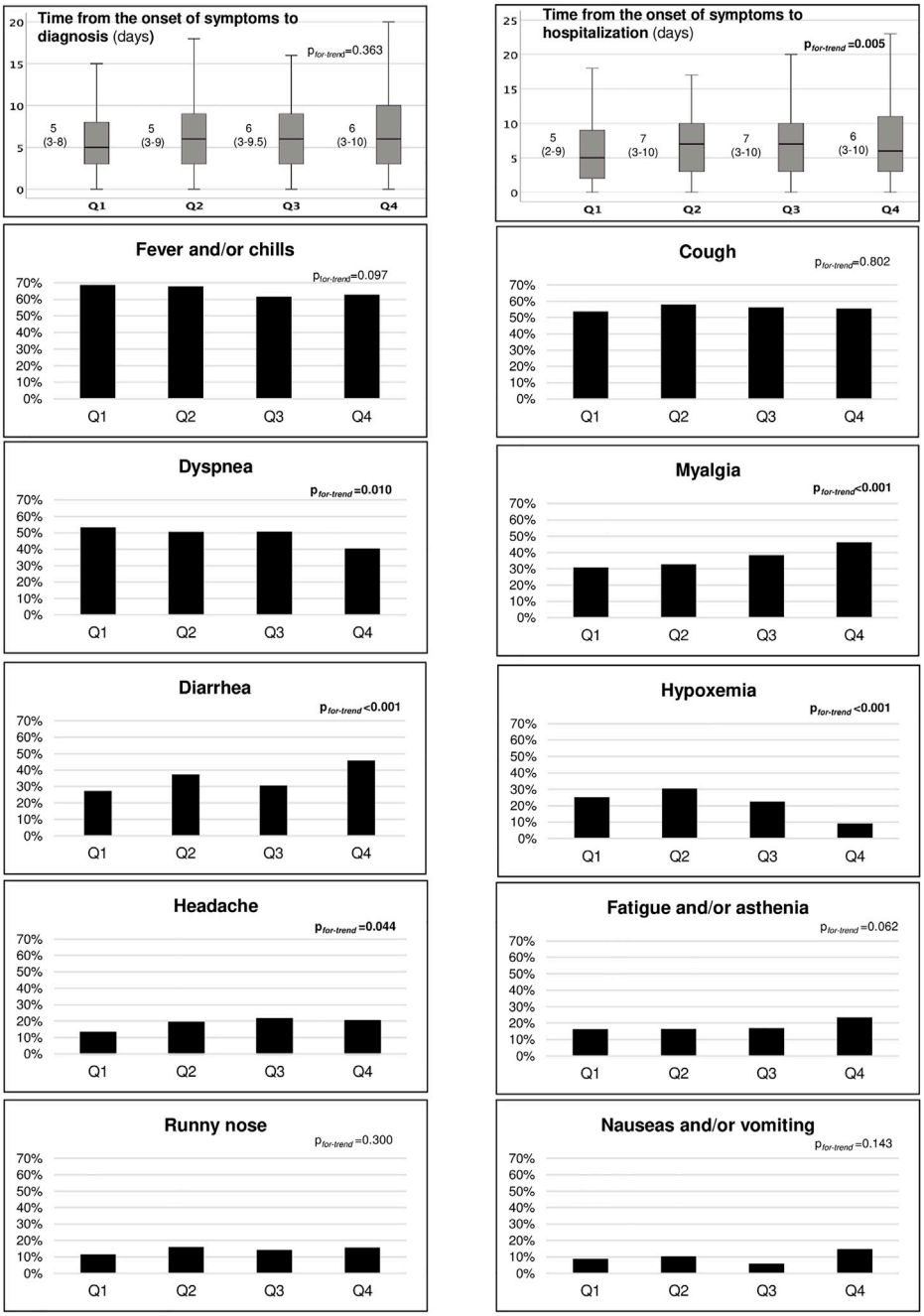


FIGURE 2 | Main signs and symptoms at COVID-19 diagnosis across the quartiles. Trend analyses were performed using Cochran–Armitage test and Jonckheere-Terpstra test.

The Clinical Presentation Across the Quartiles

The analysis across quartiles showed a decrease in the proportion of patients with dyspnea and hypoxemia at diagnosis, whereas myalgia, diarrhea, and headache progressively increased. Although the time from the onset of COVID-19 symptoms to diagnosis remained stable over time (median 6 days; IQR 3–9), a longer time until hospitalization since symptoms onset was observed, increasing from Q1 (median 5 days, IQR 2–9) to Q4 (median 6 days, IQR 3–10) ($p_{for\text{-}trend}$ = 0.005) (**Figure 2**).

