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Table of contents

Transplant Trial Watch

10 Transplant Trial Watch

DOI: 10.3389/ti.2022.10513

John M. O'Callaghan

Point of View

13 Comparing Deceased Organ Donation Performance in Two Countries that Use Different Metrics: Comparing Apples With Apples

DOI: 10.3389/ti.2022.10461

Luke Milross, Chloe Brown, Laura Gladkis, Kylie Downes, Melissa Goodwin, Susanna Madden, Mark McDonald, Lucinda Barry, Helen Opdam, Alex Manara and Dale Gardiner

Organ donation networks audit donation activity to optimise performance however this process differs between organisations making direct data comparisons difficult. This collaboration between the UK and Australian donation networks assesses the comparability of the definitions and metrics used and reflects on the potential benefits of their convergent evolution.

21 Demonstrating Benefit-Risk Profiles of Novel Therapeutic Strategies in Kidney Transplantation: Opportunities and Challenges of Real-World Evidence

DOI: 10.3389/ti.2022.10329

Ilkka Helanterä, Jon Snyder, Anders Åsberg, Josep Maria Cruzado, Samira Bell, Christophe Legendre, Hélio Tedesco-Silva Jr, Giovanna Tedesco Barcelos, Yvonne Geissbühler, Luis Prieto, Jennifer B. Christian, Erik Scalfaro and Nancy A. Dreyer

Innovative clinical trial designs incorporating real-world evidence can facilitate longitudinal transplant research and timely translation to clinical practice. Registry collaboration and harmonization along with technology innovations for data sharing with high privacy standards are needed to aid development of clinically meaningful endpoints and data models for measuring long-term patient outcomes.

Review

29 Kidney Transplantation From Hepatitis-C Viraemic Donors: Considerations for Practice in the United Kingdom

DOI: 10.3389/ti.2022.10277

Daniel T. Doherty, Varinder Athwal, Zia Moinuddin, Titus Augustine, Martin Prince, David van Dellen and Hussein A. Khambalia

Peri-operative DAAs have successfully been employed in KT to mitigate HCV transmission from viraemic donors in clinical trials. Carefully incorporating this strategy into the UK transplant framework has the opportunity to expand the donor pool and increase organ utilisation.

Systematic Review and Meta-Analysis

- 38 **Hyperammonemia After Lung Transplantation: Systematic Review and a Mini Case Series**
DOI: 10.3389/ti.2022.10433
Amir Y. Kamel, Amir M. Emtiazjoo, Lauren Adkins, Abbas Shahmohammadi, Hassan Alnuaimat, Andres Pelaez, Tiago Machuca, Mauricio Pipkin, Hyun-wook Lee, David I. Weiner and Satish Chandrashekar
Hyperammonemia after lung transplant (HALT) is rare but fatal complication. This a systematic literature review on topic for the past 25 years and our center experience using alternative successful approach to HALT management. Metabolic aminoassay analysis of index cases was also performed to determine if HALT impacted urea cycle pathway.
- 51 **Perfusate Composition and Duration of Ex-Vivo Normothermic Perfusion in Kidney Transplantation: A Systematic Review**
DOI: 10.3389/ti.2022.10236
Amir Fard, Robert Pearson, Rashida Lathan, Patrick B. Mark and Marc J. Clancy
Normothermic perfusion is an emerging strategy in kidney preservation for organ viability assessment. This systematic review outlines the current evidence on the constituents and characteristics of the perfusates used and highlights areas where more research is required.

Cover Article

- 65 **A National Survey Comparing Patients' and Transplant Professionals' Research Priorities in the Swiss Transplant Cohort Study**
DOI: 10.3389/ti.2022.10255
Sonja Beckmann, Oliver Mauthner, Liz Schick, Jessica Rochat, Christian Lovis, Annette Boehler, Isabelle Binet, Uyen Huynh-Do, Sabina De Geest, the Psychosocial Interest Group, and the Swiss Transplant Cohort Study
Based on the insights of solid organ transplant patients, we identified 13 research priorities on all 4 levels of Bronfenbrenner's ecological framework. Our results highlight the necessity to expand research beyond the patient level and examine topics related to social interactions, practice patterns and the policy level.

Original Research

- 75 **Prevalence of Blood-Borne Viruses and Predictors of Risk in Potential Organ Donors in Australia**
DOI: 10.3389/ti.2022.10395
Martin J. Dutch, Cameron J. Patrick, Peter A. Boan, Jonathan C. Knott and Helen I. Opdam
This retrospective Australian audit of potential organ donors demonstrated increased viral behaviours were associated with a higher prevalence of HCV, but not of HBV or HIV. The majority of HBV and HIV infections occurred in donors without known increased risk behaviours.

85 Radiological Screening Methods in Deceased Organ Donation: An Overview of Guidelines Worldwide

DOI: 10.3389/ti.2022.10289

K. A. Chotkan, J. W. Mensink, R. A. Pol, N. P. Van Der Kaaij, L. F. M. Beenen, W. N. Nijboer, B. Schaefer, I. P. J. Alwayn and A. E. Braat

Although organ transplantation is performed worldwide, policies regarding donor assessment and imaging are not uniform. An overview of the policies and underlying arguments in different regions of the world could provide valuable information for countries who are thinking about changing their policy. This study aim to provide such an overview.

95 Hyperspectral Imaging as a Tool for Viability Assessment During Normothermic Machine Perfusion of Human Livers: A Proof of Concept Pilot Study

DOI: 10.3389/ti.2022.10355

Margot Fodor, Lukas Lanser, Julia Hofmann, Giorgi Otarashvili, Marlene Pühringer, Benno Cardini, Rupert Oberhuber, Thomas Resch, Annemarie Weissenbacher, Manuel Maglione, Christian Margreiter, Philipp Zelger, Johannes D. Pallua, Dietmar Öfner, Robert Sucher, Theresa Hautz and Stefan Schneeberger

Normothermic machine perfusion (NMP) allows for ex vivo viability and functional assessment prior to liver transplantation (LT). Hyperspectral imaging represents a suitable, non-invasive method to evaluate tissue morphology and organ perfusion during NMP. This study provides first evidence of feasibility of hyperspectral imaging as a potentially helpful contact-free organ viability assessment tool during liver NMP.

106 Mortality and Causes of Death After Liver Transplantation: Analysis of Sex Differences in a Large Nationwide Cohort

DOI: 10.3389/ti.2022.10263

M. Trinidad Serrano, Sergio Sabroso, Luis M. Esteban, Marina Berenguer, Constantino Fondevila, Sara Lorente, Luis Cortés, Gloria Sanchez-Antolin, Javier Nuño, Gloria De la Rosa and Magdalena Salcedo, on behalf of the Spanish Liver Transplant Registry

Sex-differences in Liver transplantation outcomes: male patients have lower short-term mortality than females but higher long-term mortality. In addition, the post-LT mortality risk related to previous liver disease and the causes of mortality differ between males and females.

- 118 Postoperative Trapped Lung After Orthotopic Liver Transplantation is a Predictor of Increased Mortality**
DOI: 10.3389/ti.2022.10387
Natasha Cuk, Kathryn H. Melamed, Sitaram Vangala, Ramy Salah, W. Dwight Miller, Sarah Swanson, David Dai, Zarah Antongiorgi, Tisha Wang, Vatche G. Agopian, Joseph Dinorcica, Douglas G. Farmer, Jane Yanagawa, Fady M. Kaldas and Igor Barjaktarevic
Pleural effusions are a common complication of orthotopic liver transplantation. We found that among these effusions, trapped lung was associated with higher morbidity, mortality, and healthcare utilization. This finding should be examined in future study to better inform strategies to assess risk for transplant candidacy and optimize management after transplantation.
- 126 Incidence and Outcomes of Early Cancers After Kidney Transplantation**
DOI: 10.3389/ti.2022.10024
A. Krishnan, G. Wong, A. Teixeira-Pinto and W. H. Lim,
Early onset de novo cancers (within 12-months of kidney transplantation) occur infrequently but are associated with extremely poor survival. While post-transplant lymphoproliferative disease, urinary tract cancers and malignant melanomas were the most frequent cancer types, lung and colon cancers tend to present with metastatic disease. Nearly 1 in 3 patients die with most deaths attributed to cancer-related deaths.
- 137 Clinical Significances of Anti-Collagen Type I and Type III Antibodies in Antibody-Mediated Rejection**
DOI: 10.3389/ti.2022.10099
Sehoon Park, Seung-Hee Yang, Jiyeon Kim, Semin Cho, Jaeseok Yang, Sang-Il Min, Jongwon Ha, Chang Wook Jeong, Seong Hee Bhoo, Yong Chul Kim, Dong Ki Kim, Kook-Hwan Oh, Kwon Wook Joo, Yon Su Kim, Kyung Chul Moon, Eun Young Song and Hajeong Lee
By performing non-HLA antibody screening for antibody-mediated rejection cases and controls, this study identified that anti-collagen type I or III antibody was associated with antibody-mediated rejection and its' prognosis. Measurements for anti-collagen type I or type III antibody in kidney transplant recipients may be helpful for diagnosis of antibody-mediated rejection.
- 149 The MUC5B Promoter Polymorphism is Not Associated With Non-ILD Chronic Respiratory Diseases or Post-transplant Outcome**
DOI: 10.3389/ti.2022.10159
Tinne Goos, Stijn E. Verleden, Laurens J. De Sadeleer, Anke Van Herck, Annelore Sacreas, Arno Vanstapel, Janne Kaes, Vincent Geudens, Celine Aelbrecht, David Ruttens, Diether Lambrechts, Sascha Vermeer, Laurens J. Ceulemans, Dirk E. Van Raemdonck, Laurent Godinas, Jonas Yserbyt, Bart M. Vanaudenaerde, Geert M. Verleden, Robin Vos and Wim A. Wuyts
This study shows firstly that the MUC5B promoter polymorphism is only associated with pulmonary fibrosis and not with other chronic respiratory diseases. Secondly, recipient MUC5B promoter polymorphism does not play a role in post-transplant outcome.

Brief Research Report

158 Role of the Intramural Vascular Network of the Extrahepatic Bile Duct for the Blood Circulation in the Recipient Extrahepatic Bile Duct Used for Duct-to-Duct-Biliary-Anastomosis in Living Donor Liver Transplantation

DOI: 10.3389/ti.2022.10276

Naotaka Yamaguchi, Ryusei Matsuyama, Yutaro Kikuchi, Sho Sato, Yasuhiro Yabushita, Yu Sawada, Yuki Homma, Takafumi Kumamoto, Kazuhisa Takeda, Daisuke Morioka, Itaru Endo and Hiroshi Shimada

We examined axial-sections of the extrahepatic-bile-duct (EBD) microscopically with 5-mm-intervals of 10

formalin-fixed-deceased-livers to examine the

intramural-vascular-network of the EBD. This study demonstrated that there is the significant distributional heterogeneity of the intramural-vessels of the EBD; this can cause the ischemia of the anastomotic site of the EBD in liver-transplantation.

165 Increased Tacrolimus Exposure in Kidney Transplant Recipients With COVID-19: Inflammation-Driven Downregulation of Metabolism as a Potential Mechanism

DOI: 10.3389/ti.2022.10269

Sylvia D. Klomp, Soufian Meziyerh, Maurits F. J. M. Vissers, Dirk J. A. R. Moes, Eline J. Arends, Y. K. Onno Teng, Jesse J. Swen and Aiko P. J. de Vries

COVID-19 can result in increased tacrolimus exposure in kidney transplant recipients, potentially caused by inflammation-driven downregulation of cytochrome P450 metabolism. Consequently, during COVID-19 kidney transplantation recipients are at risk for tacrolimus overexposure, warranting increased vigilance and therapeutic drug monitoring in spite of social distancing or isolation recommendations.

In Memoriam

174 In Memoriam: Professor Paolo Muiesan (1961–2022)

DOI: 10.3389/ti.2022.10595

Constantino Fondevila, Luciano Potena, Umberto Cillo, Gabriel Oniscu and the ELITA Board, on behalf of the ESOT Council



TRANSPLANT LIVE

ONLINE EDUCATION



Transplant Live is the online education platform of the European Society for Organ Transplantation (ESOT). We are strongly committed to offering high-quality, easily accessible education opportunities to the transplant community worldwide.

A wealth of resources is available on this platform: EACCME-accredited online courses, case studies, the best content from ESOT's scientific meetings including the ESOT Congress and TLJ, a media library, and much more. Start exploring now and learn more about the educational opportunities offered by Transplant Live.



Consensus Conference

At TLJ 3.0, participants will be introduced to nine key transplantation topics following a systematic review. Delegates will convene for three days of high-quality debate, discussion and exploration in order to finalise a series of consensus reports that will be submitted for publication.

MACHINE PERFUSION IN CARDIOTHORACIC TRANSPLANTATION

HISTOPATHOLOGICAL ANALYSIS OF PRE-IMPLANTATION DONOR KIDNEY BIOPSY: REDEFINING THE ROLE IN THE PROCESS OF GRAFT ASSESSMENT

THE VALUE OF MONITORING (SUBCLINICAL) DSA'S FOR TRANSPLANT OUTCOMES

LIVER TRANSPLANTATION IN PATIENTS WITH PRIMARY SCLEROSING CHOLANGITIS (PSC) AND INFLAMMATORY BOWEL DISEASE (IBD)

CLINICAL ENDPOINTS IN LIVER TRANSPLANTATION ACCORDING TO VALUE BASED CARE

ROLE OF PANCREAS MACHINE PERFUSION TO INCREASE THE DONOR POOL FOR BETA CELL REPLACEMENT

DOWNSTAGING, BRIDGING AND IMMUNOTHERAPY IN LIVER TRANSPLANTATION FOR HCC

PREHABILITATION FOR SOLID ORGAN TRANSPLANT CANDIDATES

MOLECULAR BIOLOGY TESTING FOR NON-INVASIVE DIAGNOSIS OF ALLOGRAFT RE

and

EDUCATIONAL WORKSHOP: GUIDELINES DEVELOPMENT

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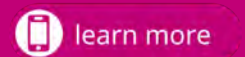
The Netherlands

3rd
ECTORS
meeting

29-30
September 2022

#ESOT_ECTORS

#ECTORSmeeting



The main topics for 3rd ECTORS meeting will be:

- Stem cells
- organoids
- machine perfusion
- regeneration

Learning Objectives:

- Hear the latest developments in clinical regeneration
- Get updated on immunomodulatory cell therapy in transplantation
- Be informed about the introduction of cell therapy in machine perfusion
- Learn about novel developments in organoid research

Target Group:

Researchers and clinicians from the transplant field interested in regenerative medicine



Transplant Trial Watch

John M. O'Callaghan^{1,2*}

¹University Hospitals Coventry and Warwickshire, Coventry, United Kingdom, ²Centre for Evidence in Transplantation, Nuffield Department of Surgical Sciences, University of Oxford, Oxford, United Kingdom

Keywords: kidney transplantation, liver transplantation, everolimus, CMV infection, machine perfusion

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

RANDOMISED CONTROLLED TRIAL 1

Incidence of Cytomegalovirus Infection in Seropositive Kidney Transplant Recipients Treated with Everolimus: a randomized, open-labelled, multicentre phase 4 trial.

by Kaminski, H., et al. *American Journal of Transplantation* [record in progress].

Aims

The aim of this study was to investigate whether everolimus (EVR)-based immunosuppression leads to a decrease in the incidence of cytomegalovirus (CMV) DNAemia and disease.

Interventions

Participants were randomised to receive either EVR or mycophenolic acid (MPA) combined with basiliximab, cyclosporin and steroids.

Participants

186 CMV seropositive renal transplant recipients.

Outcomes

The primary outcomes were CMV treatment, CMV DNAemia, patient death, graft loss and discontinuation of the study at 6 months following transplantation. The secondary outcomes were maximal viral load, the CMV treatment failure, proportion of patients with CMV disease, and the incidence of CMV mutations (UL97 or UL54) associated with a resistance to an anti-CMV therapy.

Follow-up

12 months.

CET Conclusion

This large, multicentre phase 4 RCT aimed to demonstrate whether everolimus-based immunosuppression is associated with a reduction in CMV viraemia following renal



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transplantation. Seropositive recipients were randomised to either everolimus or mycophenolic acid, in conjunction with cyclosporin and steroids. CMV prophylaxis was not used. The study used a composite primary endpoint of CMV treatment, graft loss, death and discontinuation, and showed that in intent-to-treat analysis there was a significant reduction in this endpoint with everolimus. This was driven mainly by a reduction in CMV DNAemia in the everolimus arm. The study was stopped early due to findings from the ATHENA study that CsA and everolimus is associated with increased incidence of acute rejection—a finding that was not replicated in the present study. Nonetheless, as CMV infection rates were higher than anticipated the study has sufficient statistical power to demonstrate differences in outcome. Similar to previous studies, everolimus was poorly tolerated and benefit will be limited to those patients who can tolerate and maintain treatment. It is unclear how the present strategy compares to universal prophylaxis with more standard immunosuppression.

Jadad Score

3.

Data Analysis

Modified intention-to-treat analysis.

Allocation Concealment

Yes.

Trial Registration

ClinicalTrials.gov - NCT02328963.

Funding Source

Industry funded.

RANDOMISED CONTROLLED TRIAL 2

Impact of Portable Normothermic Blood-Based Machine Perfusion on Outcomes of Liver Transplant: The OCS Liver PROTECT Randomized Clinical Trial.

by Markmann, J. F., et al. *JAMA Surgery* 2022; 157 (3):189–198.

Aims

The aim of this study was to investigate liver transplant outcomes associated with portable normothermic machine perfusion preservation of livers obtained from deceased donors.

Interventions

Participants were randomised to either the Organ Care System (OCS) group or ischemic cold storage (ICS) group.

Participants

300 recipients receiving donor livers preserved using ICS or the OCS.

Outcomes

The primary effectiveness outcome was the incidence of early allograft dysfunction (EAD). Secondary outcomes were extent of reperfusion syndrome, OCS Liver *ex vivo* assessment capability of donor allografts, incidence of ischemic biliary complications (IBCs) at 6 and 12 months, and overall patient survival posttransplant. The primary safety outcome was the number of severe adverse events related to the liver graft within 30 days following transplantation.

Follow-up

1 year.

CET Conclusions

This is an interesting and well-conducted, multicentre study in liver transplantation using a normothermic preservation machine (OCS). The study was adequately randomised and, understandably, clinicians could not be blinded to the group allocation, the comparator being standard cold storage on ice. However, good steps were taken to re-randomise patients if a first liver was subsequently not suitable for transplant. The donor population for inclusion was selected on the basis of at least one of the following criteria: 40 years of age or older; expected total cross-clamp/cold ischemic time of six or more hours; DCD donors if 55 years or younger; or macrosteatotic livers ($\leq 40\%$). The primary endpoint was early allograft dysfunction (EAD) using the Olthoff definition. Mean perfusion time on the machine was 117 min, 152/155 preserved in this way were transplanted. However, there was a significantly higher proportion of DCD livers transplanted from the OCS group than the cold storage group (51% versus 26%). There were 298 patients included in the modified intention to treat analysis, which showed a significant decrease in EAD when the OCS machine was used compared to standard cold storage. Short term patient and graft survival was equivalent but ischaemic biliary lesions were significantly reduced with OCS by 6 and 12 months (2.6% versus 9.9%) and recipients experienced fewer incidences of severe reperfusion injury.

Jadad Score

3.

Data Analysis

Per protocol analysis.

Allocation Concealment

Yes.

Trial Registration

ClinicalTrials.gov - NCT02522871.

Funding Source

Industry funded.

CLINICAL IMPACT SUMMARY

This is a well-conducted, multicentre study in liver transplantation using a normothermic preservation machine (The OCS Liver from TransMedics, MA, United States). The study took place over a period of approximately 3 years at 20 centres in the United States. The study targeted organs that had risk factors for early allograft dysfunction (EAD), such as older donor age, moderate steatosis, or anticipated long cold ischaemic time.

The study was adequately randomised and, understandably, clinicians could not be blinded to the group allocation. If a liver was found to be not suitable for transplantation, then the recipient was randomised a second time. This, to some extent, mediates any potential bias that might be introduced when clinicians could not be easily blinded to the preservation method.

Mean perfusion time on the machine was 117 min. The total preservation time for machine perfused livers was on average longer than the control group at 455 min compared to 339 min. Approximately 10% of livers randomised to OCS cross over to the other arm and were preserved with cold storage instead due to: accessory vessels, vascular reconstruction, or liver haematoma. However, the results from the intention to treat analysis were very similar to the per protocol analysis regardless, suggesting that there was no systematic bias introduced.

Reassuringly 98% of livers preserved on the machine were successfully transplanted; those not transplanted were not used following assessment on the machine, showing poor lactate clearance or fibrosis on biopsy. There was a significantly higher proportion of DCD livers transplanted from the machine perfusion group than the cold storage group (51% versus 26%).

The analysis showed a significant decrease in early allograft dysfunction (EAD, using the Olthoff definition) when the OCS machine was used. Short term patient and graft survival was equivalent but ischaemic biliary lesions were significantly reduced with OCS by 6 and 12 months (2.6% versus 9.9%).

This study shows the safety of this technology in liver preservation and how it can potentially give greater confidence to transplant livers following DCD or marginal DBD. Despite the greater proportion of DCD livers in the OCS machine group, and the longer overall preservation time, there was a lower incidence of severe reperfusion injury, EAD and ischaemic biliary lesions. This study adds weight to the improved preservation possible with normothermic machines, and the confidence in organ viability when using this platform.

AUTHOR CONTRIBUTIONS

JO'C wrote the clinical impact summary.

CONFLICT OF INTEREST

The author declares that this clinical impact summary has been written in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Comparing Deceased Organ Donation Performance in Two Countries that Use Different Metrics: Comparing Apples With Apples

Luke Milross^{1*}, Chloe Brown¹, Laura Gladkis², Kylie Downes², Melissa Goodwin², Susanna Madden¹, Mark McDonald², Lucinda Barry², Helen Opdam², Alex Manara¹ and Dale Gardiner¹

¹Organ and Tissue Donation and Transplantation, NHS Blood and Transplant, Bristol, United Kingdom, ²Organ and Tissue Authority, Canberra, NSW, Australia

Organ donation networks audit and report on national or regional organ donation performance, however there are inconsistencies in the metrics and definitions used, rendering comparisons difficult or inappropriate. This is despite multiple attempts exploring the possibility for convergently evolving audits so that collectives of donation networks might transparently share data and practice and then target system interventions. This paper represents a collaboration between the United Kingdom and Australian organ donation organisations which aimed to understand the intricacies of our respective auditing systems, compare the metrics and definitions they employ and ultimately assess their level of comparability. This point of view outlines the historical context underlying the development of the auditing tools, demonstrates their differences to the Critical Pathway proposed as a common tool a decade ago and presents a side-by-side comparison of donation definitions, metrics and data for the 2019 calendar year. There were significant differences in donation definition terminology, metrics and overall structure of the audits. Fitting the audits to a tiered scaffold allowed for reasonable comparisons however this required substantial effort and understanding of nuance. Direct comparison of international and inter-regional donation performance is challenging and would benefit from consistent auditing processes across organisations.

Keywords: transplantation, organ donation, performance, auditing, reporting, metrics, definitions



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INTRODUCTION

Organ transplantation is a lifesaving, life-transforming intervention which often is the only effective treatment available to patients with end-stage organ failure. Such patients rely on a limited supply of organs and experience high mortality and significant morbidity whilst waitlisted (1). Supply is influenced both by the size of the potential donor pool and critically the efficacy of its conversion into actual donors (2). Conversion broadly depends on healthcare system resources and cultural factors and is facilitated through donor identification, referral and approach, community attitudes to donation, donor physiological support and transplant unit acceptance practices. Countries with advanced donation systems have organ donation organisations which lead in the assessment of national/regional donation

TABLE 1 | Critical pathway for deceased donation definitions—adapted from Dominguez et al. (2011)⁶.

Common term	DBD component	DCD component
Potential	Potential DBD donor: A person whose clinical condition is suspected to fulfil brain death criteria	Potential DCD donor: A. A person whose circulatory and respiratory functions have ceased and resuscitative measures are not to be attempted or continued, or B. A person in whom the cessation of circulatory and respiratory functions is anticipated to occur within a time frame that will enable organ recovery
Eligible	Eligible DBD donor: A medically suitable person who has been declared dead based on neurological criteria as stipulated by the law of the relevant jurisdiction	Eligible DCD donor: A medically suitable person who has been declared dead based on the irreversible absence of circulatory and respiratory functions as stipulated by the law of the relevant jurisdiction, within a time frame that enables organ recovery
Actual	Actual DBD donor: A consented eligible donor: A. In whom an operative incision was made with the intent of organ recovery for the purpose of transplantation, or B. From whom at least one organ was recovered for the purpose of transplantation	Actual DCD donor: A consented eligible donor: A. In whom an operative incision was made with the intent of organ recovery for the purpose of transplantation, or B. From whom at least one organ was recovered for the purpose of transplantation
Utilised	Utilised DBD donor: An actual donor from whom at least one organ was transplanted	Utilised DCD donor: An actual donor from whom at least one organ was transplanted

conversion performance, collecting data to identify barriers to donation, direct interventions and measure the effect of their implementation.

Meaningful comparison of national/regional donation metrics might allow for sharing of best practice and overall improvement of donation performance. Countries with low conversion rates could learn from practices of countries with better performance (3). However, difficulties exist in comparisons due to inconsistencies in the definitions and metrics used as performance indicators (4). Indeed, a recent US study showed significant variability in the performance rankings of organ procurement agencies depending on which donation metrics were used (5).

The “Critical Pathway for Deceased Donation,” the outcome of a multi-national initiative held between 2008–10, was aimed to provide a solution to this issue by providing a set of common definitions to guide consistency in reporting of donation performance (6). However, while the Critical Pathway was welcomed, the goal of common international definitions has not been realised and many nations have witnessed divergent evolution in the audit of donation performance. We aimed to explore this issue through a collaboration between the national donation organisations of the United Kingdom and Australia, both countries which contributed to the development of the critical pathway. In this point of view, we will outline the critical pathway for deceased donation, the history of the development of our individual auditing tools and finally, investigate the degree of comparability between our donation definitions and metrics.

THE CRITICAL PATHWAY FOR DECEASED DONATION

The critical pathway for deceased donation was developed by a multi-national collective at the Madrid Resolution on Organ Donation and Transplantation (7) and published by

Dominguez et al. in 2011 (6). It outlines a series of definitions which enable all “possible deceased organ donors” to be quantified, including definitions for “potential” donors, “actual” donors and “utilised” donors. A similar template was recently suggested for European tissue donation (8). The value of this structured approach to donation networks is its ability to pinpoint where unrealised donation opportunities occur along the pathway. Where cases of avoidable unrealised donation are identified, interventions can be targeted to increase rates of donation.

Inclusion in the “possible deceased organ donor” pool is defined by the critical pathway as “A patient with devastating brain injury or lesion or a patient with circulatory failure and apparently medically suitable for organ donation”(6). The pathway then splits into two components, separating into donation after brain death (DBD) and donation after circulatory death (DCD) pathways. There are four major steps to each pathway (Table 1); “Potential,” “Eligible,” “Actual” and “Utilised” DBD/DCD donors.

THE DEVELOPMENT OF THE UK AND AUSTRALIAN DONATION AUDITS

The development of the potential donor audit (PDA) in the UK followed the publication of a study auditing DBD potential in intensive care units (ICUs) which estimated a possible 20% increase in deceased kidney donation based on prompt testing for brain stem death (9). Following this publication, the first UK PDA, auditing the DBD pathway, was established in 2003. Since then, the PDA inclusion criteria have been extended, firstly in 2009 to also audit the potential for DCD donation and include deaths in emergency departments (EDs), and next in 2013 when the age criteria were extended from 75 years and under to 80 years and under. Enhancements to the PDA were made in 2020 to capture more informative data on the medical suitability of eligible DCD donors and further detail on the donation

TABLE 2 | Differences in audited deaths included in the UK and Australian donation audits.

	United Kingdom	Australia
Inclusion criteria	Deaths under 80 years old occurring in intensive care OR emergency department (excluding deaths in neonatal ICU)	Deaths under 80 years old or >28 days old occurring in intensive care or emergency departments OR occurring anywhere in hospital within 24 h of presence in intensive care OR emergency department where irrecoverable brain injury present. Additional inclusion of patients >80 yr if formal request for consideration of donation placed by family and donation considered feasible by attending staff
Data pathway structure	DBD and DCD data audited separately	DBD and DCD data combined in audit
Network Organisation	National, centralised service: "Statistics and Clinical Research department, NHS Blood and Transplant"	National, centralised service: the "Organ and Tissue Authority" (OTA) which maintains a web-based auditing tool capturing approx. 98% of deceased donation activity in Australia
Data Collection and input	Specialist Nurses in Organ Donation embedded in individual hospitals	Nurse donation specialists embedded in individual hospitals or through outreach roles in smaller hospitals without permanent embedded staff

decision conversations. Since this time, data are collected via an app and can be entered in real time. Data are input and validated by Specialist Nurses in Organ Donation (SNODs), employed by NHS Blood and Transplant (NHSBT), who are embedded in the individual hospitals.

Early audits of hospital deaths occurred in several states in Australia with the aim of quantifying the potential for organ donation, focusing on identifying missed donor cases (10–12). Most missed opportunities for donation occurred in severely brain injured patients who, due to poor prognoses, had treatment withdrawn in the ED or ICU. The first national audit occurred during a National Organ Donation Collaborative from 2006–09. In 2009, a national reform began that included the establishment of a national agency, the Organ and Tissue Authority and the state-based DonateLife Network. The DonateLife Audit was developed as a monitoring tool with retrospective review of all hospital patient deaths with donor potential. A new web-based tool was implemented in 2012 that included fields for donor physiology and organ function, providing more detailed information about donor organ suitability for transplantation. The audit provides a means of optimising clinical practice both at a local and national level, identifying cases with learning points for local case review and providing national, jurisdictional and hospital level data on measures such as the donor pool, and rates of consent and donation (13). Regular internal reporting enables monitoring of clinical practice improvement including the routine referral to donation services of patients at medical consensus of end-of-life and utilisation of a best practice approach to offering donation to families (14). The audit is completed by donation specialist staff and is undertaken in most Australian hospitals with donor potential.

A COMPARISON OF UK AND AUSTRALIAN DEFINITIONS AND METRICS USED IN DONATION REPORTING

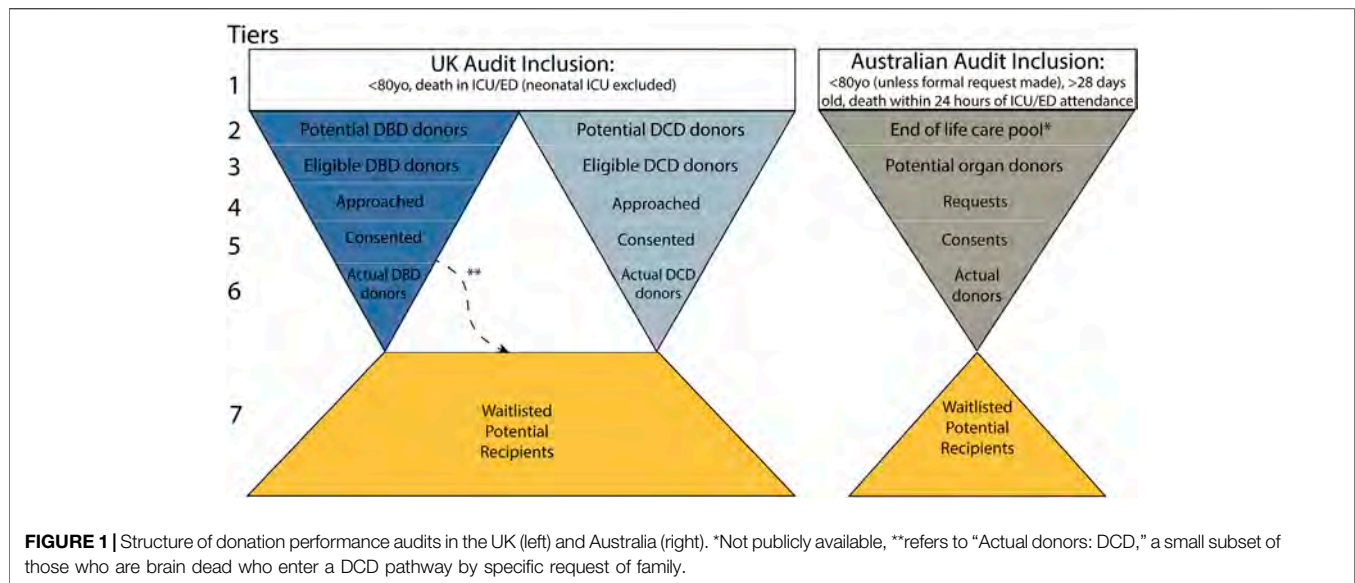
Over 2020–2021, we conducted a series of virtual meetings aiming to compare national methods, definitions and metrics used for data collection and reporting of national deceased

donation performance. Tables were created outlining the definitions used in DBD and DCD pathways set out by the "Critical Pathway for Deceased Donation" (6) in the first column, with further columns left blank for population by nearest equivalent definitions from Australian and UK official reference documents. These included the "Potential Donor Audit Report 2019–20" from NHS Blood and Transplant, UK and the "DonateLife Audit Standard Operation Procedure" used by the Organ and Tissue Authority in Australia. Side-by-side definitions allowed for in-depth discussion within the group surrounding similarities and differences between definitions used. Minutes were taken and differences and similarities synthesised through discussion across subsequent meetings.

General differences between the auditing structures were immediately apparent (Table 2). Estimating the potential donor pool is essential to any donation audit and the first challenge is that the two national audits cast differently sized nets in the denominator of audited deaths. In the UK, deaths are only audited if they physically occurred within the ICU or ED. In Australia, this is extended to deaths due to irrecoverable brain injury occurring anywhere in hospital within 24 h of being in an ICU or ED. The audits also differ slightly in age at death range captured. Both audits capture deaths from 28 days to 80 years, however the Australian audit also includes patients who were referred for consideration of organ donation outside these criteria, for example those above 80 years old where a family request was made and where donation was considered feasible by attending staff. Differing inclusion criteria mean that when it comes to comparing the possible donor pools between countries, we could only proceed by restricting inclusion to death in ICU alone.

The basic structure of the audit also differed. In the UK, when DBD and DCD cases are audited they feed into separate streams of data collection (similar to the Critical Pathway) whereas in Australia these streams are combined (Figure 1).

Despite some differences in terminology used between countries, both audits could be fitted to seven major tiers (Figure 1). The general inclusion criteria (Tier 1) already represented an uneven starting point for comparisons, and differences continued throughout the tiers. Table 3 outlines



specific differences in the UK and Australian donation audits in Tiers 2–6. Tier 2 represents the first group in each audit which is deemed to have donation potential, thus warranting inclusion for further evaluation. In the UK, potential DBD and DCD donors are separate and feed down the audit as such whereas in Australia these groups are combined into an “End-of-Life Care Pool.” The Australian end-of-life care pool contains patients confirmed brain dead (or likely to have fulfilled criteria for brain death), or had treatment withdrawn and where death was anticipated, thus combining the DBD and DCD streams.

There were differences in the inclusion criteria of potential DBD- and DCD-pathway patients. For DBD in the UK, Tier 2 contains those suspected of brain death and meet criteria for formal neurological death testing whereas in Australia Tier 2 captures both suspected and confirmed brain dead patients. For DCD in the UK, a timeframe is applied to the potential DCD donor definition with inclusion if death was anticipated within 4 hours of withdrawal of life-sustaining treatment whereas Australia includes deaths which actually occurred within 6 h of withdrawal (or longer if DCD was planned but death did not occur within 6 h).

Tier 3 represents those in Tier 2 who are then deemed medically suitable with no absolute contraindications to donation. The UK refers to these patients as “Eligible DBD/DCD donors” as per the Critical Pathway (6) whereas Australia uses the term “Potential donors.” For inclusion of those in the brain death pathway in Tier 3, confirmation of brain death by formal neurological testing is essential to both audits. Data is impacted at Tier 3 due to differences in exclusion criteria outlined by nationally accepted lists of absolute contraindications.

Tier 4 refers to the interaction between donor families and healthcare staff including donation coordinators, nurses and hospital doctors. In the UK, donation coordinators are referred to generically as Specialist Nurse-Organ Donation (SNOD) and in Australia the term Donation Specialist Nurse encompasses a number of slightly varying roles. At this tier,

differing semantics are used, however both “Approach” (UK) and “Request” (Australia) are used in the audit which refers to family approaches to offer donation. Where these definitions do differ is in their denominator, with only those deemed eligible included in the UK whereas in Australia it is all discussions held, including those which may have been raised by families or led by ICU staff where donation was initially considered feasible although ultimately the person was not suitable.

Tier 5 is the consent rate of those families approached or requested for donation. The combined DBD/DCD Australian figure means comparison of specific consent, between the two types of deceased organ donation, cannot be readily achieved such as in the UK.

Tier 6 counts where donation is considered to have taken place. In the UK, “actual donor” status is defined by organ retrieval with the intention to transplant whereas in Australia cases are included at the point of “knife to skin” of the donor, both irrespective of actual utilisation (implantation) of organs. A final difference in audit structure occurs here as the UK reports on the small proportion of those included in the DBD pathway who actually proceed down a DCD pathway due to specific requests from the family to be present when the heart stops beating. Such cases also occur in Australia in practice.

COMPARISON OF REAL DATA—WHAT CAN BE REASONABLY COMPARED?

We next examined real data collected by both national audits (Table 4). The 2019 calendar year was chosen as this was the most recent year where donation activity was not impacted by the COVID-19 pandemic. To proceed, the DBD and DCD streams in the UK audit needed to be totalled for equivalence to the corresponding Australian tiers. We were able to compare figures for the possible donor pool (Tier 1) by adjusting the catchment to include only deaths occurring within ICUs.

TABLE 3 | Specific differences in the UK and Australian donation audits.

Tier	UK—DBD	UK—DCD	Australia	Comments
2	“Potential DBD donor” A patient who meets all four criteria (coma, ventilated, fixed pupils, apnoeic) for neurological death testing excluding those not tested due to reasons “cardiac arrest despite resuscitation,” “brainstem reflexes returned,” “neonates—less than 2 months post term”	“Potential DCD donor” A patient who had treatment withdrawn and death was anticipated within 4 hours	“End-of-life care pool” Any patient who meets the following criteria: -Confirmed or suspected brain death -Withdrawal of one or more of mechanical ventilation, artificial airway, mechanical circulatory support prior to death as part of the process of end-of-life care -A decision was made regarding organ donation	-Differing terms -DBD: Australian audit combines suspected brain dead and those confirmed via testing DCD: UK places time restriction of anticipated to 4 hours -“End-of-Life Care Pool” data not publicly available
3	“Eligible DBD donor.” Patients for whom death was confirmed following neurological tests and who had no absolute medical contraindications to solid organ donation	“Eligible DCD donor” Patients who had treatment withdrawn and death was anticipated within 4 hours, with no absolute medical contraindications to solid organ donation	“Potential donor” Any of the “End-of-Life Care pool” who were medically suitable/had no absolute medical contraindications to solid organ donation	-Differing terms -Neurological tests to confirm brain death for inclusion in category in both countries -Inclusion subject to differences in lists of absolute medical contraindications/medical suitability
4	“Approached DBD donors.” Eligible DBD families approached for consent/authorisation for donation	“Approached eligible DCD donors.” Eligible DCD donor families approached for consent/authorisation for donation	“Requests” Count of all cases where organ donation was discussed with the family and a final decision of consent or decline was made. Includes all requests, regardless of age or potential donor status, except cases where family was advised of lack of donor suitability	-Differing terms -Differing denominators with UK using eligible DBD/DCD donors only -UK also uses both terms “consent” and “authorisation” owing to different legislation in Scotland
5	“Consented DBD donors.” Families or nominated/appointed representatives of eligible DBD donors approached for formal organ donation discussion where consent/authorisation was ascertained	“Consented eligible DCD donors.” Families or nominated/appointed representatives of eligible DCD donors approached for formal organ donation discussion where consent/authorisation was ascertained	“Consents” Consent for organ donation is given by the family or next of kin. Cases where the family is advised of lack of donor suitability are not included	-Congruent in inclusion of actual family donation conversations in cases which had no absolute or prior identified medical contraindications
6	“Actual donors: DBD”: Consented, eligible DBD pathway patients who became actual DBD donors as defined by organ retrieval with the intention to transplant (unless returned to donor where considered unsuitable)	“Actual DCD donors”: Consented, eligible DCD pathway patients who became actual DCD donors as defined by organ retrieval with the intention to transplant (unless returned to donor where considered unsuitable)	“Actual donors”: A person for whom the organ retrieval procedure commenced in the operating room (with surgical incision) for the purpose of transplantation. This includes donors who may have been deemed medically unsuitable during surgery or after the removal of organs	-Actual donation defined at “knife to skin” of donor in Australia and “organ retrieval with the intention to transplant” in UK. -Select few in DBD pathway in UK who became DCD donors due to specific requests of family reported in audit. This does occur in Australia however is not publicly reported

However, this by necessity, excluded deaths associated with other locations such as EDs and wards and thus underestimates the true donor pool (11). Where appropriate, data was provided in absolute numbers as well as in per million population (pmp) however we note population age distribution impacts national donation potential (15). This figure is also impacted by proportion of donation-compatible deaths, for example differing due to variable cerebrovascular disease and traffic accident mortality (16).

DISCUSSION

Direct comparison of UK and Australian deceased organ donation data was challenging due to differences in the

metrics and definitions used by the national donation networks. A tiered structure allowed approximations at each step of the pathway and subsequently, certain comparisons could be cautiously made. Interpretation of comparisons requires detailed understanding of the way data is derived, collection methods, flow and the relationships between data points.

Difficulties in comparing national donation performance is not a new issue. Jansen et al. (2009) found significant heterogeneity in definitions used for “potential organ donor” and “refusal rate” across 11 European countries (4). They concluded non-uniform definitions meant that comparisons were not appropriate and called for shared definitions. In the United States, non-standardised, inconsistent, self-reported metrics reported by Organ Procurement Organisations (OPOs)

TABLE 4 | Comparison of 2019 donation activity data in the UK and Australia across tiers. Population estimate used for per million population (pmp) calculations were 66.8 million in the UK and 25.37 in Australia for 2019.

Tier	Corresponding metric	UK (DBD + DCD)	Australia
1	Deaths in chosen location (ICU)	22688 (339 pmp)	5990 (234 pmp)
2	Potential donors (UK) or EOL care pool (Aus)	Not included	Not included (not publicly available)
3	“Eligible” (UK)/“Potential” (Aus)	5844 (87 pmp)	1309 (51 pmp)
4	“Approached” (UK)/“Requested” (Aus)	3351 (50 pmp)	1224 (48 pmp)
5	Consents	2276 (34 pmp)	756 (30 pmp)
	Consent rate	67.9%	62%
6	Actual donors	1624 (24 pmp)	548 (22 pmp)

TABLE 5 | Immediate actions and future directions.

- The most meaningful comparisons between the UK and Australian donation organisations begin at “Tier 4,” or the number “approached” or “requested” for donation. Further collaborations between our organisations should focus on downstream data comparisons including consent and conversion rates
- Invite and encourage dialogue between other organ donation organisations interested in updating or evolving their audits by establishing a working group which would routinely meet at a recurring international conference such as the International Society for Organ Donation and Procurement (ISODP) Congress
- The use of standardised definitions and metrics by databases which collect and publish data on organ donation and transplantation activity such as the Global Observatory on Donation and Transplantation (GODT)
- Encourage the use of side-by-side descriptive information alongside data points in publications which aid the reader in understanding how each data point was derived

also make interregional performance assessments problematic (5,17,18). As pointed out by Goldberg *et al.* (2019) this is an issue of fairness as these metrics inform interventions which could improve access in truly underperforming states. Canada also has difficulties with a lack of standardisation possibly due to its provincially-administered healthcare system (19).

Many initiatives have attempted to establish and promulgate a set of standard definitions and metrics which measure donation performance. Most notably, the multi-national collaborative led by Dominguez *et al.* (2011) established the “critical pathway for deceased donation” which played an important role in providing a universal framework for the process of deceased organ donation (6). However, donation practices constantly evolve, necessitating continuous reassessment of benchmarking practices. A recent ‘call to action’ from the European Kidney Health Alliance argued there is work to be done and recommended establishing appropriate comparative tools (3).

Our group attempted to take up the mantle of this work. From our minutes, “The goal is the concept of potentially using our two databases and trying to bring them together so that we can actually have comparative metrics.” It was noted that the two audits, “. . . have probably evolved in different directions.” When comparing our audits, we first noted there were several significant general differences in their structure. The starting points varied due to differing inclusion criteria in estimating the “possible” donor pool. We also note that not all ICUs and EDs report all deaths where organ donation is possible in a consistent and standardised way. To identify the full depth of this pool would require an audit of all hospital deaths nationally (11). For the purposes of our review, we approximated our data by only considering deaths in ICU though this is inconsistent with our actual practice and underestimates the donor pool. Our second major difference was that when DBD and DCD cases are audited they feed into separate streams of data in the UK whereas in Australia they are reported in a combined fashion. A strength of

separate reporting is the ease in external assessment of DCD implementation. DCD has been shown as a way to increase donation activity and contributes substantially to overall donation numbers (20) and therefore may benefit from separate monitoring. However, a weakness in stream separation lies in accounting for the small number of potential donors where the donation process was stopped prior to the point where the pathway was completely differentiated or, in the data collection phase, where it was not possible to allocate them retrospectively to a pathway.

We developed a tiered system based on the critical pathway for deceased donation to compare the definitions and metrics used by our audits. At almost every tier there were different uses of terminology and nuance in metrics. It was felt that much of the differences found were in the way data was reported rather than collected and that internal data could be produced which would more readily match the counterpart organisation’s data. Undertaking this work itself did help with interpreting each counterpart’s figures and some comparisons were felt to represent reasonable approximations.

There are several limitations with auditing donation performance in general. The audits attempt to simplify the messy real world of variably unfolding patient scenarios and different clinician practices and record-keeping. Difficulties arise in capturing scenarios outside of the expected ‘order of events’, for example where families are approached at earlier stages such as prior to brain death testing. Furthermore, the audits variably combine elements of retrospective data collection as well as data collection which is actively and purposefully collected during the donation process. For example, when recording potential DCD donors, the UK approach would be to include “A patient who had treatment withdrawn and death was anticipated within 4 hours”, this relying on the clear recording of “anticipation” of death during the donation process for later retrospective data

collection. In other words, this element of the audit is conducted prospectively but collected retrospectively. In Australia, the observation that death occurred within 6 h of withdrawal of cardio-respiratory support (or beyond 6 h if donation had been planned) is the trigger for inclusion which necessitates the retrospective approach.

We also discussed the mutual development of “quality metrics”, including tracking characteristics of the donation conversation, from formalised pre-discussion planning sessions to presence of donation specialise staff. Notably, donation coordinator nursing staff involvement in donation conversations is implicated in increasing DBD and DCD consent rates (21).

Clearly, moving towards a shared reality, “international language” and uniform metrics is desirable. **Table 5** outlines our suggestions for the immediate steps and future directions which can be taken which include further work between our organisations and others. In the future, international donation networks could audit a standardised pool of potential donors, capturing all deaths using a global coding system integrating digital time stamps and in a digitalised, user-friendly system. Metrics could then be generated from shared definitions and reported in multiple formats including absolute numbers, adjustments made for per million population and even considerations for adjustments made for population age distribution and “mortality profiles” (16).

We found that comparison of deceased organ donation data between two countries, which at first glance have similar culture and donation practice, was extremely challenging due to differences in our metrics and definitions. This would be compounded when comparing with even more countries and organ donation organisations. However, this work is essential if we are to search widely for solutions and learn from our partners when addressing the shortage of organs for transplantation. We do know that our goal is the same: the minimisation of unrealised potential donors. We therefore encourage, invite and hope to

foster larger collaborative efforts from this international audience towards the goal of convergent evolution of definitions and metrics. This work will become increasingly relevant as practices in organ donation and transplantation evolve with society and time. It’s time to compare apples with apples when reporting donation performance.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

Conceptualisation—LM, AM, and DG; Literature search—LM; Figure and table development—LM, CB, LG, KD, MG, SM, and MM; Writing—original draft—LM; Writing—review and editing, including verification of data—CB, LG, KD, MG, SM, MM, LB, HO, AM, and DG.

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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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Demonstrating Benefit-Risk Profiles of Novel Therapeutic Strategies in Kidney Transplantation: Opportunities and Challenges of Real-World Evidence

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While great progress has been made in transplantation medicine, long-term graft failure and serious side effects still pose a challenge in kidney transplantation. Effective and safe long-term treatments are needed. Therefore, evidence of the lasting benefit-risk of novel therapies is required. Demonstrating superiority of novel therapies is unlikely via conventional randomized controlled trials, as long-term follow-up in large sample sizes pose statistical and operational challenges. Furthermore, endpoints generally accepted in short-term clinical trials need to be translated to real-world (RW) care settings, enabling robust assessments of novel treatments. Hence, there is an evidence gap that calls for innovative clinical trial designs, with RW evidence (RWE) providing an opportunity to facilitate longitudinal transplant research with timely translation to clinical practice. Nonetheless, the current RWE landscape shows considerable heterogeneity, with few registries capturing detailed data to support the establishment of new endpoints. The main recommendations by leading scientists in the field are increased collaboration between registries for data harmonization and leveraging the development of technology innovations for data sharing under high privacy standards. This will

Abbreviations: AE, Adverse event; AZA, azathioprine; CADI, Chronic Allograft Damage Index; CNI, calcineurin inhibitor; EC, External comparator; eGFR, estimated glomerular filtration rate; FDA, U.S. Food and Drug Administration; GDPR, General Data Protection Regulation; GFR, glomerular filtration rate; MMF, mycophenolate mofetil; OMOP, Observational Medical Outcomes Partnership; PCORnet, Patient-Centered Outcomes Research Network; RCT, Randomized controlled trial; RLSE, reasonably likely surrogate endpoint; RW, Real-world; RWD, Real-world data; RWE, Real-world evidence; SoC, Standard of care; SONG, Standardised Outcomes in Nephrology.

aid the development of clinically meaningful endpoints and data models, enabling future long-term research and ultimately establish optimal long-term outcomes for transplant patients.

Keywords: data harmonization, extension studies, real-world evidence, registries, kidney transplantation

INTRODUCTION

While short-term survival rates of transplanted grafts and patients have improved in past decades, progress of long-term graft survival is still limited. In addition to the highly specialized surgery, long-term immunomodulatory treatment is needed to prevent rejection and allograft failure (1). The average graft half-life is around 12 years, with around one in five kidney transplant patients experiencing graft failure within the first 5 years (2, 3). Limited long-term effectiveness of immunomodulatory treatments, reduced adherence over time and long-term adverse events (AEs), calls for improvement of lasting outcomes for post-transplant patients (4).

Demonstrating superiority of novel therapies and strategies in the long-term is challenging in conventional randomized controlled trial (RCT) settings. This is due to statistical challenges presented by the requirement to demonstrate benefits with long-term follow-up and large sample sizes. Resulting in increased operational risks (e.g., costs, trial incompleteness) for sponsors, they also pose a high operational burden on patients and physicians. The need for shorter term, clinically meaningful endpoints that are predictive of longer-term outcomes has been extensively described (5–7).

Whereas regulatory hurdles limit opportunities for novel therapeutics in RCTs to demonstrate improved graft and patient survival in the short-term (e.g., limitation of recognized endpoints), studies in real-world (RW) treatment settings offer new possibilities to generate evidence. With generalizable cohorts, RW settings have a broader relevance and efficiency compared to RCTs; provided that data elements relate to accurately recognized clinical phenomena and are comparable across settings (5–7).

To expand the scientific understanding of innovative evidence generation in kidney transplantation, a scientific discussion was initiated by Novartis in 2020, including a panel of leading nephrologists, scientists, transplant registry experts, and drug development professionals. Participants were invited based on clinical research in kidney transplantation and/or experience in registry and real-world data (RWD)¹ collection. The group included representatives from identified major transplant registries interested in collaboration. This viewpoint examines the current limitations of RCTs and outlines the opportunities of employing RW evidence (RWE)² to evaluate novel drug therapies

in kidney transplantation (8). The viewpoint further elaborates on the systematic review of renal registries by Liu et al. in 2015 (9), by identifying the most relevant RWD sources to characterize the benefit-risk profile of novel therapeutic strategies in kidney transplantation, while making a critical assessment of the challenges that generating RWE entails.

Current Limitations of Conventional Randomized Controlled Trials in Kidney Transplantation

Long-term data is needed to understand patient outcomes beyond the one-to-three-year time-point usually considered in RCTs. Currently there is limited follow-up data available from clinical trials for kidney transplants, particularly in later years post-transplant, partly due to the high number of complex data elements (e.g., donor and recipient characteristics, transplantation procedure, acute rejection, antibody-mediated rejection, calcineurin inhibitor nephrotoxicity, scoring of inflammation from tissue biopsies etc.) (1, 10).

One of the issues are the high discontinuation rates (15–30%) observed in the first year of many immunosuppressive drug trials. Examples of this can be found in recent immunosuppressive drug RCTs in which the main reasons for patient discontinuation were AEs, severe refractory rejection or ineligibility (11, 12).

Classical RCT settings are unlikely to fulfil needs for long-term outcome data as they require large sample sizes leading to an operational and financial burden, resulting in very few patients, physicians, and sponsors (government, commercial or academic) being willing to participate in studies that require long years of clinical follow-up (13). RCTs also typically have restrictive inclusion/exclusion criteria, which can lead to the limited generalizability of trial results.

The current standard of care (SoC) provides excellent short-term outcomes in suitable donor-recipient combinations; therefore, it is difficult to exceed SoC outcomes in RCTs of novel treatments. The currently accepted endpoints by regulatory authorities (graft survival, graft function, or biopsy-proven acute rejection) provide mostly short-term outcomes, rather than long-term results (14). There are also ethical concerns due to impaired clinical equipoise: if a treatment shows short-term superiority, and potential for long-term benefit, it might not be considered ethical to include a control arm for long-term results (15). Yet, novel treatments and therapies need to be tested with long-term treatment outcomes and patient wellbeing in mind, which is often difficult to achieve within RCT settings. Advancements in graft survival improves patient quality of life, reducing both the risk of return to dialysis and the demand for a limited donor organ supply (1).

The authors believe that studies of sub-groups (e.g., hyperimmunized, desensitized, and perfused organs etc.), and

¹RWD are the data relating to patient health status and/or the delivery of health care, not collected through clinical trials, but rather routinely collected from a variety of sources (electronic health records, claims and billing activities, product and disease registries, patient-generated data including in home-use settings, data gathered from other sources that can inform on health status such as mobile devices) (8).

²RWE is the clinical evidence regarding the usage and potential benefits or risks of a medical product derived from the analysis of RWD (8).

non-ideal donor-recipient combinations, could demonstrate superiority of novel treatments in situations where SoC is not yet sufficient. There is also little inclusion of non-immunological aspects of kidney transplantation that should be considered (hypertension, post-transplant diabetes, renal protective therapies, hyperparathyroidism, and urinary tract infection, etc.) (16). Higher risk populations may represent an alternative to prove superiority as event rates of interest are likely to be more frequent, required sample sizes smaller, and observation periods shorter.

There is a need to improve the relevance and inclusion of patient-centric and patient reported outcomes in future research, as outlined by the Standardised Outcomes in Nephrology (SONG) initiative (17). Few trials study quality of life and patient concerns, however, some national and international registries do collect this information (18). In conjunction with strategies for better long-term follow-up, the growing need for more consistent collection of PROs, and short-term outcomes in sub-populations, RW study designs can provide alternative approaches to interventional clinical study designs.

A common understanding on surrogate endpoints in kidney transplantation is required to improve the comparability of data as these do not directly measure clinical benefits, but rather predict the likelihood of a clinical benefit (19). Some surrogate endpoints are a small subset of biomarkers, “laboratory measurements that reflects the activity of a disease process” (20), and should stem from data routinely captured in clinical practice, deemed acceptable by health authorities, and compatible with information regularly captured in RCTs (18). However, these often require a breadth of clinical data not always captured in routine healthcare data collection and/or registries (18).

Kidney transplant biomarkers were categorized by Mannon et al. as either pre-transplant, early post-transplant and late post-transplant markers (5). One pre-transplant biomarker—the Eplet-mismatch score has been accepted into the Biomarker Qualification Program, with attempts to qualify it as a prognostic biomarker (5). The iBox, an early post-transplant biomarker, is used to predict long-term allograft failure after a fairly short observation time—only 1 year (21). As an integrative risk prediction score derived from eight functional, histological, and immunological prognostic factors, in 2020 the U.S. Food and Drug Administration (FDA) also provided information to support the qualification of iBox as a reasonably likely surrogate endpoint (RLSE) in clinical trials evaluating immunosuppressive therapies in kidney transplantation (22). There is also another RLSE—the rate of decline of estimated glomerular filtration rate (eGFR) as a late post-transplant biomarker, that has been deemed acceptable by the FDA for use in a rare condition (chronic antibody-mediated rejection), however this biomarker remains to be validated for general use across clinical trials (5). Finally the Chronic Allograft Damage Index (CADI) adopts a sum score of six histopathological lesions in transplanted kidneys associated with graft function (23). CADI has been useful in clinical decision-making, by providing information on extent of chronic injury in the kidney allograft (23).

Finding accurate predictors depends on the immunological response, which can be highly variable due to immunosuppression therapies, comorbidities, and lifestyle factors. Transferring surrogate markers to new “surroundings” is also challenging as the predictive performance may not be the same and cross-validations may be necessary. For example, biomarkers evaluated in calcineurin inhibitor (CNI) based immunosuppression may not necessarily be valid in non-CNI protocols. The cost of immunosuppressive drugs and availability of follow-up visits also differ significantly across healthcare systems. Keeping these differences in mind will improve and ensure the comparability of treatment outcomes across geographies and treatment situations (24).

Opportunities of Real-World Evidence

Both RWD and RWE refer to patient related data not collected through a RCT (25). “The diverse patient population, as well as broad scope of RWD sources makes it easier to generalize long-term outcomes and risks of a treatment compared to RCT results” (25). Additionally, discontinuation rates from regular follow-up in the transplant centres, captured by registries that may be statutory or otherwise mandatory, are much lower and ensure long-term continuity of data in studies that typically have less inclusion/exclusion criteria and are less invasive.

Innovative clinical trial designs, such as those using external comparators (ECs), harness the power of RWD derived from patients treated in RW settings (26). ECs, also sometimes referred to as “synthetic control data,” are used to provide context to a single arm study where it would be impractical or unethical to design the study with a placebo or active comparator arm (27). EC studies have different approaches in utilizing RWD for contextualization of trial data, and to supplement single arm trials. ECs can be used independently, for further contextualization while having a control arm in an RCT, or to supplement a control arm in an RCT (28, 29). ECs sourced from RW settings reflect the SoC, and whilst finding these control cohorts can be challenging and resource intensive, they provide context to the benefits and risks observed in single arm studies, and can provide insight into RW patient experiences. Furthermore, EC designs are likely to shorten time frames to regulatory submissions and lessen operational risks, and are increasingly used by regulators and government payers in difficult-to-recruit areas (30). Credible RWD needs to be of high quality, obtained from relevant sources, cleaned, harmonized, and—if needed—linked to additional data sources to fill in information gaps and include relevant endpoints to be fit for purpose (26). Within kidney transplant research, RWD could drive the conduct of pragmatic trials, EC studies, or the build of registries that can be used for nested trials³. Nonetheless, it is necessary to assess fitness for use of RWD by undergoing feasibility assessments before pursuing the study design.

The potential of RWE was seen in research by Friends of Cancer Research, where several RW clinical endpoints in patients

³Trials recruiting study subjects from a larger established study population whose characteristics are known

with advanced non-small cell lung cancer treated with immune checkpoint inhibitors were compared to results of RCTs (29). Similar approaches are also in broader initiatives, notably the RCT Duplicate Initiative building an empirical evidence base through large-scale replication of RCTs (31). These pioneering projects ascertain the benefits of using RWD for extension studies and demonstrate the potential of ECs in future trial designs that study long-term outcomes to evaluate novel therapies.

There are two examples of kidney transplant studies, which followed a similar approach to an EC using extension studies (32, 33). The first compared rabbit antithymocyte globulin and basiliximab in kidney transplantation (32). To obtain 5-years follow-up data, patient trial records were matched with records in the Organ Procurement and Transplantation Network database for birth date, transplant date, sex, and transplant centre (32). This method allowed for extended follow-up, whilst also reducing costs of observation compared to prospective designs (32).

The second clinical trial is the tricontinental mycophenolate mofetil (MMF) kidney transplantation extension study, which initially recruited 503 patients that received a deceased donor kidney, and were randomized in equal groups to receive azathioprine (AZA) or MMF in combination with cyclosporine and steroids (11, 33). With 15 years of matched follow-up data from the Australia and New Zealand Dialysis and Transplant Registry, the study concluded little superiority of MMF over AZA (33). Linking the RCT to registries for long-term follow-up decreased biases compared against biases from purely observational designs (33).

RWE is increasingly required by regulators to demonstrate generalizable comparative insights, notably for: market authorization applications, line extension and post-authorization safety studies etc, (29). The non-invasive nature of RWD presents opportunities to assess long-term treatment outcomes using a combination of a properly designed clinical trials and registry outcomes data (17, 34).

Furthermore, RWD can be used to support the validation and test the predictive nature of short-term surrogate endpoints, as clinically meaningful surrogate endpoints that are predictive of final outcomes can be used and are needed for shorter term studies as well. Once such surrogate endpoints are validated, they could be used in clinical trials or other RW study designs. Specific transplant data (e.g., histology, immunology, and treatment) should be considered for consistent inclusion across registries, for example from diagnostic databases and biobanks, to expedite validation requirements.

Identification of Most Relevant Real-World Data Sources and Challenges in Generating Evidence From Them

The potential advantages of using RWD must outweigh concerns of quality and consistency (35). Not many existing registries capture sufficiently complete follow-up data for kidney transplant, which is a limitation of the RWE approach. Whilst some sources allow for nationwide assessments (e.g., cause of death), more consistent inclusion of surrogate endpoints, and

biopsies, across follow-up periods are still needed to ascertain the cause of graft loss.

A global literature search assessment was conducted by the authors in 2020, using a standard methodology described in Ekman et al., (36), to identify the most relevant RWD sources to assess treatment patterns, the clinical manifestations of AEs and validate predictive surrogate endpoints (e.g., iBox) in kidney transplantation (5). The search identified 94 RWD sources worldwide that had published research in English between 2010–2019, of which 37 were prioritized for in depth desk research based on publication record, patient and geographic coverage (**Figure 1A**). Further literature assessments for classification of data characteristics and follow-up found only 12 sources as preliminarily suitable for long-term assessments, of which five were qualitatively assessed during respective interviews with data source owners. Qualitative assessments aimed to determine database content, such as availability of variables, as well as research experience and ways of working (**Figure 2**) (36).

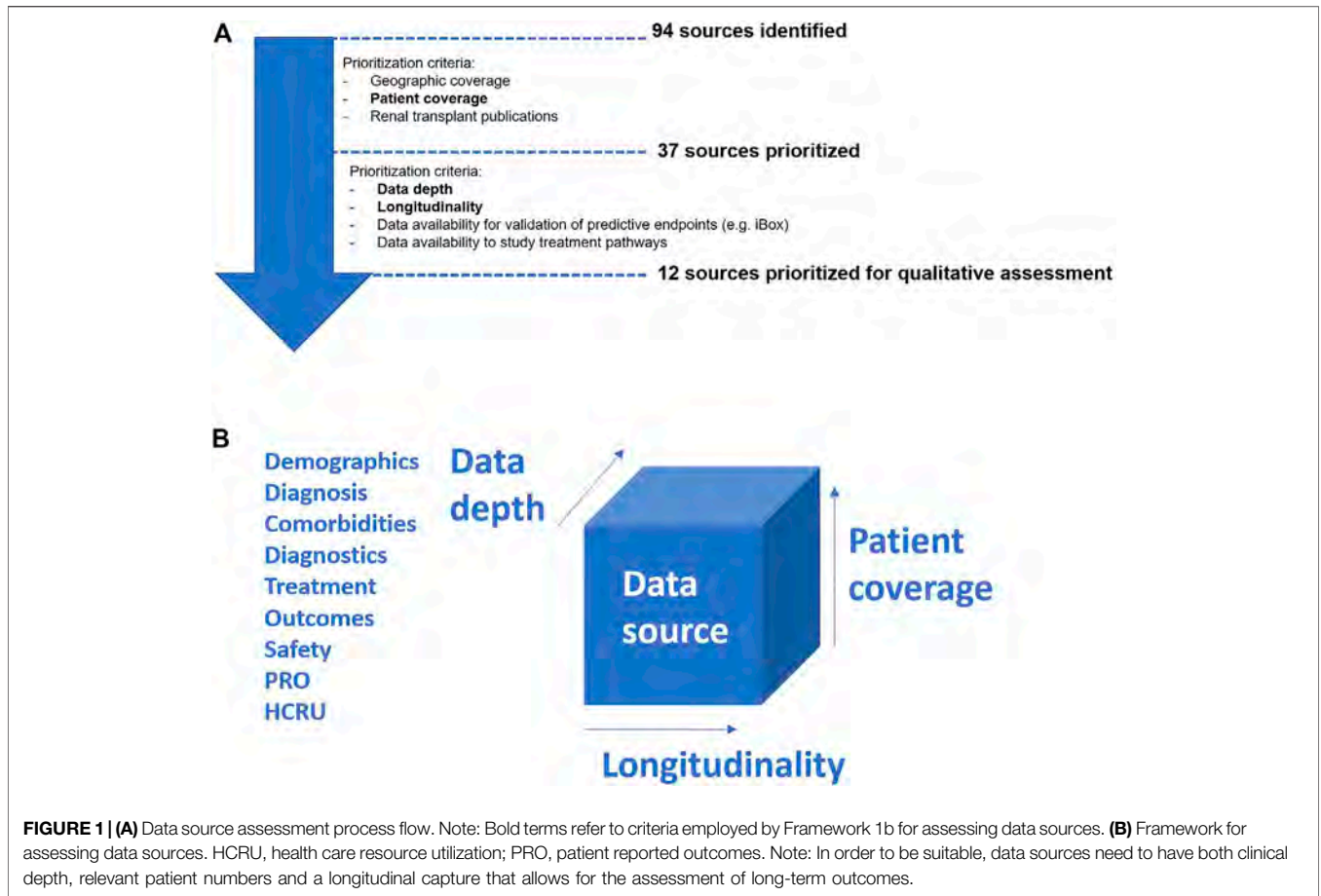
Whilst the five sources fully or partly met data requirements to assess treatment patterns, burden of disease, and validated predictive surrogate endpoints (e.g., iBox), they represent less than 10% of the kidney transplant sources identified. Hence, the assessment concluded that few kidney transplant RWD sources routinely capture data needed to derive predictive markers (e.g., tissue biopsy data for graft assessments) in greater clinical depth (**Figure 1B**) (36). Enhanced collaborations may alleviate the resource burden in order to produce and maintain long-term data, yet technical and semantic interoperability are required to overcome barriers that arise when harmonizing different sources (e.g., data standards, storage requirements, data handling procedures) (35). Failing to do so limits data utility, as seen in during the ADAPTABLE trial: divergence in data collection across facilities, and the “incomplete capture of past procedures and differences in classification of data,” limited comparison of doses of aspirin for prevention of hospitalization for myocardial infarction (35).

Identifying outcomes available across many sources, standardizing and enhancing data collection, will improve cross-source comparability to generate robust assessments. For example, more consistent glomerular filtration rate (GFR) measures would support definition of relevant surrogate endpoints for graft loss, and whilst this would likely require a shift from eGFR to standardized measured GFR assessments, this may be feasible with capillary samples and mathematical models. Ensuring such data breadth and completeness requires common definitions and sufficient time to implement changes that enforce required data quality.

Lastly, technology innovations such as Natural Language Processing⁴ and federated data models⁵, can support the building of larger cohorts with deeper structured data (37, 38).

⁴“a branch of artificial intelligence that helps computers understand, interpret and manipulate human language” (37).

⁵“Data federation is an aspect of data virtualization where the data stored in a heterogeneous set of autonomous data stores are made accessible to data consumers as one integrated data store by using on-demand data integration” (38).



Data source	Number of kidney transplant patients	Linkage	Time of data Lag	Data content								
				Demographics	Diagnosis	Comorbidities	Diagnostics	Treatment	Outcomes	Safety	PRO	HCRU
1.	• ~20k/year • ~220k total	✓	2 years	G	G	R	A	G	G	G	R	G
2.	• ~20k/year • ~300k total	✓	2 years	G	G	R	A	A	G	A	R	R
3.	• ~20k/year • ~300k total	✓	6 months	G	G	R	A	A	G	A	R	R
4.	• 250/year • ~4k total	✓	1 year	G	G	A	G	G	G	G	R	G
5.	• 290/year • ~3k total	✓	1 year	G	G	A	A	G	G	A	R	G

G Good coverage of variables of interest in a structured format
 A Limited coverage of variables of interest in a structured format
 R Information not available

FIGURE 2 | Five data sources qualitatively assessed. HCRU, health care resource utilization; PRO, patient reported outcomes.

TABLE 1 | Conclusions and Recommendations by the scientific forum.**Conclusions and Recommendations by the scientific panel**

RWD^a sources, in combination with properly designed clinical trials, offer an effective and affordable way to assess long-term transplant outcomes. The FDA released guidance for industry to be used: “RWD: Assessing Electronic Health Records and Medical Claims Data To Support Regulatory Decision-Making for Drug and Biological Products” (42)

To enhance the use and impact of RWE^b, registry collaborations and multi-country collaborative studies alike should work towards consistent selection of surrogate endpoints for increased comparability

Data harmonization that broadens patient coverage and extends follow-up should enable RWD to support the validation and test the predictive nature of short-term endpoints. Cross-source comparability assessments prior to harmonization are recommended for effective use of RWD [35]

Comparing data from different sources is possible even when pooling is difficult by leveraging technology innovations, including the use of federated models. Such approaches enable rapid and consistent assessments across data depth, coverage, and temporality of capture

Emerging innovative clinical trial designs that utilize RWD to complement trial data can provide additional benefits and shorten time frames to regulatory submissions. They require close alignment with regards to population characteristics and the definition of data collected

FDA: U.S., Food and Drug Administration; RWD, real-world data; RWE, real-world evidence.

^aRWD: data relating to patient health status and/or the delivery of health care, not collected through clinical trials, but rather routinely collected from a variety of sources (electronic health records, claims and billing activities, product and disease registries, patient-generated data including in home-use settings, data gathered from other sources that can inform on health status such as mobile devices) (3).

^bRWE, is the clinical evidence regarding the usage and potential benefits or risks of a medical product derived from the analysis of RWD (3).

Such approaches enable rapid and consistent assessments across data depth, coverage, and temporality of capture. Federated data models utilizing clinical data repositories, and public-private partnerships, such as the Observational Medical Outcomes Partnership (OMOP), Patient-Centered Outcomes Research Network (PCORnet) serve as examples of international standards for data linkage and sharing. However, the practical considerations when using federated data models, such as ensuring linkage of disparate data sources, warrant caution (31). Use of RWD cohorts in innovative trial designs need to be aligned to prospective single arm trials with regards to population characteristics and definitions of data collected (28). Thus, to maximise the utility of harmonization by robust linkage and comparability, registries should more proactively develop common data modes to enable future research (39). This should be preferably done with the support from scientific transplant societies and consensus workshops and statements.

Several practical challenges exist in implementing large multinational registries with enough granularity and validated contemporary data for RWE studies. First, such a resource would be costly, and would require innovative design to start and maintain such a registry. Some examples exist however, where regulatory authorities are involved together with the industry, in funding and initiating a wide network of RWD, such as the EU-wide DARWIN (40). Another example of a private-public partnership project is the Transplant Therapeutics Consortium, including the different transplantation societies, FDA, and the industry (41). The inclusion of clinicians and clinical researchers as owners and curators of the datasets is vital for these types of joint efforts to be successful.

Another major hurdle for registry collaboration comes from ownership of data and data sharing policies, especially within the EU with the current General Data Protection Regulations (GDPR). Although GDPR should be EU-wide, individual countries have adopted very different policies for defining concepts of data transfer, making international collaboration

sometimes challenging. One possible solution to this problem could be federated data models, described above, which allow for the generation of cohorts from different datasets without requiring data to leave.

CONCLUSION

Sub-optimal long-term graft survival highlights the need for novel therapies and ways to demonstrate their long-term benefit-risk ratio for patients. Demonstrating superiority of novel therapies is unlikely in conventional RCT's due to the financial and logistical burden of long-term follow-up. However, innovative designs have the potential to facilitate improved longitudinal transplant research by harnessing RWD sources to demonstrate both effectiveness and safety of treatment in a non-invasive, effective, and affordable way. Nonetheless, for innovative designs to bring more value to patients, a common understanding, definition, and agreement on surrogate endpoints predictive of final outcomes in kidney transplantation is required. For this to be possible, harmonization among registries via the alignment of definitions is crucial to improve the comparability and wealth of usable data across clinical practice, RCTs and registries.

The authors recognise that efforts are needed to strengthen the RWD infrastructure, thus also encourage developing studies of sub-populations and non-immunological aspects, as we believe these can demonstrate short and long-term benefits in situations where it may be methodologically hard to demonstrate superiority versus SoC in the general transplant population. Nonetheless, registry collaboration and data harmonization are considered key steps in demonstrating long-term beneficial outcomes of new therapies in kidney transplant patients (Table 1). Finally, clinicians, researchers and data owners are encouraged to explore multi-country collaborative studies that leverage

registries, uptake of technology innovations, as well as the use of federated access and linkage from trials to RWE.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation, provided applicable data protection regulations are complied with. The datasets presented in this article are not readily available because of applicable legislation protecting personal data. Requests to access the datasets should be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

IH, JS, AA, JMC, SB, CL, HT-S, GTB, YG, LP, JBC, and NAD, contributed to designing the paper, to scientific discussions on content, and gave input into the writing of the manuscript. ES contributed to analyzing data sources, and writing of the manuscript.

CONFLICT OF INTEREST

All disclosures below refer to the past 36 months. IH has received funding for Investigator-initiated research from MSD; consulting fees from Novartis, Hansa Biopharma and Takeda; payment or honoraria from Astellas, Boehringer-Ingelheim, Takeda and Novartis; support for attending meetings from Novartis; and participated on a board for Hansa Biopharma, MSD and Novartis. Further, IH is a Coordinating Committee Member of Transplant Therapeutics Consortium and an Associate Editor for the American Journal of Transplantation and Transplant International. JS's institution received research contracts from Novartis, Astellas, Atara Bio and CSL Behring; JS is on the Board of Directors for Donate Life America and Organ Donation Transplantation Alliance, and is on the Clinical Policy Board of LifeSource. AA's institution received lecture honoraria from Sandoz. SB's institution has received honoraria, and SB has received advisory board fees from Astra Zeneca. HT-S received

grants from Novartis and Natera; consulting fees from Novartis, Pfizer, Takeda and GSK; payment or honoraria from Novartis, Pfizer and CareDx; and participated on a board for Novartis. GTB, YG and LP were employed by Novartis Pharma AG at the time of working on this manuscript. YG also declares having stocks in Novartis. JBC is employed by IQVIA and is a member of the Vifor Registry Ad Board and PCORI Clinical Trials Advisory Board, with no personal payments received for this. JBC also declares having stocks and stock options in IQVIA and in GSK (former employer). ES was employed by IQVIA at the time of writing this manuscript. NAD is employed by IQVIA and received train fare fees to attend the Academy of Managed Care Pharmacists meeting in 2021 to discuss high-quality real-world evidence generation. NAD is on the Advisory Board for a study on pulmonary arterial hypertension for Janssen, and a member of the Scientific Advisory Board for The Center for International Blood and Marrow Transplant Research. NAD is also a member of the Board of Trustees of Brandeis University, Waltham, MA. NAD does not receive any personal payments for any of these board memberships.

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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Kidney Transplantation From Hepatitis-C Viraemic Donors: Considerations for Practice in the United Kingdom

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Background: Donor hepatitis-C (HCV) infection has historically represented a barrier to kidney transplantation (KT). However, direct-acting antiviral (DAA) medications have revolutionised treatment of chronic HCV infection. Recent American studies have demonstrated that DAA regimes can be used safely peri-operatively in KT to mitigate HCV transmission risk.

Methods: To formulate this narrative review, a comprehensive literature search was performed to analyse results of existing clinical trials examining KT from HCV-positive donors to HCV-negative recipients with peri-operative DAA regimes.

Results: 13 studies were reviewed (11 single centre, four retrospective). Outcomes for 315 recipients were available across these studies. A sustained virological response at 12 weeks (SVR12) of 100% was achieved in 11 studies. One study employed an ultra-short DAA regime and achieved an SVR12 of 98%, while another achieved SVR12 of 96% due to treatment of a missed mixed genotype.

Conclusion: HCV+ KT is safe and may allow increased utilisation of organs for transplantation from HCV+ donors, who often have other favourable characteristics for successful donation. Findings from US clinical trials can be applied to the United Kingdom transplant framework to improve organ utilisation as suggested by the NHSBT vision strategy “Organ Donation and Transplantation 2030: meeting the need”.

Keywords: kidney transplant, hepatitis C infection, viraemia, donor, utilisation

BACKGROUND

Historically, donor infection with hepatitis-C virus (HCV) has been a barrier to kidney transplantation (KT). This was due to concerns regarding HCV transmission in the context of immunosuppression (IS) with reports of rapidly progressive liver disease in cases of inadvertent viral transmission or glomerulonephritis, directly damaging the implanted kidney (1). Furthermore, interferon therapies, the previous mainstay of HCV treatment were linked with organ rejection (2). Developments of novel antiviral therapeutic agents over the past decade, however, are beginning to change the landscape of transplantation.

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The development of direct-acting antiviral medications (DAA) have revolutionised care of management of chronic HCV infection. Once-daily oral regimens varying between 8 and 16 weeks are very well tolerated and have shown efficacy of >95% of a sustained virological response at 12-weeks (SVR12), indicating viral clearance and cure (3). In times of increased organ demand, such developments have opened the door to a previously overlooked donor pool. Between 2005 and 2014, 3273 HCV antibody positive donors were identified in the United States. Only 37% of retrieved kidneys from this group proceeded to transplantation, the overwhelming majority in HCV-positive recipients. From this group, 4,144 kidneys were discarded although, other than HCV infection, they displayed favourable donor characteristics defined by Kidney Donor Profile Index (KDPI). Moreover, the public health crisis of non-prescribed opioid use in North America has seen a surge in deaths in intra-venous drug users under the age of 50 years old many of whom are HCV-positive and who otherwise might be considered for organ donation (4,5). As a consequence of this, the demographics of potential HCV-positive donors have altered, with the median age decreasing from 47 in 2012 to 35 in 2016 (4). Consequently, if HCV risks can be mitigated, there is the opportunity to increase the donor pool with organs with favourable characteristics for organ transplantation.

These epidemiological changes mean that consideration of HCV-positive donors will become a more commonplace scenario for the transplant clinician. Here, we will discuss how strategies have evolved to mitigate peri-transplant HCV transmission and consider how these developments which have been driven by necessity in North America can be applied to improve utilisation of organs for safe KT within the United Kingdom transplant setting.

METHODOLOGY

A comprehensive database search was performed to formulate this narrative review of the literature. Search strategies employed MedLINE, EMBASE and Cochrane databases to identify studies published up to December 2021. Searches were performed for English language texts using MeSH terms “Kidney Transplantation” AND “Hepatitis C” AND “Tissue Donors”. These terms were also used as keywords within searches. All subsequent abstracts were reviewed. Articles relating to treatment of chronic recipient HCV infection, inadvertent HCV transmission, KT in HIV/HCV co-infection, simultaneous liver-kidney transplantation and HCV+ to HCV- KT prior to the DAA era were excluded. Published articles demonstrating the use of DAA interventions to mitigate the risk of HCV transmission were included. Both prospective and retrospective studies were included. References from the identified studies were also explored to highlight additional studies. United Kingdom transplant data was taken from publicly available annual reports produced by NHS Blood and Transplant and published literature.

DIRECT-ACTING ANTIVIRAL THERAPY AND HCV-POSITIVE KIDNEY TRANSPLANTATION

HCV-Positive Testing and Definitions

Review of early studies of HCV positive donors may be confounded by changes in definition of HCV positivity. Historical and very early studies classed donors as HCV positive based on the presence of anti-HCV antibodies. The more widespread application of HCV antigen test with nucleic acid testing (NAT), by assessing viral RNA by polymerase chain reaction (PCR), allows the detection of contemporaneous viraemia. However approximately 25% of HCV antibody positive individuals will not be chronically infected and thus not viraemic due to spontaneous (innate) viral clearance (6), with a very low to no transmission risk. Furthermore, the roll out of therapeutic and public HCV elimination strategies means an increasing proportion of previous infected individuals will have now been cured of their infection. It is now consensus, that HCV-positive status, should be defined as the presence of HCV NAT viraemia, which conveys risk of transmission. Therefore, it is essential that chronic infection is defined based on detection of HCV NAT. It should also be noted, that immediately following HCV exposure, there is thought to be a window of up to 7 days in which viraemia may be present, but NAT will be negative. This is termed the eclipse window (4).

HCV+ to HCV+ Kidney Transplantation

DAA regimes have been applied successfully to KT in HCV-positive recipients in a number of North American centres. Outcomes of 40 HCV-positive recipients were examined retrospectively, 19 of whom received an HCV-positive KT. Twenty-three received Ledipasvir (LDP) and Sofosbuvir (SOF), 12 received SOF and Simeprevir (SIM) and four received LDP, SOF and Ribavirin (RIB) in combination. Thirty-six patients received 12 weeks of DAA therapy, while the remainder received 16 or 24 weeks, as directed by a transplant hepatologist. All patients achieved a sustained virologic response at 12 weeks (SVR12) with good tolerance of treatment and 100% 1 year graft survival (7). This successful approach has been echoed in another cohort of 25 HCV-positive recipients who received an HCV-positive KT, with the majority receiving a 12-week DAA regimen, initiated at a median of 125 days (IQR 100–169) post-transplant. One recipient was non-compliant, producing an intention to treat derived SVR12 of 96% (8). Critically, both of these studies noted a reduced time on the waiting list after acceptance of an HCV-positive KT (7,8). For these recipients, the developments in DAA regimens, mitigated HCV risk and was favourable when compared to a prolonged period on dialysis with its associated morbidity and mortality. These initial studies have demonstrated how recipients can benefit from the safe expansion of the donor pool with good outcomes which has now become established practice. Such studies have also encouraged other investigators to consider the safe use of HCV-positive kidneys in HCV-negative recipients.

TABLE 1 | Studies investigating HCV+/HCV- kidney transplant with DAA regimes.

Author	Sample	Donor	Recipient	Genotypes	Immunosuppression	DAA regime	SVR	Notes
Durand et al 2017 (10)	<i>n</i> = 10	100% DBD	20% female	G1a 30%	Induction: Methylprednisolone, rATG	G1a: GZR/EBR 12/52	100% at 12/52	Pre-emptive
EXPANDER	Single centre	Median 30yo (IQR 23–35)	Median 71yo (IQR 65–72)	G2 10%	Maintenance: Tacrolimus, MMF, Prednisolone	G2 and G3: GZR/EBR + SOF 12/52		No DAA SAE
	Prospective Non-randomised	KDPI 45% (IQR 32–48)	Pre-transplant dialysis 1.6 years (IQR 0–2.6)	G3 10% G1a/3 10% Indeterminate 40%				
Goldberg et al 2017 (9)	<i>n</i> = 10	80% DBD	50% female	G1a 100%	Induction: Methylprednisolone, rATG	GZR/EBR 12/52	100% at 12/52	DAA started after HCV viraemia detected POD3 1 case possible DAA FSGS
THINKER	Single centre	Median 31yo (IQR 29–42)	Median 59yo (IQR 52–63)		Maintenance: Tacrolimus, MMF, Prednisolone			
	Prospective Non-randomised	KDPI 42% (IQR 32–48)						
Molnar et al 2019 (32)	<i>n</i> = 53	89% DBD	18% female	G1a 64%	Induction: rATG	89% GLP/PTR	100% at 12/52	DAA started after HCV viraemia 4–8/52 post KTx DAA AE due to delayed treatment
	Single centre Retrospective	Mean 32.2yo (SD ± 5.3)	Mean 52.6yo (SD ± 10.9)	G1b 2% G2 6% G3 28%	Maintenance: Tacrolimus, MMF, Prednisolone	9% SOF/VPT 2% SOF/LDP		
Friebus-Kardash et al 2019 (29)	<i>n</i> = 7	57% female	57% female	G1a 28%	Induction: Basiliximab	G1a: SOF/VEL or SOF/VEL/RIB	100% at 12/52	DAA started after recipient viraemia detected; median POD7 No DAA SAE
	Single centre Retrospective	Mean 44.2 yo (SD ± 10.2)	Mean 52.8yo (SD ± 13.5)	G1b 42% G3a 28%	Maintenance: Tacrolimus, MMF, Prednisolone 1 used anti-CD40 Ab for induction in place of Tac; 1 received plasmapheresis and IVIG due to HLA pre-sensitisation	G1b: SOF/LED G3a: SOF/VEL (All 8–12/52)		
Gupta et al 2019 (17)	<i>n</i> = 50	KDPI 62% (SD ± 18)	36% female	G1a 19%	Induction: rATG	Prophylaxis 2–4/7 SOF/VEL If HCV transmission ELB/GZR 12/52 + 2nd line option if required	98% at 12/52	Pre-emptive Ultra-short course promotes DAA resistant HCV mutations
	Single centre Adaptive trial design		Median 60yo (IQR 36–76)	G2 4% G3 12% Indeterminate 8% Unknown 8%	Maintenance: Tacrolimus, MMF, Prednisolone			
Duerr et al 2019 (15)	<i>n</i> = 7	Mean 46.4 (±SD 7.8)	Mean 59.4 (±SD 8.4)	G2a 100% (of those NAT+)	Induction: Basiliximab Maintenance: Tacrolimus, MMF, Prednisolone	DCV/SOF 12/52	100% at 12/52	Pre-emptive Seroconversion (HCV Ab+) at 12/52 in 5/7 recipients
	3 HCV NAT+, 4 HCV Ab + Single centre Prospective							
Kapila et al 2020 (34)	<i>n</i> = 64	Median 32 (range 19–56)	Median age 69.5 (range 32–81)	G1 5%	Induction: Methylprednisolone, rATG	LDP/SOF 12/52 37.5%	At end of study period	DAA started after viraemia (median 72 days; range 9–198)
	Single centre	KDPI 54% (range 25–99)	68.8% male	G1a 59%	Maintenance: Tacrolimus, MMF, Prednisolone	GLP/PTR 12/52 51.2% VEL/SOF 12/52 1.6%	58 received DAA 41 SVR12 10 HCV NAT- but had not reached 12/52	3 patients did not develop viraemia 2 cases FCH
	Prospective			G2 9% G3 13%				

(Continued on following page)

TABLE 1 | (Continued) Studies investigating HCV+/HCV- kidney transplant with DAA regimes.

Author	Sample	Donor	Recipient	Genotypes	Immunosuppression	DAA regime	SVR	Notes
				G4 5% Mixed 5%			follow up 7 DAA current treatment 1 case of resistance with prolonged therapy due to resistance	
Sise et al 2020 (12)	n = 30	KDPI 53% (IQR 41–65)	30.0% female	G1a 43%	Usual standard of care	GLP/PTR 8/52	100% at 12/52	DAA started POD 2–5
MYTHIC	Multicentre	Median 33.5yo (IQR 29–38)	57yo (IQR 51–60)	G2 3%	Variation of regimes between centres			Median 6-month eGFR 57 ml/min/1.73 m ² No DAA SAE
Sise et al 2020 (13)	n = 8 Single centre	100% DBD Median 27yo (IQR 25–30)	25% female Mean 55.9yo (SD ± 9.4)	G4 3% Unknown 50% G1a 100%	Induction: Methylprednisolone, rATG Maintenance: Tacrolimus, MMF, Prednisolone	GZR/ELB 12/52	100% at 12/52	Pre-emptive No DAA SAEs
	Retrospective	KDPI 31% (IQR 29–65)						
Feld et al 2020 (20)	n = 30 Single centre	Median 36 (IQR 31–39)	77% male Median 61yo (IQR 48–66)	G1 50% G2 11% G3 28% Unknown 11%	Usual standard of care Cyclosporin avoided	EZE (10 mg) + GLP/ PTR (300mg/120 mg) 7/7	100% at 12/52	Pre-emptive 1 DAA serious AE (transient elevation of liver enzymes in KT recipient)
Jandovitz et al 2020 (16)	n = 25 Single centre	Mean age 35yo (SD ± 8.9)	76% male	G1a 60%	Induction: Basiliximab	LDP/SOF 12/52 56%	96% at 12/52	DAA start median 13 days (IQR 8–22)
	Retrospective	KDPI 49 (IQR 38–66)	Mean age 57.7yo (SD ± 10.4)	3a 28%	Maintenance: Tacrolimus, MMF, Prednisolone	VEL/SOF 12/52 32%	1 case of mixed genotype requiring re- treatment to achieve SVR12	
Durand et al 2020 (14)	n = 10 Single centre	Median age 38.5yo (IQR 20–45)	70% male	G1a 60%	Not specified	GLP/PTR 4/52	100% at 12/52	Pre-emptive
		KDPI 60% (29–76)	Median 67yo (IQR 40–75)	G1b 10% G3 20% Unknown 10%				No DAA SAE
Terrault et al 2021 (46)	n = 24 Multi-centre	Median age 36 (IQR 31–41.5)	KT recipients 45% male	Not specified	Usual standard of care	SOF/VEL 12/52	100% at 12/52	DAA start median 16.5 days (IQR 9.8–24.5) No DAA SAE in KT group
	Heterogeneous recipients (11 KT)	KDPI (52 40.5–61.5)	Median age 54 (IQR 52–57)					

Ab—antibody; AE—adverse event; DAA—direct acting antiviral; DCV—daclatasvir; ELB—elbasvir; EZE—ezetimibe; FCH—fibrosing cholestatic hepatitis; GLP—glecaprevir; GZR—grazoprevir; HCV—hepatitis C virus; HLA—human leukocyte antigen; IQR—inter-quartile range; IVIG—intravenous immunoglobulin; KDPI—kidney donor profile index; KT—kidney transplant; LDP—ledipasvir; MMF—mycophenolate mofetil; NAT—nucleic acid amplification test; POD—post-operative day; PTR—pibrentasvir; rATG—rabbit antithymocyte globulin; SAE—serious adverse event; SPK—simultaneous kidney pancreas transplant; SIM—simperavir; SOF—sofosbuvir; Tac—tacrolimus; VEL—velpatasvir.

HCV+ to HCV- Kidney Transplantation

Several centres have made significant progress in this field over the past 5 years (Table 1). Initial studies used 12 week regimens of Grazoprevir (GZR) and Elbasvir (EBR) in small single centre prospective cohorts to good effect, demonstrating 100% SVR12 ($n = 10$ and 10 respectively) (9,10). These studies used majority DBD (100% and 80%) donors with median ages [30 (IQR 23–35) and 31 (IQR 29–42)], demonstrating the advantageous demographics previously described in HCV-positive donors (5). Different timepoints for the onset of DAA regimens were used by these study groups. In the THINKER trial, Goldberg et al (9) initiated the DAA regime on post-transplant day 3 after HCV viraemia had been detected within the transplant recipients, whereas Durand et al (10) opted for a pre-emptive approach in EXPANDER. This initiated DAA therapy immediately post-transplant. These two strategies of transmit and treat versus prophylactic regimens have been mirrored in subsequent generations of peri-transplant DAA studies. As DAA studies in this field have emerged as successful and safe, investigators have sought to determine the optimal course timing and duration, without sacrificing efficacy (11).

Early studies favoured testing for HCV genotype with subsequent genotype specific treatment, whereas, more recently, small volume studies have used pangenotypic agents for long or intermediate post-transplant durations and achieved satisfactory results (Table 1) (12–14). These have mostly been used in confirmed cases of HCV NAT+ donors, but one strategy has employed the use of pangenotypic DAAs in HCV NAT- Ab+ donors in addition (15). Of note, transmit and treat strategies which do not employ pangenotypic agents are reliant on accurate genotyping, this can cause difficulty when mixed genotypes are not detected (16). Gupta et al (17) used an adaptive trial design to trial two to four doses of pangenotypic SOF and Velpatasvir (VEL) on transplant day 0–4. This was commenced immediately pre-transplant to prevent transmission in 50 recipients. Six cases across all phases of the study required 3 months of DAA treatment for HCV transmission. This regimen was associated with a lower SVR12 compared to other trials (98%), and three recipients of six cases of HCV transmission developed treatment resistant mutations. One recipient also developed acute rejection simultaneously to developing HCV viraemia, which the authors suggest could have contributed a non-specific immune response triggering rejection. Given the inferior results in comparison to widespread success with longer DAA regimens, the authors suggested that this course length should not be adopted. While such an approach may be favoured by healthcare funders, the outcomes appear inferior.

Following the use of a 4-week course of SOF/VEL producing 100% SVR12 in a cohort of 44 cardiothoracic transplant recipients (36 lung, 8 heart) receiving organ from HCV+ donors without any adverse events, a similar strategy has been applied to kidney transplantation (18). Durand et al (14) used GLP/PTR combination therapy for 4 weeks with the first dose administered prior to organ perfusion. This small study demonstrated feasibility of such an approach in renal transplant recipients with a 100% SVR12. HCV was transmitted in 50% of cases, of which all had undetectable

levels of HCV RNA 2 weeks after treatment was commenced. This strategy, although only a preliminary study, seems to balance the safety requirements required with excellent efficacy and a short duration, making prophylactic regimens acceptable for healthcare funders. It should be noted that DAAs have been well tolerated in all transplant studies to date as has been described in the literature relating to treatment for chronic HCV. In particular, toxicity is infrequent and not severe, not usually requiring treatment alteration and there are few drug-drug interactions (DDI), which is of special importance in the transplant cohort (19). Of note, cyclosporin has been avoided in previous trials due to DDI risk due associated with GLP and GZR, but is no longer generally favoured for use in immunosuppressive regimens (20,21). As such, review by pharmacist with experience in management of HCV DAAs is of importance.

The shortest regime has been applied to a heterogeneous group of 30 transplant recipients with success (10 KT, one simultaneous pancreas and kidney (SPK)) (20). In addition to a DAA regime of GLP/PTR, Ezetimibe (EZE) was also administered with eight doses (one prior to transplant and on seven subsequent post-transplant days). EZE acts as a Niemann-Pick C1-like 1 (NPC1L1) receptor antagonist, a key component of cholesterol uptake in hepatocytes, warranting its use in hypercholesterolaemia. NPC1L1, is also targeted by HCV for hepatocyte cell entry and has been demonstrated to block this *in vitro* and reduce HCV establishment of some genotypes *in vivo* mouse models (22). 67% of recipients developed transient viraemia, with HCV RNA undetectable by 14 days post-transplant and 100% SVR12. This initial transmission rate is comparable with other studies, without the use of EZE, suggesting that its role needs further investigation. These studies have changed the field, but more is required to facilitate widespread use outside of clinical trials. The studies to date are published by single specialist centres with small sample sizes and only limited follow up with regards to graft function. Many of the studies have heterogeneous organ recipients. This should be considered, as although useful for demonstrating initial safety and proof of principle, there may be important factors to observe in longer term follow up between organ recipient groups and different immunotherapeutic regimens. Treatment resistance emerged as a concern following short course DAA regimens. This is something which should be monitored closely in other larger and longer-term studies to examine whether this phenomenon also is exhibited in longer DAA regimens but has not been detected due to insufficient study power. Given the risks of chronic HCV infection to the recipient in the advent of failed viral clearance, a low threshold for treatment failure should be established in future studies and clinical practice. Reassuringly, no variation in standard immunosuppressive regimens have been employed in the existing trials to date (Table 1), such requirements would represent significant concerns for transplant clinicians and any requirement for immunosuppression alterations should be recorded in future trials and registries. Currently, kidney transplantation in the context of HCV has been performed in small volumes at a limited number of centres and more comprehensive data is not currently available.