Laboratory data and chest radiological findings at COVID-19 diagnosis are shown in **Supplementary Table S1**. An increase in the percentage of patients with normal chest radiological evaluation was observed from Q1 (2.1%) to Q4 (6.7%) ($p_{for\text{-}trend}$ = 0.015).

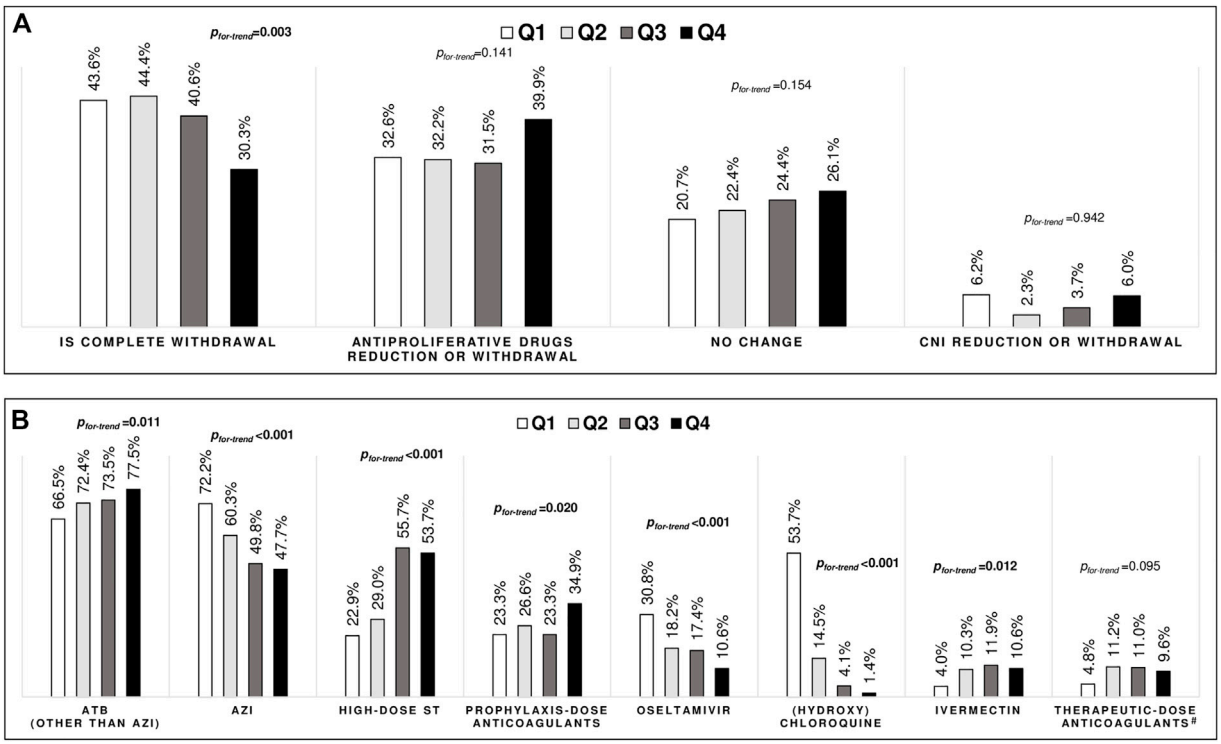


FIGURE 3 | Management of immunosuppressive drugs **(A)** and pharmacological treatments **(B)** across the quartiles. Legend: IS, immunosuppressive drugs; CNI, calcineurin inhibitor; ATB, antibiotics; AZI, azithromycin; ST, steroids. Trend analyses were performed using Cochran–Armitage test #Therapeutic-dose anticoagulants was empirically used for critically il patients with high d-dimer values, regardless of thrombosis events.

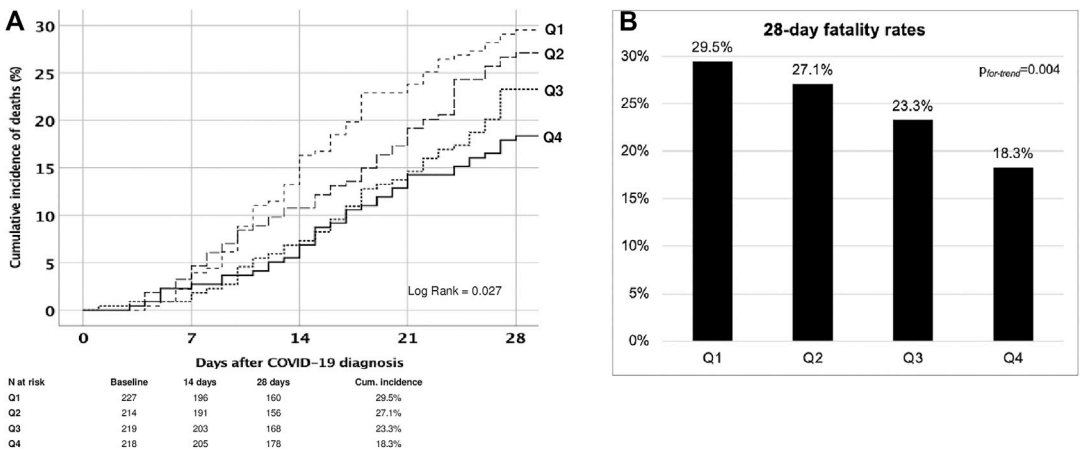


FIGURE 4 | Cumulative incidence of deaths of SARS-CoV-2-infected kidney transplant patients within 28 days. **(A)** and 28-day fatality rates **(B)** across the quartiles.

Immunosuppression and Pharmacological Treatment Across the Quartiles

Complete immunosuppressive drug withdrawal decreased from Q1 to Q4 (from 43.6 to 30.3%, $p_{for\text{-}trend}$ = 0.003), while no significant changes were observed in the percentage of patients submitted to withdrawal or reduction of the antiproliferative or calcineurin inhibitors agents, or no intervention on the immunosuppressive regimen (**Figure 3A**).

Regarding the pharmacological treatments, there was an increase in the use of antibiotics, high-dose steroids, prophylactic use of anticoagulants, and ivermectin, while the use of azithromycin, oseltamivir, chloroquine, or hydroxychloroquine decreased from Q1 to Q4 (**Figure 3B**).

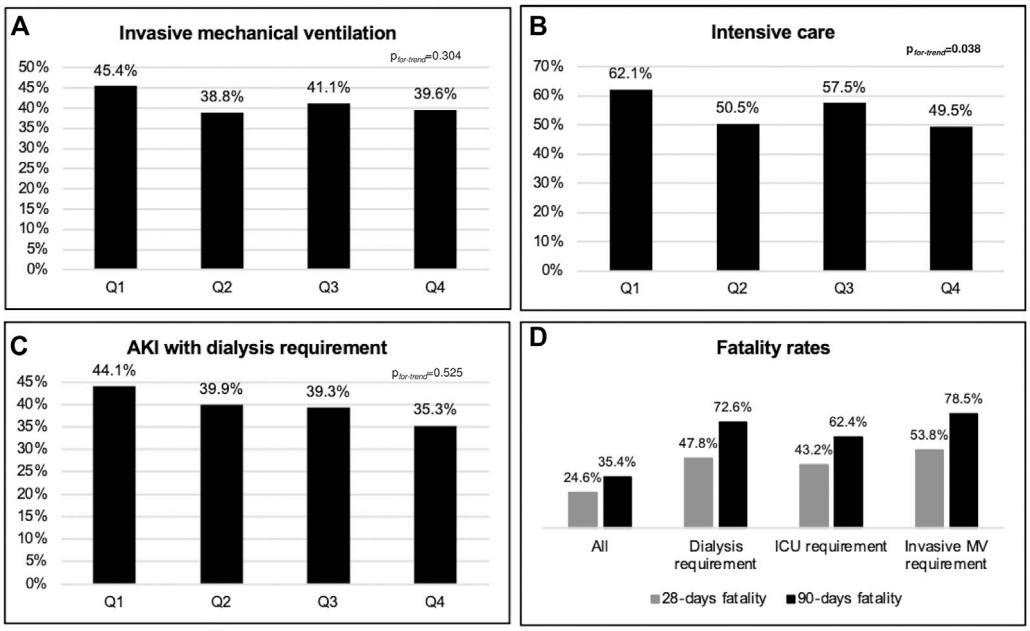


FIGURE 5 | Outcomes after SARS-CoV-2 infection in kidney transplant patients across the quartiles **(A–C)** and fatality rates **(D)**. Legend: AKI, acute kidney injury; ICU, intensive care unit; MV, mechanical ventilation. Trend analyses were performed using Cochran–Armitage test.

The Outcomes Across the Quartiles

The 28-day fatality rate was 24.6% (n = 216), with a significant downward trend over time, from 29.5% in Q1 to 18.3% in Q4 (log rank = 0.027, $p_{for\text{-}trend}$ = 0.004) (**Figures 4A,B**).

Causes of death within 28 days included septic shock (60.2%), acute respiratory failure (21.8%), cardiovascular or embolic event (5.1%), and in 13% the cause of death was not clearly defined nor registered. No difference in the distribution of the causes of death occurred from Q1 to Q4 ($p_{for\text{-}trend}$ = 0.677). Although 69.5% of deaths occurred in the first 28 days, the median time from COVID-19 diagnosis to death increased from 17 days (Q1) to 25 days (Q4) ($p_{for\text{-}trend}$ = 0.035). Within the 90-day follow-up, the overall fatality rate was 35.4% (n = 311), with a non-significant downward trend from 39.2 to 31.2% (Log-rank = 0.208, $p_{for\text{-}trend}$ = 0.073) (**Supplementary Figure S1**). Causes of death within 90 days were similar to that described for 28 days.