UNITED KINGDOM LANDSCAPE

The utilisation of organs from HCV positive donors is not established practice in the United Kingdom and the prevalence of HCV positive donors is lower than in North American populations. Between 2010 and 2014 of 8,184 potential organ donors with acquired consent for transplantation, 77 tested anti-HCV antibody positive: a prevalence of 0.94% (CI 0.74–1.18). 54 of this group were below the age of 54 with 42 having injected recreational drugs of which 21 had continued active use (23). This represents a lower volume than the United States but mirrors the typically younger age of HCV+ donors.

In 2018, 26 individuals were identified, and consent acquired for donation who also tested positive for anti-HCV antibody. Of these 26 patients, only five had organs utilised. In 2019, 50 anti-HCV antibody positive donors were identified and consented, but only 16 of these proceeded to donation. Exact reasons for this attrition are not specified but it is presumed to be due to concerns regarding transmission. Unfortunately, data on organ specific patterns are not available. The median ages of those that proceeded to donation in those years were 41.9 and 44 years, respectively, lower than the mean age of all donors of 52, demonstrating the possible benefits in utilisation (24,25). Of note, although younger than the mean United Kingdom age, this is older than the typical age seen in HCV+ donors in the United States (4).

Mitigating the risk of HCV transmission would allow a greater proportion of these donors to proceed to donation and increased organ utilisation. In 2018–2019, this would equate to a potential of 76 donors and 152 kidney recipients. Of note, HCV RNA screening is not routine for potential United Kingdom donors. Consequently, an unknown proportion of these donors may not have been HCV viraemic at time of donation, a scenario which has been demonstrated to be safe in some cohorts (26,27). The addition of HCV NAT testing in the United Kingdom, would allow improved objective assessment to allow transmission risk to be considered and potentially mitigated. Although this article analyses epidemiological factors within the United Kingdom, we anticipate that this is similarly applicable to other European populations where opiate use is less prevalent than the United States; indeed promising early German and Spanish experiences have been published (28,29).

FUTURE CONSIDERATIONS FOR UNITED KINGDOM APPLICATION

As discussed, there has been continued advance in DAA therapy to mitigate transmission from HCV viraemic donors, which has allowed increased organ utilisation in the US. The most recently published data by NHSBT in the United Kingdom suggests that there is a potentially under-utilised donor pool within the United Kingdom. Consequently, the increased use of such organs should be considered, resulting in significant benefits for patients on the transplant waiting lists. The joint United Kingdom vision statement “Organ Donation and

Transplantation 2030: Meeting the need” highlights the need to further increase organ utilisation. Instigation of recommendations from “Taking Organ Transplant to 2020” has led to an increase in the successful utilisation of older donors with more comorbidities with sustained level of outcomes nationally but opportunities remain for improvement (30). Although the number of people waiting for a kidney transplant in the United Kingdom had reduced to 2015, since then the number on the active waiting list for a cadaveric kidney transplant has plateaued around 5,000 patients (2017/18: 5,033; 2018/19: 4,977; 2019/20: 4,960), 67% of whom are still waiting beyond a year for transplantation (31). As we have described, although HCV+ positive donors have been identified, the proportion of organs utilised could be improved. Consequently, as the waiting list continues to build, utilisation of HCV+ organs with DAA regimens to mitigate transmission risk represents a feasible and sustainable strategy to achieve the goals for 2030.

Real world data from the US has demonstrated that outside of clinical trials, where regimens are supplied by manufacturers or funding for DAA therapy is guaranteed, there have been difficulties in acquiring approval from insurers following HCV transmission (16,32). Many funders are reluctant to provide cover for a pre-emptive or prophylactic DAA regimen and subsequently favour transmit and treat approaches (33). Consequently, this has led to delays in treatment (34). Such delays have the potential to induce sequelae of HCV infection, with serious implications such as fibrosing cholestatic hepatitis (35). It should be noted that treatment failure has the potential to induce devastating complications including graft loss. Concerns have also been raised regarding the increased risk of the development of BK viraemia and cytomegalovirus (CMV) and severe cases have coincided with the formation of *de novo* donor specific antibodies (32,36). Studies to date have not noted significant difference in the prevalence of such viral complications, but when such events occur, the severity has been increased (36,37). Consequently, thorough surveillance strategies will be required.

National funding strategies on medication approval based on evidence-based medicine and controlled by the National Institute for Clinical Excellence removes this consideration from the equation in the United Kingdom. As a result, prophylactic regimens which can be approved for patients nationally may be more palatable in the United Kingdom and may mean that translation from clinical trials to common practice is less challenging. The possibility of short course DAA regimens make this even more possible. From a health economics perspective, the potential to reduce waiting list time and associated long term dialysis costs are likely to offset the cost of DAA regimens, making such strategies appealing when overall cost of care for patients with ESRD are considered. The unit price for a 28 day pack of GPR-PBR is £12,993.99 as reported by NICE for use in chronic HCV (38), while estimated annual dialysis costs in the United Kingdom are £24,043 and £20,078 for haemodialysis and peritoneal dialysis respectively (39). This has been robustly demonstrated in the Canadian and US populations and agreed by the United Kingdom joint taskforce (40,41). The cost benefits for providers will also be greater if short

courses of DAA regimens as described by Durand et al (14) and Feld et al (20) can become standard care.

Despite the evidence of safety, patient perception and education regarding this novel approach is paramount. HCV for many has an associated stigma and may result in reduced uptake. However, several studies have shown that those in receipt of an HCV+ transplant have had positive experiences. For most recipients surveyed, the benefit of reduced waiting list time was important in their choice to accept an HCV+ organ. Smaller numbers reported concerns with donor lifestyle factors and a possibility that the organ they received was of lower quality and in one survey, only 9% were concerned about sexual transmission to partners although reported behavioural change, such as avoiding sharing glasses, due to concerns of transmission (42,43). In the follow up to the EXPANDER study, no patients report being victims of stigma or being treated differently and did not regret their involvement (44).

Despite the increasing amount of evidence, this remains a novel approach to care and warrants stringent observation and assessment in line with IDEAL standards (45). Through NHSBT the United Kingdom has excellent tools in place for clinical governance and registration with continued assessment of patient outcomes which is crucial as this option remains a treatment strategy which should be conducted within an investigative framework. Patients who choose to enrol in such schemes should be provided with sufficient information regarding the evidence to date including the possible consequences in order that informed consent can be acquired. In some non-publicly funded healthcare settings, it should also be necessary to determine the availability of DAA therapy prior to proceeding to transplant.

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CONCLUSION

There has been rapid progress in the development of DAA therapy after renal transplantation to facilitate the use of HCV viraemic donor organs safely in HCV non-viraemic recipients. Such strategies have been demonstrated to be safe in US clinical trials, but there have been difficulties in transforming this to become standard care. Although less than in North America, there is a potential pool of young, otherwise healthy donors with preferential characteristics for organ utilisation, if HCV transmission can be mitigated. The national funding and governance structure of United Kingdom healthcare allows evidenced based practice to be initiated with stringent assessment of outcomes to use this potential donor pool to safely reduce waiting list time for the benefit of all patients with ESRD.

AUTHOR CONTRIBUTIONS

DD, DvD, MP, and HK were responsible for study concept. DD and HK performed literature review. Original draft was produced by DD, VA and HK. All authors contributed and reviewed final version of article.

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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Hyperammonemia After Lung Transplantation: Systematic Review and a Mini Case Series

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Background: Hyperammonemia after lung transplantation (HALT) is a rare but serious complication with high mortality. This systematic review delineates possible etiologies of HALT and highlights successful strategies used to manage this fatal complication.

Methods: Seven biomedical databases and grey literature sources were searched using keywords relevant to hyperammonemia and lung transplantation for publications between 1995 and 2020. Additionally, we retrospectively analyzed HALT cases managed at our institution between January 2016 and August 2018.

Results: The systematic review resulted in 18 studies with 40 individual cases. The mean peak ammonia level was 769 $\mu\text{mol/L}$ at a mean of 14.1 days post-transplant. The mortality due to HALT was 57.5%. In our cohort of 120 lung transplants performed, four cases of HALT were identified. The mean peak ammonia level was 180.5 $\mu\text{mol/L}$ at a mean of 11 days after transplantation. HALT in all four patients was successfully treated using a multimodal approach with an overall mortality of 25%.

Conclusion: The incidence of HALT (3.3%) in our institution is comparable to prior reports. Nonetheless, ammonia levels in our cohort were not as high as previously reported and peaked earlier. We attributed these significant differences to early recognition and prompt institution of multimodal treatment approach.

Keywords: hyperammonemia, lung transplantation, ammonia, glutamine synthetase, urea cycle, ammonia scavengers, mollicutes

INTRODUCTION

Hyperammonemia after lung transplantation (HALT) is a rare but often fatal complication. It manifests as an elevated serum ammonia level that leads to encephalopathy, cerebral edema, seizure, coma, cerebral herniation, and death. Reported incidence of HALT ranges from 0.99 to 4%, with fatality rates exceeding 75% (1–3).

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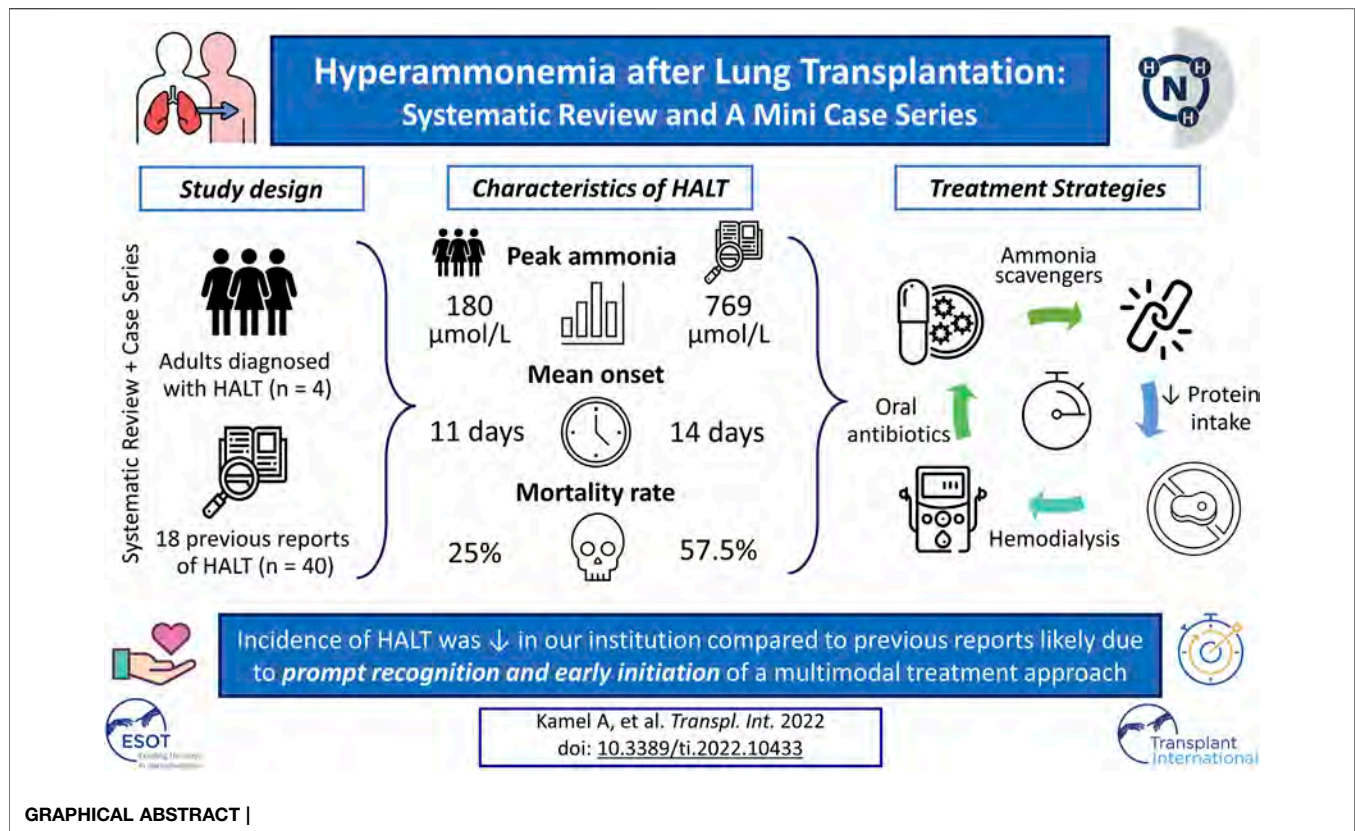
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Hyperammonemia (HA) after organ transplantation has also been described in patients who underwent bone marrow, liver, kidney, and heart transplantation (4–12). The exact etiology of HALT is unknown. Ultimately, the cause of HA may be related to excess production, decreased clearance of ammonia, or both. Disorders of glutamine synthetase (GS) have been described in patients with HALT (1,13,14). More recently, infection with urea-splitting microorganisms has been reported in patients with HA after organ transplantation (11,12,15–17).

This systematic review aims to explore potential etiologies and investigate if patients' metabolic profile supports the Urea cycle (UC) pathway involvement (Figure 1). Additionally, we report our center's successful experience managing four HALT cases, emphasizing an alternative approach to therapy. In one of the cases, we examined the liver tissue obtained at biopsy, which showed a significant downregulation of GS, suggesting a potential role for the GS pathway in HALT.

MATERIALS AND METHODS

Data Sources

A health science librarian performed a systematic search of the medical literature on the occurrence of HA in lung transplants. An initial examination of six bibliographic databases and grey literature sources, CINAHL, Clinical Trials.gov, Cochrane Library, Embase, International Pharmaceutical Abstracts (IPA), PubMed, and Web of Science, was performed. The search strategy

combined database-specific controlled vocabulary, truncated, and phrase-searched HA and lung transplantation keywords limited to English language full-text and publication dates of 1995–2020 (Supplementary Material S1).

Study Selection Criteria

To be included in the systematic review, a study had to 1) be performed in adults that developed HALT requiring treatment, combined cases of other organs were not included, and 2) have full text available in English.

Data Extraction

Extracted data included author name, year of publication, number of patients, time to peak ammonia level, peak ammonia level, treatment with bowel decontamination, nitrogen scavengers and renal replacement therapy, outcome, and hypothesized etiology of HA.

Our Experience

We performed a retrospective review of all lung transplants performed at the University of Florida Health hospital between 1 January 2016, and 31 August 2018. We screened for HA episodes by extracting serum ammonia levels from the electronic medical record obtained on and after the transplantation. Hyperammonemia was defined as any plasma ammonia level higher than the upper limit of normal as previously described (18). At our center 60 µmol/L is the upper limit of normal.

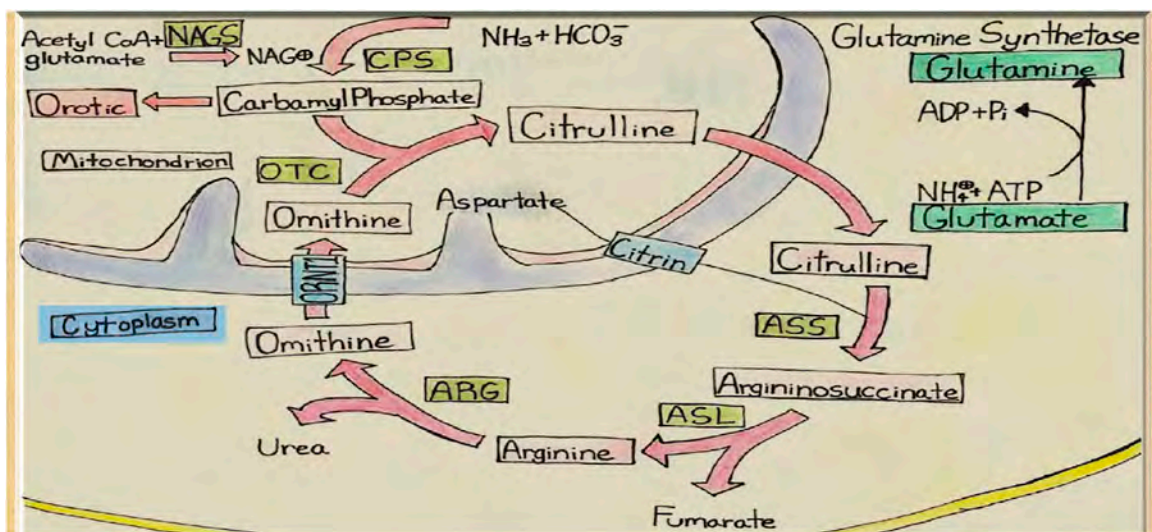


FIGURE 1 | Urea Cycle and Hepatic Glutamine Synthetase. NAGS, N-acetyl glutamate synthetase; CPS, Carbamoyl Phosphate Synthetase; OTC, Ornithine Transcarbamoylase; ASS, Argininosuccinate Synthetase; ASL, Argininosuccinate Lyase; ARG, Arginase; ORNT1, Ornithine Translocase.

In addition to hyperammonemia, our definition of HALT required meeting at least three out of the following criteria: 1) absence of cirrhosis, liver failure, or history of liver transplantation; 2) the presence of encephalopathy; 3) administration of specific treatment for HA; and 4) agreement of at least two independent reviewers (AK, AE, SC). We extracted the following data: demographic information, induction and maintenance immunosuppression used, ammonia levels, metabolic profile, baseline laboratory, treatment with bowel decontamination, nitrogen scavengers, RRT modality, and patient outcomes. The University of Florida Institutional Review Board approved this study.

Statistical Analysis

Statistical software JMP (SAS v 15) was used to analyze the data. Descriptive analyses were applied for demographic variables and medical condition variables. Means and standard deviations were calculated for continuous variables; frequencies and percentages were calculated for categorical variables.

Glutamine Synthetase Immunohistochemistry

Liver biopsy obtained from the first case was stained for GS enzyme activity using mono and polyclonal antibodies and compared to healthy liver tissue control (Figure 2).

RESULTS

Characteristics of the Systematic Review

A flow chart of screening and selection for inclusion is presented in Figure 3. After filtering out duplicate studies, our search resulted in

18 studies, including 40 individual cases that met inclusion for full-text review. Details of previously published HALT cases are reported in Table 1. Time from transplantation to peak ammonia level ranged from 1 to 45 days (mean 14.1 days) of the 35 cases with reported values. Peak ammonia levels ranged from 55 to 5,000 $\mu\text{mol/L}$ (mean 760.2 $\mu\text{mol/L}$) of the 35 cases with reported values. Of the 40 cases, 17 (42.5%) survived and 23 (57.5%) died.

Treatment and Outcome

The majority of patients who received bowel decontamination were administered lactulose, rifaximin, metronidazole, or neomycin. Besides arginine or levocarnitine, ammonia scavengers were used in at least 15 of the reported cases, with few cases using up to four different agents. Continuous veno-venous hemodialysis (CVVHD) was the primary RRT in 15 patients. Intermittent hemodialysis (iHD) was used in eight cases. In six patients, the combination of the two was used. One case used continuous arterio-venous hemodialysis (CAVHD), and two cases used a molecular adsorbent recirculating system (MARS) in combination with plasmapheresis, extracorporeal oxygenation (ECMO), and RRT.

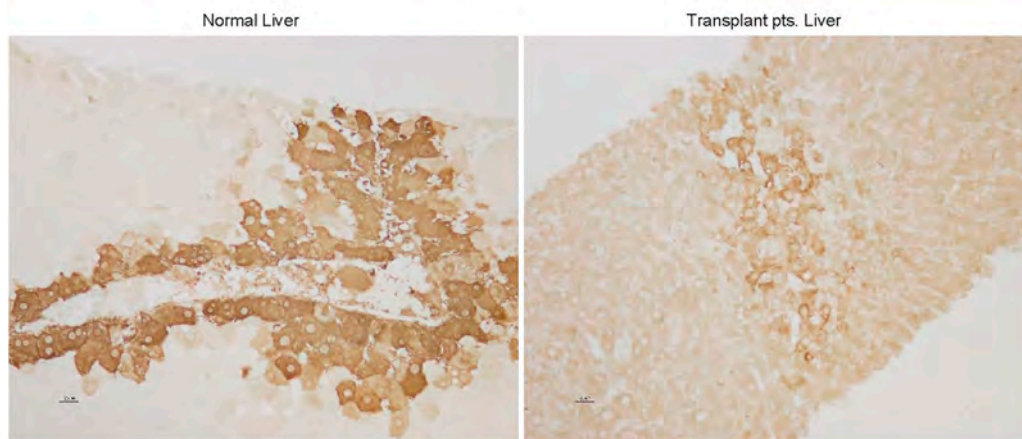
The majority of reported etiology was idiopathic (26 cases), followed by Mycoplasma/ureaplasma infection (nine cases) and GS deficiency (two cases). One case attributed etiology to inhibition of carbamoyl phosphate synthase by valproic acid, and two others did not have etiology reported.

EXPERIENCE AT OUR CENTER

Case 1

A 68-year-old man with end-stage idiopathic pulmonary fibrosis underwent bilateral sequential LT. The patient developed severe encephalopathy on post-op day 2. His clinical condition further

Glutamine synthetase- Monoclonal antibody



Glutamine synthetase- Monoclonal antibody

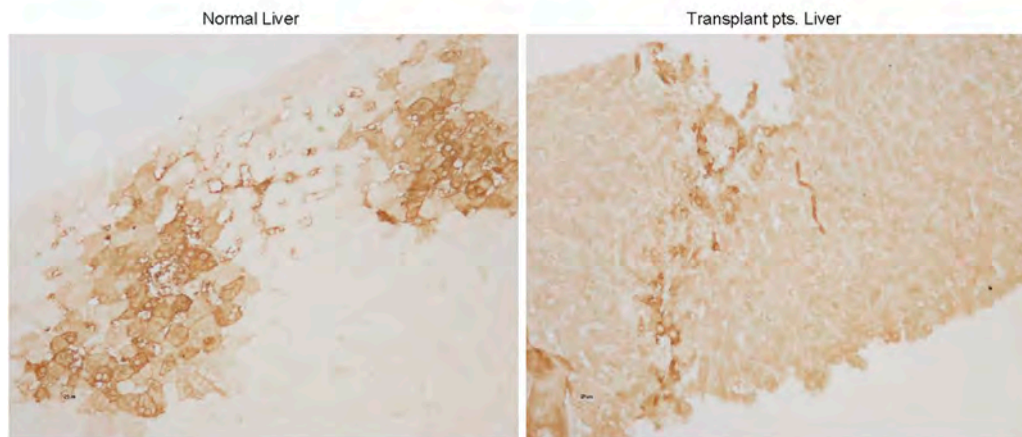
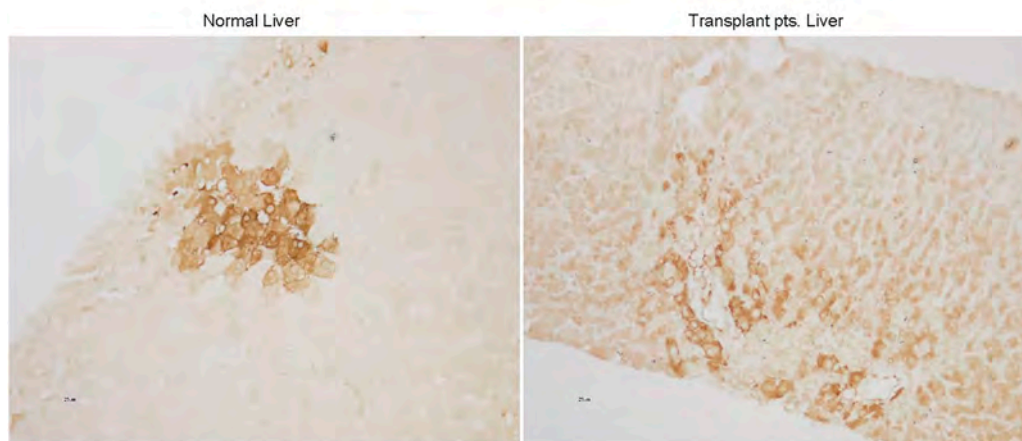


FIGURE 2 | (Continued).

deteriorated, and he went into distributive shock, requiring several vasopressors. Antimicrobial coverage was broadened to include levofloxacin, metronidazole, and micafungin. Baseline lab (Table 2) and computed tomography of the head, chest, and abdomen were unrevealing. Electroencephalogram was negative for seizures. Serum ammonia (NH_3) level on post-op day six was $245 \mu\text{mol/L}$, which was elevated from the immediate post-transplant level of $55 \mu\text{mol/L}$ (normal $<60 \mu\text{mol/L}$). A comprehensive workup for an infection that included blood cultures for urea-splitting organisms, bacteria, and fungi was negative. Serum ammonia continued to rise despite the implementation of RRT and aggressive bowel decontamination with lactulose and metronidazole. The patient's distributive shock

persisted. At this juncture, the possibility of a urea cycle (UC) disorder was considered, and intravenous sodium benzoate, sodium phenylacetate, and arginine were initiated. With the above therapies, the patient's serum ammonia returned to normal over the next 3 days. This coincided with the normalization of his hemodynamics, serum lactate level, and resolution of his altered mental status. Unfortunately, the patient subsequently developed severe septic shock from a perforated cecum that required exploratory laparotomy, colectomy, and placement of ileostomy. Following this, a transjugular liver biopsy was performed for elevated liver enzymes, and this was complicated by hemorrhagic shock. At this point, the patient's family withdrew care.

Glutamine synthetase- Polyclonal antibody



Glutamine synthetase- Polyclonal antibody

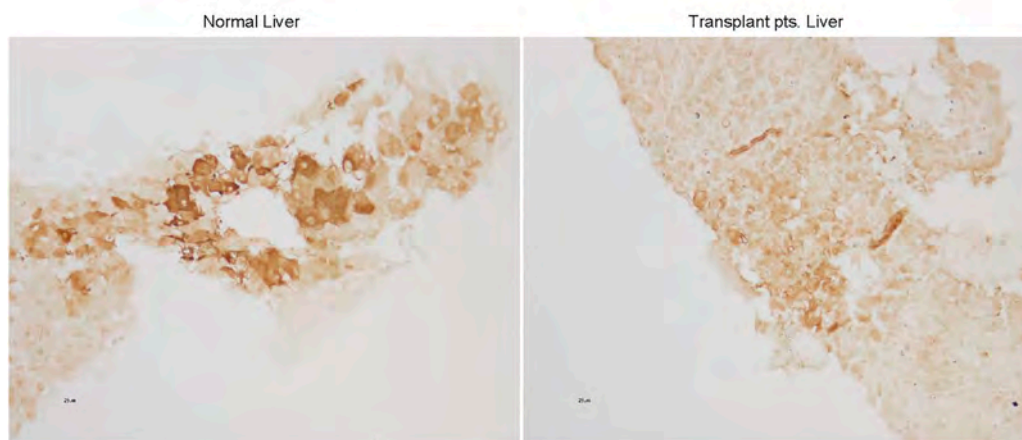


FIGURE 2 | (Continued). Hepatic glutamine synthetase monoclonal and polyclonal antibody immunohistochemistry.

Case 2

A 64-year-old man with end-stage idiopathic pulmonary fibrosis, diabetes, and sleep apnea, underwent uncomplicated bilateral sequential LT. Cultures from the donor bronchus grew methicillin-sensitive staph aureus, which was treated with cefazolin for 7 days. Postoperative ammonia level on day 2 was 155 $\mu\text{mol/L}$ with a follow-up level of 77 $\mu\text{mol/L}$. Levofloxacin to cover urea spitting organisms and metronidazole to provide bowel decontamination was started. Ammonia level decreased to 35 $\mu\text{mol/L}$ on post-op day 3, and he was extubated the following day. However, the ammonia level increased to 77 $\mu\text{mol/L}$. Azithromycin was added for dual coverage of ureaplasma and mycoplasma. All sources of oral protein intake were stopped for 24 h. Enteral sodium

phenylbutyrate, rifaximin, lactulose, and intravenous arginine in dextrose with micronutrients (**Supplementary Material S2**), and intravenous lipid as a source of calories were initiated. Serum amino acid profile and urine orotic acid levels that were checked to detect underlying UC disorders were negative (**Table 3**). The patient's ammonia level continued to fluctuate between 60 and 80 $\mu\text{mol/L}$ despite the above interventions. The patient was, however, asymptomatic without any signs of encephalopathy. Protein in the diet was introduced 48 h later (0.25 g/kg/d initially) and gradually increased to prevent catabolism. Ammonia level eventually started to downtrend with this multimodal therapy. The patient continued to improve and was ultimately discharged to a rehabilitation facility on post-transplant day 17.

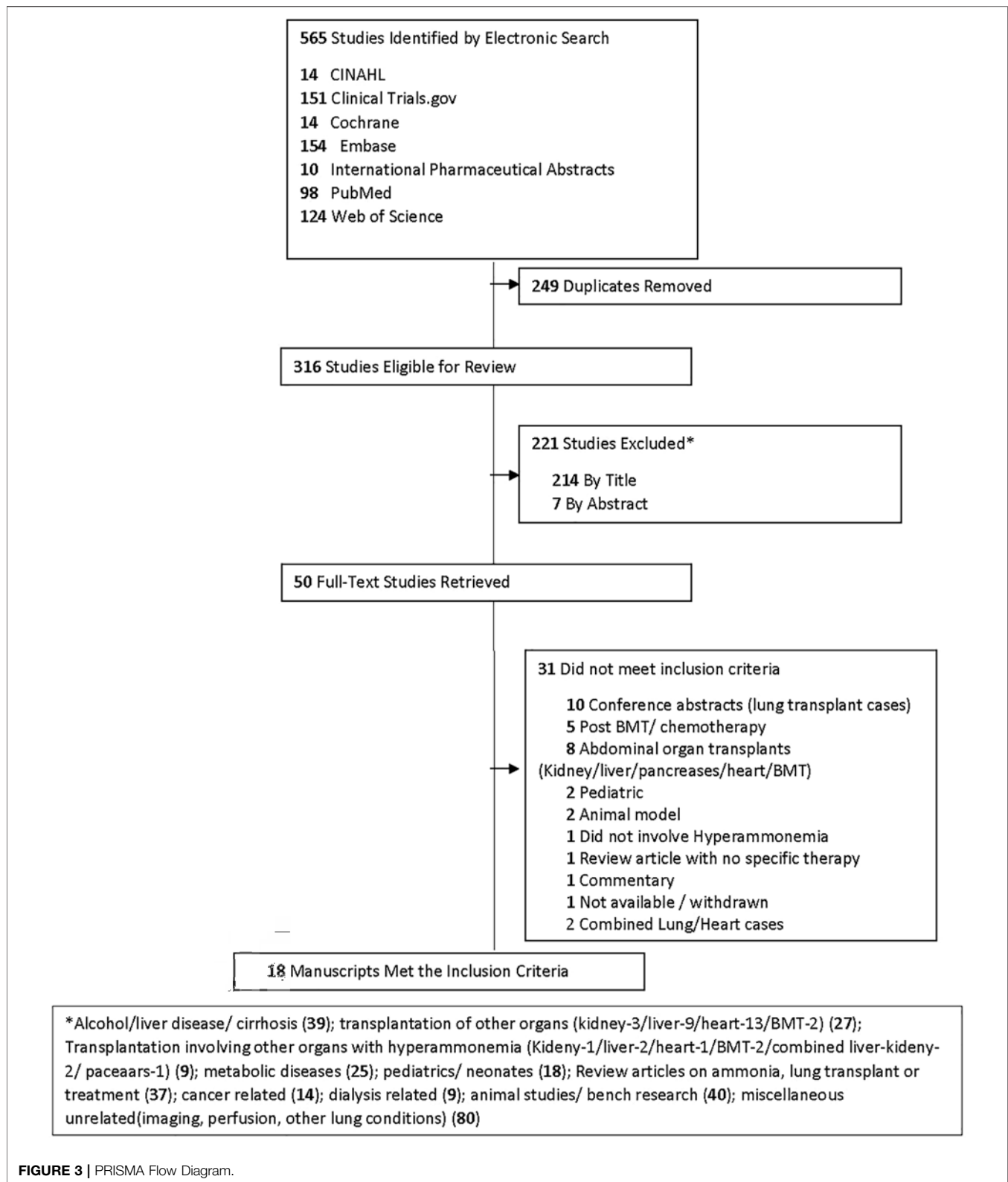


FIGURE 3 | PRISMA Flow Diagram.

TABLE 1 | Previous Reported Cases of Post lung Transplant Hyperammonemia.

References	Gender	Case (s)	POD to peak	Peak NH ₃	Bowel decontamination	N ₂ scavengers	RRT	Outcome	Hypothesized etiology
(31)	F	1	7	3,207	L	NR	CAVHD	Died	Glutamine synthetase deficiency
(19)	F	1	35	535	L, N	SB	IHD	Died	Glutamine synthetase deficiency
(32)	M	1	1	269	L, N	AR,SB, SP	Hemoperfusion with charcoal + IHD	Survived	NR
(1)		4							
Patient 1	NR	—	29	5000	L +/- N	NR	None	Died	Idiopathic
Patient 2	NR	—	27	900	L +/- N	SB, SP	None	Died	Idiopathic
Patient 3	NR	—	5	3136	None	NR	None	Died	Idiopathic
Patient 4	NR	—	20	1800	L +/- N	SB, SP	IHD	Survived	Idiopathic
(33)	M	1	22	338	L, ME, R	AR, LC, SB, SP	CWHD	Survived	Idiopathic
(14)	F	1	5	>1,200	L, R	NR	CWHD	Died	<i>Mycoplasma hominis</i>
(34)	F	1	7	704	L, R	NR	CWHD	Died	<i>Mycoplasma hominis</i>
(35)		4							
Patient 1	NR	—	NR	NR	L	SB, SP	CWHD	Died	Idiopathic
Patient 2	NR	—	NR	NR	L	SB, SP	CWHD	Died	Idiopathic
Patient 3	NR	—	NR	NR	L	SB, SP	CWHD	Died	Idiopathic
Patient 4	NR	—	NR	NR	L	SB, SP	CWHD	Survived	Idiopathic
(27)		3							
Patient 1	M	—	9	269	L, N	AA, AR, SB, SP	CWHD+IHD	Survived	Idiopathic
Patient 2	M	—	12	330	R	AR, LC	CWHD	Died	Idiopathic
Patient 3	M	—	9	475	L, R, ME	AR, LC, SP	CWHD, IHD	Survived	Idiopathic
(11)		5							
Patient 1	M	—	10	291	L, R	LC, SB, SP	CWHD	Died	<i>Ureaplasma urealyticum</i> infection
Patient 2	M	—	10	NR	NR	NR	NR	Died	<i>Ureaplasma urealyticum</i> infection
Patient 3	M	—	12	549	NR	NR	NR	Died	<i>Ureaplasma urealyticum</i> infection
Patient 4	M	—	9	NR	NR	NR	NR	Survived	<i>Ureaplasma parvum</i> infection
Patient 5	F	—	10	>200	L, R	AR, LC, SB	IHD	Survived	<i>Ureaplasma parvum</i> infection
(2)		6	—						
Patient 1	M	—	—	1,597	L	NR	IHD	Died	Idiopathic
Patient 2	F	—	—	479	L, N	NR	CWHD	Died	Idiopathic
Patient 3	M	—	—	366	L, N	AR, LC	IHD	Died	Idiopathic
Patient 4	M	—	—	289	L, ME, R	AR, LV, SP	IHD	Died	Idiopathic
Patient 5	F	—	—	304	L, ME, R	AR, LV, SP	IHD	Survived	Idiopathic
Patient 6	M	—	11	374	NR	NR	CWHD	Died	Idiopathic
(12)	F	1	5	80	NR	NR	NR	Survived	<i>Ureaplasma</i>
(36)	M	1	29	246	L, ME, R	SP	NR	Survived	<i>Ureaplasma</i>
(37)	F	1	8	399	L, R	SB	CWHD/IHD	Died	Idiopathic
(18)	—	6	—						
Patient 1	M	—	18	312	L, ME, R	AR, LC, SB	CWHD	Survived	Idiopathic
Patient 2	M	—	8	341	L, ME, R	AR, LC, SB, SP	CWHD + IHD	Survived	Idiopathic
Patient 3	M	—	11	55	L, ME, R	AR, LC, SB, SP	CWHD	Died	Idiopathic
Patient 4	M	—	45	189	L, ME, R	AR, LC, SB, SP	CWHD	Died	Idiopathic
Patient 5	M	—	6	198	L, ME, R	AR, LC, SB	IHD	Survived	Idiopathic
(38)	M	1	8	830	L, R	NR	CRRT + HD	Died	<i>CPS I inhibition by VPA</i>
(39)	M	1	32	506	L, R	SB, SP	CWHD	Survived	NR
(40)	—	2	—						
Patient 1	F	—	3	144	L, R, ME	LC, SB	MARS	Survived	Idiopathic
Patient 2	F	—	9	95	L, R, ME	NR	MARS/ECMO/PP/RRT	Survived	Idiopathic

AA, acetohydroxamic Acid; AR, arginine; CA, carbaglumic acid; CAVHD, continuous arteriovenous hemodialysis; CVVHD, continuous veno-venous hemodialysis; CPS I, carbamoyl phosphate synthase I; ECMO, extracorporeal membrane oxygenation; F, female; IHD, intermittent hemodialysis; L, lactulose; LC, levocarnitine; M, male; MARS, molecular adsorbent recirculating system; ME, metronidazole; N, neomycin; NR, not reported; PP, plasmapheresis; POD, Post-operative day; R, rifaximin; RRT, renal replacement therapy; SB, sodium benzoate; SP, sodium phenylacetate; VPA, valproic acid.

TABLE 2 | Index patients' laboratory results at presentation.

	Reference Range	Case 1	Case 2	Case 3	Case 4
Sodium	136–145 mmol/L	141	143	137	140
Potassium	3.3–5.1 mmol/L	4.6	4.2	4.8	3.9
Chloride	98–107 mmol/L	96 (L)	102	95	102
Carbon dioxide	22–30 mmol/L	29	24	31	28
Urea nitrogen	6–20 mg/dl	13	19	13	8
Creatinine	0.80–1.20 mg/dl	1.05	1	0.83	0.79
Glucose	65–99 mg/dl	125 (H)	92	319	87
Calcium	8.4–10.2 mg/dl	9.7	9.9	10	9.6
Anion gap	8–16 mmol/L	16	17	11	10
Total protein	6.4–8.3 g/dl	7.3	8	8	7.8
Albumin	3.5–5.0 g/dl	4.2	4.3	4.4	3.8
Phosphorus	2.7–4.5 mg/dl	3.0	5.2	2.3	3.5
AST	0–37 U/L	13	21	20	16
ALT	0–41 U/L	15	17	14	6
Total bilirubin	0.0–1.0 mg/dl	0.3	0.4	0.3	0.4
Alkaline phosphatase	35–129 U/L	78	45	104	88
Magnesium	1.5–2.8 mg/dl	1.5	1.8	2.1	1.9
WBC	4.0–10.0 thou/cu mm	12.3 (H)	8.3	19.5	9.6
Hemoglobin	13.0–16.5 g/dl	15.3	17.2	12.6	11.3
Platelet count	150–450 thou/cu mm	227	180	482	199

AST, aspartate aminotransferase; ALT, alanine aminotransferase; WBC, white blood cell.

TABLE 3 | Metabolic profile and assay results.

	References range	Case 1	Case 2	Case 3	Case 4
Citrulline	10–60 mmol/L	27	16	13	32
Argininosuccinate	0–2 mmol/L	0	<2	<2	<2
Arginine	40–160 mmol/L	72	48	61	48
Ornithine	20–135 mmol/L	74	42	67	94
Aspartate	0–25 mmol/L	4	4	10	6
Glutamine	410–700 mmol/L	287	539	614	488
Urinary Orotic acid	0.2–1.5 mol/mol	Not tested	0.9	0.3	0.4
BAL or Blood PCR	NA	Neg	Neg	Neg	Neg
Mycoplasma Culture	NA	No sent	Neg	Neg	Neg
Ureaplasma Culture	NA	Not sent	Neg	neg	Neg
Specimen Type	NA	Not sent	Blood ^a	BAL Fluid ^b , (41,42)	Blood ^a

BAL, bronchoalveolar lavage, D, donor, NA, not applicable, Neg, Negative, PCR, polymerase chain reaction, R, recipient.

^aTest performed in ARUP laboratories.

^bTest performed in Mayo Clinic laboratories.

Case 3

A 54-year-old woman with end-stage COPD received bilateral LT. The patient was successfully extubated on post-op day 1. However, she developed primary graft dysfunction grade 3 on post-op day two, requiring initiation of ECMO. During that time, she became confused and encephalopathic, and her ammonia level was found to be 122 $\mu\text{mol/L}$. She was started on levofloxacin, rifaximin, and doxycycline for empiric treatment of mycoplasma and ureaplasma infections. The PCR assays for these organisms, however came back negative. All protein in the diet was discontinued. Oral sodium phenylbutyrate and intravenous arginine, dextrorse, and carnitine were started (**Supplementary Material S2**). Ammonia levels continued to fluctuate between 100–140 $\mu\text{mol/L}$ with a peak of 146 $\mu\text{mol/L}$ on day 18 despite the above treatment regimen. Intermittent hemodialysis was

started for 4 h daily with no appreciable change in the ammonia level. Oral sodium benzoate was added to the treatment. The duration of HD was increased to 6 h daily. CVVH was added in between the HD. These measures decreased the ammonia levels to an average of 40–60 $\mu\text{mol/L}$. The patient recovered and was discharged home.

Case 4

A 64-year-old woman with rheumatoid arthritis-related interstitial lung disease underwent bilateral LT. The patient was extubated on day 1. She became febrile with elevated white blood cell count on post-op day 3. Ammonia level in the blood sample was elevated at 162 $\mu\text{mol/L}$. The patient was reintubated for respiratory failure on post-op day 4. Pressors were started, and antibiotics were broadened to include rifaximin, lactulose, azithromycin, levofloxacin, micafungin,

TABLE 4 | Summary of patient characteristics, and clinical course.

	Case 1	Case 2	Case 3	Case 4
Age	68	64	54	64
Gender	M	M	F	F
Race	CA	CA	CA	AA
Presenting Symptoms	Encephalopathy, fever, shock/PRESS	Asymptomatic	Confusion	Unable to assess as patient intubated for respiratory failure
Transplant indication	IPF	IPF	PE/PH	RA ILD/IPF
Transplant type	BL	BL	BL	BL
CMV Donor D/Recipient R	-/-	+/-	-/+	+/-
EBV	I/+	+/+	+/+	+/+
Induction	B	B	B	B
Maintenance	MMF, MP, T	MMF, MP, T	MMF, MP, T	MMF, MP, T
Post Op complications	AMS/Sepsis/bleeding/MV/MOF	A fib, Left subclavian DVT Wound dehiscence s/p debridement and wound vac placement	Primary graft dysfunction grade 3	Trached but weaned, clam shell wound dehiscence
—	—	—	ECMO	ECMO
Initial ammonia value ^a	55	29	54	39
Day ammonia Peaked	10	6	18	10
Peak ammonia value	245	155	146	176
Antimicrobial agents at time of diagnosis	AZ, DO CE/V/ME/L/MI	AZ, L ME	AZ, DO, L CE/V/	AZ, L CE/V/ME/ME

AA, african american; A Fib, Atrial fibrillation; AMS, altered mental status; AZ, azithromycin; B, basiliximab; BL, bilateral; CA, caucasian american; CE, cefepime; CMV, cytomegalovirus, D, donor, DO, doxycycline; DVT, deep vein thrombosis; EBV, epstein barr virus; ECMO, extracorporeal membrane oxygenation; F, female; H, hispanic; I, intermediate; IPF, idiopathic pulmonary fibrosis; L, levofloxacin; M, male; ME, metronidazole; MI, micafungin; MMF, mycophenolate mofetil; MP, methylprednisolone; MV, mechanical ventilation; MOF, multiorgan failure; PE, pulmonary emphysema; PH, pulmonary hypertension; PRESS, posterior reversible encephalopathy syndrome; RA ILD, rheumatoid arthritis interstitial lung disease; R, recipient; T, tacrolimus; V, vancomycin.

^aAmmonia level at the day of transplantation.

metronidazole inhaled tobramycin. Cultures from both initial and follow-up bronchoalveolar lavages remained negative. Hyperammonemia protocol was initiated (**Supplementary Material S2**). All protein sources in the diet were stopped. Intravenous arginine in dextrose and oral sodium phenylbutyrate were started. Daily extended iHD for 6 h was launched with CVVHD in-between. The ammonia level decreased to 82 $\mu\text{mol/L}$. Ureaplasma and Mycoplasma PCR assays were negative (**Table 3**). The patient was discharged to the rehabilitation center on day 66 post-transplantation.

DISCUSSION

Whether previous cases reporting etiology as idiopathic or more recently ones attributed it to mollicutes, none took a metabolic focus to describe if HA impacted UC or GS pathways or reported aminoassay to better assess if UC or GS were implicated in HALT. **Table 3; Figure 2** and **Supplementary Material S3** describe our metabolic analyses and illustration of index cases.

Induction immunosuppression was the same in all four cases and included basiliximab administered on the day of surgery and a second dose on post-op day 4 per our center's protocol (**Table 4**). As ammonia levels started to increase in the above cases, all sources of protein intake in the diet were stopped. Broad antimicrobial coverage targeting mycoplasma and ureaplasma was initiated. Despite that, ammonia continued to increase (**Supplementary Material S3**).

Intravenous lipid and dextrose were used as a source of calories for the first 24–48 h post-diagnosis. This was followed by a gradual introduction of protein (starting at 0.25 g/kg and increased to target protein intake). The intravenous ammonia scavenger used in the first case was successfully replaced with oral agents in the other three cases. In patients with a rapid increase in ammonia levels, RRT was promptly instituted. In hemodynamically stable patients, iHD was preferred over CRRT. In patients who continued to have higher ammonia levels despite iHD, strategies that include a longer duration of iHD up to 6 hours and adding CRRT between iHD sessions were adopted. Amino acid profile (all four cases) and urinary Orotic acid (case 2, 3, and 4) were within normal limits or negative, indicating that urea cycle disorders (UCD) or metabolic diseases are less likely to be the underlying triggers (**Table 2**). Ureaplasma PCR in all the cases was negative. The increase in ammonia levels started on post-transplant days 11, 1, 3, and 4 (in cases 1, 2, 3, and 4, respectively). Ammonia level peaked at 245, 155, 146, and 176 $\mu\text{mol/L}$ (cases 1, 2, 3, and 4, respectively). The use of oral or enteral ammonia scavenger instead of intravenous was effective and was associated with significant cost savings.

The incidence of HALT in our institution (3.3%) is consistent with previous reports (1–3). Our mean peak ammonia level was 185 $\mu\text{mol/L}$, much lower than reported previously. The median days to peak ammonia level was also shorter (11 days compared to 14.1). These differences could be related to early recognition and prompt institution of a multimodal treatment plan (**Supplementary Material S2**).

ETIOLOGY

Underlying Urea Cycle Disorder

HALT was initially thought to be caused by unmasking of partial UC defect under the metabolic stress of transplantation (1,13,14). However, in all our four cases, amino acid profile and orotic acid results did not suggest the presence of a UCD. Glutamine, citrulline, arginine, ornithine in all cases, and orotic acid levels in cases 2, 3, and 4 were normal. Moreover, there were no reported cases of UCDs in patients with HALT in the lung transplant literature. In particular, quantitative analysis of UC enzyme expression in HALT's fatal case showed no evidence of loss of urea cycle enzyme expression (1). Moreover, our cases' glutamine levels were either normal or below normal, indicating the less likelihood of UCDs as an etiology in this patient population (19).