No changes were observed in the percentage of patients receiving invasive mechanical ventilation. However, the time from the onset of symptoms to orotracheal intubation increased from 8 to 11 days in median ($p_{for\text{-}trend}$ = <0.001), and fewer patients were admitted to intensive care units (ICU) over time (from 62.1 to 49.5%, $p_{for\text{-}trend}$ = 0.038) (**Figures 5A,B**). No significant trend was observed in the percentage of patients requiring dialysis therapy (**Figure 5C**).

Fourteen (1.6%) patients lost the graft within the 90 days follow-up, most of them with advanced chronic kidney disease at the time of COVID-19 diagnosis (median baseline eGFR 16.9 ml/min/1.73 m^2, IQR, 9.5–24.3) (**Supplementary Table S2**). **Figure 5D** shows the 28 and 90-day fatality rates in patients requiring dialysis therapy, ICU admission, and invasive mechanical ventilation.

Patients with COVID-19 diagnosis 140 days after the index case (Q4) showed a 35% reduction risk in 28-day mortality (HR 0.65, 95% CI 0.44–0.97, p = 0.037). Each month after March 3rd was associated with 10% reduction in the fatality (HR 0.90, 95% CI 0.82–0.99), p = 0.024). Age and presence of three or more comorbidities in addition to chronic kidney disease were also risk factors associated with increased risk of death, whereas the use of mTOR inhibitor and the increasing baseline glomerular filtration rate were associated with decreased risk of death (**Table 2**; **Supplementary Table S3**). The impact of timing on 90-day fatality was not clearly demonstrated (**Supplementary Table S4**).

DISCUSSION

This national multicenter cohort suggests that COVID-19-associated fatality decreased over the first 6 months after the beginning of the pandemic. Changes in the demographic profile of infected patients, in the clinical presentation at diagnosis, and in pharmacological and non-pharmacological treatment options might explain this result.

The overall fatality rate was high and similar to that described in international published cohorts (15, 16, 18, 22). As a novelty, this cohort showed that the cumulative incidence of death within 28 days after diagnosis significantly decreased over time, and deaths occurred later. Changes in the demographic profile, mainly the reduction in the percentage of patients with multiple comorbid conditions, probably contributed to this finding, since the number of comorbidities was an independent risk factor for death (3). Despite the statistically

TABLE 2 | Risk factors for 28-days fatality after COVID-19 infection in KT recipients.

***N* = 878**	**Univariable HR (95%CI), *p* value**	**Multivariable HR (95%CI), *p* value**
Age (×10 years-old)	1.49 (1.31–1.69), <0.001	**1.50 (1.32–1.70), <0.001**
Male gender	0.76 (0.57–1.00), 0.050	0.76 (0.58–1.00), 0.051
BMI (kg/m^2)	1.01 (0.98–1.04), 0.443	—
Afro-Brazilian or mixed-race ethnicity	0.92 (0.69–1.22), 0.568	—
Living donor	0.83 (0.57–1.19), 0.307	—
Timer after KT (years)	1.01 (0.98–1.03), 0.627	—
Number of comorbidities		
None	REF	**REF**
1 or 2	1.27 (0.75–2.16), 0.370	1.34 (0.80–2.23), 0.260
≥3	1.81 (1.00–3.28), 0.050	**1.96 (1.10–3.48), 0.022**
IS regimen – ST	0.72 (0.42–1.25), 0.248	—
IS regimen – CNI	0.90 (0.49–1.65), 0.722	—
IS regimen – MPA/AZA	1.15 (0.63–2.08), 0.649	—
IS regimen – mTORi	0.44 (0.26–0.75), 0.003	**0.44 (0.27–0.72), 0.001**
ST pulse therapy ≤3 months	1.55 (0.68–3.57), 0.297	—
rATG ≤3 months	1.10 (0.39–3.05), 0.860	—
RAS blockade	1.22 (0.89–1.67), 0.209	—
Baseline eGFR (×10 ml/min/1.73 m^2)	0.88 (0.82–0.94), <0.001	**0.87 (0.82–0.93), <0.001**
Quartiles of time after index case		
Q1: <72 days	REF	REF
Q2: 72–104 days	1.03 (0.72–1.48), 0.863	1.04 (0.73–1.48), 0.843
Q3: 105–140 days	0.75 (0.52–1.10), 0.145	0.80 (0.55–1.15), 0.228
Q4: >140 days	0.60 (0.40–0.90), 0.014	**0.65 (0.44–0.97), 0.037**

BMI, body mass index; KT, kidney transplant; IS, immunosuppressive; ST, steroid; MPA, mycophenolate; AZA, azathioprine; CNI, calcineurin inhibitor; mTORi, mammalian target of rapamycin inhibitor; rATG, rabbit anti-thymocyte globulin; RAS, renin-angiotensin system; eGFR, estimated glomerular filtration rate; HR, hazard ratio; CI, confidence interval; REF, reference. Bold values denote statistical significance at the p < *0.05 level.*

significant trend for higher BMI over time, we believe that this finding is not clinically relevant. The reasons for the changes in the demographic profile over the months are not clear. The wide dissemination of the worst prognosis on the elderly, and patients with comorbidities might have resulted in intensification of protective measures in these individuals.

Other factors that might have impacted outcomes were the changes in the recommendations of the health care organizations, the higher availability of diagnostics tests, and the learning curve about disease diagnosis and management, leading to earlier and broader diagnosis, properly referred hospitalization, or better management of pharmacological and non-pharmacological interventions. In fact, the reduction in the percentage of patients with dyspnea, hypoxemia, and radiological chest findings suggest earlier demand for medical assistance, earlier clinical suspicion and diagnosis, and/or earlier hospitalization. The median time until intubation was prolonged by 3 days, suggesting improvements in the optimal use of non-invasive ventilation techniques. Unfortunately, we did not capture information about ventilatory management before invasive mechanical ventilation. Noteworthy, the interpretation of the downward trend in ICU admission must be cautious, since the availability of ICU beds is not uniform across the country's centers and regions (2).

Interestingly, the improvement in the 90-day fatality was not evident. We believe that the 28-day mortality rate reflects disease severity, and prompt and proper diagnosis and treatment. In turn, 90-day mortality also seems to reflect intra-hospital care, such as preventing nosocomial infections, thromboembolic events, and other adverse events related to health care, malnutrition, and immobilization. Although these processes have probably also improved over the period, our study was not empowered to show this trend.

A clear change in the pharmacological supporting treatments was observed, which might also have impacted outcomes, mainly the higher use of high-dose steroids and anticoagulants (8, 9). The retrospective nature of a registry study, the absence of data on the onset of all interventions, and the diversity of COVID-19 management protocols in our continental country preclude any definitive conclusion about the efficacy of these strategies. We could not access information of patients who did not have access to medical care. The overwhelmed health system during the peaks of the pandemic could have hindered the arrival of more severe COVID-19 patients at the hospital, leading to deaths before hospitalization. In addition, despite the homogeneous number of patients in each quartile, groups have different duration, potentially hampering to capture the workload of periods with a higher incidence of cases and the effect of overwhelmed hospitals.

As another limitation, this study was limited to the first wave of the pandemic in Brazil, and reflected the pre-vaccination period. We do not have information on the viral genotype, which also might influence the clinical presentation and outcomes. However, at that time, the variants of concern leading to potential changes in the clinical profile and patients outcomes had not been identified yet (23). The imprecise definition of death cause in more than 10% of patients also impaired a better understanding of the reasons behind the reduction in fatality rates, as well as hampered the precise distinction between related and non-related COVID deaths.

It is also notable that a lower percentage of patients had their immunosuppressive regimen completely withdrawn over the study time. Despite plenty of *in vitro* studies suggesting the potential benefit of immunosuppressive drugs on the clinical outcomes of coronavirus infection (24–29), no clinical study supports robust conclusions. In the multivariable analysis, the use of mTOR inhibitors in the maintenance immunosuppressive regimen was associated with lower death risk. The reduction in SARS-CoV-2 replication after the inhibition of the Akt/mTOR/HIF-1 signaling pathway was previously demonstrated by a recently published *in vitro* study (29). However, no conclusion in this regard is feasible considering the limitation of the study design. Finally, despite the statistically significant linearly increasing trend through time, complex dynamics observed in some variables, such as the time between COVID-19 diagnosis and hospitalization, do not necessarily reflect clinically relevant changes.

Notwithstanding the above-mentioned limitations, inherent to registry data analysis, our study has important strengths: to the best of our knowledge, this is one of the largest multicenter national registers on COVID-19 in KT patients; the national representation is consistent with site activities and with COVID-19 incidence in the Brazilian States; a robust center-adjusted analysis was performed to minimize site-effect; and the selection of hospitalized patients only, excluding patients with mild COVID-19 forms, makes our sample more homogeneous as to the initial severity criterion.

In conclusion, this study suggests that the COVID-associated fatality in KT patients requiring hospitalization improved over the six first months of the pandemic. Prospective studies are of utmost needed to better understand the impact of each intervention on outcomes.