Ureaplasma/Mycoplasma

Bharat et al. utilized specialized culture, polymerase chain reaction, and molecular resistance profiling to provide evidence supporting a causal relationship between *Ureaplasma* infections and HA in LT recipients (11,12). Empiric dual antibiotic coverage that includes levofloxacin, azithromycin, and or doxycycline was initiated in all four cases. Despite the timely initiation of antibiotics, ammonia levels continued to rise. Ureaplasma and mycoplasma PCR assays from BAL or blood were sent in all but the first case (Table 2) and were negative. These tests are performed in few specialized labs in the country. Moreover, the turnaround time for the results ranges from 3 days to a week. Hence the clinician will have to start empiric antibiotics even before the results of the tests are available.

Medication-Induced

Seizures resulting from intravenous calcineurin use and leading to the development of HA have been described. None of our cases developed seizures while on calcineurin inhibitors.

Hepatic Glutamine Synthetase Deficiency

Glutamine synthetase (GS) is a cytosolic enzyme that catalyzes ammonia and glutamate condensation to produce glutamine, which is a substrate for various metabolic pathways and is essential for many organs. GS also plays a crucial role in 1) protecting the neurons by capturing ammonia and glutamate in the glial cells; and 2) supplying glutamine for glutamate and GABA synthesis in the glutamine-glutamate-GABA cycle, regulating the excitatory and inhibitory synaptic transmission of neurons (20–22).

Urea cycle-ammonia detoxifying effect accounts for only 35%. Using GS knockout/liver, control mice, and stepwise increments of enterally infused ammonia, Hakvoort et al. showed that the other 35% of ammonia is detoxified by GS while the remaining 30% is not cleared by the liver (23). They further showed, through genetic and pharmacologic approaches to modulate GS activity, that stepwise increments detoxification of intravenously infused ammonia is almost totally dependent on GS activity (23). GS deficiency causes only mild to moderate hyperammonemia

(24,25), as it might be the case in some of the HALT. Previous studies have shown decreases in hepatic GS enzyme activity to 12% and 28% of the mean value of controls in two cases (1,19). In one patient in which we were able to obtain liver tissue, there was near-complete loss of hepatic GS expression (Figure 2). Further supporting a critical role of hepatic GS, rather than a UCD in the pathogenesis of HALT. Analysis of systemic amino acid profiles in several patients in our case series showed normal amino acid profiles, which is atypical for a primary urea cycle disorder. Thus, to our knowledge, every case of HALT, which has examined hepatic GS expression, has identified substantial hepatic GS deficiency. Therefore, these findings are consistent with the possibility that LT induces a transient down-regulation of hepatic GS expression in susceptible individuals, leading to the development of HA.

MANAGEMENT

Enteral Versus Intravenous Therapy

To our knowledge, this is the first case series reporting the successful use of combination oral ammonia scavengers in patients with HALT. In our second, third, and fourth cases, we successfully used oral sodium phenylbutyrate (Supplementary Material S2) instead of intravenous sodium benzoate/sodium phenylacetate. In the third case, we added oral benzoate at a dose of 5.5 g/m² to sodium phenylbutyrate for more aggressive clearing of ammonia in addition to dialysis and observed no identifiable complication. This has not been described before, as it was thought that both medications have similar mechanisms of action. Diarrhea might cause suboptimal absorption of the ammonia scavengers. Diarrhea is common in HALT patients due to the use of bowel decontamination agents. Similarly, CRRT and extended iHD might result in augmented clearance of the medication. Due to the above two concerns, sodium benzoate was added. We have also added carnitine and micronutrients to the arginine-dextrose to help substrate utilization (see below).

Role of Dialysis

Dialysis and dialytic modalities are an integral part of HALT management, though in some cases, dialysis may not be required. Currently, there is no consensus of appropriate timing to initiate dialysis, but some clinicians suggest considering dialysis if the ammonia level exceeds three times the upper limit of normal in the absence of liver disease (26). The main goal is to reduce the ammonia level as quickly as possible. Lag time between diagnosis and initiation of dialysis may contribute to adverse outcomes (27). Intermittent hemodialysis with a large surface area dialyzer is a more efficient modality over CRRT and peritoneal dialysis or charcoal hemoperfusion. Extended dialysis session of ≥6 h, a blood flow rate of 400 ml/min, and a fluid flow rate of 800 ml/min is more efficient in clearing ammonia. If iHD is not an option due to hemodynamic instability, sustained low-efficiency dialysis or continuous veno-venous hemodialysis at the rate of 250 ml/kg/h and 40–50 ml/kg/h, respectively, should be considered with the

highest possible blood flows (27). High flux daily HD, in addition to other adjunct therapies, should be considered.

Early Diagnosis

At our center, we routinely check daily ammonia levels for the first-week post LT and also when there is any sign of mental status change, thus diagnosing this condition at a very early stage. Early diagnosis strategy has enabled us to institute early treatment, thus preventing ammonia levels from getting very high. This has resulted in better survival of our patients.

Role of Micronutrients

The direct and toxic effects of HA on the astrocytes within the brain (such as oxidative/nitrosative stress due to disturbance of the NO pathway), creatine deficiency, and inhibition of the tricarboxylic acid cycle have been described. These toxic effects can lead to secondary mitochondrial failure, and thus, energy deficiency; hence adding micronutrients to arginine and intravenous continuous dextrose infusion may help better substrate utilization (**Supplementary Material S2**) (21,28,29).

PERSPECTIVE

While some reports have described the casual association between mollicutes and HA (11,12,14–17,30), there is no conclusive evidence that this is the only possible etiology for HALT. We initiated dual antibiotics targeting these mollicutes very early in our case series. Despite the continued use of these antimicrobials, we witnessed a worsening in ammonia levels prompting multimodal interventions. Additionally, the tests for all the mollicutes have been negative (**Table 3**, **Supplementary Material S3**). All these seem to indicate that there is possibly more than one etiology for HALT. The metabolic and biochemical analyses of our index cases are unique to our study to show LT effect on UC as described above (**Table 3**). While Baharat et al. (11), showed disseminated ureaplasma/mycoplasma as the cause for HALT, it is unclear how these infections would impact UC or GS pathways.

While mortality rate of patients who develop HA is historically very high. Our case “series” mortality was lower, indicating early detection and early multimodal treatment might have contributed to improved survival. However, such an approach may also result in slightly more patients being treated with this multimodal strategy. Since historically, the mortality with this disease has been so high, the benefits of treatments outweigh the decision to delay treatment or the decision not to treat. Our center has developed a very safe and comprehensive protocol to treat this disease with highly favorable results. As there is no single exact etiology for this condition and probably several mechanisms at play, a multimodal treatment approach appears to be the best.

The cause of HALT remains elusive. It may be related to reduced GS activity and unmasking of partial UC defects. Early diagnosis of this syndrome and implementing a multidimensional therapeutic approach is paramount for a successful outcome. A very efficacious and cost-effective successful multimodal strategy for the treatment of HALT is described here. Practical issues include provider and nursing education and proper handling of the ammonia specimen.

Based on our experience, we suggest early testing and close monitoring of ammonia levels. Multimodal strategies to manage HALT include stopping all protein in the diet for the first 24 h, early initiation of ammonia scavenger medications, dialysis, and broad-spectrum antibiotic with mycoplasma and ureaplasma coverage and utilization of dextrose, lipid, and micronutrients as a source of calories in the acute phase. We believe that this will improve the survival of patients with this condition.

AUTHOR CONTRIBUTIONS

AK, AE and SC equally contributed to the conception and design of the research; AK, AE, LA, AS, HA, AP, TM, MP, H-WL, DW and SC contributed to the design of the research; AK, AE, LA, H-WL, DW and SC contributed to the acquisition and analysis of the data; AK, AE, AS, HA, AP, TM, MP, DW and SC contributed to the interpretation of the data; and AK, AE and SC drafted the manuscript. All authors critically revised the manuscript, agree to be fully accountable for ensuring the integrity and accuracy of the work, and read and approved 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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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10433/full#supplementary-material>

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Perfusate Composition and Duration of *Ex-Vivo* Normothermic Perfusion in Kidney Transplantation: A Systematic Review

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Ex-vivo normothermic perfusion (EVNP) is an emerging strategy in kidney preservation that enables resuscitation and viability assessment under pseudo-physiological conditions prior to transplantation. The optimal perfusate composition and duration, however, remain undefined. A systematic literature search (Embase; Medline; Scopus; and BIOSIS Previews) was conducted. We identified 1,811 unique articles dating from January 1956 to July 2021, from which 24 studies were deemed eligible for qualitative analysis. The perfusate commonly used in clinical practice consisted of leukocyte-depleted, packed red blood cells suspended in Ringer's lactate solution with Mannitol, dexamethasone, heparin, sodium bicarbonate and a specific nutrient solution supplemented with insulin, glucose, multivitamins and vasodilators. There is increasing support in preclinical studies for non-blood cell-based perfusates, including Steen solution, synthetic haem-based oxygen carriers and acellular perfusates with supraphysiological carbogen mixtures that support adequate oxygenation whilst also enabling gradual rewarming. Extended durations of perfusion (up to 24 h) were also feasible in animal models. Direct comparison between studies was not possible due to study heterogeneity. Current evidence demonstrates safety with the aforementioned widely used protocol, however, extracellular base solutions with adequate oxygenation, supplemented with nutrient and metabolic substrates, show promise by providing a suitable environment for prolonged preservation and resuscitation.

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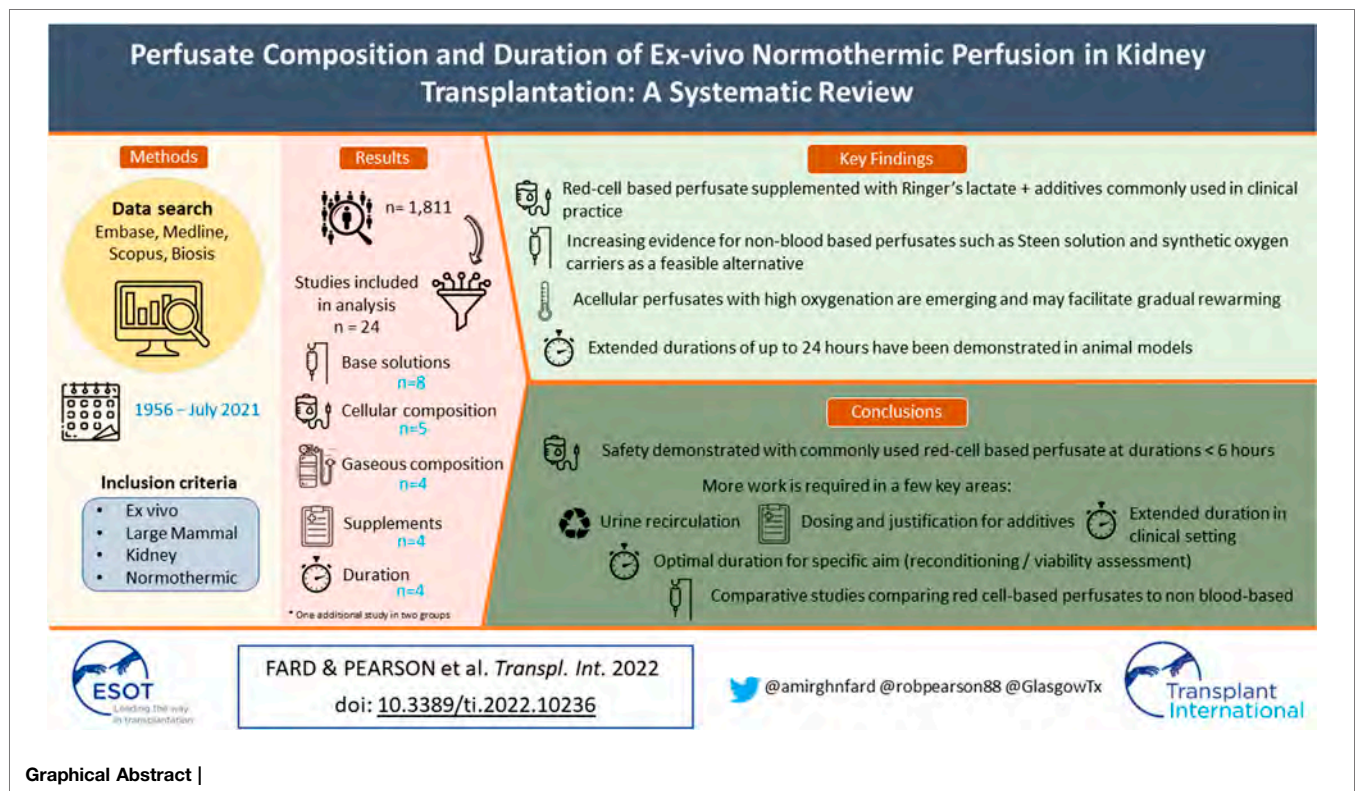
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Keywords: review, kidney, perfusion, normothermic, perfusate

Abbreviations: CO, carbon monoxide; DBD, donation after brain death; DCD, donation after circulatory death; DGF, delayed graft function; ECD, extended-criteria donor; EPO, erythropoietin; ESRD, end stage renal disease; EVNP, *ex vivo* normothermic perfusion; H₂S, hydrogen sulfide; HBOCs, Heam-based oxygen carriers; HMP, hypothermic machine perfusion; IRI, ischaemic reperfusion injury; RBC, red blood cells; ROS, reactive oxygen species; SCS, static cold storage; UW, University of Wisconsin.



INTRODUCTION

Kidney transplantation is the gold standard treatment for end stage renal disease. The mainstay of organ preservation has traditionally focused on reducing metabolism by utilising hypothermic conditions with static cold storage (SCS) or, more recently, hypothermic machine perfusion (HMP) (1). The continued donor organ shortage has necessitated increased use of kidneys from donation after circulatory death (DCD) and “extended criteria” donor (ECD), (2) which are more susceptible to the effects of ischaemia reperfusion injury (IRI). IRI is multifactorial process that results in an increase in reactive oxygen species (ROS) and inflammatory mediators which stimulate vascular permeability leading to oedema and vascular endothelial damage (3–5). Furthermore, the effects of IRI are associated with higher rates of acute rejection, delayed graft function (DGF), and reduced long-term allograft survival (4). Preservation techniques to mitigate against the effects of IRI are therefore of increasing importance.

One emerging strategy is *ex-vivo* normothermic perfusion (EVNP). This involves rewarming the graft to normothermic conditions (37°C) with a perfusate that replicates the pseudo-physiological environment. Thus, facilitating the restoration of energetic substrates (e.g., ATP), metabolism and repair processes, whilst also facilitating graft viability assessment. Recently, the safety and feasibility of EVNP has been established in human clinical studies (6,7). Although unlikely to entirely counteract the process of IRI, EVNP has the potential to mitigate these deleterious effects during the period of perfusion (6).

The ideal perfusion characteristics including perfusate composition and duration remain undefined. Common clinical protocols employ a nutrient-enriched, red blood cell (RBC)-based perfusate to deliver nutrients and oxygen during 1-hour of perfusion (6,8). In addition to prolonging the duration of EVNP, variations in composition, such as synthetic and acellular preparations with varying base media, have been proposed in preclinical studies and established in liver and lung clinical protocols. However, major deviations have yet to be clinically implemented in kidneys, and limited evidence exists for the impact of different perfusion characteristics. The aim of this review was to summarise the evidence for the roles of perfusate constituents and the effects of different perfusion durations in optimising clinically relevant outcomes in the context of renal EVNP.

MATERIALS AND METHODS

Data Sources and Search Strategy

For this systematic review, we followed the methods proposed by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement, (9) and the Cochrane Handbook for Systematic Reviews of Interventions. This review was registered with PROSPERO (CRD42021231381) (10).

A limited search of the literature was conducted to identify keywords, followed by an extensive literature search on the following databases: Embase (Ovid) 1947-Present; Ovid

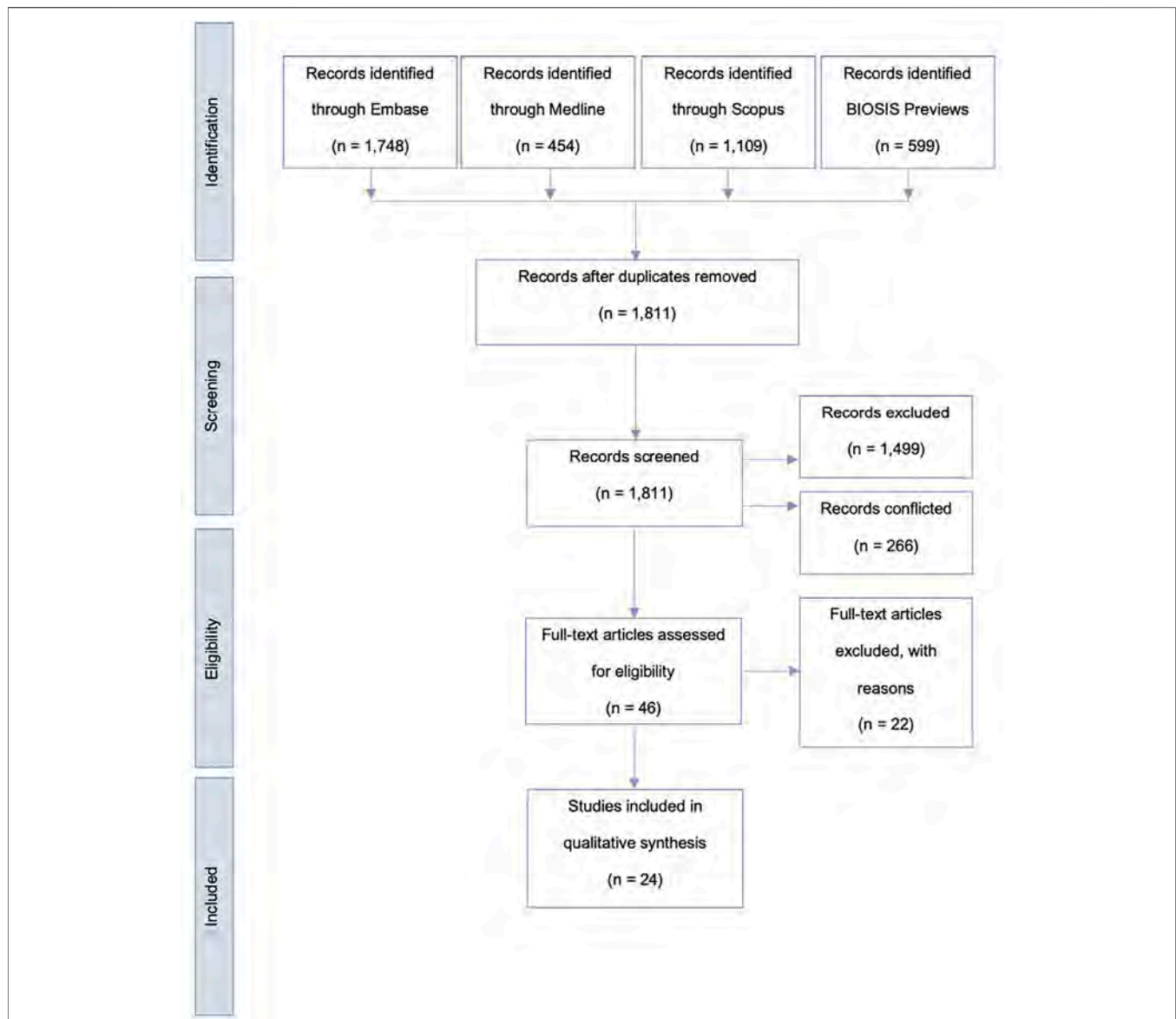


FIGURE 1 | Search strategy flow diagram; adapted from the preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow diagram (9).

Medline[®] without Revision; Scopus; and BIOSIS Previews. The keywords used to identify relevant studies included normothermic perfusion and evnp and kidney; a comprehensive description of the search strategy can be found in **Supplementary Appendix S1**. Results were imported into Rayyan QCRI web application, where duplicate articles were removed, then two main reviewers independently and blindly screened the titles and abstracts based on predefined eligibility criteria. Thereafter, selected studies were read in full. Bibliographies of the selected articles were screened to identify landmark trials.

Eligibility Criteria

The eligibility criteria were agreed based on the study objectives and specific research question: what are the roles of various perfusate constituents, and what are the effects of different

durations of perfusion on clinically relevant outcomes in renal EVNP?

Eligible studies included preclinical and clinical, published and abstract publications from any year and any region, where English translations were available. Studies that were unpublished and those concerning *in vivo* perfusion methods, non-large mammal studies, non-kidney studies, assessment of perfusate biomarkers, and therapeutic interventions were excluded. Articles relating to sub-normothermic perfusion methods were only included where specific rationale for perfusate composition was discussed.

Data Extraction and Analysis

The most recently dated studies were read in full first to identify up-to-date knowledge and previous related studies. Study characteristics, including name, year, design, subjects,

TABLE 1 | Summary characteristics of perfusate composition studies qualitatively assessed.

Theme	Study	Design	Subject model	Objectives	Main outcome measures	Key findings
Whole perfusates and base solutions	Hosgood SA et al; 2011(6)	Published; clinical case report	Human patient (<i>n</i> = 1)	First EVNP in human renal transplantation	Renal hemodynamics (renal blood flow, resistance, urine output); post-transplant serum creatinine; graft function	EVNP with plasma-free red cell-based perfusate is feasible
	Nicholson ML et al; 2013(7)	Published; clinical study	Human patients (<i>n</i> = 18)	First clinical series EVNP in human renal transplantation	Graft primary nonfunction; delayed graft function (DGF)—need for dialysis; graft failure—need for nephrectomy or RRT	DGF was 5.6% in EVNP group vs. 36.2% in SCS group (<i>p</i> = 0.014); no difference of graft or patient survival at 12 months
	Hosgood SA et al; 2016(8)	Published; clinical case report	Human patients (<i>n</i> = 2)	First clinical EVNP transplantation of DCD kidneys deemed untranslatable	Graft hemodynamics; posttransplant graft function; serum creatinine	Serum creatinine at 3 months was 1.2 mg/dl and 1.62 mg/dl in the recipient of the left and right kidney—EVNP rescued kidneys previously deemed unsuitable for transplantation
	Hosgood SA et al; 2017(11)	Published; Protocol of clinical trial	Human patients (<i>n</i> = 400 for recruitment)	1-hour renal EVNP in kidneys from DCD donors versus SCS	Primary: DGF (need for dialysis in first 7-day); Secondary: renal function, hospital stay, graft & patient survival at 1 year; acute rejection; blood chemistry biomarkers	Study suspended during COVID-19 pandemic and preliminary results not yet available
	Horiuchi T et al; 2009(14)	Published; preclinical	Canine kidneys	Pyridoxalated hemoglobin-polyoxyethylene (Php) addition to UW solution for normothermic preservation	Oxygen consumption; histopathological assessment	Php added to UW during 12-hour normothermic preservation increased oxygen consumption, reduced damage of tubular epithelium and edematous degeneration compared to UW alone
	Kaths JM et al; 2015(35)	Published; preclinical	Beating-heart porcine kidneys (<i>n</i> = 6)	EVNP using erythrocyte-based Steen solution diluted with LR perfusate	Renal hemodynamics; blood gas analysis; histopathological assessment	10-hour DCD porcine perfusion using erythrocyte-based Steen solution diluted with ringer's lactate demonstrated stable hemodynamics, active renal metabolism and minimal renal injury
	Urcuyo D et al; 2017(12)	Published; preclinical	Porcine kidneys (<i>n</i> = 15)	Whole-blood at normothermia, whole-blood with Steen solution at normothermia, and acellular Steen solution at sub-normothermia, on prolonged preservation	Primary: Hemodynamic stability and histological damage Secondary endpoints: Urine production, perfusate potassium and arterial pH	Acellular Steen solution at 21°C supported low and stable vascular resistance with adequate histological preservation during 24-hour perfusion; whole blood diluted with Steen solution at normothermia was successful but resulted in acidosis and necrosis. Whole blood alone at normothermia was unsuccessful beyond 5-hours
	Horn CV et al; 2021(15)	Published; preclinical	Porcine Kidneys (<i>n</i> = 12)	New preservation solution Custodiol-MP for <i>ex vivo</i> reconditioning of kidney grafts compared to Belzer MPS solution	Primary: renal haemodynamics Secondary: Molecular markers of renal injury and histology	No statistically significant difference in outcomes between Custodiol-MP and Belzer MPS solutions. Custodiol-MP was safe and applicable for short-term kidney perfusion
	Pool MBF et al; 2021(36)	Published; preclinical	Porcine Kidneys (<i>n</i> = 20)	Comparison of four different perfusate solutions	Perfusion parameters, Urine and perfusate analysis, Markers of renal injury, Histology	All four perfusates were feasible but with differences in outcome measures.

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TABLE 1 | (Continued) Summary characteristics of perfusate composition studies qualitatively assessed.

Theme	Study	Design	Subject model	Objectives	Main outcome measures	Key findings
						Individual influence of perfusate components remain unclear
Cellular Composition	Harper S et al; 2006(16)	Published; Preclinical	Porcine kidneys (n = 12)	Leukocyte-depleted blood versus whole blood-based perfusates	Serum creatinine, urine output, renal blood flow, oxygen consumption, acid-base homeostasis, histological features	Leukocyte-depleted blood significantly improved post-ischemia renal function; lower serum creatinine, higher creatinine clearance and urine output ($p = 0.002$ for all)
	Aburawi MM et al; 2019(17)	Published; Preclinical	Discarded human kidneys (n = 14)	Hemoglobin-based oxygen carriers (HBOC) versus packed red blood cell-based perfusates	Renal artery resistance, oxygen extraction, metabolic activity, energy stores and histological features	Lactic acid levels in kidneys pRBC group was higher than HBOC group ($p = 0.007$); other outcomes were similar
	Minor T et al; 2019(13)	Published; preclinical	DCD Porcine kidneys (n = 12)	RBC-based perfusate versus acellular perfusate versus control during controlled rewarming	Renal hemodynamics and histological assessment	Controlled organ rewarming is superior to immediate rewarming in terms of creatinine clearance, sodium excretion, oxygen extraction, urinary protein loss and innate immune activation; inclusion of RBC added no benefit
	Minor T et al; 2019(18)	Published; clinical case report	Human Patient (n = 1)	First controlled rewarming with an acellular Steen perfusate in human renal transplantation	Post-transplant immediate graft function; serum creatinine; urine output; patient outcomes	Postoperative course was event-free, and patient was discharged after 16 days with a serum creatinine of 143 $\mu\text{mol/L}$; Acellular controlled oxygenated rewarming was successful
Gaseous Composition	Adams TD et al; 2019(19)	Published; preclinical	Porcine kidneys (n = 43)	Effects of reducing perfusate oxygenation on renal function and oxygen kinetics during EVNP and reperfusion	Renal function and hemodynamics; blood gas analysis; biomarkers of renal injury (NGAL)	Reducing partial pressure of oxygen significantly reduced oxygen extraction during EVNP ($p = 0.037$) however showed no significant difference in urine output, sodium excretion, creatinine clearance or NGAL during reperfusion
	Maasseen H et al; 2019(21)	Published; preclinical	Porcine kidneys (n = 10)	Hydrogen sulphide versus control	Renal function and hemodynamics; oxygen kinetics; histopathological assessment; metabolic activity	Hydrogen sulphide significantly reduce oxygen consumption, by 61%, ($p = 0.047$) without directly affecting tissue ATP levels. Renal function was unchanged
	Bagul A et al; 2008(20)	Published; preclinical	Porcine kidneys (n = 4)	Effect of carbon monoxide	Renal function and hemodynamics	Carbon monoxide improved renal blood flow ($p = 0.002$), creatinine clearance ($p = 0.006$), and urine output ($p = 0.01$). Higher concentrations had negative effects
	Smith SF et al; 2017(22)	Published; preclinical	Porcine kidneys (n = 18)	70% argon versus 70% nitrogen versus 95% O ₂ 5% CO ₂ during EVNP	Renal function and hemodynamics; inflammatory mediators and histopathological assessment	Argon did not mediate any significant effects during EVNP nor reperfusion during functional parameters, inflammatory mediators or histological changes

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TABLE 1 | (Continued) Summary characteristics of perfusate composition studies qualitatively assessed.

Theme	Study	Design	Subject model	Objectives	Main outcome measures	Key findings
Supplementary Composition	Bleilevens C et al; 2019(23)	Published; preclinical	Porcine kidneys (n = 10)	Vitamin C versus placebo in an <i>in vitro</i> ischemia-reperfusion porcine kidney EVNP model	Perfusate analysis (blood gas, serum chemistry, oxidative stress markers); renal hemodynamics; histological analysis	Vitamin C significantly increased antioxidant capacity and hemoglobin concentrations ($p = 0.02$), reduced oxidative stress ($p = 0.002$) however did not improve creatinine clearance, fractional sodium excretion or renal histology
	Hosgood SA et al; 2017(25)	Published; preclinical	Porcine kidneys (n = 10)	Effect of a CytoSorb heme-adsorber in an isolated kidney perfusion system	Tissue and blood markers of inflammation and renal function	In the cytosorb group, interleukin-6/8, prostaglandin E2 and thromboxane were significantly lower during reperfusion ($p = 0.023$, $p = 0.0001$ and $p = 0.005$ respectively) and renal blood flow was significantly higher ($p = 0.005$); creatinine clearance was not significantly difference ($p = 0.109$)
	Brasile L et al; 2003(26)	Published; preclinical	Canine kidneys (n = 32)	Feasibility of cobalt protoporphyrin (CoPP) on heme-oxygenase (HO-1) expression during acellular warm perfusion	HO-1 activity; Renal hemodynamics	Induction of HO-1 during warm acellular perfusion by CoPP is feasible within clinical timeframe
	Yang B et al; 2011(24)	Published; preclinical	Porcine kidneys (n = 6)	Impact of EPO addition to 2-hour RBC-based EVNP	Renal hemodynamics; immunohistochemistry, histopathological assessment	EPO in EVNP significantly facilitated inflammation clearance and improved and urine output

EVNP, Ex-vivo normothermic perfusion; SCS, Static cold storage; DGF, Delayed graft function; UW, University of Wisconsin solution; LR, lactate Ringer's solution; Php, Pyridoxalated hemoglobin-polyoxyethylene; DCD, Donation after circulatory death; ECD, Expanded criteria donor; HBOC, hemoglobin-based oxygen carriers; pRBC, Pack red blood cells; CoPP, Cobalt Protoporphyrin; HO-1, Heme-oxygenase 1; EPO, Erythropoietin; IRI, ischemia-reperfusion injury.

objectives, perfusate composition, perfusion duration, main outcome measures and key findings were recorded.

RESULTS

The search identified 3,910 articles, 2099 of which were duplicates, giving 1,811 unique articles, dating from January 1956 to July 2021. Following blinded screening by two independent reviewers, 1,499 articles were deemed ineligible, with 266 decisions conflicted. A third reviewer was used to address conflicts. Of the articles selected, 46 met the eligibility criteria. Full-text assessment reasoned a further 22 articles ineligible for qualitative analysis. Only studies utilizing human or large mammal tissue were included. **Figure 1** illustrates the search process in full.

Included studies were grouped according to common themes: Whole perfusates and base solutions ($n = 8$); cellular composition ($n = 5$); gaseous composition ($n = 4$); supplementary composition ($n = 4$); and perfusion duration ($n = 4$), with one study applicable to both whole perfusate and base solutions, and perfusion duration. Studies comprised 5 clinical studies on human patients and 19 preclinical studies. Key findings were recorded

and summarised in **Table 1** for perfusate composition and **Table 2** for perfusion duration.

Qualitative analysis found the perfusate commonly implemented in clinical renal EVNP consisted of Ringer's lactate, O-negative packed red blood cells (pRBC), Mannitol 10%, dexamethasone 8 mg, heparin, Sodium bicarbonate 8.4% as the main components, and a specific nutrient solution with insulin, multivitamins, prostacyclin 0.5 mg and glucose 5% as supplementary components, for a perfusion duration of 1-hour following SCS, pioneered by Nicholson et al. in Cambridge (7).

Preservation solutions are broadly categorised into intracellular and extracellular solutions, pertaining to whether the potassium and sodium concentrations mirror that of the intra- or extra-cellular milieu. Regarding the base solutions used for perfusate at normothermia, extracellular electrolyte compositions such as Ringer's lactate have demonstrated safety and feasibility when implemented in human clinical studies; although lacking robust data, the perfusion pressure maintained in human trials thus far ranges from 65 to 75 mmHg (6,8,11). In addition, Steen-based solutions, with or without RBCs, have been shown to support prolonged perfusion up to 24-hour of EVNP of DCD porcine kidneys (12,13). One

TABLE 2 | Summary characteristics of kidney perfusion duration studies qualitatively assessed.

Study	Design	Subject model	Objectives	Duration groups	Main outcome measures	Key findings
Kaths JM et al; 2016(52)	Published: preclinical	SCD Porcine kidneys (n = 10)	Safety and feasibility of 8-hour EVNP versus SCS	(A) SCS (8 h) (B) EVNP (8 h)	Perfusate injury markers (AST, LDH); Renal function (serum creatinine, 24-hour creatinine clearance); Histological assessment	Continuous EVNP is feasible and safe in good quality beating-heart donor kidney grafts
Kaths JM et al; 2017(28)	Published: preclinical	DCD Porcine kidneys (n = 20)	Brief EVNP following SCS versus prolonged, continuous EVNP in DCD porcine kidney autotransplantation	(A) 16 h SCS (B) 15 h SCS + 1 h EVNP (C) 8 h SCS + 8 h EVNP (D) 16 h EVNP	Perfusate injury markers (AST, LDH); Renal function (serum creatinine, 24-hour creatinine clearance), Histological assessment	Prolonged EVNP significantly decreased serum creatinine, LDH, and apoptotic cells following DCD kidney transplantation compared to SCS or short EVNP after SCS.
Kaths JM et al; 2017(27)	Published: preclinical	DCD Porcine kidneys (n = 35)	Brief versus intermediate versus prolonged EVNP following 8-hours SCS in DCD porcine kidney autotransplantation	(A) 8 h SCS (B) 8 h SCS + 1 h EVNP (C) 8 h SCS + 8 h EVNP (D) 8 h SCS + 16 h EVNP	Renal function and hemodynamics; Histological assessments 8 days post-transplantation	Intermediate and prolonged EVNP were significantly superior to brief EVNP following SCS. Brief EVNP resulted in a higher serum creatinine compared to SCS alone
Urcuyo D et al; 2017(12)	Published: preclinical	DCD Porcine kidneys (n = 15)	Whole-blood at normothermia versus whole-blood with Steen solution at normothermia, and acellular Steen solution at sub-normothermia, on prolonged preservation	(A) 24 h EVNP with whole blood (B) 24 h EVNP with whole blood + Steen solution (C) 24 h sub-normothermic preservation with acellular Steen solution	Primary: Hemodynamic stability and histological damage Secondary endpoints: Urine production, perfusate potassium and arterial pH	Acellular Steen solution at 21°C supported low and stable vascular resistance with adequate histological preservation during 24-hour perfusion; whole blood diluted with Steen solution at normothermia was successful however resulted in acidosis and necrosis. Whole blood alone at normothermia was unsuccessful beyond 5-hour

SCD, Standard criteria donor; SCS, Static cold storage; EVNP, Ex-vivo normothermic perfusion; AST, Aspartate transaminase; LDH, Lactate dehydrogenase; DCD, Donation after cardiac death.

TABLE 3 | Perfusate composition commonly used for clinical renal ex-vivo normothermic perfusion; adapted from the nicholson protocol (6, 7, 11).

	Constituent	Volume
Components	Ringer's lactate solution	300–400 ml
	O-negative packed red blood cells (leukocyte depleted) from blood bank	1 Unit
	Mannitol 10%	25 ml
	Dexamethasone 8 mg	Direct to circuit
	Sodium Bicarbonate 8.4%	25 ml
	Heparin 1,000 iu/ml	2 ml
Supplement	Nutrient solution (Nutriflex or Synthamin)	20 ml/h infusion
	Sodium Bicarbonate 8.4%	20 ml/h infusion
	Insulin 100 iu	20 ml/h infusion
	Multivitamins (Cernevit)	20 ml/h infusion
	Prostacyclin 0.5 mg	5 ml/h infusion
	Glucose 5%	5 ml/h infusion
	Ringer's lactate solution	Replace urine output ml for ml

study on isolated canine kidneys showed that addition of pyridoxalated haemoglobin-polyoxyethylene (Php) to UW solution enhanced oxygen consumption and reduced oedematous damage of tubular epithelium during 12-hour normothermic preservation, however, no studies have yet translated this into clinical models (14). Custodiol-MP

solution was safe and feasible for short-term perfusion of porcine kidneys, and non-inferior to clinically established Belzer MPS solution. Head-to-head comparison of four different perfusates showed feasibility in all settings during 7-hour EVNP of porcine DCD kidneys, but with substantial differences in perfusion and injury parameters (15). In this

TABLE 4 | Clinical Perfusate Constituent Options summary; Adapted from of Qualitative Analysis of Studies.

	Component role	Clinical constituent options
Base Solution	Fluid and electrolyte balance	Ringer's Lactate Steen solution
	Elevation of osmolality pH Buffer Calcium Buffer Immune suppression Anticoagulation	Mannitol 10% Sodium Bicarbonate 8.4% Calcium Gluconate 10% Dexamethasone 8 mg Heparin 1,000 iu/ml
Cells	Oxygenation	Plasma free, leukocyte-depleted packed Red Blood Cells (1 unit) Synthetic Heme-based oxygen carriers Acellular with no oxygen carrier
Gases	Oxygenation	Carbogen gas mixture (95% O ₂ , 5% CO ₂)
	Hypo-metabolite Vasodilation	Hydrogen sulphide (H ₂ S) Carbon Monoxide (CO)
Supplementary Component	Nutrition	Nutrient solution (Nutriflex) Synthamin 17 (500 ml)
	pH Buffer Energetic & metabolic substrates substrate	Sodium Bicarbonate 8.4% (25 ml) Insulin 100 iu Glucose 5%
	Nutrition solution Vasodilation	Multivitamins (Cernevit) (1 vial) Prostacyclin 0.5 mg Verapamil 0.25 mg/h
	Replace fluid lost in urine output Inflammatory suppression	Ringer's Lactate (ml for ml) Heme-oxygenase-1 (HO-1)

instance, the influence of individual perfusate components remains unclear.

For cellular composition, leukocyte-depleted blood significantly improved post-ischaemia renal function by measure of serum creatinine and urine output ($p = 0.002$) in porcine kidneys (16). Perfusates utilising synthetic haemoglobin-based oxygen carriers (HBOCs) were found to be non-inferior to whole blood perfusates with regard to histological injury, vascular resistance, oxygen consumption and tissue ATP, and exhibited significantly lower lactic acid levels ($p = 0.007$) during perfusion.(17) Controlled oxygenated rewarming without any oxygen carriers resulted in successful transplantation with good immediate renal function, in a recent human clinical case study.(18).

Evidence for gaseous composition supported 95% oxygen (O₂), 5% carbon dioxide (CO₂) mixtures. Reducing oxygen levels to normoxia significantly reduced oxygen consumption during EVNP ($p = 0.037$), however showed no difference in urine output, sodium excretion, creatinine clearance or markers of injury during reperfusion (19). The addition of carbon monoxide (CO) improved renal blood flow ($p = 0.002$), creatinine clearance ($p = 0.006$), and urine output ($p = 0.01$), however higher concentrations had negative effects (20). Despite being commonly known for its toxicity, the infusion of hydrogen sulfide (H₂S) to the perfusate was found to induce a hypometabolic state, significantly reducing oxygen consumption by 61%, ($p = 0.047$) without directly impacting tissue ATP levels, and renal function was unchanged (21). Argon did not mediate any significant effects during EVNP or during reperfusion (22).

Evidence for supplementary additives was limited. While vitamin C significantly increased antioxidant capacity, haemoglobin concentrations ($p = 0.02$), and reduced oxidative stress ($p = 0.002$); it was not shown to improve creatinine clearance, fractional sodium excretion or histological markers of renal tubular injury (23). In a porcine model EPO was found to be anti-inflammatory and anti-apoptotic, demonstrating improved urine output with the mechanism attributed to caspase-3 and IL-1 β (24). Reduction of inflammatory mediators was also demonstrated to be achieved by filtration via CytoSorb haemadsorption, which significantly reduced interleukin (IL)-6/8, prostaglandin E2 and thromboxane during reperfusion ($p = 0.023$, $p = 0.0001$ and $p = 0.005$ respectively), and increased renal blood flow ($p = 0.005$) without significantly altering creatinine clearance ($p = 0.109$).(25) In addition, induction of haem-oxygenase-1 (HO-1) was demonstrated in canine kidneys however evidence for clinical impact is yet to be elucidated (26). Commonly used protocol for clinical use and prominent variations in perfusate constituents, along with their roles, are summarized in **Tables 3, 4**, respectively.

Continuous EVNP, with and without complete exclusion of SCS, was feasible and superior to brief EVNP (27,28). 8-hour and 16-hour durations showed significantly lower post-transplant serum creatinine compared to 1-hour EVNP ($p = 0.027$), with no significant difference between the former (28). Acellular Steen solution at 21°C supported low and stable vascular resistance with adequate histological preservation during 24-hour perfusion, compared to whole blood alone at normothermia, which was unsuccessful beyond 5-hour (12).

DISCUSSION

In this systematic review, the most recent evidence for roles of various EVNP perfusate constituents and durations in optimising clinically relevant outcomes of kidney transplantation were reviewed and summarised.

Fundamentals of Perfusate Composition and Current Clinical Practice

Preservation of organs at normothermia requires a physiological milieu with adequate oxygen, nutrition, and metabolic substrates to replace depleted energy resources. Furthermore, it is necessary that the solution stabilises electrolyte balance and cell fluid content to reduce oedema and reduce free radical peroxide scavengers to diminish oxidative injury (29). Accordingly, the protocol most commonly utilised in clinical practice, (6,7) comprises a nutrient enriched, red cell-based solution, with physiological buffers and added supplementary constituents such as vitamins, insulin, glucose and vasodilators (14,30,31).

Base Solutions

Early evidence has shown that, under normothermic conditions, colloid solutions with high-sodium, low-potassium compositions like that of extracellular fluid, such as Ringer's lactate, are superior to UW, which has a low-sodium, high-potassium composition like that of intracellular fluid, by reducing temperature-dependent oedema during IRI (31). This is consistent with evidence that clinical implementation of renal EVNP using Ringer's lactate solution is feasible (6,7,11). Further work is required to elucidate optimal mean arterial pressure (65–75 mmHg non-pulsatile is most commonly reported as target pressure), particularly in the setting of high resistance kidneys where some groups describe increasing pressure to 100 mmHg to promote perfusate flow (11).

Steen solution is alternative plasma-like solution that was initially utilised for EVNP of the lungs in the Toronto Protocol (32), and has since been developed in liver EVNP (33,34). It contains dextran and a high albumin concentration that provides oncotic force to drive water out of swollen endothelial cells, helping sustain high perfusion flow rates (12). For use with EVNP, it can remain acellular or be supplemented with RBCs. Recent studies using similar protocols in kidneys have shown that Steen solution-based perfusates can support low and stable vascular resistance during prolonged perfusion, superior to red cell-based perfusates (12). Gaining popularity is Ringer's lactate diluted with Steen solution, which has been successfully implemented in porcine kidneys for up to 10 h of EVNP, both with RBCs (35) and without (12). Further research is required to compare these different base solutions at normothermia, and to explore the potentially protective effects of Php.

Another emerging product is Custodiol-MP solution, which is reported to have antioxidant properties, specifically designed for aerobic or oxygenated machine perfusion. Compared to Belzer MPS, Custodiol-MP was deemed safe for short-term kidney perfusion, and while there were no statistically significant

differences in renal hemodynamic outcomes, it remains an attractive solution which may benefit from testing in further models, as it allows flexible addition of colloids, specific to the requirements of each organ, potentially enabling wider clinical application (15).

Few studies to date have conducted head-to-head comparisons of perfusates for EVNP. A recent publication from Pool et al., however, compared four different perfusates during 7-hour EVNP of porcine kidney in a DCD model (36). While all four perfusates demonstrated feasibility, there were apparent differences between electrolyte levels, renal function parameters, and injury markers in the four groups. Perfusate 1, consisting of RBCs in Williams' Medium E-based solution, and Perfusate 2, consisting of RBCs, albumin and balanced electrolyte solution, were similar in terms of EVNP flow patterns, whereas Perfusate 3, consisting of RBCs with clinically established solution used by Hosgood et al., (7) and Perfusate 4, consisting of RBCs and a 0.9% sodium chloride-based medium (successfully used in porcine autotransplantation, (37) showed lower but more stable flow rates. This may be explained by a lack of vasodilator use in Perfusates 1 and 2. Notably, Perfusate 2 resulted in significantly lower levels of injury marker N-acetyl- β -D glucosaminidase compared to Perfusate 3 and 4, and where Perfusate 3 had the highest levels, indicating greatest tubular damage. Ultimately, this study highlighted the significant influence of different perfusate compositions on EVNP outcomes, and the importance of a harmonious protocol to enable consistent interpretation of EVNP data. The need for further comparative studies to assess these perfusate protocols is self-evident in order to further this perfusion technology.

Cellular Composition

Most preclinical studies to date have used red cell-based perfusates; however, it is important to note that whole blood is a finite resource, particularly given that type O packed erythrocytes is most commonly used. Furthermore, the blood may contain antibodies, clotting factors, activated leukocytes and thrombocytes which potentially exacerbate IRI through generation of inflammatory mediators and activation of complement cascade (16). Accordingly, plasma-free and leukocyte-depleted perfusates have been well-established in both preclinical and clinical studies (7, 8). However, there is limited data on whether or not plasma-based perfusates, or the use leucocyte depletion filters, have a role in wider clinical use.

Nevertheless, adequate oxygenation remains a vital prerequisite, which can be delivered by several means: RBCs, synthetic HBOCs or simple diffused oxygen by carbogen gas mixtures. While RBC-based perfusates are proven, they are limited by poor availability, high cost and short-shelf life, with potentially increased risk of infection transmission and haemolysis (17). HBOCs are more accessible with reduced infection and haemolysis risks (17). Recently, preclinical studies on discarded human kidneys have demonstrated that HBOCs are non-inferior to pRBCs in terms of renal hemodynamics and histological damage (17), suggesting that HBOCs may indeed offer a logistically more convenient alternative to pRBC in EVNP of human kidneys. Further

studies, however, demonstrating improved clinical outcomes in appropriate transplant models are required.

Acellular perfusates, without any haem-based oxygen carriers, may offer a unique benefit as they better enable gradual rewarming of the organ to normothermia. At present, EVNP is performed at the receiving site after a period of SCS transport from the donor hospital. This abrupt restoration of normothermia and rise in metabolic turnover has been implicated as a secondary cause of IRI (5). This is thought to be due to disrupted cellular homeostasis at the mitochondrial level (5) and to RBCs losing their deformability in cold, leading to impaired microcirculation and tissue oxygenation, and can be mitigated by gently rewarming the organ from SCS using an acellular perfusate (13). It has been demonstrated (data presented at ATC 2019) that EVNP may be feasible without haem-based oxygen carriers for up to 6 h in discarded human kidneys (38). In this instance, the perfusate, with 95% O₂, 5% CO₂, sustained stable renal haemodynamics and restored tissue ATP levels similar to concentrations in a red cell-based perfusate. Acellular EVNP of porcine kidneys has also been shown to fully saturate venous haemoglobin when the partial pressure of oxygen was maintained above 500 mmHg (13). The same group later reinforced these findings in a first-in-man clinical case-study, in which controlled oxygenated rewarming without any oxygen carriers resulted in successful transplantation with good immediate renal function (18). Increasingly, evidence suggests that oxygen carriers may not be required to achieve adequate oxygenation during short-term renal perfusion (17,38,39).

Although beyond the scope of this review that concentrated on normothermic perfusion, there is growing evidence in favor of gradual rewarming. Comparing controlled oxygenated rewarming with continuous up-front perfusion in a porcine transplant model using steen-based solution with 95% oxygen and 5% CO₂, both methods effectively restored renal function after SCS to the same level, with controlled oxygenated rewarming significantly reducing tenascin C expression in tissue—a glycoprotein induced during injury—compared to SCS (40). Heat-shock proteins are well known as a defense mechanism induced by stressful stimuli such as hypoxia or hyperthermia (41,42). Minor et al. demonstrated that with gradual rewarming (or “controlled hyperthermia”), they found a 50% increase of heat-shock proteins, which correlated to improvement of tubular reabsorption of sodium and glucose upon reperfusion, and reduced loss of urinary protein compared with controls, meriting further exploration of this technique in preclinical models (43). As a result of this work, there is emerging evidence that avoiding the abrupt temperature changes may be protective against IRI.

Gaseous Composition

Supraphysiological concentrations of oxygen, in the form of 95% O₂, 5% CO₂ gas mixtures, have been utilised in most EVNP protocols. However, excess oxygenation may exacerbate IRI through increased production of ROS (4). A porcine kidney transplant model comparing EVNP with 95%, 25% and 12% O₂ with 5% CO₂, found that while oxygen extraction was

significantly reduced, reducing oxygen levels to normoxia did not significantly influence functional parameters or biomarkers of renal injury during reperfusion (19). This directly contradicts previous studies that advocate hyperoxemia (13,18). Importantly, the latter studies used acellular perfusates, signifying that higher oxygen concentrations may be necessary in the absence of oxygen carriers. In either case, theoretically neither hypoxemia nor hyperoxemia should alter renal vasomotor tone in constant CO₂ concentrations (44); thus, reducing oxygen tensions would not be expected to influence renal function. Further characterisation of oxidative stress in the context of EVNP may enhance this field of research.

Gases are easily absorbed into the blood, and therefore can be utilised as additives to enhance the protective effects of EVNP. In human-sized porcine kidneys, hydrogen sulfide (H₂S) infusion after 30 min of EVNP reduced oxygen consumption which was restored rapidly after cessation without any short-term indications of histological or biochemical damage (21). With further corroborating evidence, H₂S supplementation may offer potential in reducing the extent of oxygenation required, facilitating the use of acellular perfusates or normoxic gas mixtures; further work is required, particularly, to exclude any potential long-term toxicity prior to clinical translation.

Other gases that have been utilised include carbon monoxide (CO), which has shown to significantly reduce IRI in experimental models by promoting vasodilation (20); and argon, which despite suggestion that it may potentially reduce IRI by inhibiting IL-8, did not influence renal function when administered during EVNP of porcine kidneys (22), consistent with EVNP models in porcine lungs (45). These findings may be explained by the longer durations of perfusion permitted in the experimental studies, and that benefits of argon may only be quantifiable after prolonged periods.

Supplementary Composition

Metabolic and energetic substrates are essential for restoration of normal metabolism. Clinical perfusates have been most commonly supplemented with a nutrient solution with insulin, glucose 5%, sodium bicarbonate 8.4%, multivitamins and extracellular fluid (Ringer’s lactate) to replace urine output (6,7,11). Moreover, blood-based perfusates include anticoagulants to prevent clotting within the perfusate tubing circuitry and to reduce risk of graft thrombosis, and vasodilators to reduce transient vascular constriction upon reperfusion with RBCs (46). Furthermore, liver studies have shown that maintenance of optimal microcirculatory homeostasis using vasodilators is a key factor in EVNP (34). There has been limited research, however, evaluating the impact or need for anticoagulants and vasodilators, particularly in the context of acellular perfusates.

Other supplements in the literature have aimed to further ameliorate IRI. Currently, reduction of inflammatory mediators is achieved through integration of hemadsorption technology (CytoSorb) into the EVNP circuit (25). However, such broad-spectrum hemadsorption may potentially remove important anti-inflammatory mediators. An alternative method proposed to reduce oxidative stress is the utilisation of endogenous HO-1;

a heat shock protein that catalyses degradation of haem, exerting cytoprotective effects (42). Naturally, HO-1 decreases during SCS due to reduced protein expression under hypothermia (25,26). However, one study showed that addition of cobalt protoporphyrin (CoPP) during normothermic preservation successfully induces HO-1 within clinically appropriate timeframes (26). Of note, some degree of toxicity, presented as reduced urine output and increased proteinuria, was observed at higher concentrations of CoPP, without further increases in HO-1. Therefore, optimal HO-1 inducers and concentrations need to be explored further. Vitamin C is known to prevent apoptosis, reduce inflammation and endothelial permeability, in addition to enhancing microcirculation. However, in 6-hour animal EVNP models, no improvements in clinical parameters were observed despite a significant reduction in oxidative stress (23), consistent with negative findings of small clinical studies (47). Finally, EPO supplementation has been speculated to lessen IRI by modulation of apoptotic mediators: caspase-3, interleukin-1 β and HSP70 (24). In porcine kidneys subjected to 2-hour of haem-based EVNP, addition of EPO reduced apoptotic cells in tubular lumens and interstitial areas and facilitated renal tissue remodelling (48). While encouraging, these studies were limited by lack of clinically relevant outcome measures and did not address potential adverse effects.

Of note, no data was found on the use of antibiotics or the specific dosing of the aforementioned additives. Additionally, the administration of therapeutics such as regenerative cell therapies was deemed beyond the scope of this review.

Duration of Perfusion

Optimising perfusion duration may be a critical step in augmenting the benefits of suitably engineered perfusates. As successfully demonstrated in clinical studies, a short period of EVNP (up to 2-hour) is acceptable following a period of SCS (6,7,11,49). However, continuous normothermic perfusion from time of retrieval may permit complete avoidance of cold ischaemic injury. Recent DCD porcine studies have verified the feasibility and safety of prolonged EVNP with near complete exclusion of SCS using whole-blood perfusates for 10-hour in livers (33,50) and acellular Steen solution for 12-hour in lungs (51). Initial evidence in kidneys showed that continuous, 8-hour EVNP is feasible and safe in good quality beating-heart donor kidney grafts, (52) and in a follow-up study on DCD porcine kidneys, the same group demonstrated that continuous 16-hour EVNP with near complete exclusion of SCS was superior to brief EVNP following SCS (28). Furthermore, sub-normothermic 24-hour preservation using acellular Steen solution has been shown to support low and stable vascular resistance whilst providing adequate histological preservation in DCD porcine kidneys (12). Notably, in this study EVNP beyond 5-hour was not feasible when whole blood alone was used, and when diluted with Steen solution, acidosis, hyperkalaemia and necrosis resulted (12). This study was limited by variable warm ischaemic times, use of inconsistent vasodilators, and lack of post-transplant reperfusion outcome measures; however, it may be of interest to further investigate the effects of different perfusates at varying durations.

Despite this emergent potential, no portable devices are yet available for continuous renal EVNP during transportation, unlike the OrganOx metra device that has shown to continuously preserve donor livers for up to 24-hour (50). Logistical burden of machine failure during transport, health-care costs, and complicated transportation procedures would also require consideration. Therefore, to evaluate outcomes of prolonged EVNP in current clinical context, brief, intermediate and prolonged EVNP following 8 h of SCS were compared in similar DCD porcine models (27). All durations maintained stable hemodynamic parameters, however posttransplant serum creatinine was significantly lower after intermediate and prolonged EVNP compared to the brief EVNP. Noticeably, serum creatinine was higher after 1-hour EVNP compared to SCS alone. This may be explained by several mechanisms: 1) 1-hour is insufficient for repair mechanisms; 2) rapid warming from hypothermia is harmful in short-term, as previously discussed; or 3) discrepancies exist due to different transplant models. Despite the higher tier evidence provided by human clinical studies (7), future studies should assess protein expression during prolonged EVNP to ascertain the specific molecular processes, whilst also exploring the feasibility of portable renal EVNP machines.

Debate remains regarding the recirculation of urine versus replacement of urine losses with colloid solution, particularly in the context of longer perfusion durations. Weissenbacher et al. demonstrated that the recirculation of urine permitted stability over a 24-hour normothermic perfusion period with urine recirculation. The control group ($n = 3$) with fluid replacement as per urine loss were unable to be perfused beyond 4–6 h due to an inability to maintain a physiological pH (53). Subsequent work by the same group has confirmed these findings in a porcine model in which urine circulation aided the maintenance of physiological arterial pressure and acid-base homeostasis (54). Proteomic data also suggests urine recirculation may increase glucose metabolism, which may indicate an increase in metabolic activity, potentially protective against IRI (55).

Study Strengths and Limitations

Due to the exploratory nature of this review, there lacked clear uniformity in the study designs, objectives, and outcome measures evaluated. Furthermore, high study heterogeneity precluded a meta-analysis. Moreover, a large proportion of the selected studies were experimental, yielding lower strengths of evidence and limiting our use of the recognised Cochrane bias risk assessment tool for randomised controlled trials. However, our efforts in screening a large number of databases, with wide eligibility criteria, provided some safeguard against missing relevant studies. Further identification of potentially relevant studies may have been achieved by expanding the eligibility criteria to include studies of sub-normothermic perfusion methods. The term “EVNP” was used throughout this manuscript, however, we acknowledge that the terms normothermic *ex-vivo* kidney perfusion (NEVKP), sub-normothermic kidney perfusion (SNMP), normothermic machine perfusion (NMP) are also used in the literature.

Standardisation and reproducibility of terms is an important part of collaboration with new technologies and techniques; importantly, our search strategy accounted for these additional terms.

Overall Context and Future Direction

EVNP is a technology used for multiple reasons in the solid organ transplant field. “Optimisation” may represent different factors to different ends. For the purposes of kidney viability assessment, short-term perfusion may provide valuable information. Rapid transplantation places the kidney in a more physiological environment and may make longer perfusion undesirable. Prolonged EVNP clearly has the potential to recondition kidneys and regenerate their injured cells/tissue, not to mention the untapped potential for immunomodulation. Prolonged regeneration and immunomodulation would appear likely to require a more adaptive and physiological environment, perhaps with natural biological homeostats such as a liver in circuit, or with advanced sensors and chemical modulation beyond anything applied in the studies discussed in this review. It will perhaps be the adaptability and sensitivity of the circuit in regulating its perfusate composition, that allows the full potential of this therapy to be realised. There remains room for vast innovation and automation in this field even beyond a device such as Organox which is being taken up rapidly in the liver transplant arena.

CONCLUSION

EVNP is an evolving technology which has the opportunity to resuscitate and evaluate kidneys prior to transplantation, and the elucidation of ideal perfusate constituents and perfusion duration remain key in the optimisation of this clinical tool. Our findings suggest that Ringer’s lactate or Steen solution supplemented with nutrient and metabolic substrates provide a suitable environment for preservation at normothermia. Given logistical implications, under current protocols, blood-based perfusates may be suboptimal if synthetic HBOCs or acellular perfusates with carbogen gas mixtures are proven to support adequate oxygenation and enable gradual rewarming where continuous renal EVNP to completely bypass SCS is in development. Particularly given that longer perfusion durations (beyond 6 h) may be harmful with the use of red cell-based perfusates. However, this may relate to the limited homeostasis of established EVNP circuits and will clearly need re-evaluation

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as the many other biochemical parameters of kidney EVNP are optimised by improved technology. There are also emerging roles for supplementary constituents that reduce metabolism and suppress inflammation which are beyond the scope of this review. *Ex-vivo* modulatory interventions represent a brave new world of therapy in transplantation. Extensive further research is required, however, in appropriate transplant models to ascertain clinical benefits.

It is clear that co-ordinated research aims and better collaboration between the many groups involved in this emerging technology would be beneficial to progress. In conclusion, while current clinical protocols have been feasible, there is increasing evidence that there is potential to better define perfusion composition, in particular with use of non-blood-based perfusates, and prolonged duration, to optimise organo-protective benefits of EVNP.

AUTHOR CONTRIBUTIONS

AF—screened articles, data collection, manuscript writing; RP—designed research, screened articles, manuscript writing; RL—resolved conflicts in article screening, manuscript preparation; PM—manuscript preparation and review; MC—designed research, manuscript review.

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

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A National Survey Comparing Patients' and Transplant Professionals' Research Priorities in the Swiss Transplant Cohort Study

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We aimed to identify, assess, compare and map research priorities of patients and professionals in the Swiss Transplant Cohort Study. The project followed 3 steps. 1) Focus group interviews identified patients' ($n = 22$) research priorities. 2) A nationwide survey assessed and compared the priorities in 292 patients and 175 professionals. 3) Priorities were mapped to the 4 levels of Bronfenbrenner's ecological framework. The 13 research priorities (financial pressure, medication taking, continuity of care, emotional well-being, return to work, trustful relationships, person-centredness, organization of care, exercise and physical fitness, graft functioning, pregnancy, peer contact and public knowledge of transplantation), addressed all framework levels: patient ($n = 7$), micro ($n = 3$), meso ($n = 2$), and macro ($n = 1$). Comparing each group's top 10 priorities revealed that continuity of care received highest importance rating from both (92.2% patients, 92.5% professionals), with 3 more agreements between the groups. Otherwise, perspectives were more diverse than congruent: Patients emphasized patient level priorities (emotional well-being, graft functioning, return to work), professionals those on the meso level (continuity of care, organization of care). Patients' research priorities highlighted a need to expand research to the micro, meso and macro level. Discrepancies should be recognized to avoid understudying topics that are more important to professionals than to patients.

Keywords: organ transplantation, patient involvement, research priorities, qualitative methods, registry-based study

Abbreviations: STCS, Swiss Transplant Cohort Study.

A national survey comparing patients' and transplant professionals' research priorities in the Swiss Transplant Cohort Study



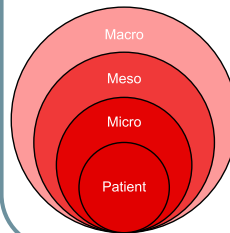
Rationale

To identify, assess, compare and map research priorities of Swiss organ transplant patients and professionals

Sequential methods

- 1) **Focus group interviews** in 22 patients identified research priorities
- 2) **Survey** in 292 patients and 175 professionals rated importance of research priorities
- 3) **Mapping** the priorities on 4 levels of Bronfenbrenner's ecological model

Results



- Transplant patients were prime informants to identify **13 research priorities** on all 4 levels
- **Continuity of care** (meso level) received highest importance rating by both groups
- **Discrepancies**: patients emphasized patient level priorities (emotional well-being, graft functioning, return to work), professionals those on the meso level (continuity of care, organization of care)

Conclusion

- Research must be expanded beyond the patients' level and cover also the micro, meso and macro level priorities
- Recognition of discrepancies may avoid understudying topics that are more important to professionals than to patients



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GRAPHICAL ABSTRACT |

INTRODUCTION

Setting research priorities with patient involvement is key to optimizing resources, reducing research waste and producing relevant and warranted evidence that improves not only clinical practice but also the quality of life of those affected. When setting research priorities, an increasing number of initiatives promote the involvement not only of clinicians and researchers but also of patients and other stakeholders (1–7). These efforts have been essential in determining the research agenda (1), conducting research toward the needs of those who live with a certain condition (8), performing research with the greatest public health benefit and enhancing the societal return-on-investment of research funding (9, 10). Within the research team, patients contribute perspectives that may be based on the lived experience and therefore complement the scientific view. former Chief Medical Officer for England, Professor Dame Sally Davis, aptly highlighted the beneficial effect of diverse perspectives: “No matter how complicated the research, or how brilliant the researcher, patients and the public always offer unique, invaluable insights. Their advice when designing, implementing and evaluating research invariably makes studies more effective, more credible and often more cost efficient as well” (11).

In the transplant setting, a large international study revealed that patients and clinicians differ considerably in their opinions about relevant research outcomes that should be assessed (12). The discrepancy highlights the necessity to thoroughly understand patients' needs and opinions in order to add their perspective in the process of setting research priorities. A

systematic review examined 28 transplant research priority setting projects involving different stakeholders such as patients, healthcare providers, policymakers and researchers (13). Tong et al. found that only nine projects (32%) reported patient or caregiver involvement, restricted to projects in kidney and heart transplantation. The nine projects used different methodologies to identify research priorities such as surveys, interviews or workshop discussions. Importantly, only one project started the priority setting process from the patients' perspective (14).

The Swiss Transplant Cohort Study (STCS), a nation-wide prospective cohort study started in 2008, has currently involved more than 6300 patients. The STCS collects a broad set of biomedical, genetic and psychosocial variables, including patient-reported outcomes, before and after transplantation (15, 16). In 2017, driven by the international and national call for patient involvement in research priority setting (1, 4, 8), the STCS launched a patient involvement project. This study is part of that project, which followed the stages of the research cycle as recommended by the INVOLVE report and started to first identify and prioritize research topics (1). Given that an individual is not isolated but surrounded by a wider community and society, Bronfenbrenner ecological framework suggests four levels (i.e., patient, micro, meso and macro level) to examine interactions and relationships (17, 18). Therefore, the aims of this study were to identify, assess, and compare the research priorities of Swiss transplant patients and transplant professionals and to map the priorities according to Bronfenbrenner's framework.

PATIENTS AND METHODS

Design

This study was a sequential multi-methods project. First, we conducted focus group interviews with organ transplant patients to identify research priorities from the patient perspective. Second, we conducted a survey to assess and compare the importance of research priorities in transplant patients and transplant professionals. Third, we conceptually mapped the research priorities according to the ecological framework by Bronfenbrenner (17, 18). The study received a declaration of no objection from Swissethics (EKNZ Req-2017-00279).

Part 1: Focus Group Interviews With Patients Sample and Setting

We conducted 3 focus group interviews to identify research priorities. To facilitate the journey to the interviews, recipients could choose from three locations (Zurich, Basel or Geneva). Inclusion criteria were age ≥ 18 years and having received a multi-organ transplant or a single kidney, liver, heart or lung transplantation. People who were not able to speak German or French were excluded.

Data Collection and Analysis

The interviews were conducted in April and May 2017. Eligible participants of all transplant centers were asked by the local STCS data manager or members of the study team to participate. In advance, they received from the researchers oral and written information about the purpose of the discussion, the voluntary nature of their participation and the use of their contributions. Prior to the discussion, all participants were informed, that the discussion content would be treated confidentially and were asked to agree to an audio-recording of the interview.

At the beginning of each interview, participants were encouraged to talk to each other, and interactions within the group were stimulated. The discussions were guided by a semi-structured guideline, which was sent to the participants in advance to facilitate preparation. The guideline included three open-ended questions: 1) What is important for you, or what concerns do you have when dealing with your transplantation? 2) Which questions should researchers focus on to improve life with a transplantation? 3) Which topics are important for you following your transplantation? Probe questions on specific transplant topics (e.g., psychosocial issues, psychological and social support, comorbidities) guided further discussions if necessary.

The knowledge mapping technique was used for analysis, allowing an organized, condensed and visualized presentation of the issues emerging from each focus group interview (19, 20). While the main moderator guided the interview, the co-moderator identified and grouped important topics in the maps. At the end of each interview, the co-moderator explained and summarized the knowledge maps to the participants. The visualization highlighted relationships and allowed related themes to be developed. This procedure and the resulting discussion served to validate the topics and was

TABLE 1 | Patient and professional characteristics.

	Valid n	Patients, n = 292
Male gender, n (%)	256	171 (58.6)
Age in years, median (IQR)	246	58 (27-65)
Time after Tx in years, median (IQR)	263	4.37 (0.6-12.4)
Tx organ	255	
Kidney, n (%)		160 (55)
Liver, n (%)		48 (16)
Heart, n (%)		38 (13)
Lung, n (%)		1 (0.3)
Other, n (%)		5 (2)
Combined, n (%)		3 (1)
Tx center	254	
Basel, n (%)		79 (27)
Zurich, n (%)		48 (16)
Bern, n (%)		44 (15)
Lausanne, n (%)		37 (13)
St. Gallen, n (%)		22 (8)
Geneva, n (%)		24 (8)
	Valid n	Professionals, n = 175
Male gender, n (%)	158	81 (46)
Age in years, median (IQR)	158	42.5 (36-51)
Time working in Tx in years, median (IQR)	158	10 (3.75-17)
Profession	158	
Physician, n (%)		119 (68)
Nurse, n (%)		17 (10)
Researcher, n (%)		10 (6)
Data manager, n (%)		5 (3)
Other, n (%)		7 (4)
Specialization	157	
Nephrology, n (%)		51 (29)
Transplant surgery, n (%)		28 (16)
Hepatology, n (%)		12 (7)
Infectiology, n (%)		10 (6)
Pulmonology, n (%)		6 (3)
Cardiology, n (%)		2 (1)
Other, n (%)		48 (27)

SD: standard deviation, IQR: interquartile range, Tx: transplant.

considered as the first step of data analysis. Afterwards, the knowledge maps of all three focus group interviews were reviewed and analyzed by research team members to identify common topics and research priorities.

Part 2: Survey Among Patients and Professionals

The focus groups generated 13 research priorities, represented by 34 example statements. The 34 statements formed one section of a 95-item questionnaire on research priorities and patient involvement in transplant research. The questionnaire's two other sections covered the importance of patient involvement (5 items) and factors to be assessed in STCS (56 items), which were not the focus of this analysis. The questionnaire was translated by native speakers from German to English and French.

Setting and Sample

The questionnaire was distributed among a convenience sample of patients and professionals in all six transplant centers and their respective solid organ transplant outpatient clinics in Switzerland. Inclusion criteria for the patients were: age ≥ 18 years, having

TABLE 2 | The top 10 research priorities with corresponding example statements and the level of the ecological framework for each group.

Top 10 Ratings by patients (n = 292)		% (Valid n)	Top 10 Ratings by professionals (n = 175)		% (Valid n)
1	Continuity of care—Meso level	92.2 (282)	1	Continuity of care—Meso level	92.5 (161)
	Care begins even before the transplant takes place.			Care begins even before the transplant takes place.	
2	Continuity of care—Meso level	91.2 (285)	2	Organization of care—Meso level	90.6 (160)
	It's nice if you can always call the same people at the hospital. Then they know you.			I would like a telephone number where I can get a sensible answer if I call. A point of contact where I can clarify whether I need to go to hospital or not.	
3	Person-centeredness—Micro level	87.9 (173)	3	Organization of care—Meso level	89.9 (159)
	My life does not consist solely of the transplant. A good doctor is one who sees the person as a whole, who sees you as a complete person and not just as a "transplanted organ".			I discovered that I did not have a contact person at the hospital. There is nobody that I can relate to, and I miss that.	
4	Public knowledge of transplantation—Macro level	82 (272)	4	Continuity of care—Meso level	85.7 (161)
	The general public needs to be better educated about organ transplantation. People have strange ideas.			It's nice if you can always call the same people at the hospital. Then they know you.	
5	Emotional well-being—Patient level	81.4 (269)	5	Person-centeredness—Micro level	83.8 (160)
	How you deal with the illness is important. How you find a balance between anxiety, the consequences of the transplant and the desire to live.			My life does not consist solely of the transplant. A good doctor is one who sees the person as a whole, who sees you as a complete person and not just as a "transplanted organ".	
6	Graft functioning—Patient level	78.3 (263)	6	Continuity of care—Meso level	83.4 (157)
	I worry about how long my graft will last. I don't know what to expect. I'd like to see research focused on ways to make grafts last longer.			I had a new doctor every time. He had never seen me before and I had to explain everything all over again. This usually took up most of the appointment time.	
7	Emotional well-being—Patient level	77.2 (272)	7	Trustful relationships—Micro level	82.5 (160)
	It is my motivation: what progress can I see for myself from day to day. It just needs a lot of discipline. Otherwise, it doesn't work.			In hospital they said I should go to my GP. But he is so overwhelmed with my case that it makes me even more uncertain, and I have lost confidence in the hospital and in my GP.	
8	Emotional well-being—Patient level	76.9 (268)	8	Continuity of care—Meso level	81.1 (159)
	Not everybody, especially younger people, can master it in the same way. Attention should be paid to psychological care as well as to medical care.			Prior to the transplant there is too little information about what happens afterwards.	
9	Return to work—Patient level	73.8 (244)	9	Return to work—Patient level	79.9 (159)
	Many young people who have not worked or were unable to do training prior to the transplant later have great difficulty getting back into work.			I am still very tired during the day and I have difficulty concentrating. Now I've been given notice and the application for disability insurance is pending. But at 56 you're really gone - and I don't know what will happen now.	
10	Organization of care—Meso level	73.6 (269)	10	Exercise and physical fitness—Patient level	76.9 (160)
	I would like a telephone number where I can get a sensible answer if I call. A point of contact where I can clarify whether I need to go to hospital or not.			Since the transplant, exercise is very important to me. I enjoy it immensely.	

The 4 matching example statements among the groups are highlighted with bold rank numbers and % values. The shades of gray represent the ecological framework levels.

received a multi-organ transplant or a single kidney, liver, heart or lung transplantation. Patients in the immediate perioperative period, meaning those, who were still hospitalized after transplantation, were excluded. Inclusion criteria for the professionals were: age ≥ 18 years, being a member of the STCS (i.e., researcher, data manager) or being a professional who cares for transplant patients in one of the six transplant centers

(i.e., nurse, physician). Patients and professionals unable to speak German, English or French were excluded.

Data Collection and Management

Data were collected in November and December 2017. Patients were recruited and informed about the study during their follow-up appointment in the transplant centers' outpatient

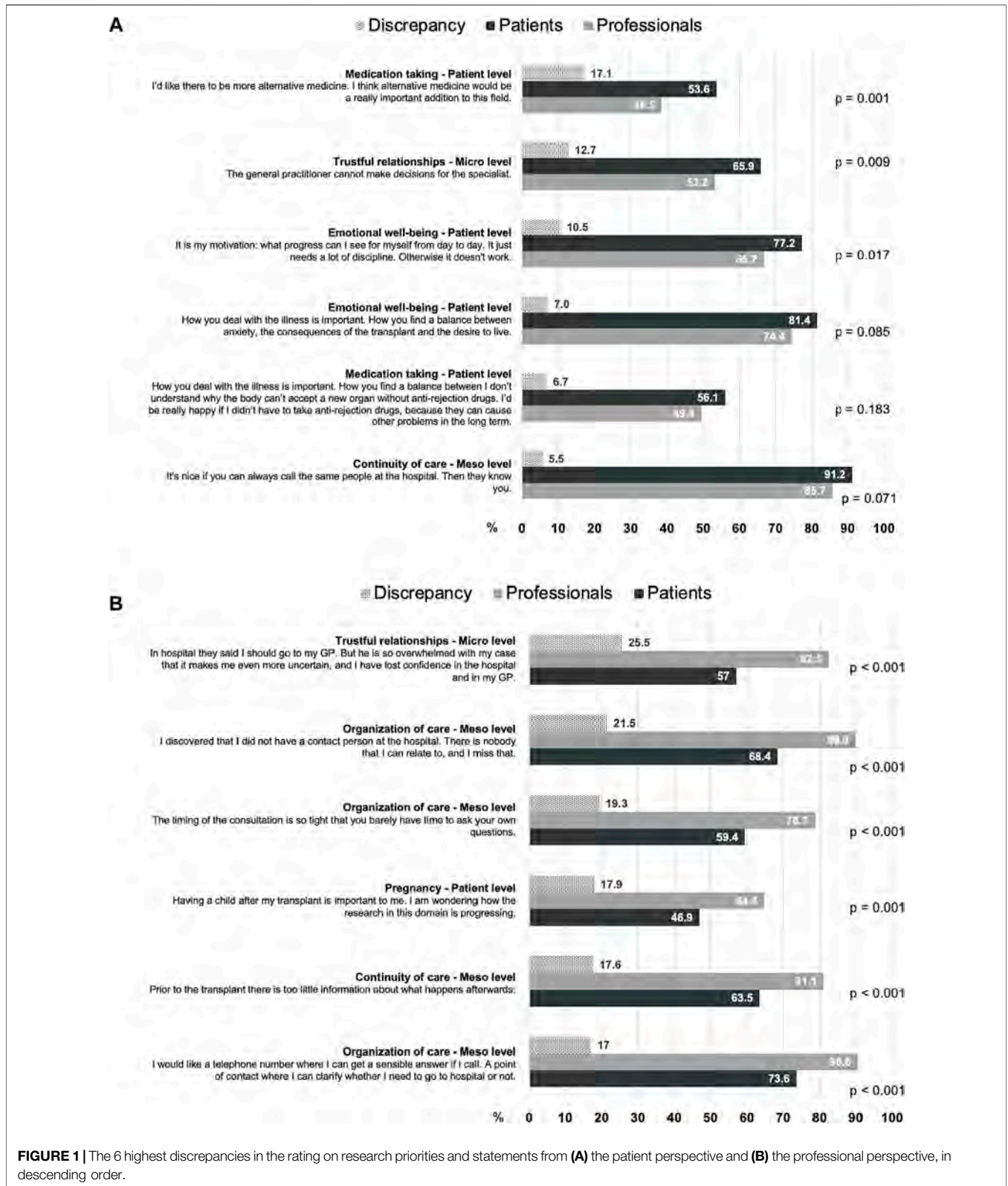
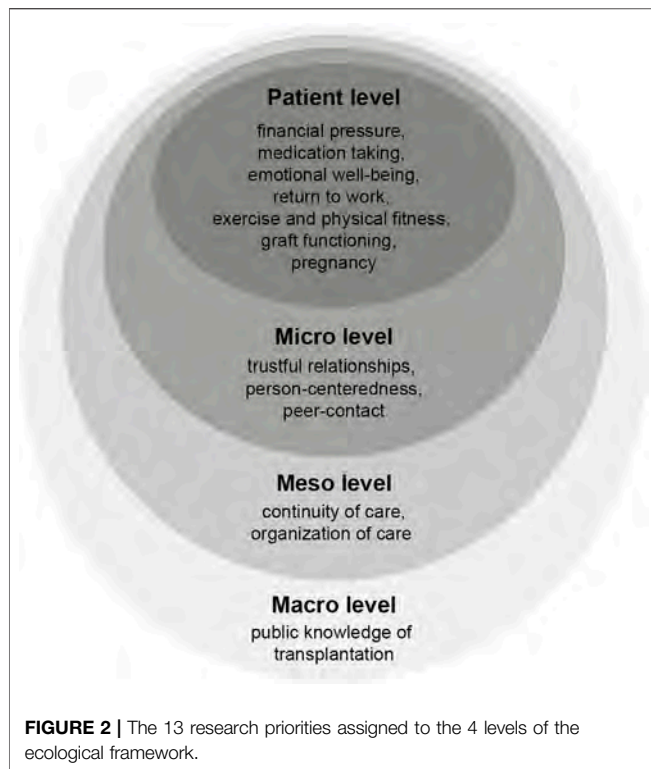


FIGURE 1 | The 6 highest discrepancies in the rating on research priorities and statements from (A) the patient perspective and (B) the professional perspective, in descending order.

clinics by the nurses and physicians. Interested patients received a hard copy of the questionnaire in their preferred language and a pre-stamped envelope to return the

questionnaire to the study team. Patients who preferred to participate online received a link to the electronic version of the survey.



All professionals in the STCS and in the six transplant centers were invited via e-mail to participate in the online survey in their preferred language. The e-mail with written study information and the link was distributed by the key stakeholders in the STCS and each transplant center. At the end of the data collection, the online data were transferred to a statistical software program. Two team members individually entered the data of the paper questionnaires in the statistical software program and double checked each entry for potential mistakes.

Variables and Measurements

The 34 items in our survey were rated regarding their importance for transplant research on a 9-point Likert-scale from 1 (not at all important) to 9 (very important) with the additional answer option “unsure”. The ratings from the continuous scale were dichotomized (cutoff at 7) for further analysis: items with values ≥ 7 were considered “important” to the participants. For each item, we noted the proportion of the “important” rating. The answer option “unsure” was considered as a missing value. The following general information was collected from patients: gender, age in years, transplanted organ, date of first transplant and transplant center; and from professionals: gender, age in years, years working in the field of transplantation, profession and specialization.

Data Analysis

Descriptive statistics included frequencies and percentages, mean and standard deviation, as well as median and interquartile range (IQR) as appropriate. Seventeen items had missing values $> 10\%$, which were not imputed. Discrepancies among patients and professionals in importance scores were calculated by

subtraction. Scores were compared using a Chi square test. A two-tailed p -value < 0.05 was considered statistically significant. Statistical analyses were conducted using IBM SPSS Version 25.0 for Mac (Armonk, NY: IBM Corp).

Part 3: Mapping Transplant Research Priorities

The research priorities (and corresponding example statements) were subsequently mapped according to the 4 levels of Bronfenbrenner’s ecological framework (17, 18): Patient level was defined as individual issues and characteristics such as knowledge, attitudes or behavior. Micro level was related to social support, interpersonal relationships and interactions between patient, family and healthcare providers. Meso level represented practice patterns and characteristics of the transplant center or the health care organization where patients were treated. Macro level covered issues related to the healthcare system and at policy level.

RESULTS

Part 1: Identification of Patients’ Research Priorities

Twenty-two patients participated in the focus groups (Zurich $n = 7$, Basel $n = 10$, Geneva $n = 5$). They had received an organ transplant between 1998 and 2017 (kidney $n = 9$, 43%, liver $n = 6$, 29%, heart $n = 4$, 19% and lung $n = 2$, 9%). The majority was female ($n = 12$, 57%) and the mean age was 53 years. Patients discussed a broad variety of issues, with congruous issues being discussed in each of the 3 focus groups. We identified 13 research priorities, represented by 34 example statements: financial pressure ($n = 5$ example statements); medication taking, continuity of care (each $n = 4$); emotional well-being, return to work, trustful relationships, person-centeredness, organization of care (each $n = 3$); exercise and physical fitness ($n = 2$); graft functioning, pregnancy, peer contact, and public knowledge of transplantation (each $n = 1$). A list of all research priorities and example statements is provided in the supplemental digital content (**Supplementary Table S1**).

Part 2: Assessment and Comparison of Patient and Professional Research Priorities

Across the 6 transplant centers, 16 outpatient clinics recruited patients. One kidney transplant outpatient clinic did not participate due to high workload. Of the 735 questionnaires distributed to patients, 292 were returned (response rate 39.7%). The online survey was completed by 175 professionals. The response rate was not calculated given the unknown denominator. Patient and professional characteristics are shown in **Table 1**.

The complete ranking of research priorities and example statements by patients and professionals is provided in the supplemental digital content (**Supplementary Table S1**). **Table 2**, with the top 10 research priorities for both groups, shows that both groups agreed in their highest rating on continuity of care (“Care

begins even before the transplant takes place”), which was important to 92.2% of the patients (n = 282) and 92.5% of the professionals (n = 181). Otherwise, patients and professionals had only 3 more matches in their top 10 ratings. The overall priorities of both groups differed as patients mostly chose statements relating to emotional well-being (n = 3 statements) while professionals emphasized statements relating to continuity of care (n = 4 statements).

Figure 1 shows the 6 highest ranked discrepancies from each perspective. From the patient perspective, the highest discrepancy in research priorities (17.1%) was medication taking, which was important to 53.6% of the patients and to only 38.5% of the professionals (**Figure 1A**). From the professional perspective, the highest discrepancy in research priorities (25.5%) was trustful relationships, which was important to 82.5% of the professionals and to only 57% of the patients (**Figure 1B**).

Part 3: Mapping Research Priorities According to the Ecological Framework

Figure 2 shows the mapping of the 13 research priorities addressing all 4 levels of the ecological framework: 7 patient level priorities (financial pressure, medication taking, emotional well-being, return to work, exercise and physical fitness, graft functioning, pregnancy), 3 micro level priorities (trustful relationships, person-centeredness, peer contact), 2 meso level priorities (continuity of care, organization of care), and 1 macro level priority (public knowledge of transplantation).

Patients and professionals focused on different research priority levels (**Table 2**). Within the top 10 research priorities for each group, the biggest proportion of patients' priorities was on the patient level (n = 5), such as emotional well-being, graft functioning and return to work. In addition, patients' priorities covered all 4 levels of the ecological model. Professionals' priorities were most often on the *meso level* (n = 6), such as continuity of care and organization of care, while they only chose 2 patient level priorities such as return to work and exercise and physical fitness.

The discrepancies between the groups revealed the same distribution of research priority levels (**Figure 1**). From the patient perspective, 4 out of the 6 discrepancies were related to the patient level priorities emotional well-being and medication taking (**Figure 1A**). From the professional perspective, 4 out of the 6 discrepancies were related to the meso level priorities organization of care and continuity of care (**Figure 1B**).

DISCUSSION

Our project identified 13 transplant research priorities covering a broad range of topics on all levels of the ecological framework. Setting research priorities informed by the patients' perspectives has gained increased importance over the last decade, also in transplantation (13). Our findings strengthen and expand this movement, especially as we focused on transplant patients as prime informants to determine research priorities.

We chose this approach to maximize the patients' inputs from the beginning; however, there are other methods. The James Lind Alliance, for example, suggested identifying research priorities

based on the equal voices of various stakeholders (8). So far, the approach of working with a mixed stakeholder group instead of patients only seemed to be the more common practice in research into priorities for solid organ transplantation. A systematic review has examined 28 research priority setting projects, 27 of which identified priorities based on the combined inputs from patients, caregivers, clinicians, researchers or policy makers (13). While the inclusion of diverse stakeholders is commendable, the authors also observed in the included studies a lack of details on the process of identifying the research priorities. Using a reporting checklist such as the GRIPP2 (Guidance for Reporting Involvement of Patients and the Public) (21) may enhance the quality of reporting and provide transparent information on the process of stakeholder involvement.

We mapped the research priorities according to the ecological model to enhance the interpretation of our results. Overall, the majority of our survey's example statements and research priorities were assigned to the patient level. The importance of patient-oriented topics was also highlighted by other projects, which primarily identified patient level priorities such as transplant outcomes, graft or recipient complications, immunosuppressive medication, fertility/pregnancy or organ donation criteria (13, 22). However, our results add to previous evidence because patients and professionals highlighted the need to expand the research to the micro, meso and macro level. Few transplantation studies integrated the transplant center or healthcare system level perspectives to examine transplant outcomes. A recent study used data from a multi-continental project in heart transplantation to examine nonadherence with immunosuppressive medication (23). Besides patient level factors, the authors also considered variables on the micro level (e.g., social support, trust in the healthcare team), the meso level (e.g., duration of visit in the outpatient clinic, care by a multidisciplinary team) and the macro level (e.g., health insurance covering costs for immunosuppressants). The multiple logistic regression identified 6 correlates from all ecological levels as associated with immunosuppressant nonadherence, which broadened the picture and increased understanding of medication nonadherence. We therefore encourage future transplant studies to follow this inclusive approach. Considering the micro, meso and macro level perspectives is likely to enlarge the evidence and therefore potentially improve patient outcomes and quality of care.

Another finding from our study supports the expansion beyond patient level factors because both parties agreed on their most important research priority continuity of care, which belongs to the meso level. Continuity of care is a broad concept, which can be characterized by three elements: longitudinal care with as few professionals as possible, a caring patient-professional relationship and coordinated care (24). It relates to the other meso level priority, organization of care, which was second most chosen by professionals. Patients and professionals therefore identified the need to consider the principles of chronic illness management in transplant research. In numerous chronically ill populations, the re-organization of care delivery according to the components of chronic illness management has improved outcomes such as reducing hospital admissions, improving health behaviors or a better quality of life (25). As researchers and clinicians have already called to adapt follow-up care to the principles of chronic illness

management, which better reflects the complex needs of solid organ transplant recipients (26), our results emphasize the importance of accompanying this process in transplantation by research.

Our results on the ranking of research priorities, however, revealed perceptions to be more diverse than congruent among patients and professionals. Within the top 10, the groups shared only 4 common research priorities, and our immediate comparison of research priorities of each groups' perspectives revealed additional discrepancies. Overall, patients chose more patient level priorities, professionals those on the meso level. Patient level priorities in our study covered various elements such as graft functioning, emotional well-being and return to work, thereby highlighting the need to expand transplant research beyond purely clinical or medical topics to psychosocial topics. Prioritizing psychosocial topics was also a finding in a systematic review, although this research priority scored comparatively low in their ranking since only 7 of the 28 reviewed studies mentioned psychosocial and lifestyle topics (13).

Dissenting views on research priorities among stakeholders have also been observed in other priority setting projects. Knight et al. used the James Lind Alliance method to identify and prioritize unanswered research questions in the field of kidney transplantation (22). Professionals and non-professionals initially identified 497 questions covering all parts of transplantation. After a process of surveying, grouping, refining and validating, a final set of 25 top ranked questions was discussed in a workshop with patients, carers and healthcare professionals. The groups agreed on the importance of improving long-term transplant outcomes; however, patients prioritized questions about immunosuppression, organ preservation and equity of access while professionals emphasized medical aspects such as the assessment of patient and organ suitability as well as the management of antibody mediated rejection. A systematic review reported the same pattern with patients focusing on person-centered topics (e.g., patient and family education, reducing side-effects of medication, quality of life) and professionals prioritizing technical or policy aspects of transplantation (e.g., HLA antibodies and sensitization, allocation, pharmacokinetics of immunosuppression) (13). While our study also revealed discrepant views among patients and professionals, the topics differed from the previous examples. The reason might be that, in our project, the research priorities were initially determined by patients. Since the survey did, therefore, not include procedures, medical or technical topics related to transplantation, participants, and especially professionals, could not choose research priorities from these domains.

Regardless of whether the process of identifying research priorities was initiated by mixed stakeholders or patients only, an important finding from our study and previous projects is that discrepancies between stakeholders occur and should therefore be recognized and used to an advantage. Emphasizing research priorities with high importance to patients but less importance to professionals may reduce the risk of understudying those issues in research. This emphasis also strengthens the necessity to involve patients early in the research process to combine the perspectives of lived experience and science (1). Indeed, combining complementary views in research priority settings can be positive and productive as it reduces the risk of a mismatch between the research being conducted and the research

expected by all parties (1, 8). However, it seems as if this combining is easier said than done. A recent study found that only 27% of the published articles in two main transplant journals considered research priorities as identified by patients, caregivers and researchers (27). More effort will be needed if the priorities and the research conducted are to be better matched. Importantly, the transplant community already started activities to support this movement. The newly established European Transplant Patient Organization, initiated by the European Society for Organ Transplantation, is considered to function as a platform to support mutual understanding, learning and collaborative partnership between transplant professionals and solid organ recipients (28).

Our study was conducted within the research framework of the STCS, and the results will shape the future STCS research agenda towards more diverse perspectives. We identified research priorities on each level of the ecological model. They will now support the development of specific research questions, guided by specific evidence and the needs of each solid organ transplant group separately. This process will again involve transplant patients because evidence suggests that patient involvement enhances the significance of research projects and the impact of study findings (29–31).

While the STCS will take further actions based on the results of this study, some limitations should be noted. First, dichotomizing the answer categories might have resulted in a loss of variability compared to using mean values. Second, perspectives from participants speaking languages other than German, French or English are missing. Especially patients from other cultural or ethnic backgrounds probably deal with different issues, which were not highlighted in our nationwide survey. This perspective should be examined in future research.

In conclusion, patients identified research priorities, which were compared and assessed in a nationwide survey with patients and professionals and mapped according to the ecological framework. Our results highlight the need to expand research to cover not only patient level but also micro, meso and macro level topics. However, comparing the research priorities revealed diverse perspectives that should be acknowledged. Patients focused on patient level priorities related to psychosocial issues while professionals emphasized meso level priorities related to the principles of chronic illness management. Our findings add a crucial patient perspective to the STCS research agenda and the broader transplant research community. Combining the perspectives of lived experience and science will facilitate future research that is of high priority to both patients and professionals.

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DATA AVAILABILITY STATEMENT

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

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ETHICS STATEMENT

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

AUTHOR CONTRIBUTIONS

SB, OM, LS, JR, CL, and SG designed the research; SB, OM, LS, and JR conducted the research; SB, OM, and SG analyzed the data; SB, OM, LS, JR, CL, AB, IB, UH-D, and SG wrote 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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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10255/full#supplementary-material>

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Prevalence of Blood-Borne Viruses and Predictors of Risk in Potential Organ Donors in Australia

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Internationally, the designation of a patient as an increased viral risk organ donor has been associated with lower utilisation rates. The actual prevalence of blood borne viruses in Australian potential organ donors, and the predictive performance of questionnaires administered to stratify this risk, remains unknown. We conducted a retrospective review of all patients who commenced workup for donation on the national database between 2014–2020. The prevalence of HIV, Active HBV and Active HCV in 3650 potential organ donors was 0.16%, 0.9%, and 2.2%, respectively. The behavioural risk profile was assessed in a subset of 3633 patients. Next-of-kin reported increased risk behaviours were associated with an increased prevalence of HCV but not of HIV or HBV (OR 13.8, $p < 0.01$, OR 0.3, $p = 0.42$, OR 1.5, $p = 0.14$). Furthermore, the majority of HIV and HBV infections occurred in potential donors without a disclosed history of increased risk behaviours. In this series, donors had a higher prevalence of HCV, and similar rates of HBV and HIV to the broader community. Behavioural transmission risks were poorly predictive of HIV and HBV. Rather than pre-transplantation behavioural risk screening, routine post-transplant recipient screening may provide a more powerful tool in mitigating the consequences of unexpected viral transmission.

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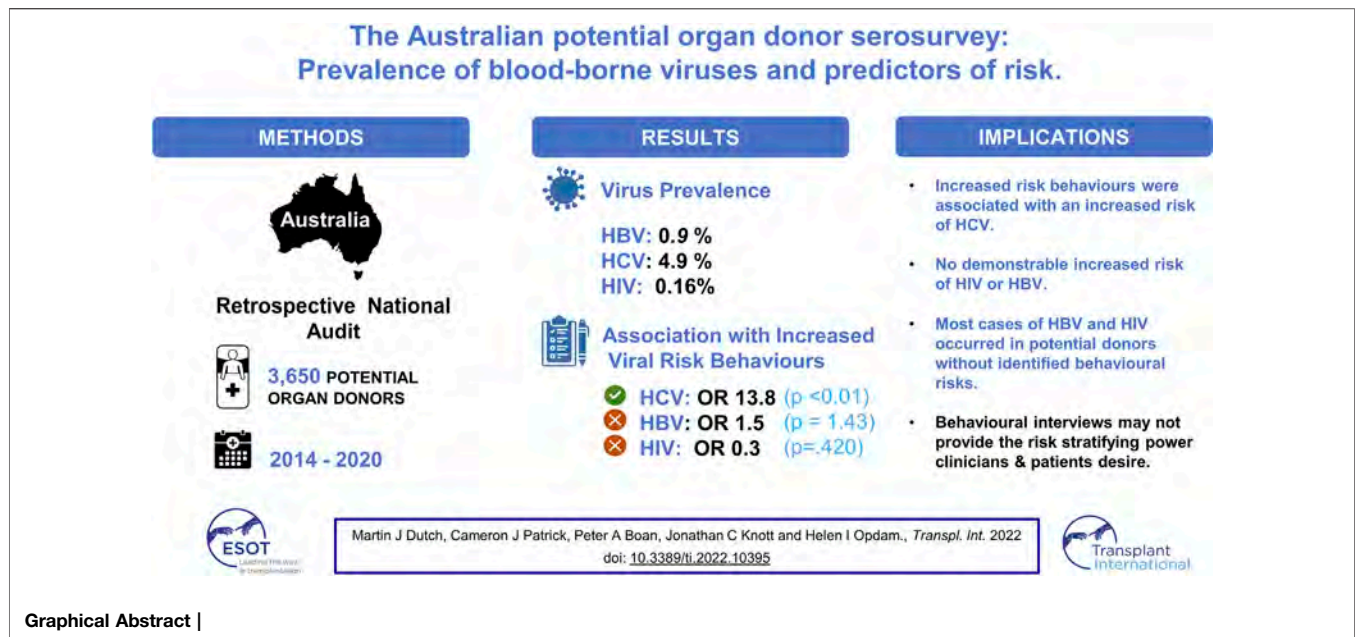
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Abbreviations: BBV, blood-borne virus; BRAQ, behavioural risk assessment questionnaire; EDR, electronic donor record; HBcAb, hepatitis B core antibody; HBsAb, hepatitis B surface antibody; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HIV Ag/Ab, HIV antigen/antibody combination test; IDU, injecting drug use; IRB, increased viral risk behaviour; IVRD, increased viral risk donor; MSM, men who have sex with men; NAT, nucleic acid test; PWID, person who injects drugs; TSANZ, transplantation society of Australia and New Zealand; US, United States; UK, United Kingdom.