CAPSULE SENTENCE SUMMARY

This multicenter national Brazilian study accessed the fatality rates of COVID-19 among kidney transplanted patients over the first 6 months after the beginning of the pandemic. Using trend analysis, we could observe a decrease in the fatality rates from March to August 2020. A center-adjusted analysis was performed to explore the reasons for the improvement in the outcomes. Differences in demographics, clinical presentation, and treatment options might be involved in this trend.

THE COVID-19-KT BRAZIL STUDY GROUP

Beyond the authors, the COVID-19-KT Brazil Study Group includes the following participants: Roger Kist[8], Aline Lima Cunha Alcântara[2], Maria Luiza de Mattos Brito Oliveira Sales[3], Mario Abbud Filho[9], Katia Cronenberge Sousa[10], Roberto Ceratti Manfro[11], Tomás Pereira Júnior[12], Maria Eduarda Heinzen de Almeida Coelho[13], Marilda Mazzali[21], Marcos Vinicius de Sousa[21], Juliana Bastos Campos[14], Nicole Gomes Campos Rocha[15], Tania Leme da Rocha Martinez[17], Joao Egidio Romao Junior[17], Maria Regina Teixeira Araújo[17], Sibele Lessa Braga[17], Marcos Alexandre Vieira[16], Elen Almeida Romão[22], Miguel Moysés Neto[22], Juliana Aparecida Zanocco[23], Auro Buffani Claudino[23], Gustavo Guilherme Queiroz Arimatea[19], Tereza Azevedo Matuck[20], Alexandre Tortoza Bignelli[24], Maria Ferneda Puerari[24], José Hermógenes Rocco Suassuna[25], Suzimar da Silveira Rioja[25], Rafael Lage Madeira[26], Sandra Simone Vilaça[26], Carlos Alberto Chalabi Calazans[27], Daniel Costa Chalabi Calazans[27], Patricia Malafronte[28], Luiz Antonio Miorin[28], Larissa Guedes da Fonte Andrade[29], Filipe Carrilho de Aguiar[29], Fabiana Loss de Carvalho Contieri[30], Karoline Sesiuk Martins[30], Helady Sanders Pinheiro[31], Emiliana Spadarotto Sertório[31], André Barreto Pereira[32], David Jose[9]; Barros Machado[33], Carolina Maria Pozzi[34], Leonardo Viliano Kroth[34], Lauro Monteiro Vasconcellos Filho[36], Rafael Fabio Maciel[37], Amanda Maíra Damasceno Silva[38], Ana Paula Maia Baptista[39], Pedro Augusto Macedo de Souza[40], Marcus Lasmar[41], Luciana Tanajura Santamaria Saber[42], Lilian Palma[43].

[21]Hospital de Clínicas da Universidade de Campinas-UNICAMP, Campinas, SP, Brazil; [22]Divisão de Nefrologia, Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo (FMRP-USP), Ribeirão Preto, SP, Brazil; [23]Hospital Santa Marcelina, São Paulo, SP, Brazil; [24]Hospital Universitário Cajuru, Curitiba, PR, Brazil; [25]Hospital Universitário Pedro Ernesto, Rio de Janeiro, RJ, Brazil; [26]Hospital Felício Rocho, Belo Horizonte, BH, Brazil; [27]Hospital Marcio Cunha, Ipatinga, MG, Brazil; [28]Santa Casa de Misericórdia de São Paulo, São Paulo, SP, Brazil; [29]Hospital das Clínicas da UFPE Universidade Federal de Pernambuco, Recife, PE, Brazil; [30]Hospital do Rocio, Campo Largo, PR, Brazil; [31]Hospital Universitário da Universidade Federal de Juiz de Fora, Juiz de Fora, MG, Brazil; [32]Hospital Marieta Konder Bornhausen, Itajai, SC, Brazil; [33]Hospital Alemão Osvaldo Cruz, São Paulo, SP, Brazil; [34]Hospital Evangélico, Curitiba, PR, Brazil; [35]Hospital São Lucas da PUCRS, Porto Alegre, RS, Brazil; [36]Hospital Meridional, Cariacica, ES, Brazil; [37]Hospital Nossa Senhora das Neves, João Pessoa, PB, Brazil; [38]Hospital Antonio Targino, Campina Grande, PB, Brazil; [39]Hospital São Rafael, Salvador, BA, Brazil; [40]Santa Casa de Misericórdia de Belo Horizonte, Belo Horizonte, MG, Brazil; [41]Hospital Universitário Ciências Médicas, Belo Horizonte, MG, Brazil; [42]Santa Casa de Misericórdia de Ribeirão Preto, Ribeirão Preto, SP, Brazil; [43]Centro Médico de Campinas, Campinas, SP, Brazil.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions 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 Institutional Review Board (IRB) of the Hospital do Rim/Fundação Oswaldo Ramos, from where the study was coordinated and for the National Commission for Research Ethics (approval number 4.033.525). All participating centers also obtained local IRB approval before data collection. Informed consent or its exemption followed specific national legislations,

the local IRB recommendations, and the guidelines of the Declaration of Helsinki. Patient records and information were anonymized and de-identified before the analysis.

AUTHOR CONTRIBUTIONS

Participated in research design, in the performance of the research, in the writing of the paper, and data analysis and analytic tools: TS-F, MC, LR-M, LA, LV, JM-P, HT. Participated in the performance of the research and in the reviewing of the paper: VG, CO, RE, PL, IC, TF, RF, KC, DS, GF, VS, RA, LD, AS, IN, LO, DC, RO.

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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.

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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.2022.10205/full#supplementary-material

Supplementary Figure S1 | Cumulative incidence of deaths of SARS-CoV-2-infected kidney transplant patients within 90 days.

Supplementary Table S1 | Laboratory tests and chest radiological findings of kidney transplanted patients at COVID-19 diagnosis across quartiles of time.

Supplementary Table S2 | Graft losses after COVID-19 diagnosis.

Supplementary Table S3 | Risk factors for 28-days fatality after COVID-19 infection in KT recipients.

Supplementary Table S4 | Risk factors for 90-days fatality after COVID-19 infection in KT recipients.

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LETTER TO THE EDITOR
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Fatal Early-Onset Aspergillosis in a Recipient Receiving Lungs From a Marijuana-Smoking Donor: A Word of Caution

Eleonora Faccioli[1], Federica Pezzuto[2], Andrea Dell'Amore[1], Francesca Lunardi[2], Chiara Giraudo[3], Marco Mammana[1], Marco Schiavon[1], Antonello Cirnelli[4], Monica Loy[1], Fiorella Calabrese[2] and Federico Rea[1]*

[1]Thoracic Surgery and Lung Transplant Unit, Department of Cardiac, Thoracic, Vascular Sciences and Public Health, University of Padua, Padua, Italy, [2]Pathology Unit, Department of Cardiac, Thoracic, Vascular Sciences and Public Health, University of Padua, Padua, Italy, [3]Radiology Unit, Department of Medicine, University of Padua, Padua, Italy, [4]Forensic Pathology Center "CML", Portogruaro, Italy

Keywords: lung transplantation, Aspergillosis, marijuana-smoking donor, infection, fatal outcome

Dear editors,

We are aware that donors tobacco smoking history is quite common in the lung donor pool and several studies have investigated this aspect in order to understand whether this habit may influence the outcomes of recipients transplanted with lungs from smoking-donors (1,2). At the same time, there is very little literature focusing on donors' marijuana smoking history as a factor affecting lung transplant (LTx) outcomes with conflicting results on early and intermediate (3,4) lung transplant outcomes.

We would like to focus the attention of the clinicians involved in LTx on a case of a 50 years-old patient, affected by idiopathic pulmonary fibrosis in therapy with nintedanib, who underwent bilateral lung transplantation at our Unit.

The donor was a 21 year-old male patient, admitted to the Intensive Care Unit (ICU) for a traumatic brain hemorrhage, with an unremarkable medical history except for cannabis abuse. Oto Score was 0 and all microbiological tests were negative.

The lung transplantation was performed with the usual surgical technique and peri and post-operative antibacterial prophylaxis was performed with combined antibiotics.

Antifungal and Cytomegalovirus prophylaxis and immunosuppressive therapy were based on aerosolized amphotericin B, ganciclovir and corticosteroids, mycophenolate mofetil, and cyclosporine respectively.

During the post-surgical phase, one blood culture was positive for *Staphilococcus Epidermidis* and two bronchial aspirates were positive for *Acinetobacter baumannii* and *Klebsiella pneumonia*, respectively.

Since the clinical conditions of the recipient were progressively improving, he was considered ready to be discharged. Before discharge, he underwent a bronchoscopy to perform surveillance trans-bronchial biopsies. The sample was insufficient. The histological examination showed diffuse alveolar damage and organizing pneumonia, as signs of ischemia reperfusion injury, while neither acute cellular rejection/lymphocytic bronchiolitis, infection, or marijuana-related lesions were detected.

The day after the procedure, the recipient presented a massive hemoptysis with cardiac arrest that required re-intubation and re-admission to the ICU. Since then, numerous episodes of hemoptysis have occurred and the patient died 10 days later because of hypovolemic shock.