INTRODUCTION

The potential for donor derived infections of human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) is an important consideration in the medical suitability assessment of any organ donor. In addition to routine pathology screening, the structured exploration of donor increase-viral-risk behaviours (IRBs) is a routine component in the assessment of risk for transmission of blood-borne viruses (BBVs) (1).

Conducting a structured behavioural interview is a significant undertaking. The potential donor has often died in a sudden and unexpected manner and acutely bereaved family members are requested to engage in an extensive screening interview of their relative's medical history and behaviours. The Australian interview contains over 40 questions and covers a variety of sensitive subjects including the deceased's sexual health, illicit drug use, forensic and psychiatric histories.

The identification of what constitutes an increased-viral risk behaviour (IRB) has historically been derived from a combination of discerning biologically plausible mechanisms for transmission, self-reported behaviours in ecological studies and expert opinion (2).

Potential donors who have no evidence of BBV exposure on blood testing but are thought to have engaged in recent IRBs are designated as increased-viral-risk donors (IVRDs). The underlying premise being engagement in recent IRBs is thought to produce a clinically meaningful elevation in the risk of window period infection when compared to standard risk organ donors.

Designation as an IVRD may have significant implications. International experience shows IVRD designation is associated

with lower utilisation of organs (3, 4), resulting in less patients being transplanted. This is despite evidence that the objective risk of transmission is extremely low (5–7), and that IVRD organs come from donors who are on average younger, and have less comorbidities (8, 9). Recipients who accept an IVRD organ offer, have fewer post-transplant complications, and in some series, improved long-term survival (10–13).

A recent study, from New South Wales, Australia, highlighted that a significant portion of potential donors did not proceed to donation, based solely on the presence of increased risk behaviours (14). In some instances, the decision not to progress with donation workup occurred prior to pathology screening.

The prevalence of BBVs and IRBs in a national cohort of Australian potential organ donors has not been previously described. The external validity of IRBs derived from US populations has also not been tested in an Australian context (15).

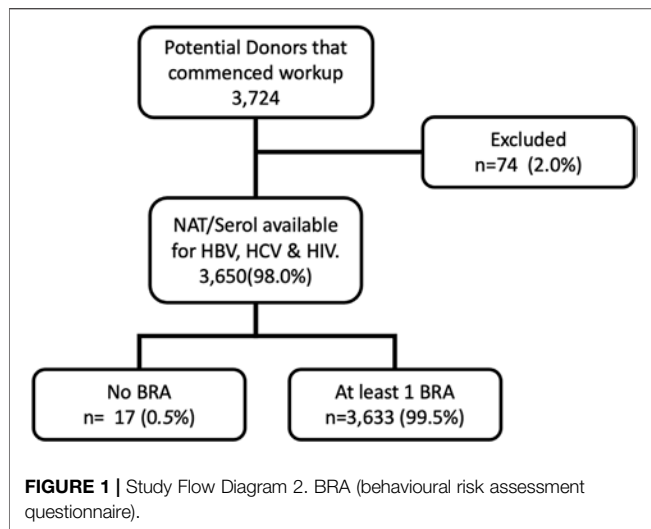
Given the effort required to elicit a history of behavioural risks, and the sequelae of a designation of increased risk, it is important to confirm that currently utilised questions successfully risk stratify potential organ donors.

This study aims to determine the prevalence of BBVs within potential organ donors in Australia and determine the utility of currently used behavioural questions to differentiate risk within this cohort.

PATIENTS AND METHODS

Design

A retrospective audit of the national electronic donor record (EDR) database was undertaken to identify all potential organ



donors referred over a 6-year period between March 2014 and March 2020. The database is hosted by the Australian Organ and Tissue Authority (AOTA).

The project was approved by the Melbourne Health human research ethics committee (QA2019030), the AOTA Data Governance Committee, and undertaken with the approval of each of the eight state and territory jurisdictions. The study was conducted in accordance with the ethical standards laid down in the Declarations of Helsinki and Istanbul.

Setting

Australia is a multicultural nation with 30% of the population having been born overseas and 46% of Australians having at least one parent born overseas (16, 17). The prevalence of HBV is 0.9% (18), with most cases occurring in migrants from higher prevalence countries. The prevalence of HIV and anti-HCV are 0.1% (18) and 2.3% respectively (19).

The AOTA coordinates the DonateLife network, which includes the organ procurement entity in each state and territory, and a network of over 90 donation hospitals. In 2019, Australia had an estimated population of 25.6 million and a donation rate of 21.6 deceased organ donors per million population (20).

In partnership with AOTA, the Transplantation Society of Australia and New Zealand (TSANZ) author donor evaluation policy and issue national guidelines on the requirements for testing for BBV in potential organ donors (1). It is then up to individual transplant clinicians and patients to determine the risk benefit of an individual organ offer.

Testing for Blood-Borne Viruses

There has been an evolution in mandatory and recommended testing for BBV since 2014. From 2014 mandatory tests were HIV antibody, Hepatitis B surface antibody (HBsAb), Hepatitis B surface antigen (HBsAg), Hepatitis B core antibody (HBcAb) and Hepatitis C antibody (HCV Ab). Nucleic acid testing (NAT) for HCV and HIV was recommended for IVRDs. From April 2016, Hepatitis B NAT was also recommended for IVRDs (1). From May 2019 the testing requirements specified that the HIV

serology testing be a combination antigen/antibody assay and also stated that prospective NAT for HIV, HBV and HCV was required wherever this was logistically feasible and was strongly advised for IVRDs. However, if serological screening results were negative, and awaiting NAT results would represent an unreasonable delay, transplantation could proceed at the discretion of the transplant team and with appropriate recipient consent (1). The majority of national deceased organ donor serology and NAT testing is undertaken by Australian Red Cross Lifeblood in dedicated state-based processing centres.

Administration of the Behavioural Risk Assessment Questionnaire

As part of the workup for donation, specialist donor coordinator nursing staff conduct interviews with family members and close associates of the potential organ donor. A behavioural risk assessment questionnaire (BRAQ) is utilised, with occasionally more than one administered if separate interviews are required according to family circumstances. The BRAQ includes more than 40 questions, and records respondent's answers both dichotomously (yes/no), and with free text fields. Answers are recorded in the EDR.

Study Population and Sampling

The target population for this study were patients who commenced workup for organ donation in Australia.

We included all patients who had an EDR commenced and excluded those who did not progress to BBV testing for all three viruses (Figure 1). EDR commencement occurred when provisional family consent was obtained and prior to the administration of the BRAQ or testing for BBVs. Patients were excluded if they did not progress to BBV testing or did not have a BRAQ administered.

Data Collection and Classification of Cases

Basic demographic data, results of the BRAQ, and pathology results for HIV, HBV and HCV were extracted for analysis.

Blood-Borne Virus Exposure Status

Blood specimens were initially classified by their haemodilution status. Specialist donor coordinator nurses audited the administration of intravenous therapy and blood product transfusions received in the 48 h prior to blood sampling for BBV testing. Pathology specimens were classified as potentially haemodiluted if the volumes of crystalloids, colloids and blood products, as a percentage of total plasma and blood volumes, exceeded a prescribed threshold (Supplementary Figure S1).

For the purpose of this study, a case was classified as having an unknown viral status, when there was either:

1) No serology or NAT undertaken for the virus.

OR

2) All tests were undertaken on haemodiluted samples AND all sample results were negative for the virus.

A case was classified as “exposed” to a virus, when serology or NAT indicated either current or past infection, with one the following tests being positive: HIV serology, HIV NAT, HBcAb, HBsAg, HBV NAT, HCV Ab, or HCV NAT. “Active” infection was defined by one of the following tests being positive: HIV serology, HIV NAT, HBsAg, HBV NAT, or HCV NAT.

Inactive HBV infection may result in reactivation and clinically significant disease in liver transplant recipients (1). Inactive HBV was defined as any evidence of prior HBV exposure (HBcAb) but no evidence of active replication (HBsAg -ve, HBV NAT -ve).

Inactive HCV infection may result from spontaneous clearance or successful treatment (1). Inactive HCV was defined as evidence of previous HCV exposure (anti-HCV positive), with no detectable HCV RNA on NAT.

This classification held, even if the specimen was flagged as haemodiluted. In cases where the test was repeated and found to subsequently be negative, the case was still classified as exposed (**Supplementary Table S4**).

As such we have adopted a conservative case definition where an exposed case may indicate current infection, past infection or a false positive.

“Any exposure to BBV” was defined as a positive test result for exposure to any BBV, and “Any active BBV” was defined as positive test result for active BBV infection.

Hepatitis B immunity was defined as being HBsAb positive, with negative HBcAb, HBsAg and HBV NAT.

A case was classified negative for a virus when a valid, non-haemodiluted sample was analysed, and all NAT and acute and chronic serological markers were negative.

Presence of Increased-Viral-Risk Behaviours

Within Australia, patients must fulfill at least one of 11 criteria to be designated an IVRD. These criteria consist of eight IRBs, and an additional three clinical scenarios that may confer increased risk which are not included in our analysis:

- 1) Where the potential donor is already known to have a BBV
- 2) Where the medical and behavioural history cannot be obtained
- 3) When a non-haemodiluted blood specimen cannot be obtained

Eight IRBs were screened for during the administration of the BRAQ. They are:

- 1) Person who injects drugs (PWID) by intravenous, intramuscular, or subcutaneous route for non-medical reasons
- 2) Men who have sex with men (MSM)
- 3) People who have been in lockup, jail, prison, or a juvenile correctional facility for more than 72 consecutive hours
- 4) People who have had sex in exchange for money or drugs
- 5) People who have had sex with a person in any of the above groups

TABLE 1 | Characteristics of Potential Organ Donors who have undertaken testing for blood borne viruses.

Demographics (n = 3,650)			
Age	51	IQR	(36–62)
Gender	n	%	
Male	2,141	58.7	
Female	1,509	41.3	
Donation Outcome	n	%	
Proceeded to donation	2,847	78.0	
Did not proceed to donation	803	22.0	
Virus Exposure	n	%	% CI
HIV	6	0.2	(0.1–0.4)
HBV			
Active	33	0.9	(0.6–1.2)
Inactive	181	5.0	(4.4–5.8)
Active and Inactive	214 ^a	5.9	(5.1–6.7)
Vaccine Immunity ^b	1,061	30.9	(29.3–32.4)
HCV			
Active	73	2.2	(1.7–2.8)
Inactive	92	2.9	(2.3–3.5)
Active and Inactive	179 ^a	4.9	(4.2–5.7)
At least 1			
Active Infection ^c	106	3.24	(2.6–3.9)
Any Exposure	344	9.4	(8.5–10.4)
Increased Risk Behaviours (n = 3,663)	n	%	
1. PWID	187	5.2	(4.5–5.9)
2. MSM	45	1.2	(0.9–1.7)
3. Detention	340	9.4	(8.4–10.4)
4. Sex Worker	23	0.6	(0.4–0.9)
5. Increased Risk Partner	1289	35.5	(33.9–37.1)
6. STI	81	2.2	(1.8–2.8)
7. Child (IRM)	0	0.0	(0.0–0.1)
8. Breastfed (IRM)	1	0.0	(0.0–0.2)
At least 1 IRB identified	1,365	37.6	(36.0–39.2)

HIV = human immunodeficiency virus; HBV Hepatitis B Virus, HCV Hepatitis C Virus.

PWID, person who injects drugs; MSM, men who have sex with men, Detention = Admission to a lock up, prison or psychiatric facility, STI, sexually transmitted infection; IRM, Increased risk mother; IRB, increased risk behaviour.

^aIncludes serologically positive patients who did not have NAT testing. Not the sum of active and inactive cases.

^bHbSAb, in absence of HBcAb or other markers. Available in 3,438 cases.

^cIncludes all HIV exposed patients, NAT+ve for HCV or HBV patients, and those with HBsAg.

- 6) People who have been newly diagnosed with, or have been treated for, syphilis, gonorrhoea, chlamydia, or genital ulcers
- 7) A child who is 18 months old or younger and born to a mother known to be infected with, or at increased risk for, HIV, HBV or HCV infection
- 8) A child who has been breastfed within the preceding 6 months, and the mother is known to be infected with, or at increased risk for, HIV, HBV or HCV infection

At the commencement of the study period, these IRBs were recorded in the EDR if they occurred within the last 12 months, or in the case of injecting drug use, had ever occurred. In line with changes by the TSANZ, after April 2016, the database recorded these behaviours only if they occurred within the last 10 weeks. A composite variable “Any predictors” was utilised as the presence of at least one IRB designating an IVRD (**Supplementary Tables S5–S7**).

TABLE 2 | Comparison of patient with and without increased risk behaviours ($n = 3,663$).

		IRB $n = 1,365$ (37.6%)	No IRB $n = 2,268$ (62.4%)	p
Age	median (IQR)	43 (31–53)	56 (43–65)	<0.001
Gender: Male	n (%)	927 (67.9)	1,199 (52.9)	<0.001
BBV exposure	n (%)	204 (15.0)	136 (6.0)	<0.001
Proceeded to Organ Donation	n (%)	1,040 (76.2)	1,805 (79.6)	0.016

IRB, Increased risk behaviour; BBV, Blood-borne virus; BRAQ, behavioural risk assessment questionnaire.

Analysis

A priori 2-sided sample size calculations were undertaken (alpha 0.05, power 0.80). Modelling used the prevalence of BBVs in Australian deceased tissue donors (21) and examined a range of potential risk-factor prevalence (1–30%) (**Supplementary Table S1**).

Results demonstrate that for an expected case series of 3,288, the sample size would be suitable to reveal clinically significant increases (OR 10) in prevalence for HBV (for risk factors frequencies >1%) and HCV (risk factor frequencies $\geq 5\%$), but were likely to be underpowered to find associations with HIV for all but the most prevalent risk factors (**Supplementary Tables S2, S3**).

The composite IRB variable (“Any predictors”) was analysed for its prediction of “Any BBV exposure” through calculation of sensitivity, specificity, positive predictive value, negative predictive value and odds ratio.

Statistical analysis was performed using STATA/IC 15.1 (StataCorp LLC).

The prevalence of each disease and risk factor was determined. Statistical comparison of proportions was undertaken using Chi-square analysis with Fisher’s exact method (alpha 0.05). Difference between non-parametric variables were analysed using Wilcoxon rank-sum. Confidence intervals for proportions were calculated using the binomial exact method.

RESULTS

Between March 2014 and March 2020, 3,724 individuals were referred to DonateLife and had an EDR commenced. Seventy-four individuals had workup for donation terminated prior to testing for all three viruses. Typical reasons for discontinuation include medical instability, identification of a contraindication to donation, and withdrawal of consent to proceed (**Figure 1**).

In total, 3,650 patients underwent pathology testing for BBV exposure. A combination of both NAT and serology screening was undertaken in the vast majority of cases (89.5%). Serology testing without NAT occurred in 10.5% of patients for HIV, 9.8% of patients for HBV, and 9.7% of patients for HCV. Over the study period, the fraction of potential donors that underwent combined NAT and serology testing increased from 86% to 95%.

The average potential donor age was 51 years and they were more commonly male (59%), and were referred from all states and territories.

Approximately three in every four patients in this study proceeded to organ donation (**Table 1**). Of patients who did not proceed, IRBs or BBV exposure were more prevalent (IRB: Proceed 36.5% vs Did not proceed 40.4%, $p = 0.041$, BBV: Proceed 3.6% vs Did not proceed: 13.8%, $p < 0.001$).

The majority of patients who failed to proceed to donation were being considered for donation via the donation-after-circulatory-death pathway (74%). Death not occurring within the time period required for successful donation and transplantation has previously been shown to be a common reason for failure of donation to proceed in these patients (22).

In the study cohort of 3,650 patients who had undergone pathology testing 99.5% of potential donors who had BBV testing had at least one BRAQ administered (see diagram 1). In some cases, more than one questionnaire was administered.

Blood-Borne Virus Exposure Prevalence

Nearly ten percent of potential donors who commenced workup for organ donation had evidence of exposure to a BBV. Exposure to HBV was the most prevalent (5.85%), followed by HCV (4.98%), then HIV (0.16%).

In total, 106 (3.24%) potential donors had active infection with a BBV.

A sizable proportion of patients with a BBV exposure were co-infected. Of the 214 patients with either active or inactive HBV, 50 (23%) had HCV co-exposure. Two patients had exposure to all three viruses.

The majority of HBV infections were inactive with active infection occurring in less than 1% of potential donors. There was serological evidence of previous vaccination in 30% of potential donors.

Prevalence of IVRBs

During the study period 4,009 BRAQs were administered to the families and associates of 3,633 potential organ donors.

Over one third of potential donors who commenced workup for organ donation had a history of engaging in one or more IRBs (**Table 1**).

The most commonly identified IRBs were having a sexual relationship with an IRB partner (35%), followed by a history of being in detention in a lockup, jail, prison, or a juvenile correctional facility (9%) (**Table 1**).

Potential donors with IRBs were a median of 13 years younger than those without IRBs, were more likely to be male (68%), and more likely to have evidence of BBV exposure and less likely to proceed to donation (**Table 2**).

TABLE 3 | Frequency of blood-borne viruses in potential donors with increased risk behaviours.

Increased risk behaviour	HIV			Active HBV			Inactive HBV			Active HCV			Inactive HCV		
	Cases in patients with IRB	Cases in patients without IRB	p	Cases in patients with IRB	Cases in patients without IRB	p	Cases in patients with IRB	Cases in patients without IRB	p	Cases in patients with IRB	Cases in patients without IRB	p	Cases in patients with IRB	Cases in patients without IRB	p
	n/total (%)	n/total (%)		n/total (%)	n/total (%)		n/total (%)	n/total (%)		n/total (%)	n/total (%)		n/total (%)	n/total (%)	
PWID	1/187 (0.53)	5/3446 (0.15)	0.272	9/187 (4.81)	23/3446 (0.67)	<0.001	40/188 (21.28)	141/3469 (4.06)	<0.001	37/166 (22.29)	35/3127 (1.12)	<0.001	67/129 (51.94)	25/3070 (0.81)	<0.001
MSM	1/45 (2.22)	5/3588 (0.14)	0.072	0/45 (0.00)	32/3588 (0.89)	1.000	5/45 (11.11)	176/3612 (4.87)	0.069	1/43 (2.33)	71/3250 (2.18)	0.616	5/41 (12.2)	87/3158 (2.75)	0.006
Detention	0/340 (0.00)	6/3293 (0.18)	1.000	3/340 (0.88)	29/3293 (0.88)	1.000	18/341 (5.28)	163/3316 (4.92)	0.793	23/316 (7.28)	49/2977 (1.65)	<0.001	40/292 (13.7)	52/2907 (1.79)	<0.001
Sex Worker	0/23 (0.00)	6/3610 (0.17)	1.000	0/23 (0.00)	32/3610 (0.89)	1.000	6/23 (26.09)	175/3634 (4.82)	<0.001	1/22 (4.55)	71/3271 (2.17)	0.386	4/22 (18.18)	88/3177 (2.77)	0.003
Increased Risk Partner	1/1289 (0.08)	5/2344 (0.21)	0.432	11/1289 (0.85)	21/2344 (0.90)	1.000	64/1291 (4.96)	117/2366 (4.95)	1.000	57/1165 (4.89)	15/2128 (0.7)	<0.001	81/1101 (7.36)	11/2098 (0.52)	<0.001
STI	0/81 (0.00)	6/3552 (0.17)	1.000	0/81 (0.00)	32/3552 (0.90)	1.000	3/81 (3.70)	178/3576 (4.98)	0.797	3/68 (4.41)	69/3225 (2.14)	0.185	0/64 (0)	92/3135 (2.93)	0.262
Breastfed (IRM)	0/1 (0.00)	6/3632 (0.17)	1.000	0/1 (0.00)	32/3632 (0.88)	1.000	0/1 (0.00)	181/3656 (4.95)	1.000	0/1 (0)	72/3292 (2.19)	1.000	0/1 (0)	92/3198 (2.88)	1.000
Any IRB	1/1366 (0.07)	5/2284 (0.22)	0.421	16/1351 (1.17)	17/2273 (0.74)	0.207	74/1367 (5.41)	107/2290 (4.67)	0.344	59/1228 (4.8)	13/2065 (0.63)	<0.001	86/1162 (7.4)	6/2037 (0.29)	<0.001

HIV, Human immunodeficiency Virus; HBV, Hepatitis B Virus; HCV, Hepatitis C Virus; PWID, Person who injects drugs; MSM, Men who have sex with men; Detention, Admission to a lock up, prison or psychiatric facility; STI, sexually transmitted infection; IRM, Higher risk mother; IRB, Increased risk behaviour.

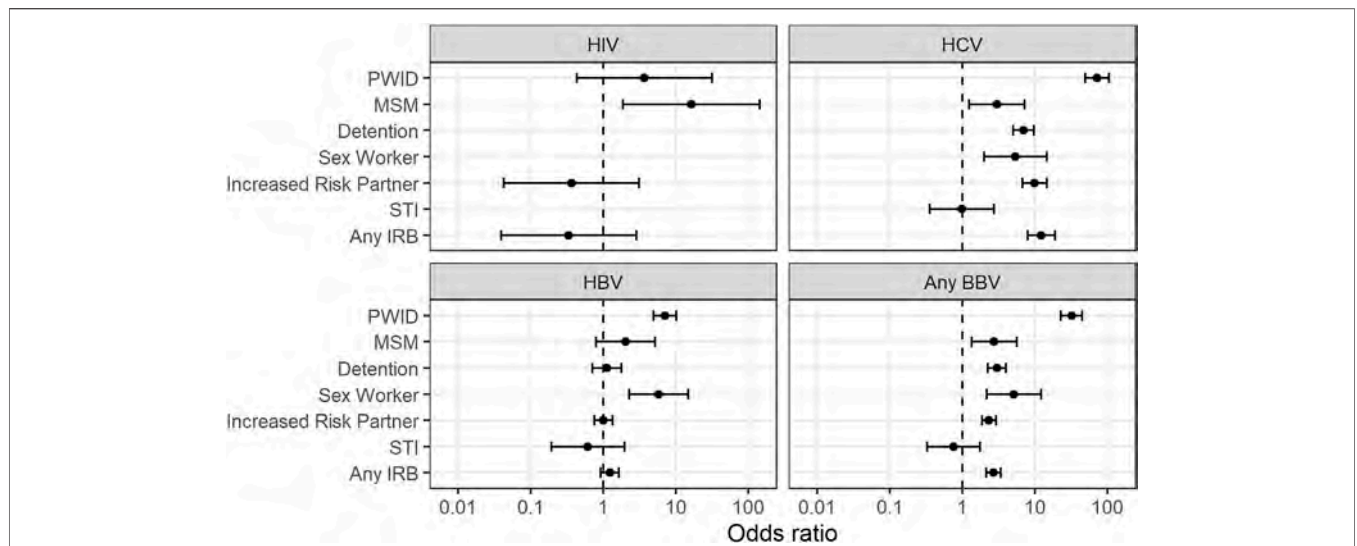


FIGURE 2 | Association between increased risk behaviours and bloodborne virus exposure 7. PWID = Person who injects drugs. MSM = Men who have sex with men. STI = Sexually transmitted infection. IRB = Increased risk behaviour. BBV = blood-borne virus (HIV, HCV or HBV).

TABLE 4 | Predictive performance of increased risk behaviours.

Virus	Sensitivity (%)	Specificity (%)	PPV (%)	OR	(95% CI)	p	
HIV	17	62	0	0.33	0.00	2.15	0.420
HBV	47	63	1	1.47	0.74	2.92	0.143
HCV	88	65	11	13.80	8.74	21.8	<0.001
Any Active BBV	71	64	6	4.21	2.76	6.42	<0.001
Any BBV	60	65	15	2.75	2.19	3.46	<0.001

PPV, Positive predictive value; OR, Odds ratio. HIV- Human immunodeficiency Virus. HCV, Hepatitis C Virus. HBV, Hepatitis B Virus. BBV, bloodborne virus.

Increased-Viral Risk Behaviours Associated With Blood-Borne Virus Exposure

Only six potential donors with HIV were referred during the study period (Table 1). None of the IRBs were associated with a significantly increased prevalence of HIV over the study period.

While inactive HBV was shown to have a higher prevalence in both PWID and persons who engaged in sex work, only injecting drug use was associated with a higher prevalence of active HBV (PWID 4.81% vs non-PWID 0.67%, OR 7.56, $p < 0.001$).

Several IRBs were associated with exposure to HCV (Table 3). These included being a PWID, being in detention, sex work, being a MSM or having a sexual partner of any of the preceding groups.

A history of a sexually transmitted infection such as syphilis, gonorrhoea or herpes was not associated with an increased prevalence of HIV, HBV or HCV (Figure 2, Supplementary Table S8).

“Any IRB” was associated with an increased prevalence of HCV (OR 12.7) but not HIV or HBV in potential organ donors (Table 4).

In this study, “Any IRB” had only modest sensitivity and positive predictive power. One in every five potential donors with a BBV did not have any IRB identified by the BRAQ. Furthermore only 1 in every 8 patients identified as being IVRD had evidence of exposure to a BBV.

DISCUSSION

This is the first study to describe the prevalence of BBVs and IRBs amongst a national cohort of persons who have commenced workup for deceased organ donation within Australia.

The study has shown that potential organ donors in Australia have a higher prevalence of HCV, but similar rates of HIV and HBV when compared to the general population (18, 19).

Whilst a reported history of any IRB was common and associated with exposure to HCV, it was not associated with exposure to HBV or HIV.

Blood-Borne Virus Exposure Prevalence

The significantly higher prevalence of HCV exposure seen in this study, when compared with the broader Australian population, is likely to derive from the over-representation

of risk behaviour within the organ donor pool (4.9% vs 2.3%, $p < 0.001$).

Our study demonstrated that 5.2% of potential donors had a history of IDU, in contrast to 1.5% in the broader Australian population (23). IDU is the primary risk factor for HCV infection. The prevalence of HCV in PWID in Australia has been estimated to be 49% (18).

Our findings are consistent with this pattern of illness burden, with 62% of HCV exposed potential organ donors having a history of IDU and a 59% prevalence of HCV exposure in PWID.

Compared with international potential organ donor populations, Australia has similar rates of HCV when compared to US and Canada (4.98% vs 5.14% & 10.34%) and generally similar rates of HIV (0.16%) compared to with United States, Canada and the United Kingdom (0.21%, 0.00% and 0.06%) (24–26).

In contrast, our study showed higher rates of HBV when compared to potential organ donor cohorts in other nations (24–26). This is likely due to the higher overall prevalence of HBV within Australia, when compared to the UK and the US (27) (HBcAb Prevalence: 6.9%, vs 3.8% and 5.4%) (27, 28). Our reported prevalence of both active and inactive HBV in potential organ donors were similar to those published in a previous Australian national serosurvey (active HBV 0.9% and inactive HBV 4.95% in our study, versus HbsAg 0.8% and HBcAb 6.9% overall in Australia) (28).

Ninety-four percent of organ donors in Australia are adults, and newly acquired HBV infection in adulthood is uncommon. In Australia the majority of HBV infections are acquired during childhood, and occur most commonly in migrants from nations with higher endemicity (18). The attributable burden of disease associated with IRBs is thought to be only modest (PWID making up 5.7% and MSM making up 4.5% of those with HBV in Australia) (18). It is therefore unsurprising that the majority of in-active HBV infections in our study, occurred in individuals with no history of IRBs, and the ability of IRBs to predict acute HBV was poor.

IRB Prevalence

Overall, IRBs appeared more common than previous estimates (3). However, direct comparisons with other studies are difficult due to variations in recency of exposure criteria required in differing international jurisdictions.

Our study reported that potential donors with IRBs were significantly younger than those without, and this finding is consistent with other studies of IVRDs and is an important fact as transplanted organs from younger donors have superior outcomes (8).

Our study showed an association between the presence of IRBs and a lower likelihood of progression to donation surgery. Future studies should better define the relationship between IVRD designation and organ utilisation in Australia.

Virus Prevalence in IRB Cohorts

Overall, the prevalence of BBV exposures were similar to those reported in community-based cohorts who seemingly engage in the same IRBs (**Supplementary Tables S9–S11**). We differ in

reporting lower rates of HCV in those with increased risk sexual partners (OR 0.47, $p = 0.01$), and those with a history of detention (OR 0.39, $p < 0.001$), and lower rates of active HBV in those with a history of detention (OR 0.3, $p = 0.03$) (see **Supplementary Material** for full analysis).

We report a strikingly high incidence of inactive HBV in potential donors who have had sex in exchange for money or drugs, when compared with HBcAB prevalence in community cohorts of predominantly commercial sex workers (7) (OR 16, $p < 0.001$). However in our series, sex work was not associated with active HBV. It may be that potential organ donors with these reported IRBs may represent a more culturally diverse cohort, a cohort with a higher number of migrant workers (29), or a higher proportion of sex-workers from the unregulated sector-any of which may be less represented in community cohort studies.

Exploration of the exact reasons for these differences in prevalence is beyond the scope of this study, but the finding provides a cautionary note when inferring risks of disease transmission from community-based cohorts.

Our study did not demonstrate an association between sexually transmitted infections and an increased prevalence of any of the BBVs. It is noteworthy that U.S. Department of Health and Human Services has recently removed this risk behaviour from their assessment of BBV transmission risk in organ donors (30). Our study would support a similar removal of this risk criteria within the Australian context.

Implications

In this study, the presence of one or more IRBs was predictive of a higher prevalence of HCV, but not of HBV or HIV in potential organ donors. In an attempt to improve sensitivity, authorities have recently introduced a new, locally modified, IRB questionnaire, which has undergone cognitive evaluation overseas (31).

For HCV, where IRB screening is predictive, the clinical ramifications of unexpected donor-derived HCV infection are rapidly diminishing. Direct acting antivirals are well tolerated and successfully cure HCV in solid organ transplant recipients (32–35). The majority of patients with HBV or HIV did not have elicited IRBs.

This leads one to question the value of existing IRB screening. Routine donor NAT screening has shortened the diagnostic window considerably, and none of the IRBs sufficiently predict window period infection because it is uncommon even for the highest risk IRB [Death with a history of IVDU: Risk of undetected infection estimated as ~1:50,000 for HIV, ~1:2000 for HbsAg, ~1:450 for HCV (7)].

If there is jurisdictional agreement to routinely use IVRD donors with negative NAT BBV tests, the more logical approach seems to be undertaking NAT in all recipients so that in the uncommon event of donor derived BBV infection it is detected and able to be treated before there are clinical ramifications. This approach has recently been adopted in the United States (30). The acceptability of such an approach within the broader Australian transplant community remains unknown.

Our study shows the prevalence of BBVs for some IVRD cohorts may be significantly different from previous estimations

(7). It may not be appropriate to extrapolate prevalence rates from other cohorts to Australian donors, and inferences of residual risk may be affected. Further studies are required.

Limitations

Given the rarity of window-period unexpected disease transmission from IVRDs and the overall small number of donors within Australia, it was not practical to design an appropriately powered study to assess the predictive power of IRBs on actual unexpected transmissions. Instead, a surrogate measure, the ability to predict established infection was used. It is possible, although though we feel unlikely, that IRBs may better predict very recent infection over established infection.

We adopted a conservative definition of exposure, and this may lead to overestimation of the prevalence of BBV in potential organ donors.

Several IRBs were rarely reported, and HIV had low prevalence. The study was, therefore, underpowered to reveal a significantly increased viral prevalence in sex-workers or in children of IRB mothers, and was underpowered to identify individual IRBs associated with HIV infection. Despite this, the study did demonstrate statistically significant correlation between sex work and exposure to HBV, and was adequately powered to detect higher prevalence of HIV in the composite IVRD cohort (OR 10 threshold).

Our study examined IRBs during a finite period of time preceding commencement of workup for organ donation rather than a history of having ever undertaken IRBs, and this may have affected the concordance with population studies. Additionally, in 2019 the TSANZ revised IRB exposure windows from “the last 12 months” to “the last 10 weeks”. This will in effect reduce the fraction of potential donors classified at IVRD (1). It is therefore likely that our study would have a higher rate of IVRD designation compared to a future case series.

Whilst not consistent with national guidelines, some potential organ donors are rejected prior to commencement of formal donation workup, either through self-censoring by the referring clinician, or based on cursory assessment by a donation service. The later having previously been documented within the local context (14). This may reduce the assessed predictive power or IRBs. Conversely, our findings of viral prevalence in potential donors are more likely to be more indicative, when compared to series where individual who do not proceed to donation are excluded (36).

Caution should be applied when extrapolating our findings to other jurisdictions or differing populations. Our study examined non-self-reported behaviours, in a potential organ donor cohort in Australia. These behaviours may have differing predictive performance when self-reported (e.g., blood donors) or in settings with higher community prevalence, or in countries where the BBV burden is distributed differently according to specific IRBs.

Conclusion

This study demonstrated that Australian potential organ donors had significantly higher rates of HCV and similar rates of HBV and HIV when compared to the broader population. Currently utilised risk behaviour assessment questionnaires were only moderately predictive of exposure or active infection with a blood borne virus. The utility of behavioural questionnaires in

stratifying the risk of unexpected disease transmission may not provide the reassurance clinicians are seeking. Eliciting IRBs may be a redundant practice if organs from NAT negative IVRDs are routinely utilised and early BBV screening is performed in all recipients.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: Individual Australian States and Territories retain governance over data in the DonateLife Electronic Donor Record (EDR). Requests to access these datasets should be directed to Australian Organ and Tissue Authority, enquiries@donatelife.gov.au.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Melbourne Health human research ethics committee (QA2019030). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

MD: conception, design, acquisition, interpretation, analysis, and writing; CP: analysis and writing; PB: interpretation and writing; JK: conception, design, and writing; HO: acquisition, interpretation, and writing.

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.2022.10395/full#supplementary-material>

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Radiological Screening Methods in Deceased Organ Donation: An Overview of Guidelines Worldwide

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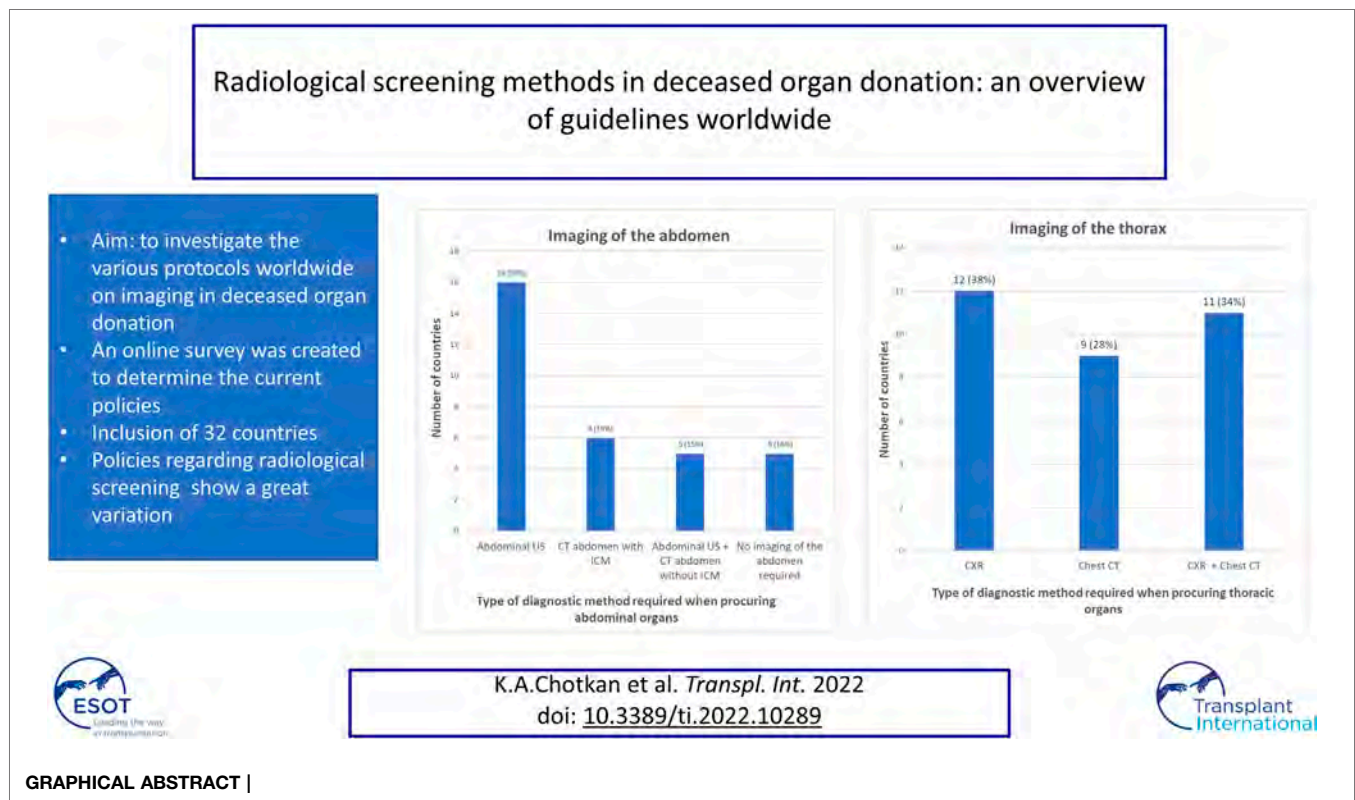
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Organ transplantation is performed worldwide, but policies regarding donor imaging are not uniform. An overview of the policies in different regions is missing. This study aims to investigate the various protocols worldwide on imaging in deceased organ donation. An online survey was created to determine the current policies. Competent authorities were approached to fill out the survey based on their current protocols. In total 32 of the 48 countries approached filled out the questionnaire (response rate 67%). In 16% of the countries no abdominal imaging is required prior to procurement. In 50%, abdominal ultrasound (US) is performed to screen the abdomen and in 19% an enhanced abdominal Computed Tomography (CT). In 15% of the countries both an unenhanced abdominal CT scan and abdominal US are performed. In 38% of the countries a chest radiographic (CXR) is performed to screen the thorax, in 28% only a chest CT, and in 34% both are performed. Policies regarding radiologic screening in deceased organ donors show a great variation between different countries. Consensus on which imaging method should be applied is missing. A uniform approach will contribute to quality and safety, justifying (inter)national exchange of organs.

Keywords: screening, transplantation, ethics, organ donation, organ procurement, imaging, guidelines, transplant ethics



INTRODUCTION

Organ transplantation is a lifesaving treatment for patients with end-stage organ failure but is not without risk for the recipient. The comprehensiveness and quality of donor assessment contribute to adequate risk management, applicable to individual and vulnerable recipients. Optimal donor assessment provides important information on organ quality and anatomy. Donor assessment includes interviews with relatives, assessment of the medical and social behavior history, full physical examination, laboratory tests, and complementary tests (in particular imaging) (1). In Netherlands (part of the Eurotransplant region), radiological screening in deceased organ donors consists of at least a chest radiography (CXR) and abdominal ultrasound (US). Various studies in the past have advocated for the inclusion of the use of chest and abdominal Computed Tomography (CT) scans to optimally prepare a donor and identify risk factors (2–4). Possible advantages of the use of CT scans are more accurate screening for malignancies and other significant diseases, mapping of aberrant (vascular) anatomy, enhanced assessment of organ quality, and improved size matching in liver and lung transplantation.

More detailed imaging may also have a downside; incidental findings on chest and (un)enhanced abdominal CT scans have a prevalence ranging from 40% to 75%. Of these, 3%–20% findings require additional investigations (5–8). This could possibly lead to more (invasive) diagnostic procedures with potential risks and could delay the procurement and allocation process. On the other

hand, when being informed pre-operatively of these findings, biopsies can be obtained before procurement.

Also, to perform an enhanced CT scan, intravenous contrast medium (ICM) must be administered, which leads to exposure of donor kidneys to a potential nephrotoxic contrast medium. A recent publication of Magnus et al., containing a retrospective analysis of 709 kidney donors who received ICM, showed no difference in serum creatine levels in the donor, delayed graft function (DGF) or graft loss in the recipients compared to 685 kidney donors who did not receive ICM (9). This group only contained Donation of Brain death (DBD) donors and no Donation after Circulatory Death (DCD) donors. The DGF rate in DCD kidneys is known to be significantly higher compared to DBD kidneys (10). The added effect of ICM may therefore have an even higher (negative) impact on outcome by inducing acute kidney injury (AKI). Finally, transport to the radiology department of a critically ill patient adds additional risks.

Although organ transplantation is performed worldwide, policies regarding donor assessment and imaging are not uniform. An overview of the policies and underlying arguments in different regions of the world could provide valuable information for countries who are thinking about changing their policy. A uniform approach will contribute to quality and safety, justifying (inter)national exchange of organs.

This study therefore aims to provide an overview on the various protocols for radiological screening in deceased organ donation worldwide.

TABLE 1 | Overview of the screenings method used in which country.

Country	Screening of the thorax when only thoracic organs are being procured	Screening of the abdomen when only thoracic organs are being procured	Screening of the thorax when only abdominal organs are being procured	Screening of the abdomen when only abdominal organs are being procured	Number of deceased donors PMP (per million people) in 2019	Guidelines used in the whole country
Australia/ New Zealand	Chest X-ray (for lung donors only if they meet certain criteria a chest CT is performed)	No Imaging performed of the abdomen	Chest X-ray	No Imaging performed of the abdomen	Australia: 20.10 New Zealand 12.40	Yes
Austria	Chest X-ray	Abdominal ultrasound	Chest X-ray	Abdominal ultrasound = minimal mandatory In daily practice abdominal ultrasound and CT	20.30	Unknown
Belarus	Chest CT	Abdominal ultrasound	Chest X-ray	Abdominal ultrasound	26.20	Unknown
Belgium	Chest X-ray and chest CT	Abdominal ultrasound	Chest X-ray and chest CT	Abdominal ultrasound	27.20	Yes
Canada	Chest X-ray	None	Chest X-ray	None (Abdominal imaging is only advised in those with age >50, comorbid conditions, high BMI or clinical history of malignancy)	21.87	Yes (But every transplant region can ask for additional examinations)
Croatia	Chest X-ray	→ very rarely only thoracic organs, but if it happens, abdominal ultrasound	Chest X-ray	Abdominal ultrasound	31.20	Unknown
Czech Republic	Chest X-Ray and Chest CT (→ due to COVID)	Abdominal ultrasound	Chest X-Ray and chest CT	Abdominal ultrasound + CT abdomen without ICM	24.98	Yes
Ecuador	Chest X-ray and chest CT	Abdominal US	Chest X-ray	Abdominal ultrasound + CT abdomen without ICM	7.78	Unknown
Estonia	Chest X-Ray and chest CT	Abdominal ultrasound + CT abdomen without ICM	Chest X-Ray and chest CT	Abdominal ultrasound + CT abdomen without ICM	18.87	Yes
Finland	Chest CT	None	Chest X-ray and CT thorax	CT abdomen with ICM	25.51	Yes (only one transplantation centre in Finland)
France	Chest CT	CT abdomen with ICM	Chest CT	CT abdomen with ICM	33.25	Yes
Germany	Chest X-ray (if CT/MRT is done, it is always covering thorax and abdomen)	Abdominal ultrasound	Chest X-ray (if CT/MRT is done, it is always covering thorax and abdomen)	Abdominal ultrasound (CT/MRT whenever possible, ICM depends on the individual situation)	10.8	Yes
Greece	Chest CT	Abdominal Ultrasound	Chest X-ray	Abdominal ultrasound	5.0	No
Hungary	Chest X-ray and Chest CT	Abdominal ultrasound	Chest X-ray	Abdominal Ultrasound	18.11	Yes
Iran	Chest X-ray and Chest CT	Abdominal ultrasound	Chest X-ray	Abdominal ultrasound	14.34	Yes
Israel	Chest CT	CT abdomen with ICM	Chest CT	CT abdomen with ICM	10.43	Yes
Italy	Chest X-Ray and Chest CT	Abdominal ultrasound	Chest X-ray	Abdominal ultrasound	22.80	Yes

(Continued on following page)

TABLE 1 | (Continued) Overview of the screenings method used in which country.

Country	Screening of the thorax when only thoracic organs are being procured	Screening of the abdomen when only thoracic organs are being procured	Screening of the thorax when only abdominal organs are being procured	Screening of the abdomen when only abdominal organs are being procured	Number of deceased donors PMP (per million people) in 2019	Guidelines used in the whole country
Japan	Chest X-ray	Abdominal Ultrasound	Chest X-ray	Abdominal ultrasound and CT abdomen without ICM	0.98	No
Netherlands	Chest X-ray	Abdominal ultrasound	Chest X-ray	Abdominal ultrasound	14.47	Yes
Norway	Chest X-ray and Chest CT	CT abdomen without ICM	Chest X-ray and chest CT	CT abdomen with ICM	18.18	Yes
Slovenia	Chest X-ray	Abdominal ultrasound	Chest X-ray and chest CT	Abdominal ultrasound	18.26	Yes
South Africa	Chest X-ray	No standard imaging of the abdomen required		No standard imaging of the abdomen required	1.29 (2016)	No
South Korea	Chest X-Ray and Chest CT	Abdominal ultrasound + CT abdomen without ICM	Chest X-Ray and CT thorax	Abdominal ultrasound + CT abdomen without ICM	8.68	Yes
Spain	Chest X-ray + Chest CT	Abdominal ultrasound	Chest X-ray	Abdominal ultrasound	49.61	Yes
Sweden	Chest CT	CT abdomen without ICM	Chest CT	CT abdomen with ICM	18.51	Yes
Switzerland	Chest X-ray + Chest CT (→ criteria defined by the lung expert group)	Abdominal ultrasound (→ when CT thorax is included, a CT abdomen is asked as well)	Chest X-ray	Abdominal ultrasound	19.30	Yes
Thailand	Chest X-ray	None	Chest X-ray	Abdominal Ultrasound (if indicated)	4.51	Yes
United Kingdom	Chest X-ray	No Imaging performed of the abdomen	Chest X-Ray	No Imaging performed of the abdomen	23.01	Yes
United States	Chest X-ray	Abdomen→ none	Chest X-ray	None	36.88	Yes (But every transplant region can ask for additional examinations)

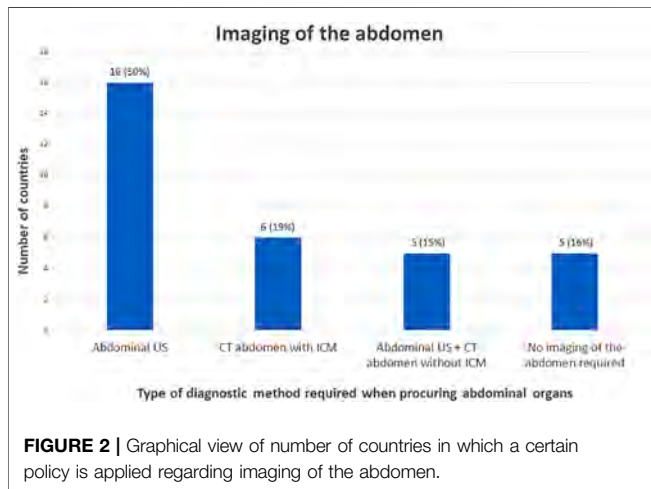
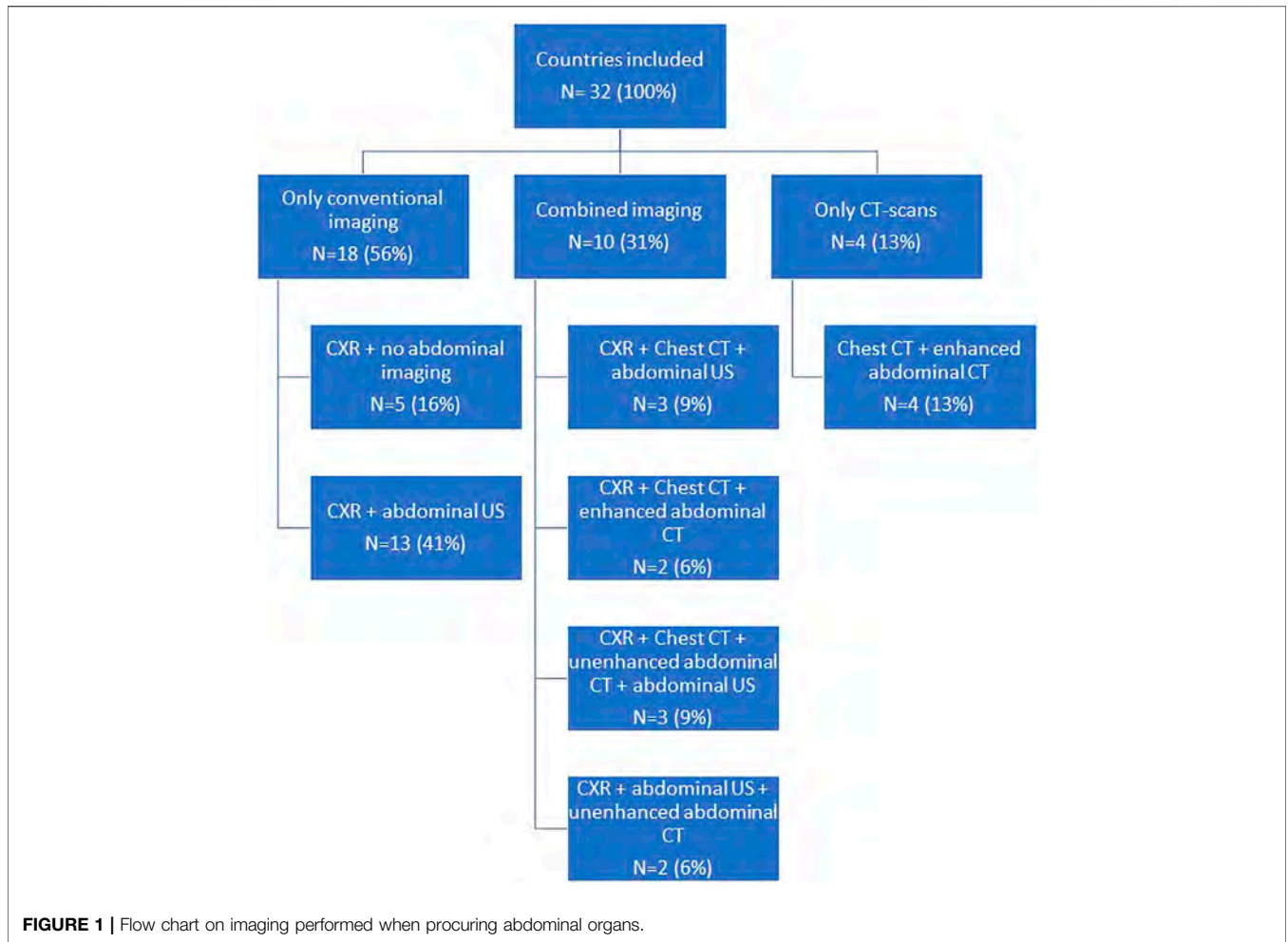
Only the countries who gave permission to name their country were included in this table.