Abbreviations: ICU, intensive care unit; IPA, invasive pulmonary aspergillosis; LTx, lung transplantation

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Correspondence:
Eleonora Faccioli
faccioli.eleonora@gmail.com
eleonora.faccioli@unipd.it

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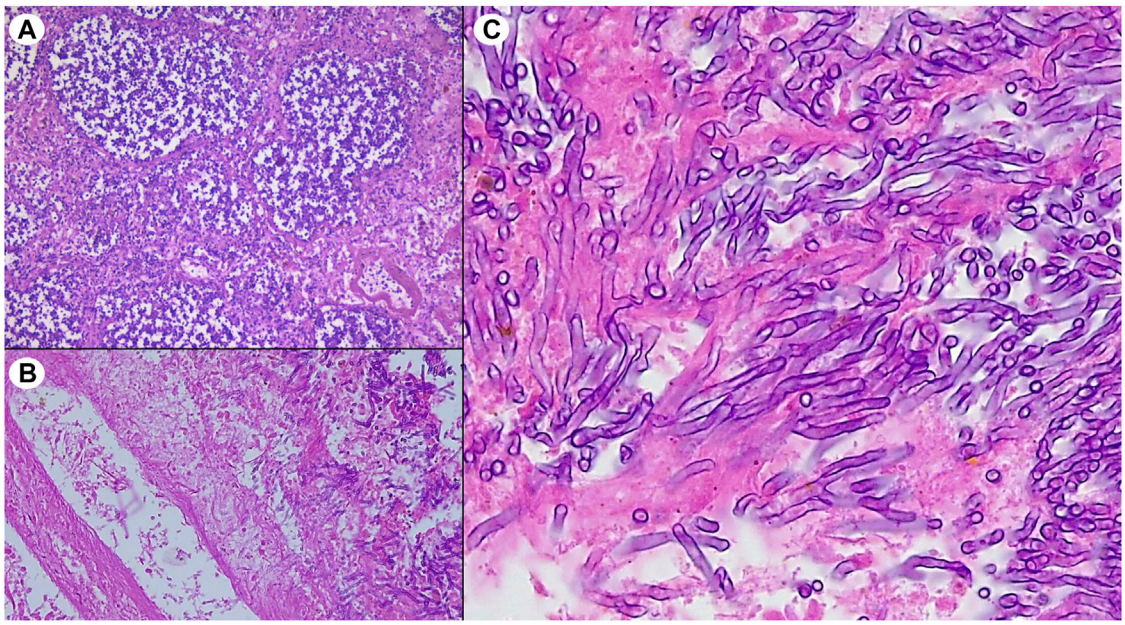

FIGURE 1 | Histological lung sections from recipient's autopsy showing **(A)** multiple foci of bronchopneumonia, **(B)** vascular erosion associated with widespread blood extravasation and, **(C)** well recognizable fungal branched hyphae compatible with *Aspergillus spp*.

A CT scan, performed the day before the exitus, showed multiple bilateral nodules which have been due to the hemorrhagic episodes and a small wedge-shaped cavitated lesion (arrow) could suggest, ex post, a possible aspergillosis (**Supplementary Figure 1**). An autopsy was then performed and histological examination of the lungs revealed an invasive pulmonary aspergillosis (IPA) (**Figure 1**) and smoking-related lesions (chronic bronchiolitis/bronchitis with infiltration of heavily pigmented macrophages) in the few evaluable areas. A timeline describing all the events is represented in **Supplementary Figure 2**.

A correlation between inhalation of marijuana and IPA has already been reported in renal (5) and bone marrow recipients (6) but, to the best of our knowledge, this is the first report of fatal early onset IPA in a patient who received lungs from a donor with ongoing marijuana use. We believe that, in our patient, a correlation between the donor's marijuana smoking history and IPA could be supposed since no other explanation justified the development of such clinical picture.

However, it must be taken into account that such a clinical manifestation is anecdotal also considering the increasing prevalence of cannabis use between 2010 and 2019 in Europe (+27% in the population between 15 and 64 years) (7).

Despite this, since organ donors are often included in this age group, we would like to raise awarness in clinicians suggesting an accurate evaluation of the lungs retrieved from donors with ongoing marijuana abuse.

In case of young donors with cannabis smoking history, the pre-emptive research of fungi (especially Aspergillus) on biological samples should always be encouraged. At the same time, more sensitive tools, like polymerase chain reactions, could help in the early detection of Aspergillus in recipients with bleeding unrelated to the surgical procedure undergone.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

EF and FP designed the research and wrote the paper. AD'A, FL, CG, MM, MS, AC, and ML collected the data. FC and FR revised the paper.

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.2022.10070/full#supplementary-material

Supplementary Figure 1 | CT scan iperformed the day before the exitus

Supplementary Figure 2 | timeline with representation of the events

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Editorial Office

Avenue du Tribunal Fédéral 34
CH – 1005 Lausanne
Switzerland

Tel +41 (0)21 510 17 40
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