MATERIALS AND METHODS

To investigate whether an overview of the different policies in organ donor screening was available, a literature search of PubMed was performed, using Mesh terms; diagnostic imaging, tissue donors, tissue and organ procurement (**Supplementary Appendix S1**).

Additionally, an online survey was created in Survey Monkey to obtain country specific information (**Supplementary Appendix S2**). For information on countries with an active deceased organ donation program, and the annual number of (deceased) donors, the website International Registry in Organ Donation and Transplantation (IRODaT) was consulted (11). From 71 countries with a deceased organ donation program, transplant authorities were selected if they reported a total of at

least 30 deceased donors per year (donation activity), based on the numbers of 2019, since 2020 is not representative due to the SARS-CoV-2 pandemic. This led to an inclusion of 48 countries. The value of a minimum of 30 deceased donors per year was chosen to include a large diversity of countries, including smaller countries, but to exclude countries which do not have deceased donation on a regular basis (and most likely do not have standardized guidelines for deceased organ donation). Contact information of these selected countries was obtained from Eurotransplant International, the Dutch Transplant Foundation and websites of the competent authorities of organ donation or donation professionals. Between May and July 2021, these contacts were approached by email to fill out the questionnaire.



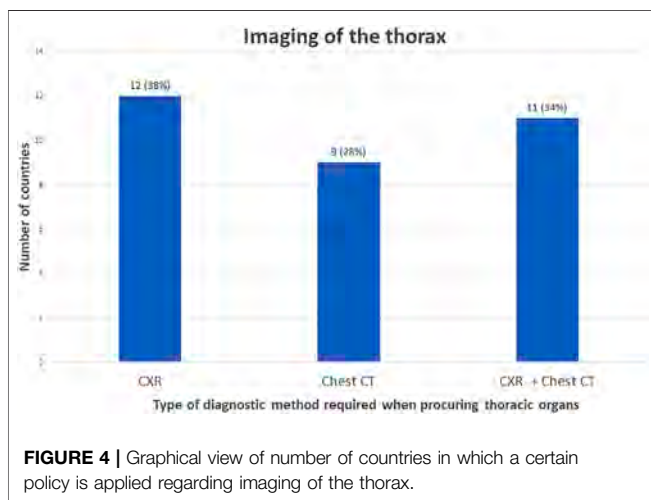
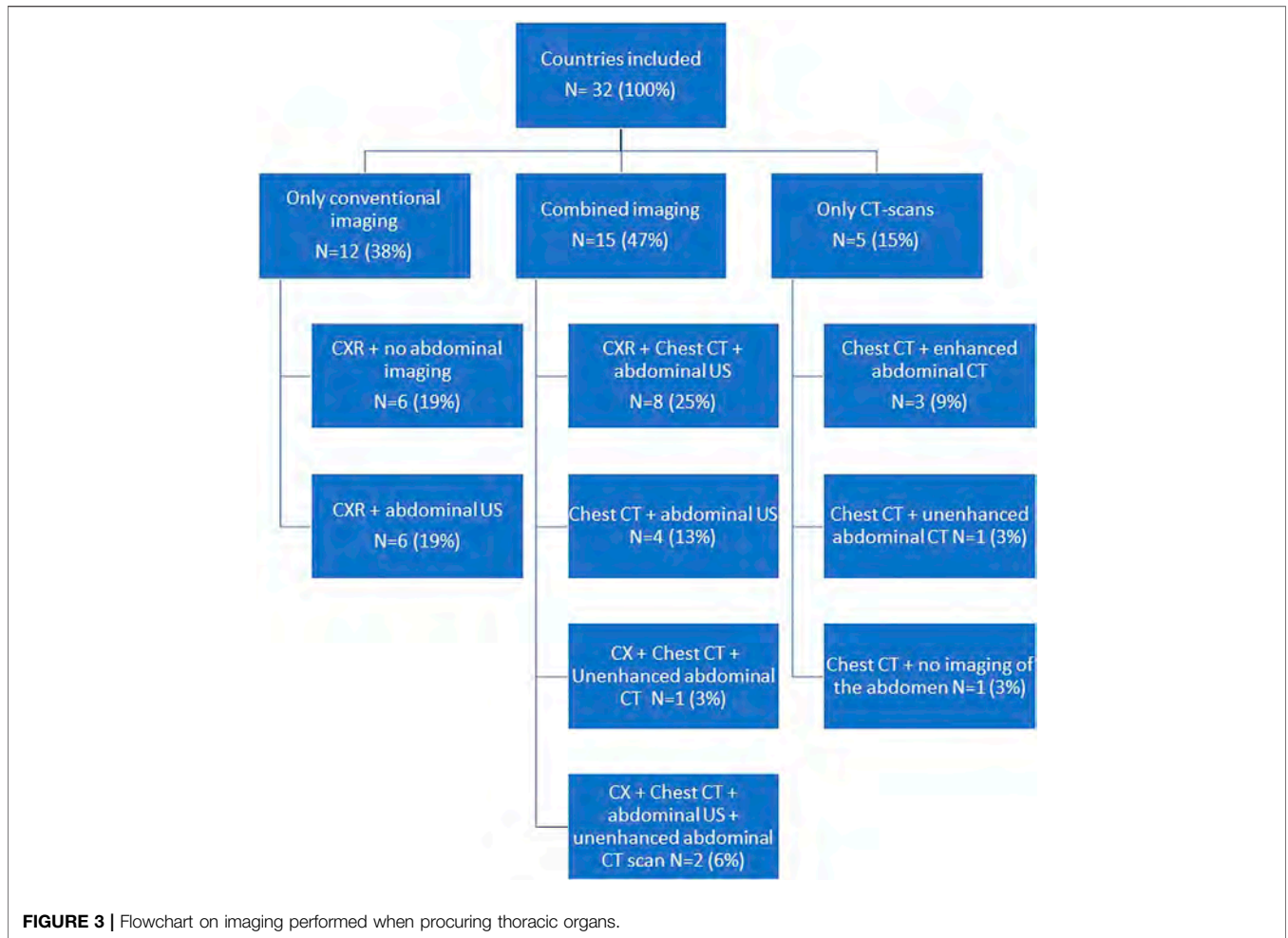
To answer the question of whether imaging policies were associated with donor rate and donation activity, statistical analyses were performed using IBM SPSS Statistics for Windows (IBM Corp. Released 2017. Version 25.0. Armonk, NY). Shapiro-Wilk tests were used to assess the distribution of

donor rate/donation activity between the imaging groups. To compare skewed numerical data the Kruskal Wallis test was used.

RESULTS

An overview of different guidelines regarding radiological screening in deceased organ donation was not found in PubMed. The Guide to the quality and safety of organs for transplantation from the council of Europe (1) has a specific chapter on donor imaging. In this chapter it is advised that at minimum, an up-to-date CXR and abdominal US should be included at the time of donation. Further radiological tests are advised to be performed when thorough donor evaluation is required, for example in patients with suspected malignancies or in donors in whom it is thought that appropriate intra-operative examination of the thoraco-abdominal cavities cannot be adequately carried out.

Thirty-two out of 48 countries on six continents responded to the questionnaire (response rate 67%). **Table 1** gives an overview of all the diagnostic screening methods reported in the survey, including the number of deceased donors PMP (per million



Although these are not included in **Table 1**, their answers were analysed anonymously. Some countries replied that the guidelines were region dependent and do not apply to the whole country. This is also included in **Table 1**. Also, three respondents mentioned that guidelines describe the minimal requirements and that the accepting transplant centre could ask for additional examinations.

Procurement of Abdominal Organs

For the assessment of abdominal organ quality, CXR and abdominal US is considered the preferred screening method in 41% countries (**Figures 1, 2**). In 9% an abdominal US is performed in combination with a chest CT instead of a CXR. In 13% of the countries a chest and abdominal CT scan is part of the regular screening of deceased donors, in 6% next to these two imaging methods also a CXR is performed. In Finland, Norway, Sweden, France, and Israel an enhanced abdominal CT is made, excluding donors with existing or high risk for acute kidney injury (AKI). Unfortunately, the definition of what was considered a high-risk kidney donor was not further explained. In 15% of the countries an abdominal US as well as an unenhanced abdominal CT is performed. In 16% of the countries there are no minimal requirements regarding

people) per country. **Supplementary Datasheet 3** provides an overview of how many countries per region have been approached and the response rate per region. Three organizations did not give permission to publish their answers.

abdominal imaging prior to procurement and only a CXR is considered necessary.

Procurement of Thoracic Organs

To determine suitability of thoracic donor organs only a CXR is required in 19% of the countries, with no requirements of imaging of the abdomen. (Figures 3, 4). A CXR and abdominal US were considered the preferred screening method in 19% of the countries. In 25% a CXR, chest CT and abdominal US is performed. In 13% both a chest CT and abdominal US is carried out. In 9% of the countries chest CT and enhanced abdominal CT scan is performed. In 3% a CXR, chest CT and an unenhanced abdominal CT scan is made. A CXR, chest CT, an unenhanced abdominal CT scan plus abdominal US are performed in 6% of the countries. In 3% a chest CT and unenhanced abdominal CT scan was required, and another 3% required only a chest CT and no imaging of the abdomen.

Summary of Preferences

Most countries (81% of the respondents) report that there are no objections against using CT scans in the screenings process of deceased donor organ donation. The reasons CT-scans are preferred are to facilitate the detection of malignancies (76% of the respondents were in favour of CT scans), and provide information about (aberrant) anatomy of the donor (68%). Sixty-four percent also reported CT scans have a value in providing information about organ quality, for example liver steatosis, renal atrophy, severe atherosclerosis, or pulmonary embolism.

Six respondents (16%) replied that there are objections for the routine use of CT scans in the screening process but addressed concerns regarding incidental findings that would unintentionally lead to donor rejection. Other objections were the logistic challenges associated with performing a routine donor CT, i.e., transporting the donor to the CT and increasing costs of the donation process.

If an abdominal CT scan is not part of the standard screening protocol, 76% of the respondents replied that the main reasons for performing an abdominal CT scan is for the purpose of trauma screening, or suspected anomalies detected on the conventional imaging (24%).

If a chest CT scan is not part of the standard screening protocol, 45% of the respondents replied that the main reason for performing a chest CT scan is also for the purpose of trauma screening or suspected anomalies on the conventional imaging (36%). Two respondents replied that reasons for making a chest CT scan was intended for screening for SARS-CoV-2.

Donor rate versus imaging policy was plotted, to investigate whether there is an association between imaging policies before procurement and donation rate (Supplementary Datasheet 4). No clear association was seen between these two using eyeball estimation. Using the Kruskal Wallis test, since the data was not normally distributed, no significant difference in donor rate between the different imaging policy groups was found ($p = 0.37$).

Donation activity (the total number of deceased donors per year) versus imaging policy was also plotted, to investigate whether there is

an association between imaging policies before procurement and donation activity (Supplementary Datasheet 5). No clear association was seen between these two using eyeball estimation. Using the Kruskal Wallis test, since the data was not normally distributed, no significant difference in donation activity between the different imaging policy groups was found ($p = 0.61$).

DISCUSSION

This study shows a large difference between policies regarding diagnostic screenings methods in deceased organ donation in different transplant regions. The current literature lacks a consensus regarding imaging of deceased donors. No significant association between donor rate and imaging policy groups before procurement was found, nor a significant association between donation activity and imaging policy groups. The donor rate of the countries included ranged from 1 to 50 deceased donors PMP. The donation activity of the countries included ranged from 44 deceased donors per year to 11.870 deceased donors per year.

In the Eurotransplant International region (including eight European countries), the age of the donor population is increasing and with it also the comorbidity rate (12). Since this has impact on the incidence of malignancies and organ quality, an intensified assessment using radiological imaging has become increasingly important (13). Also the proportion of DCD donors has increased through the years, a donor pool historically known for its comorbidity and a rapid and mainly cold dissection, without proper perfusion feedback, in which prior knowledge of the anatomy significantly aids to the operative plan (14, 15).

In Finland, Norway, Sweden, France and Israel imaging is performed using chest and enhanced abdominal CT scan. On the contrary, Australia, the United Kingdom and South Africa do not require imaging of the abdomen before procurement of abdominal organs. In the United States and Canada there is no national policy on imaging of the abdomen, but the different Organ Procurement Organisations do have their own policies. In South Africa there is no screening of the abdomen because of costs and logistic challenges, but in Australia this is a well-considered choice because the procuring surgeon always performs an examination of the abdominal cavity and organs. The idea is that the added yield of abdominal imaging is low and could potentially extend the donor work up time (due to evaluation of any abnormalities). The United Kingdom stated that, in their opinion, performing an abdominal US has no additional value. Detection of malignancies depends on exploration of the abdomen by the procuring surgeon, an approach that might work for large tumors but is expected to have a low sensitivity and specificity for smaller of intraparenchymal lesions. With the shift in the donor population towards more older and extended criteria donors, we as professionals should start asking the question of whether it is time for a paradigm shift. Furthermore, it is interesting to note that English-speaking countries tend to avoid imaging prior to procurement, which could suggest there might be a cultural or historical reason for this.

There were conflicting ideas reported regarding the risk of administrating ICM to potential kidney donors. France, Israel, Sweden, and Norway (all four using enhanced abdominal CT scans) are only reluctant giving donors with a marginal kidney function ICM. But what is considered a marginal donor is often poorly reported or defined. Except for Israel, which uses a specific definition, in which donors with an increase in serum creatinine of more than or equal to 50% from baseline, a creatinine level of $>150 \mu\text{mol/L}$ or a reduction in urine output to less than 0.5 ml/kg/h for more than 12 h despite adequate hydration, are excluded. (Of note; this is slightly different from the AKI classification of AKI stage 1/2) (16). None of these four countries have reported any data regarding negative effects on graft function in the recipients of the kidneys exposed to ICM. Estonia performs an unenhanced abdominal CT scan and abdominal US on all their donors. The idea behind this policy is that with an unenhanced abdominal CT the donor is being screened for any abnormalities or pathological findings (and if indicated, this is supplemented with an enhanced CT scan), while doppler ultrasound is used to assess renal vascularization.

Since the introduction of CT scans in the 1970s, it has become an important tool offering fast and reliable diagnosis of various diseases, which accelerated the application within a broad framework in daily medical practice (17–20). The technique of ICM was introduced even before the invention of the CT scanner, but the chemical properties changed through the years; high osmolarity contrast media were replaced, because of its nephrotoxic properties, by low osmolarity contrast and iso-osmolar contrast agents (21).

In donor assessment, the use of CT scans has several (potential) advantages, namely an accurate detection of malignancies and more accurate assessment of organ quality (i.e., liver steatosis, renal atrophy, severity of atherosclerosis, or pulmonary embolism) compared to conventional modalities. In 2019, Mensink et al. performed a retrospective study to assess the additional value of CT scans in donor screening and concluded that, if a CT scan was added to the screening protocol, at least 7 unnecessary procurements (0.5% of all procurements) could be prevented, over a 5 year period, due to the identification of malignancies (22).

Also, in detecting aberrant (vascular) anatomy, for example the kidney and the liver, CT scans will provide valuable information. Multiple renal arteries are not a rarity with a reported incidence of 24%–28% and their presence causes a higher risk of potential complications at procurement with subsequent graft loss or DGF (23–26). The incidence of variants in hepatic arteries is even higher and ranges from 25% to 45%, insufficiently recognized aberrant anatomy could increase the risk of surgical injury during procurement (27–29). In living donor liver and kidney transplantation CT-scans are already routinely performed and proven essential for measuring total and residual liver volume and assess the anatomy (30). These same advantages could be gained in deceased donors and improve transplant outcome and graft survival (30–33). In lung transplantation, matching of the donor lung and recipient thorax is important to prevent size mismatch. Performing a chest CT results in better prediction of the total lung capacity, which therefore benefits the optimal matching and preoperative planning (4, 34).

However, every advantage has its disadvantage. If more accurate imaging is applied, the risk of incidental findings increases, resulting in additional tests and thereby prolonging duration of donor assessment or even cessation of a donor procedure. The extent of this risk is currently unknown and must be weighed against the likelihood of malignancy transmission. On the other hand, not performing a CT scan because of the fear of finding anomalies of unknown significance and a chance of leading to cessation of the donor, means the physicians are taking a calculated risk for transplanting a malignancy. From an ethical perspective, this could raise the question of whether it is safe to transplant these organs.

Also, transporting a potential donor that might be hemodynamically unstable to the CT could also be a challenge. In case of a DCD II (unsuccessful resuscitation) and DCD IV (cardiac arrest in a patient who is brain dead), performing a CT scan is probably in most of the cases impossible.

A CT scans is associated with higher costs compared in comparison to CXR and abdominal US; a chest and abdominal CT scan in Netherlands cost approximately €400 together, while the costs of a CXR and abdominal US together are less than €150 (35). But despite the extra costs, it could be more cost effective by timely cessation of a donor procedure in case of malignancy. Yet this assumption should also be considered in future studies.

This study has a few limitations that need to be addressed. First, not all countries approached replied to our survey and the majority of the countries were from Europe. However, several large and influential transplant organizations did respond. The response rate was 67%, which is in accordance with the response rate in patient and health care professional surveys in surgery (the average response rate was 53%, SD 25%) (36). Since only the countries that replied to the survey could be included, a certain selection bias should be considered. The survey was created by the author itself and reviewed by several procuring surgeons, which could have led to missing questions. For example, the survey did not contain the option to fill out whether chest CT is performed with or without ICM. Nevertheless, none of the respondents commented chest CT was performed using ICM. To define the countries to be approached the IRODaT registry was used instead of the international figures from the Global Observatory on Donation and Transplantation WHO-ONT, since the author was familiar with the IRODaT Registry. After comparing the data from both databases, in 80% of the countries the number of deceased donors was the same in both databases. In 20% of the countries the numbers differed by only a few numbers.

In conclusion, this overview shows that policies regarding radiologic screening in deceased donor organ management are quite different between various countries and transplant organizations throughout the world, based on different views on (the safety of) organ transplantation. Future research should focus on interviewing specific transplant centers or Organ Procurement Organisations regarding their policies. This study shows there is a need to prospectively investigate the value of CT scans in deceased organ donation. In such a study, we would suggest the following outcome measurements; changes in acceptance of

the grafts based on the diagnostic imaging, better matching of donor-recipient (size measure for long and/or liver transplantation) and the incidence of detecting malignancies before procurement. This type of research could contribute to making decisions on policy changes evidence-based and well considered.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

AB, RP, WN, BS, and KC participated in designing the survey. KC participated in data collection. All authors participated in construction and critical revision of the article.

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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.2022.10289/full#supplementary-material>

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Hyperspectral Imaging as a Tool for Viability Assessment During Normothermic Machine Perfusion of Human Livers: A Proof of Concept Pilot Study

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Normothermic machine perfusion (NMP) allows for *ex vivo* viability and functional assessment prior to liver transplantation (LT). Hyperspectral imaging represents a suitable, non-invasive method to evaluate tissue morphology and organ perfusion during NMP. Liver allografts were subjected to NMP prior to LT. Serial image acquisition of oxygen saturation levels (StO₂), organ hemoglobin (THI), near-infrared perfusion (NIR) and tissue water indices (TWI) through hyperspectral imaging was performed during static cold storage, at 1h, 6h, 12h and at the end of NMP. The readouts were correlated with perfusate parameters at equivalent time points. Twenty-one deceased donor livers were included in the study. Seven (33.0%) were discarded due to poor organ function during NMP. StO₂ ($p < 0.001$), THI ($p < 0.001$) and NIR ($p = 0.002$) significantly augmented, from static cold storage (pre-NMP) to NMP end, while TWI dropped ($p = 0.005$) during the observational period. At 12–24h, a significantly higher hemoglobin concentration (THI) in the superficial tissue layers was seen in discarded, compared to transplanted livers ($p = 0.036$). Lactate values at 12h NMP correlated negatively with NIR perfusion index between 12 and 24h NMP and with the delta NIR perfusion index between 1 and 24h ($r_s = -0.883$, $p = 0.008$ for both). Furthermore, NIR and TWI correlated with lactate clearance and pH. This study provides first evidence of

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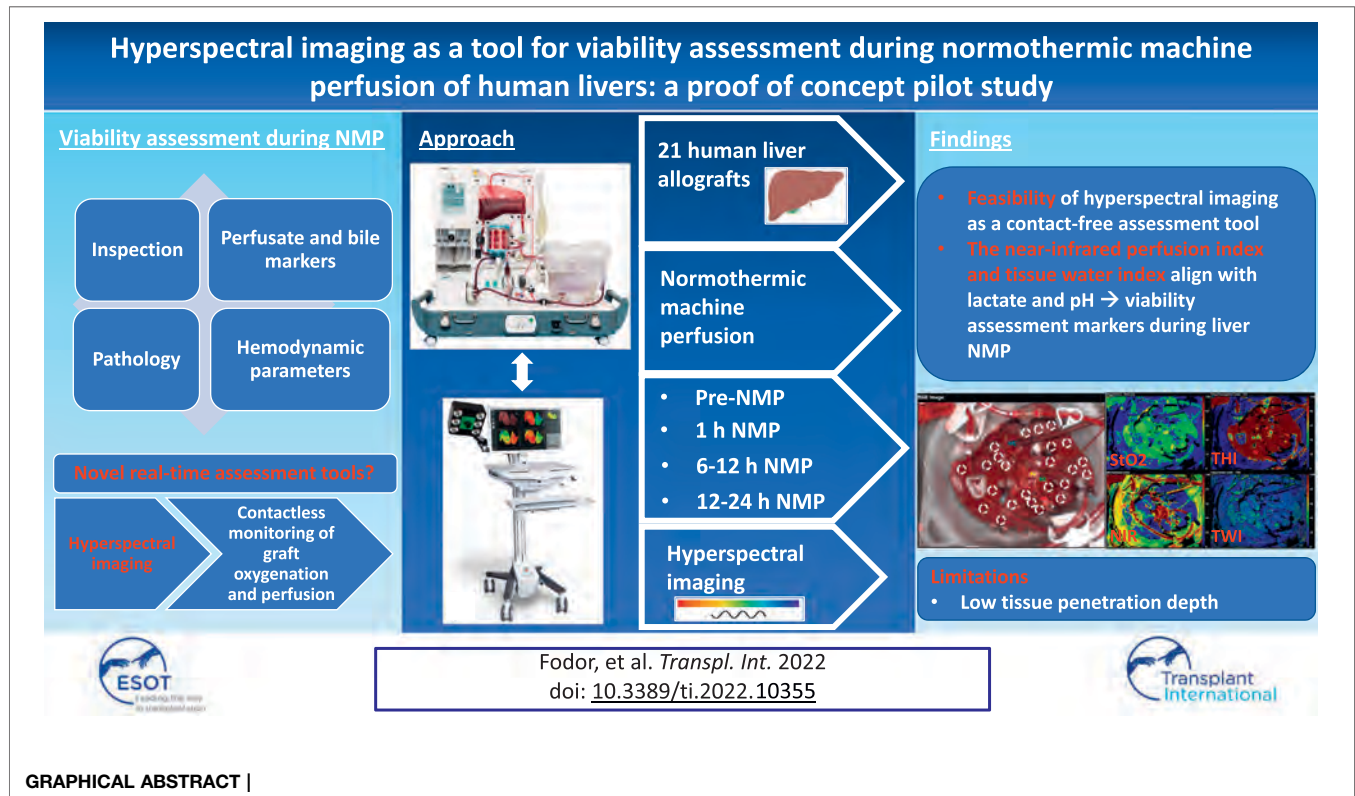
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Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BAR, balance of risk score; CIT, cold ischemia time; DBD, donation after brain death; DCD, donation after cardiac death; EAD, early graft dysfunction; ECD, extended criteria donors; HSI, hyperspectral imaging; HTK, histidine-tryptophan-ketoglutarate; ICU, intensive care unit; IGL-1, Institut Georges Lopez; IQR, interquartile range; IRI, ischemia reperfusion injury; ITBL, ischemic type biliary lesions; L-GrAFT, Liver Graft Assessment Following Transplantation; LT, Liver transplantation; MEAF, Model for Early Allograft Function; MELD, model for end-stage liver disease; MP, machine perfusion; NIR, near-infrared perfusion index; NMP, normothermic machine perfusion; PNF, primary non-function; RGB, red-green-blue; ROI, region of interest; StO₂ (%), relative blood oxygenation index; THI, tissue hemoglobin index; TWI, tissue water index; UW, University of Wisconsin.

feasibility of hyperspectral imaging as a potentially helpful contact-free organ viability assessment tool during liver NMP.

Keywords: transplantation, perfusion, normothermic, imaging, liver, hyperspectral, machine



INTRODUCTION

In the light of a shortage of donor liver organs, the use of extended criteria donors (ECD) continues to rise. This poses a risk of increased rates of early allograft dysfunction (EAD), primary non-function (PNF) and biliary complications (1–10). Compared to standard criteria donor grafts, ECD livers are more susceptible towards ischemia-reperfusion injury (IRI). In the light of these developments, machine perfusion (MP) has emerged as a procedure aiming to limit IRI. Normothermic machine perfusion (NMP) is also suitable for prolongation of preservation and a comprehensive assessment of livers *ex-vivo*. While this concept is uniquely appealing, the identification of techniques and biomarkers for a meaningful determination of the quality and function of an organ remains to be established. Essentially, NMP mimics physiologic liver perfusion. During a period of up to 24 h, the liver is accessible for inspection, biopsy, perfusate and bile sampling (11). Contemporarily, viability assessment is performed by measuring biochemical parameters and synthetic function in the perfusate and bile (12–17). Further to this, innovative liver graft viability and injury markers have been applied. However, whether they are acceptable predictors of

the outcomes after LT remains to be proven (2, 4, 11, 12). Novel non-invasive methods for the estimation of organ quality during NMP are necessitated. Hyperspectral imaging (HSI) represents a potentially suitable contactless tool to assess tissue morphology and organ perfusion. This technology allows a real-time quantitative evaluation of graft oxygenation and micro-perfusion, as well as organ hemoglobin and water concentration. Previous studies showed that HSI is suitable for monitoring of the oxygen saturation distribution and identifying areas with a reduced oxygen supply (18–20). This may help to detect and quantify impaired, inhomogeneous or deteriorating perfusion (18–27). We herein designed a study demonstrating the feasibility and the potential of HSI in the setting of liver NMP as a non-invasive, simple viability assessment tool.

MATERIALS AND METHODS

Study Design

Liver allografts accepted for transplantation were procured and subjected to NMP. The decision to apply NMP at our center was based on a previously developed concept (6). NMP was applied

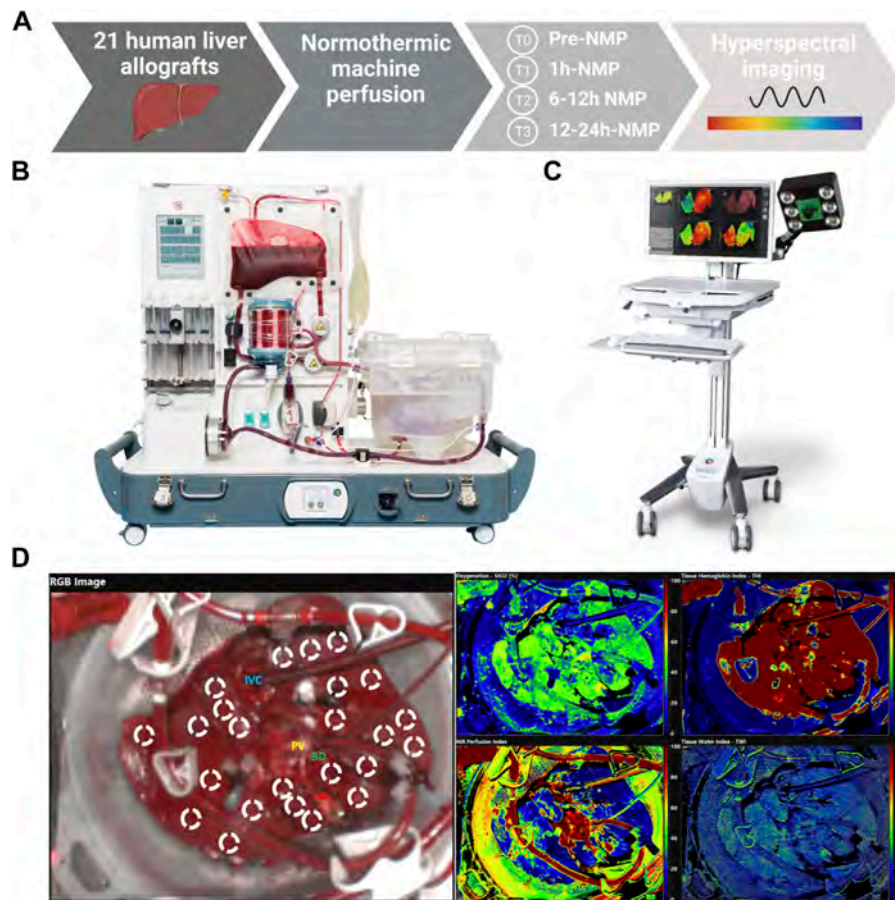


FIGURE 1 | (A) Overview describing the methodology and sample collection of the study; **(B)** OrganOx metra[®] system used for normothermic machine perfusion (14, 16, 52); **(C)** TIVITA[®] Tissue System used for hyperspectral imaging (21); **(D)** Images acquired during liver normothermic machine perfusion: RGB image, hyperspectral images for oxygenation (StO₂), perfusion (NIR perfusion), hemoglobin (THI), and water concentration (TWI), with region of interest (ROI) markers within the parenchyma of liver allografts IVC, Inferior vena cava; PV, Portal vein; HA, Hepatic artery; BD, Bile duct; StO₂, Tissue Oxygen Saturation; THI, Tissue Hemoglobin Index; NIR, Near-Infrared Perfusion Index; TWI, Tissue Water Index.

for the following indications: (I) uncertain organ quality (II) complex recipient, and (III) logistics. MP was performed using the OrganOx metra[®] system according to a local protocol (6), details are specified in the **Supplementary File**. Perfusion time on the OrganOx metra[®] system depended on the time required for assessment, decision-making and logistics. The choice to discard or transplant an organ was based on key quality parameters (6, 14): preservation of physiological pH values (7.3–7.45) without sodium bicarbonate supplementation after 2 h of NMP, a prompt decline and maintenance of lactate to physiological values (≤ 18 mg/dl), as well as bile production and bile pH > 7.45 are considered indicators for appropriate organ function. The decision to transplant or discard a liver graft was made after a minimum of 6 h NMP. Further to this, high aspartate aminotransferase (AST), alanine aminotransferase (ALT) ($>20,000$), and lactate dehydrogenase ($>20,000$) levels are calling for caution (1, 6). To assess the dynamics of HSI parameters during liver NMP and their correlation with perfusate parameters, serial measurements were performed before NMP (during static cold storage), at 1, 6, 12 h and at

the end of NMP (**Figure 1**). HSI data points were assessed longitudinally and in reference to the established biomarkers mentioned above. Donor, recipient and NMP characteristics, transplant procedural data as well as post-operative follow-up data were collected.

Ethics Statement

The study protocol was approved by the local institutional review board.

Study Population

A total of 21 donor livers were enrolled in this study between December 2020 and May 2021. The majority of these livers were ECD livers. For definition of ECD, the Eurotransplant criteria were applied (28). These include liver grafts with severe macrosteatosis (>30 or $>40\%$), prolonged cold ischemia (>12 h), DCD and high donor age (>80 years). Notably, a number of criteria that could characterize ECDs specifically for LT have been identified, but the impact of each of these remains to be defined (29). From the 21 livers studied in this trial,

TABLE 1 | Demographic data.

	Total (n = 21)	Transplanted (n = 14)	Not transplanted (n = 7)	p-value ^a
Donor data				
Age (y) ^b	61 (48–70)	66 (56–70)	46 (43–56)	p = 0.031
Gender				p = 0.011
• Man	13 (61.9)	6 (42.9)	7 (100)	
• Woman	8 (38.1)	8 (57.1)		
BMI (kg/m ²) ^b	26 (24–28)	26 (24–28)	28 (23–31)	p = 0.585
ICU time (d) ^b	3 (2–7)	4 (2–7)	2 (2–7)	p = 0.585
CIT (h) ^b	6 (5–8)	6 (5–8)	7 (5–9)	p = 0.856
Cause of death				p = 0.290
Cerebrovascular	15 (71.4)	10 (71.4)	5 (71.4)	
Circulatory	2 (9.5)	1 (7.1)	1 (14.3)	
Trauma	1 (4.8)		1 (14.3)	
Other	3 (14.3)	3 (21.4)		
ECD donor	16 (76.2)	10 (71.4)	6 (85.7)	p = 0.469
Donor Type				p = 1.000
DBD	15 (71.4)	10 (71.4)	5 (71.4)	
DCD	6 (28.6)	4 (28.6)	2 (28.6)	
DRI ^b	2.119 (1.610–2.435)	2.268 (1.728–2.482)	1.760 (1.480–2.220)	p = 0.263
Hypertension	7 (33.3)	4 (28.6)	3 (42.9)	p = 0.289
Alcohol Abuse	4 (19)	1 (7.1)	3 (42.9)	p = 0.102
Malignancy	2 (9.5)	1 (7.1)	1 (14.3)	p = 0.599
Steatosis hepatis	11 (52.4)	6 (42.9)	5 (71.4)	p = 0.279
• Mild (<40%)	10 (47.6)	5 (35.7)	5 (71.4)	
• Moderate (40%–80%)	1 (4.8)	1 (7.2)	0 (0)	
• Severe (>80%)	0 (0)	0 (0)	0 (0)	
NMP indication				
• Complex recipient	2 (9.5)	2 (14.3)		p = 0.293
• Marginal donor	18 (85.7)	11 (78.6)	7 (100)	p = 0.186
• Logistics	8 (38.1)	6 (42.9)	2 (28.6)	p = 0.525
NMP time (h) ^b	15 (11–20)	15 (13–20)	12 (7–22)	p = 0.535
Total preservation time (h) ^b	20 (17–27)	21 (17–27)	19 (9–30)	p = 0.799
Recipient data and post-operative outcome				
Age (y) ^b		62 (58–65)		
Gender				
• Man		10 (71.4)		
• Woman		4 (28.6)		
BMI (kg/m ²) ^b		25.7 (21.8–28.2)		
MELD ^b		17 (8–21)		
Time on waiting list (d) ^b		52 (37–197)		
BAR score ^b		7 (7–10)		
BAR score ≥ 8		6 (42.9)		
Total hospital stay (d) ^b		28 (21–46)		
ICU stay (d) ^b		6 (4–18)		
Early allograft dysfunction		6 (42.9)		
MEAF score ^b		5.67 (4.02–6.90)		
L-Graft score ^b		−0.73 (−1.33–0.07)		
Clavien Dindo ≥3		11 (78.6)		
90—days readmission rate (unplanned)		4 (28.6)		
Biliary complications		9 (64.3)		
• ≤ 30 d		6 (42.9)		
• > 30 d		3 (21.4)		
• Biliary leakage		4 (28.6)		
• Anastomotic stricture		4 (28.6)		
• Biliary cast syndrome		1 (7.1)		
Arterial complication		2 (14.3)		
Patient survival (d) ^b		106 (82–163)		
Graft survival (d) ^b		106 (82–163)		
Patient death		2 (14.3)		

Values in parentheses are percentages unless indicated otherwise

^aChi-square for categorical variables and Mann-Whitney-U Test for continuous variables.

^bValues are median (i.q.r.).

BMI, body mass index; ICU, intensive care unit; CIT, cold ischemia time; ECD, extended criteria donor; DBD, donation after brain death; DCD, donation after cardiac death; DRI, donor risk index; NMP, normothermic machine perfusion; MELD, Model for End-Stage Liver Disease; BAR, balance of risk; MEAF, model of early allograft function; L-Graft, Liver Graft Assessment Following Transplantation.

14 were transplanted, while seven livers were discarded based on the above-mentioned performance quality criteria during NMP. An overview summarizing the most important characteristics of donors, recipients, liver allografts and MP times are displayed in **Table 1**.

Hyperspectral Imaging of Human Liver Allografts

For the acquisition of HSI data, a contactless and non-ionizing radiation imaging system (TIVITA[®] Tissue System, Diaspective Vision GmbH, Am Salzhaff, Germany) was used under standardized conditions and previously reported settings (24, 30). The software (TIVITA Suite Tissue) provides a red-green-blue (RGB) image and four false color images illustrating physiologic parameters of the recorded tissue area, which quantified values of the parameters from blue (low values) to red (high values). The relative blood oxygenation in the microcirculation of superficial hepatic tissue layers (approximately 1 mm) is represented by StO₂ (%), whereas the near-infrared (NIR) perfusion index (0–100) represents tissue layers in 4–6 mm penetration depth. The indices THI (0–100) and TWI (0–100) display the relative distribution of hemoglobin and water in the investigated tissue area, respectively. Serial HSI measurements were performed according to our center specific NMP protocol: before NMP, at 1h, 6h, 12 h and at the end (with a maximum of 24 h) of NMP. **Supplementary Figure S1** show the different perfusion times of the liver grafts. For the assessment protocol, circular areas, representing the ROI (10 mm diameter markers, 3 markers per liver segment), were defined within the acquired hyperspectral images (**Figure 1**). The index average was calculated from the values collected from the ROI for each image. Details regarding the application of HSI in this analysis are illustrated in the **Supplementary File**.

Feasibility and Follow-Up

We primarily assessed the dynamic change of perfusion and oxygenation of liver tissue during NMP. Further to this, we have investigated 1) the differences in HSI dynamics between livers discarded and transplanted as well as 2) the correlations between HSI indices and perfusion parameters.

The clinical follow-up of transplanted patients included the assessment of patient survival, graft survival, Clavien Dindo post-operative complications rate, EAD, Model of Early Allograft Function (MEAF) score, Liver Graft Assessment Following Transplantation (L-Graft) score, biliary and vascular complications, 90 days readmission rate, ICU and total hospital stay.

Statistical Analysis

To examine the Gaussian distribution, we used the D'Agostino-Pearson normality test. The data were analyzed as proportions and medians with interquartile ranges (IQR), because they were consistent with a skewed distribution. Chi-square and Fisher's exact tests for categorical variables and Mann-Whitney-U tests for continuous variables were used to compare the HSI values in the transplanted and non-transplanted groups. Ordinal variables were analyzed as continuous variables. Using the t or F

distributions, Mann-Whitney-U tests were approximated for ordinal variables. The Friedman test and Sing tests were applied for paired non-parametric tests. To correlate HSI parameters and laboratory values measured in the perfusate during NMP, Spearman rank correlation tests were performed. Two-tailed *p*-values < 0.05 were considered significant throughout the entire analysis. Statistical analysis was performed using SPSS Statistics Version 27.0 for Macintosh (IBM Corporation, Armonk, NY, United States).

RESULTS

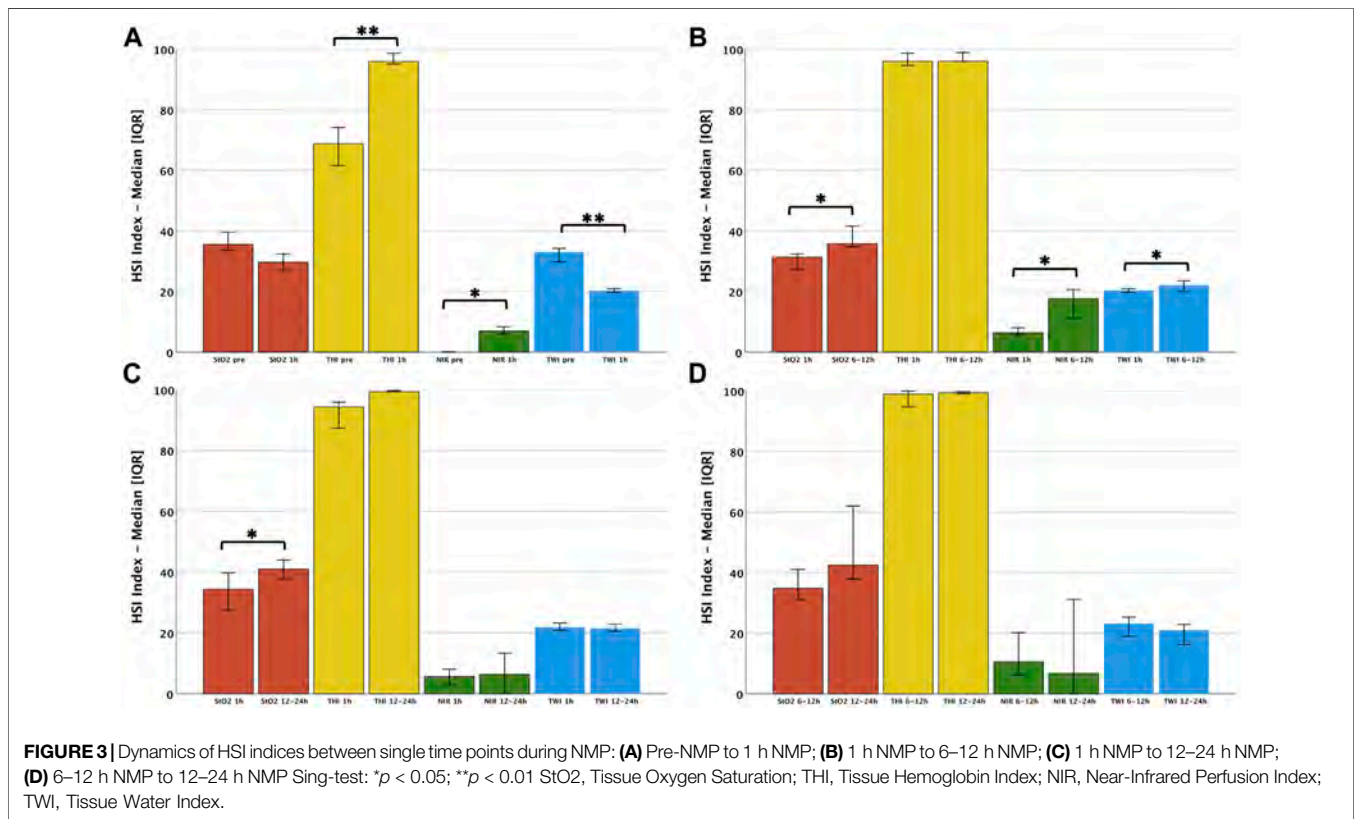
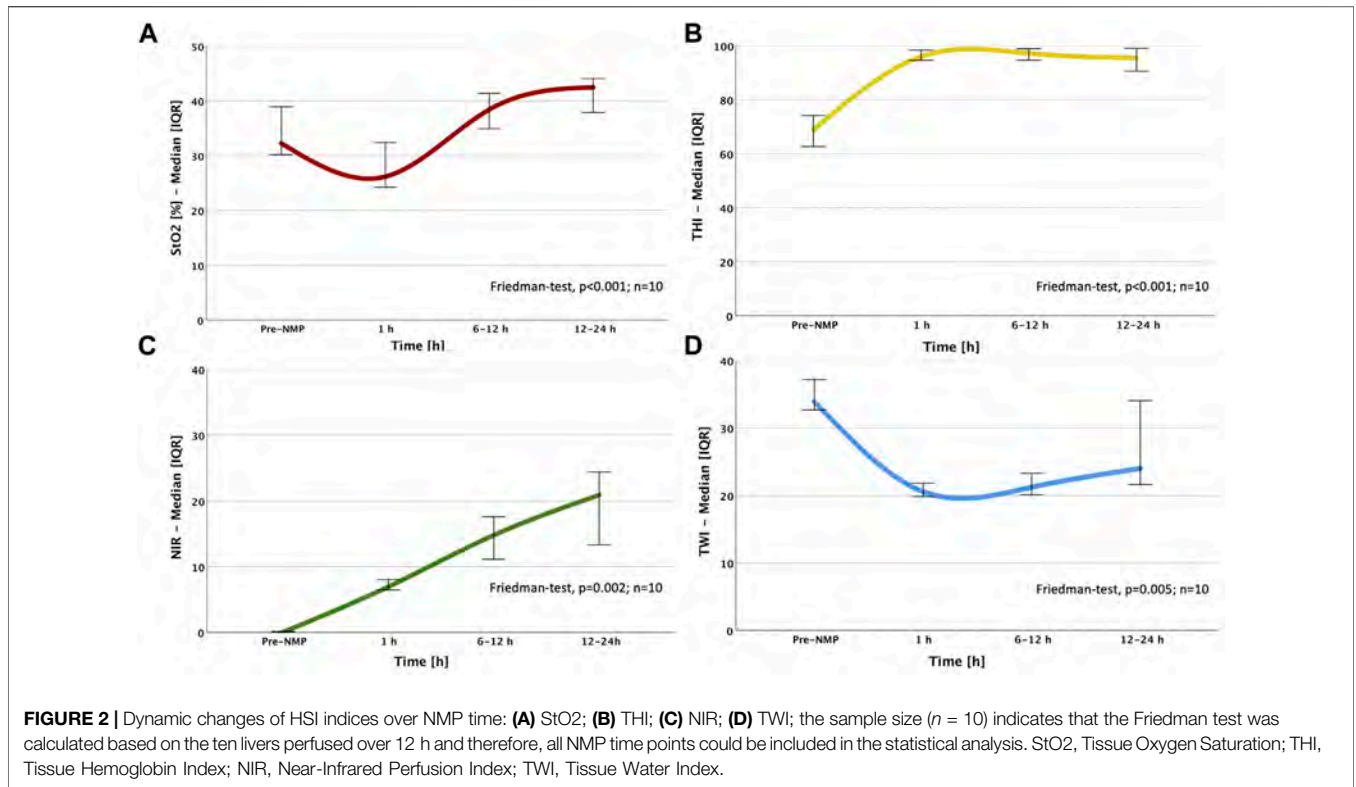
Patient Characteristics and Flow Parameters During Normothermic Machine Perfusion

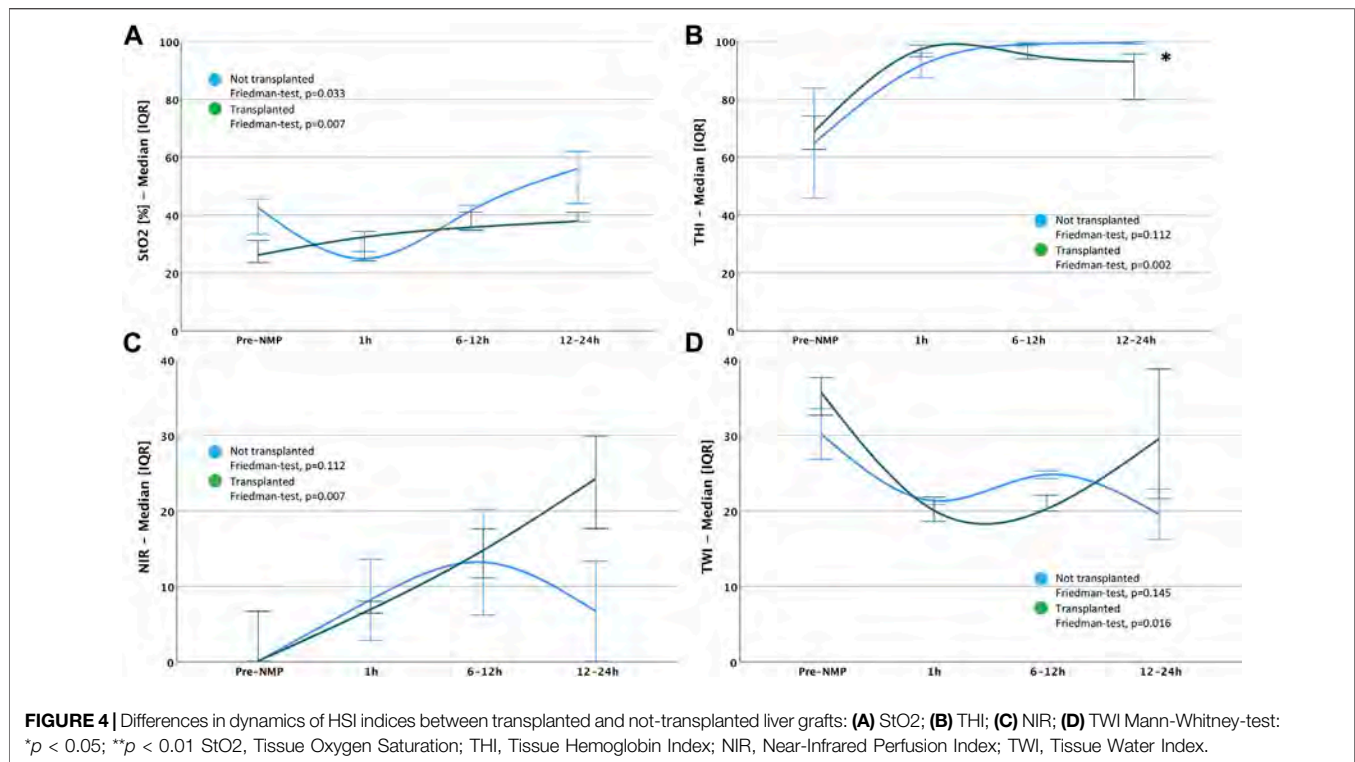
During the study period, a total of 21 deceased donor livers were preserved via NMP. Night-time procedures were avoided and NMP time did not exceed 24 h. Seven (33.0%) were discarded after NMP, due to insufficient organ quality and performance. The median donor age was 61 years (48–70 years) and the median Donor Risk Index was 2.119 (1.610–2.435). Cold ischemia time (CIT) was 6 h (5–8 h) and total NMP time was 20 h (17–27 h). Six (28.6%) grafts derived from DCD donors (Maastricht category III), the remaining grafts from DBD donors. Median recipient MELD and Balance of risk (BAR) scores were 17 (8–21) and 7 (7–10), respectively. The median recipient age was 62 years (58–65 years). The median donor age of transplanted vs. discarded livers was 66 (56–70 years) vs. 46 years (43–56 years) (*p* = 0.031). All discarded liver allografts were from male donors, while 8 (57.1%) transplanted liver allografts were from female donors (*p* = 0.011).

The median ICU and total hospital stay were 6 (4–18) and 28 days (21–46), respectively. Six patients (42.9%) developed EAD, the median MEAF and L-Graft scores were 5.67 (4.02–6.90) and –0.73 (–1.33 – (–0.07)). Clavien-Dindo grade ≥3 complications occurred in 11 (78.6%) of 14 patients. Arterial complications occurred in two (14.3%) patients (one anastomotic stricture, one anastomotic aneurysm). Early (≤30 days) biliary complications were detected in six (42.9%) while late biliary complications (>30 days) in three (21.4%) patients. No patients developed non-anastomotic strictures, ischemic type biliary lesions (ITBL) or primary non-function. No patients were listed for re-transplantation. Two patients died due to multi-organ failure. The median follow-up was 106 (82–163) days. Recipient and donor demographics, as well as post-operative outcome parameters are described in **Table 1**. NMP hepatic artery and portal vein flows were >150 ml/min and >500 ml/min, for all livers during the entire course.

Perfusion and Oxygenation of the Liver Parenchyma During Normothermic Machine Perfusion

The liver parenchyma was analyzed by HSI in cold-stored organs, at 1, 6, 12 h and at the end of NMP. The StO₂, THI and NIR perfusion indices significantly increased (*p* < 0.001, *p* < 0.001 and





$p = 0.002$ respectively), while the TWI drastically decreased ($p = 0.005$) during the observational period (**Figure 2**; **Supplementary Table S1**). In the interval between static cold storage (pre-NMP) and 1 h NMP, we observed a significant augmentation of the THI and NIR (69 vs. 96, $p < 0.001$ and 0 vs. 7, $p = 0.003$, respectively), while the TWI dropped (33 vs. 20, $p < 0.001$). Contrarily, StO₂ mainly remained constant. The dynamics of perfusion and oxygenation over the entire NMP period (between 1 h and 12–24 h) illustrated a significant augmentation of StO₂ (31 vs. 39, $p = 0.006$), while the remaining HSI parameters remained stable. A longitudinal assessment of the tissue during NMP showed a substantial increase of the relative blood oxygenation StO₂ (31 vs. 41, $p = 0.008$), the NIR perfusion index (7 vs. 17, $p = 0.008$) and the water distribution (TWI) (20 vs. 21, $p = 0.008$) during the first 6 h of NMP, while HSI values remained stable after this time (**Figure 3**; **Supplementary Table S2**).

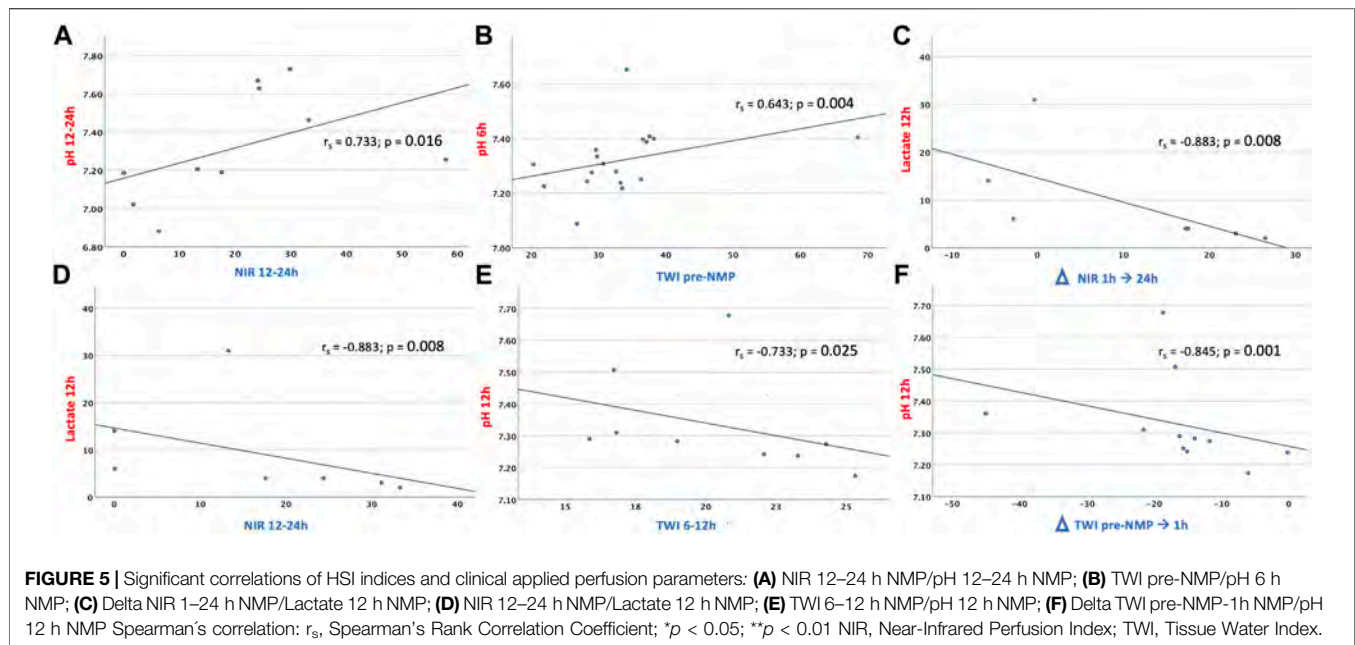
Discrimination of HSI Dynamics in Transplanted and Discarded Liver Grafts

Liver allografts subjected to NMP and transplantation revealed a significant escalation of the StO₂, THI and NIR perfusion index ($p = 0.007$, $p = 0.002$ and $p = 0.007$, respectively) over the entire observational period, while the tissue water concentration (TWI) drastically decreased ($p = 0.016$). Livers undergoing NMP without subsequent transplantation also displayed a significant augmentation of the relative blood oxygenation (StO₂%) ($p = 0.033$). However, the other HSI parameters remained mainly constant during the study period. Notably, at the end of perfusion (12–24 h), a significantly higher hemoglobin concentration (THI)

in the superficial tissue layers was seen in discarded, compared to transplanted livers ($p = 0.036$). In contrast, StO₂, THI, NIR perfusion index and TWI parameters did not differ during the early course of NMP (**Figure 4**; **Supplementary Tables S3, S4**). Discriminations of HSI findings between livers from DBD vs. DCD and SCD vs. ECD, as well as livers with sufficient vs. insufficient lactate clearance during the first 6 hours of NMP were performed. The **Supplementary File** displays these additional findings (**Supplementary Figures S2–S4**; **Supplementary Tables S5–S10**).

Correlation of HSI Indices With Perfusion Parameters During Normothermic Machine Perfusion

There is currently limited evidence about the predictive value of individual perfusion parameters (12). Several biomarkers have been proposed to determine optimal clinical and metabolic liver responses during *ex vivo* NMP, including perfusate lactate clearance, or maintenance of a stable perfusate pH value (31). Lactate has traditionally been used as a marker of sepsis. Lactatemia can subsequently develop in tissue hypoxia (31). In this context, the liver is responsible for removing about 50% of circulating serum lactate, which rises in the liver in case of reduced blood flow/oxygen delivery. In line with the consideration that lactate should be interpreted as a surrogate marker of hypoxic injury and impaired hepatocyte functionality (32–34), our data displayed a negative correlation of increasing lactate values at 12 h NMP with a high NIR perfusion index between 12 and 24 h NMP and with an improved delta NIR



perfusion index between 1 and 24 h ($r_s = -0.883$, $p = 0.008$ for both). The perfusate pH has been introduced as a viability criterium in the context of NMP, given the association with lactic acidosis, most commonly resulting from an imbalance between oxygen delivery and oxygen demand (12). Concomitant to this assumption, our analysis revealed a positive correlation of perfusate pH with the NIR perfusion index over 12 h NMP ($r_s = 0.733$, $p = 0.016$). The TWI concomitated a decrease in lactatemia and the rising pH. Liver oedema and the related parenchymal damage as detected with HSI during cold storage decreased during NMP. In accordance, the TWI between 6 and 12 h and between cold storage and 1 h NMP were negatively associated with the pH at 12 h ($r_s = -0.733$, $p = 0.025$ and $r_s = -0.845$, $p = 0.001$, respectively), while a high TWI during static cold storage correlated with a high pH at 6 h ($r_s = 0.643$, $p = 0.004$), (Figure 5; Supplementary Tables S11–S13). These findings suggest that the NIR perfusion index and the TWI are potential markers to estimate the severity of impaired perfusion and oxygenation in livers during NMP.

DISCUSSION

This pilot study was conducted with the intent to investigate HSI in the clinical setting of liver NMP. NMP allows to push the boundaries of organ transplantation, including the use of ECD grafts and longer preservation times (12, 13, 15, 35–37). HSI represents a user friendly imaging technology allowing for a quick and contactless, real-time viability assessment (22). *In vivo*, HSI can detect alterations at the early stages of NMP. While intra-operative haemodynamic monitoring has been limited to systemic measurements, a more organ-specific approach reflecting local oxygen delivery and microcirculatory perfusion has gained interest (38–40). In

the field of hepatobiliary surgery, different imaging techniques were tested in order to evaluate liver parenchymal perfusion (41, 42). Indocyanine green fluorescence was examined as a technology aiding with intraoperative navigation, useful to detect patients at risk for developing EAD after LT (42). Moreover, this method may be utilized as tool to define boundaries of ischaemic areas by capillary flow diffusion in gastrointestinal surgery (38). Intraoperative changes in the oxygenation state of liver grafts were previously measured by near infrared spectroscopy. Mean hepatic oxygen saturation of hemoglobin in the liver was positively correlated with portal flow rate, indicating heterogeneous tissue oxygenation. This parameter was also predictive of EAD (43). Sidestream dark field imaging, a microscopic technique using polarized light to visualize erythrocytes through capillaries, was experimented as non-invasive method to visualize the microvessel architecture (38). In contrast to commonly used methods for determining the oxygenation status, HSI allows a pixel-wise analysis of chemical changes. The additional information on oxygenation status and perfusion quality, might facilitate the decision-making process in transplantation (18–20, 22, 23, 30). Currently utilized HSI parameters like StO₂, NIR perfusion index and THI might be of lesser importance if measured during cold storage. However, in the context of MP, HSI may provide useful data on organ viability and performance (22). Moreover, the continuous monitoring of liver micro-perfusion, oxygenation and water content offers an early identification of functional/technical limitations during MP. For the entire observational period, we observed a significant increase in oxygen saturation, tissue hemoglobin concentration and micro-perfusion, while the organ water amount drastically diminished. Furthermore, a subgroup discrimination between transplanted and discarded liver

allografts showed an enhanced micro-perfusion in transplanted grafts, mainly after 6–12 h NMP. We observed that tissue oxygenation and micro-perfusion are specifically augmented during the first 12 h of NMP, while lesser dynamic changes were displayed in the late phase of NMP. Current liver graft evaluation is either based on scoring systems involving donor and recipient parameters, or on the invasive assessment of the parenchyma (44–51). Histopathologic examination of liver biopsies represents the current gold standard in the evaluation of liver quality in transplantation, however, several limitations such as time requirement, work-up procedure, reproducibility, intraoperative variance, inappropriate sampling, as well as the invasive nature of the retrieval represent important limitations. Further, histopathology may not always be a reliable indicator of graft quality, since this procedure only captures a snapshot of the morphological but not the functional condition (5). Other assessment technologies include perfusate/bile flow biomarkers as well as hydrodynamic parameters (11, 12). It remains to be determined, if they can be used as long-term indicators of graft outcomes (11). For livers rejected for transplantation based on particular viability criteria, no postoperative data are available and the direct comparison remains elusive (12). A decision-making process based on NMP endpoints poses the risk of incorrectly discarding organs suitable for transplantation. A definitive viability validation would require a well-powered multicenter randomized controlled trial (11). In an attempt to assess if HSI indices correspond with the perfusate biomarkers, our primary findings suggest, that NIR and TWI align with lactate and pH, considered as viability assessment markers during NMP. Based on the limited number of cases analyzed in this study, no conclusions toward an immediate clinical application can be drawn. HSI cannot replace histopathology or the viability markers currently applied. While clinical endpoints in LT trials such as EAD, MEAF and L-Graft score were applied in this study, the restricted number of transplanted patients and the selection applied through assessment during NMP did not permit the identification of discrimination towards the outcome by HSI.

The strengths of the HSI technology as applied during NMP are the immediate applicability and the comprehensive assessment of the perfusion state of an organ over the entire exposed surface (22). Integrating of a real-time imaging procedure into a clinical MP setting would require optimal acquisition distance settings and automated use under sterile conditions (22). Further to the use during NMP, utilization during donor surgery for quality assessment before cold perfusion and procurement could be of interest (30).

In addition to the small cohort analyzed in this study, the different perfusion times and the overall heterogeneity of the liver grafts represent apparent limitations. Further to these, HSI has a relatively low tissue penetration depth, which precludes the detection of injuries in deeper regions, or the potential transcutaneous measurement after transplantation. All in all, HSI during NMP appears promising and feasible and its apparent simplicity makes it attractive for clinical use, but validation in large clinical trials is needed before

establishing routine application. All analyses are explorative and p -values ≤ 0.05 were termed significant for descriptive reasons only.

To the best of our knowledge, HSI has not yet been applied previously in the field of liver NMP. We herein proved the technical feasibility of the combination of HSI and NMP. This real-time perfusion imaging may contribute to pre-transplant viability assessment.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The study protocol was approved by the review board of the Medical University of Innsbruck.

AUTHOR CONTRIBUTIONS

Conceptualization: MF and SS; Data curation: MF; Formal analysis: MF and LL; Investigation: MF; Methodology: MF, LL, JH, GO, and RS; Project administration: MF and SS; Resources: MF and SS; Software: MF and LL; Supervision: SS; Validation: MF and SS; Visualization: MF, LL, JH, GO, MP, BC, RO, TR, AW, MM, CM, PZ, JP, TH, DÖ, RS, and SS; Writing—original draft: MF, LL, and SS; Writing—review and editing: MF, LL, JH, GO, MP, BC, RO, TR, AW, MM, CM, PZ, JP, TH, DÖ, RS, and SS.

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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.2022.10355/full#supplementary-material>

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Mortality and Causes of Death After Liver Transplantation: Analysis of Sex Differences in a Large Nationwide Cohort

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on behalf of the Spanish Liver Transplant Registry

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In the last few years, several studies have analyzed sex and gender differences in liver transplantation (LT), but none have performed a disaggregated analysis of both mortality and causes of death. Data from 15,998 patients, 11,914 (74.5%) males and 4,069 (25.5%) females, transplanted between 2000 and 2016 were obtained from the Liver Transplantation Spanish Registry. Survival analysis was applied to explore recipient sex as a risk factor for death. The causes of death at different follow-up duration were disaggregated by recipient sex for analysis. Short-term survival was higher in males, whereas long-term survival was higher in females. Survival at 1, 5 and 10 years post-transplant was 87.43%, 73.83%, and 61.23%, respectively, in males and 86.28%, 74.19%, and 65.10%, respectively, in females ($p = 0.05$). Post-LT mortality related to previous liver disease also presented sex differences. Males had 37% increased overall mortality from acute liver failure ($p = 0.035$) and 37% from HCV-negative cirrhosis ($p < 0.001$). Females had approximately 16% increased mortality when the liver disease was HCV-positive cirrhosis ($p = 0.003$). Regarding causes of death, non-malignancy HCV+ recurrence (6.3% vs. 3.9% of patients; $p < 0.001$), was more frequently reported in females. By contrast, death because of malignancy recurrence (3.9% vs. 2.2% of patients; $p = 0.003$) and *de novo* malignancy (4.8% vs. 2.5% of patients; $p < 0.001$) were significantly more frequent in male recipients. Cardiovascular disease, renal failure, and

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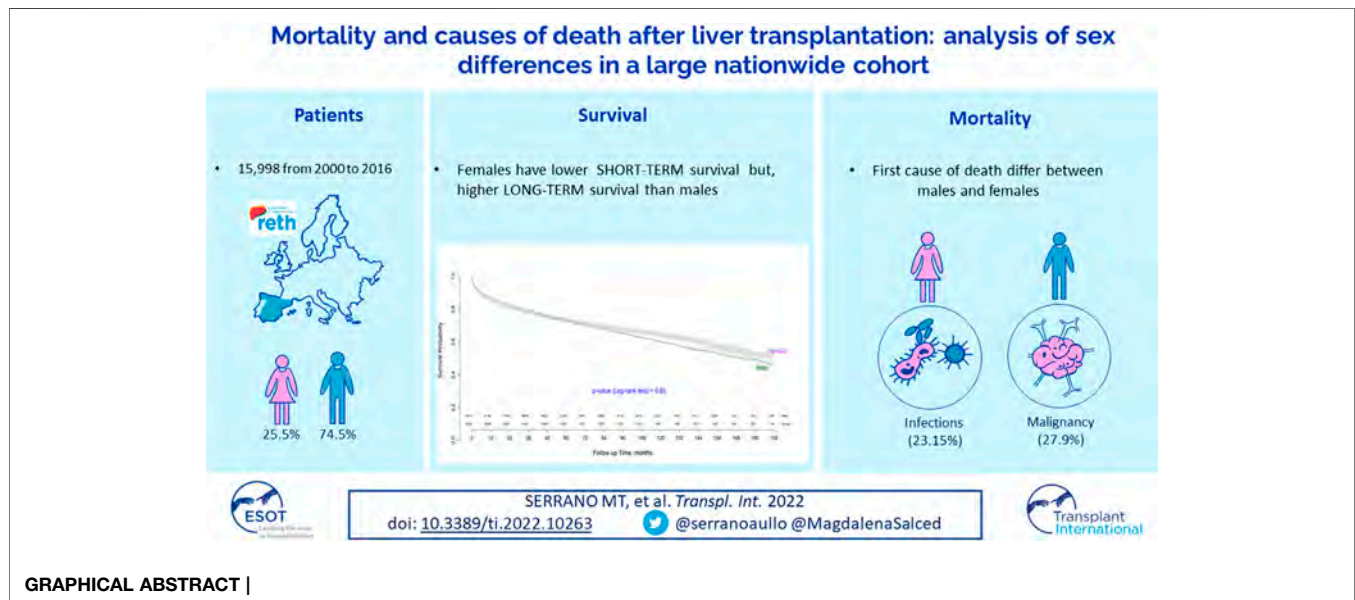
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Abbreviations: ALD, alcoholic liver disease; CVD, cardiovascular disease; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HR, hazard ratio; IQR, interquartile range; KF, kidney failure; LT, liver Transplantation; MELD, model for end-stage liver disease; MR, malignancy recurrence; NLD, *De Novo* liver disease; NM, *De Novo* malignancy; NMR, non-malignancy recurrence; OR, odds ratio; RETH, Registro Español de Trasplante Hepático.

surgical complications were similar in both. In summary, male patients have lower short-term mortality than females but higher long-term and overall mortality. In addition, the post-LT mortality risk related to previous liver disease and the causes of mortality differ between males and females.

Keywords: liver transplantation, mortality, survival, sex differences, cause of death



INTRODUCTION

Liver transplantation (LT) is the best treatment for patients with end-stage liver disease. Advances in surgical techniques and medical management of patients have markedly improved outcomes. However, short-term mortality remains at 10%–15%, and no clear improvement in long-term mortality has been achieved in the last few years (1). Interest in causes of mortality after LT and how they vary with time is increasing. Boganate et al. (2) described short-term mortality occurring mainly due to infections and circulatory disease in the first 90 days after LT. Regarding long-term mortality, Watt et al. (3) analyzed a large cohort of patients and concluded that the most frequent causes of death were graft failure, malignancy, cardiovascular disease, and kidney failure. Subsequent studies corroborated these findings and, in recent years, special programs have been launched for the early detection and prevention of cardiovascular and cancerous diseases (4,5).

Sex- and gender-disaggregated data analyses are important for reducing health inequities in medicine and many recent studies have analyzed sex and gender differences in liver disease. For example, sex imbalances in MELD predictor, waiting list mortality, and survival after LT have been studied (6–8). However, no studies have analyzed mortality after LT from the perspective of sex and gender. Gathering sex-disaggregated mortality data could provide even greater insight into

differential outcomes that are associated with biological differences and behaviors linked to gender norms. These studies are very likely essential to improving outcomes and developing sex and gender-specific short- and long-term risk prevention policies.

Sex depends on biological attributes and gender refers to social roles, behaviors and constructed identities. Given that databases typically collect only sex-related data, we will focus our analysis on sex differences. Therefore, the objective of this study was to analyze sex differences in short- and long-term mortality after LT in a large cohort of patients with long-term follow-up. In addition, we analyzed the specific causes of death from the perspective of sex recipient and calculated the cumulative incidence of mortality from specific causes in relation to the follow-up.

PATIENTS AND METHODS

Study Design and Population

We retrospectively explored data collected from the Spanish Liver Transplant Registry (Registro Español de Trasplante Hepático, RETH). RETH is a multicenter registry that recruits data from all liver transplant units in Spain with periodic auditing. The inclusion criteria were transplants performed on patients older than 16 years, from January 2000 to December 2016 with follow-

up to November 2017. Multi-visceral transplantations were excluded.

Data from the 15,998 liver transplant recipients were stratified by sex on the characteristics age of recipient, MELD, donor sex, age of donor, number of transplants, type of transplant, cold ischemia time, presence of hepatitis C virus (HCV), presence of HIV, and main liver disease (acute liver failure, cholestasis, cirrhosis HCV+, cirrhosis HCV-, liver cancer, or other causes).

Causes of death were captured in the post-transplant period, and the number of deaths in different periods were stratified by sex and cause. Causes of death were classified into the following categories: surgical complications, infections, recurrence of HCV-positive liver disease, recurrence of HCV-negative liver disease, tumor recurrence, *de novo* malignancy, circulatory disease, kidney failure, *de novo* liver disease, rejection, and others.

Statistical Analysis

Data were descriptively analyzed; continuous variables were summarized as median and interquartile range and categorical variables as absolute and relative frequencies. Significant differences by sex of the recipient were established by the Mann-Whitney or chi-squared test as appropriate.

Survival analysis was applied to analyze recipient sex as a risk factor for overall mortality. Kaplan-Meier curves and log-rank tests were used to study the differences between male and female recipients. Regarding predictors of mortality, we used univariate and multivariate Cox proportional regression models to estimate the hazard ratios and 95% confidence intervals for prognostic variables in order to predict 1 month (early), 1 year (short-term), 5 years (long-term) and overall mortality. We also performed a sub-analysis to study differences between the sexes by main disease (acute liver failure, cholestasis, cirrhosis, cirrhosis HCV-positive, cirrhosis HCV-negative, liver cancer, or other causes), sex and age of the recipient, MELD, sex and age of the donor, and the presence of HIV in the recipient in order to predict mortality. The significance of the differences between male and female recipients was determined by a test of proportions.

The causes of death at different follow-up duration (from 1 to 10 years) were analyzed overall and disaggregated by recipient sex.

In addition, the relationship between cause of mortality and main disease was analyzed using a heatmap showing the correlation between groups of both variables by sex.

Analyses were performed using R v.4.0.3 (The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Clinical characteristics of the recipients by sex are shown in **Table 1**. Our dataset consisted of 11,914 (74.5%) males and 4,069 (25.5%) females, with a longer median follow-up in females (4.6 years vs 4.2, $p = 0.009$). The median age of patients at time of LT [55 (IQR 49–61) and 56 (IQR 46–62) years, respectively] was not different. Donor sex was predominantly male (61.1%) among male recipients and female (50.2%) among female recipients ($p < 0.001$). Donation after circulatory death

was infrequent in this series (1.1% of male and 0.9% of female; $p = 0.282$). The median donor age was significantly lower for female recipients than for male recipients (55 (IQR 40–68) vs. 57 (IQR 43–69) years, $p < 0.001$) and the MELD value at LT was slightly higher for females than males (18 (IQR 12–22) vs. 17 (IQR 12–21), $p = 0.022$). A total of 860 (7.8%) men and 359 (8.8%) women received more than one liver transplant ($p = 0.003$). Regarding the urgency of the procedure, 5.8% and 12.1% of liver transplant procedures were urgent in males and female, respectively ($p < 0.001$). The median cold ischemia time was 365 min (IQR 290–471) in males and 360 min (IQR 280–470) in females ($p = 0.007$). More males than females had HIV infection (2.47% vs 1.65%; $p = 0.003$), but no differences in HCV-related liver disease were found. Differences were found in the distribution of the main liver disease (**Table 1**). The most frequent diseases were HCV-positive liver cirrhosis in women and HCV-negative liver cirrhosis in men.

Survival Analysis

Patient survival according to recipient sex showed small differences in the short- and long-term. Short-term survival was higher in males, whereas overall and long-term survival were higher in females. Male survival at 1, 5, and 10 years post-transplant was 87.43%, 73.82%, and 61.23%, respectively, while that of female patients was 86.28%, 74.20%, and 65.10%, respectively. Sex-based survival probability after transplant is depicted as a Kaplan-Meier curve in **Figure 1**, which also provides the number of patients at risk. As shown, survival curves intersect in the follow-up period and the log-rank test shown no statistical significant differences between groups ($p = 0.05$).

The analyses of recipient sex as a risk factor for mortality, or stratified by main disease are shown in **Table 2**. Female sex was found to be a risk factor for early (HR = 1.219, $p = 0.019$) and short term mortality (HR = 1.131, $p = 0.014$), while male was a risk factor (HR = 1.065, $p = 0.050$) for overall mortality, specifically when the main disease was acute liver failure (HR = 1.370, $p = 0.035$) and HCV-negative cirrhosis (HR = 1.375, $p < 0.001$). Male sex was a protective factor (HR = 0.884, $p = 0.014$), particularly when the main liver disease was HCV-positive cirrhosis (HR = 0.759; $p = 0.002$). In all other main liver diseases, no significant values were obtained. All of these results are depicted in detail in **Table 2** and the forest plot in **Figure 2**.

Regarding the interaction between sex of recipient and MELD, urgent transplantation, donor age, recipient age, cold ischemia time, and HIV positivity, results are shown in **Table 3**. MELD score was a predictive risk factor for early and overall mortality (HR = 1.030, $p = 0.014$ and HR = 1.013, $p = 0.017$, respectively), but the interaction of MELD with recipient sex was not significant, thus we did not find differences by sex in the association of MELD with mortality.

Other potential risk factors and their interaction with recipient sex were also analyzed. Recipient sex showed a significant interaction with the age of the recipient (HR = 1.004, $p = 0.011$), age of the donor (HR = 1.004, $p = 0.006$) and urgency of transplant (HR = 2.173, $p = 0.009$) on early mortality.

TABLE 1 | Clinical characteristics of the patients by sex.

Feature/sex	Male (N = 11,914)	Female (N = 4,069)	p-value
Follow-up, years			0.009
Median (IQR)	4.189 (1.170–8.832)	4.630 (1.109–9.663)	
Age of recipients, years			0.349
Median (IQR)	55 (49–61)	56 (46–62)	
MELD			0.022
Median (IQR)	17 (12–21)	18 (12–22)	
Donor sex			<0.001
Male	4,624 (38.88%)	2,022 (49.79%)	
Female	7,270 (61.12%)	2,039 (50.21%)	
Age of donors			<0.001
Median (IQR)	57 (43–69)	55 (40–68)	
Number of transplants			0.004
1	11,054 (92.78%)	3,710 (91.18%)	
2	801 (6.72%)	326 (8.01%)	
3	54 (0.45%)	31 (0.76%)	
4	5 (0.04%)	2 (0.05%)	
Type of transplant			<0.001
Elective	11,102 (94.18%)	3,551 (87.94%)	
Urgent	686 (5.82%)	487 (12.06%)	
Ischemia time, minutes			0.007
Median (IQR)	365 (290–471)	360 (280–470)	
HCV	4,349 (39.06%)	1,460 (38.37%)	0.460
HIV	294 (2.47%)	67 (1.65%)	0.003
Main disease			<0.001
Acute liver failure	273 (2.49%)	385 (10.36%)	
Cholestasis	293 (2.67%)	514 (13.83%)	
HCV-positive Cirrhosis	2,585 (23.60%)	991 (26.67%)	
HCV-negative Cirrhosis	4,149 (37.87%)	815 (21.93%)	
Liver cancer	3,314 (30.25%)	711 (19.13%)	
Other	341 (3.11%)	300 (8.07%)	

Regarding overall mortality we only found a significant interaction with recipient sex in the urgency of transplant (HR = 0.662, $p < 0.001$). All of these results are depicted in detail in **Table 3**.

In the multivariate analysis for the predictors of overall mortality (**Table 4**), recipient and donor age, number of transplants and the presence of HCV remained as independent predictors of mortality in both sexes while MELD was, prognostic factor only in the male population.

In **Table 5**, we show the results for the predictors of early mortality (1 month); number of transplants was an independent prognostic factor for mortality in both men and women. In addition recipient age, ischemia time and main disease were risk factors in the male population whereas MELD was a risk factor among females.

Mortality Analysis

Mortality and overall causes of death are shown in **Table 6**. In our cohort, a total of 3,723 (31.5%) male patients and 1,241 (30.8%)

female patients died, with important differences in the causes of mortality. The different causes of death throughout the follow-up and according to recipient sex are shown in **Table 7**. Surgical complications, infections, and cardiovascular diseases were the most frequent causes of mortality in the short-term while infections, recurrence of HCV-positive liver disease, and *de novo* malignancy were the most frequent causes of mortality in the long-term.

By sex, the main causes of death were infections and non-malignancy HCV-positive recurrence in females (23.2% and 20.7% of events) and infections and *de novo* malignancy in males (18.7% and 15.3% of events).

The cumulative relative frequency of different causes of death for male and female recipients are presented in **Figure 3**. Non malignancy HCV-positive recurrence (6.3% vs 3.9% of patients; $p < 0.001$) was more frequent in female than male recipients. By contrast, death because of malignancy recurrence (3.9% vs 2.2%; $p = 0.003$) and *de novo* malignancy (4.8% vs 2.5%; $p < 0.001$) were significantly more frequent in male recipients. In turn,

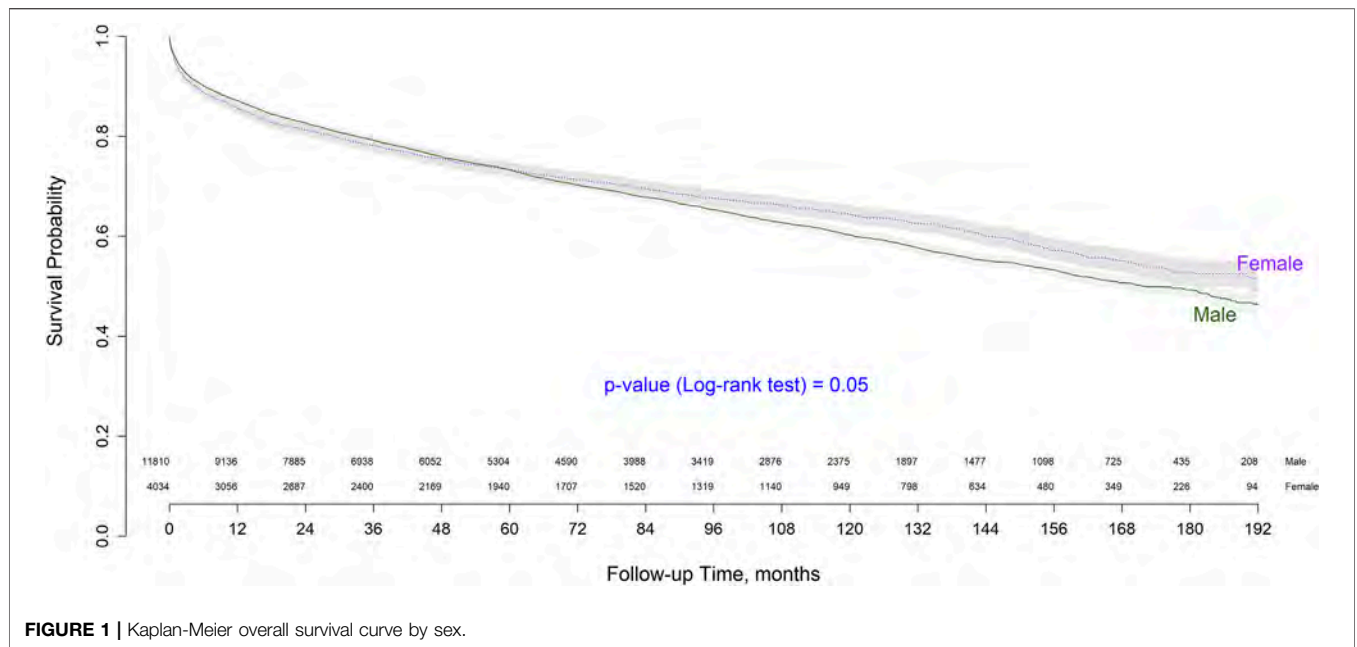


FIGURE 1 | Kaplan-Meier overall survival curve by sex.

TABLE 2 | Survival analysis according to recipient sex considering the main liver diseases.

Risk factor	1-month hazard ratio (95% CI)	p-value	1-year hazard ratio (95% CI)	p-value	5-year hazard ratio (95% CI)	p-value	Overall hazard ratio (95% CI)	p-value
Sex (male:female)	0.820 (0.695–0.968)	0.019	0.884 (0.802–0.975)	0.014	0.972 (0.902–1.046)	0.445	1.065 (1.000–1.137)	0.050
Main disease (male:female)								
Acute liver failure	1.811 (1.149–2.857)	0.011	1.511 (1.061–2.152)	0.022	1.549 (1.123–2.134)	0.008	1.370 (1.023–1.836)	0.035
Cholestasis	1.419 (0.821–2.452)	0.210	1.345 (0.879–2.058)	0.171	1.299 (0.911–1.852)	0.148	1.295 (0.954–1.758)	0.096
HCV positive-Cirrhosis	0.914 (0.657–1.272)	0.594	0.759 (0.638–0.902)	0.002	0.788 (0.692–0.897)	0.003	0.842 (0.751–0.943)	0.003
HCV negative-Cirrhosis	1.132 (0.740–1.730)	0.568	1.153 (0.902–1.472)	0.256	1.223 (1.011–1.478)	0.038	1.375 (1.176–1.607)	<0.001
Liver cancer	0.665 (0.427–1.034)	0.070	0.803 (0.637–1.010)	0.061	0.867 (0.744–1.010)	0.068	0.931 (0.815–1.065)	0.299
Other	1.623 (0.803–3.280)	0.177	1.782 (1.13–2.805)	0.013	1.796 (1.233–2.615)	0.002	1.691 (1.220–2.345)	0.016

Comparison of 1-month, 1-year, 5-year, and overall.

cardiovascular disease, renal failure, recurrence of HCV-negative liver disease and surgical complications were similarly distributed as causes of death in men and women. Importantly though, 35% of women and only in 11.7% of men with mortality due to recurrence of HCV-negative disease had been transplanted for a cholestatic disease ($p < 0.0001$).

We illustrate the relationship between causes of mortality and main diseases by sex in Figure 4. A heat map shows the differences by sex in the correlation between mortality and main disease; differences can be appreciated in the gradation of the color scale by sex.

DISCUSSION

Our study analyzed mortality data disaggregated by sex after LT in a very large sample of patients with long follow-up. We found that patient survival varies significantly according to recipient sex and the time after LT. Male patients have lower short-term mortality than

females but higher long-term and overall mortality. In addition, the post-LT mortality risk related to previous liver disease is different between male and female patients, with different causes of mortality.

Differences in Survival After Liver Transplantation

Our data show that although women have a significantly increased risk of early mortality after LT, with an overall 18% higher probability of dying in the first month after LT than males, they have better long-term survival, with males having a 6% overall higher probability of dying compared to females. A recent study based on the European Transplant Registry reported longer survival of transplanted women but did not find differences in short-term survival (9). In contrast, similar to the present study, Bruns et al. (10) reported higher mortality in women in the short-term after LT (OR 3.2), particularly among women with high MELD scores. Our results do not show a different impact of MELD according to sex on short-term mortality.

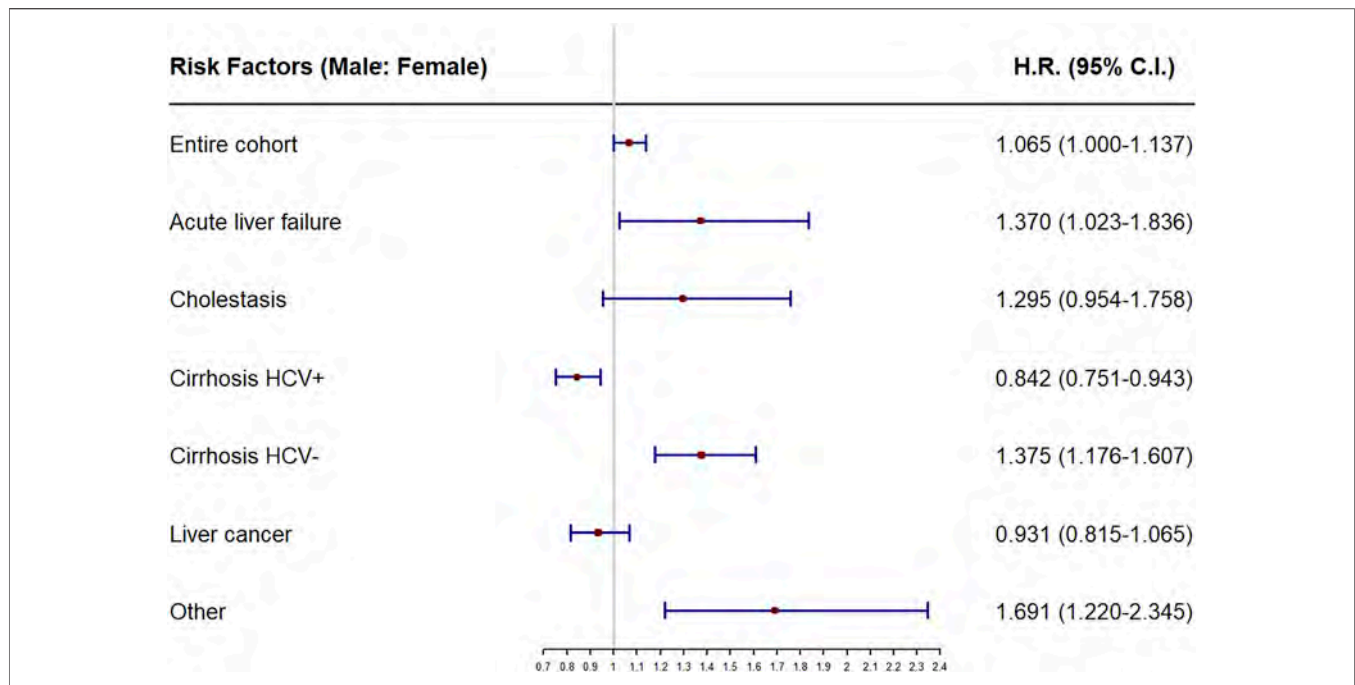


FIGURE 2 | Forest plot of recipient sex as prognostic factor of overall mortality for the entire cohort and the subcohorts stratifying by main disease. HR, hazard ratios.

TABLE 3 | Interaction of main risk factors with recipient sex.

Risk factor	1-month HR (95%CI)	p-value	1-year HR (95%CI)	p-value	5-year HR (95%CI)	p-value	Overall HR (95%CI)	p-value
Age of recipient	0.986 (0.994–1.003)	0.121	1.010 (1.006–1.015)	<0.001	1.016 (1.012–1.020)	<0.001	1.021 (1.018–1.025)	<0.001
Age of recipient: Sex (female: male)	1.004 (1.001–1.007)	0.011	1.002 (1.001–1.004)	0.007	1.001 (1.000–1.002)	0.211	0.999 (0.997–1.000)	0.106
MELD	1.030 (1.006–1.055)	0.014	1.026 (1.012–1.040)	<0.001	1.015 (1.005–1.027)	0.006	1.013 (1.001–1.024)	0.017
MELD: Sex (female: male)	1.012 (0.995–1.030)	0.164	1.003 (0.992–1.013)	0.621	0.997 (0.988–1.006)	0.473	0.998 (0.989–1.006)	0.563
Age of donor	0.998 (0.994–1.003)	0.508	1.005 (1.003–1.008)	<0.001	1.008 (1.006–1.010)	<0.001	1.010 (1.008–1.012)	<0.001
Age of donor: Sex (female: male)	1.004 (1.001–1.007)	0.006	1.002 (1.001–1.004)	0.004	1.001 (1.000–1.003)	0.045	1 (0.998–1.001)	0.633
HIV (positive: negative)	0.923 (0.508–1.678)	0.793	0.932 (0.641–1.360)	0.714	1.080 (0.882–1.323)	0.455	1.266 (1.038–1.544)	0.020
HIV = positive: Sex (female: male)	1.174 (0.328–4.209)	0.805	1.056 (0.461–2.418)	0.897	0.735 (0.445–1.217)	0.232	0.769 (0.476–1.243)	0.284
Type of transplant (urgent: elective)	0.971 (0.612–1.542)	0.900	1.123 (0.879–1.434)	0.353	1.167 (1.012–1.340)	0.029	1.571 (1.386–1.780)	<0.001
Type of transplant = urgent: Sex (female: male)	2.173 (1.219–3.876)	0.009	1.311 (0.932–1.844)	0.120	1.072 (0.875–1.313)	0.502	0.662 (0.538–0.816)	<0.001
Ischemia time	1.001 (1–1.001)	<0.001	1 (1–1.001)	0.002	1 (1–1.001)	<0.001	1.0003 (1–1.001)	<0.001
Ischemia time: Sex (female: male)	1 (1–1.001)	0.081	1 (1–1.001)	0.057	1 (0.999–1)	0.542	0.999 (0.998–1)	0.067

Each row shows the hazard ratio (HR) associated with each risk factor, and below is the HR added to that risk factor because of an interaction with recipient sex.

The multivariate analysis of risk factors for overall mortality found similar prognostic factors, with few exceptions. This may indicate that other factors not

included in our registry, such as previous comorbidities and lifestyle, likely play an important role in mortality, mainly in the long-term.

TABLE 4 | Multivariate Cox regression model of long-term mortality prognosis.

Risk Factor	Male (C-index = 0.60)		Female (C-index = 0.64)	
	Coefficient (95% CI)	p-value	Coefficient (95% CI)	p-value
Age of recipient	1.388 (1.221–1.577)	<0.001	1.263 (1.144–1.393)	<0.001
MELD	1.210 (1.080–1.356)	<0.001	—	n.s.
Age of donor	1.321 (1.158–1.507)	<0.001	1.480 (1.350–1.624)	<0.001
Number of retransplants transplants= 1	Ref	—	Ref	—
2	2.132 (1.587–2.862)	<0.001	1.746 (1.434–2.126)	<0.001
≥3	11.834 (3.977–35.209)	<0.001	5.953 (3.768–9.405)	<0.001
HCV-negative	Ref	—	ref	—
HCV-positive	1.524 (1.291–1.789)	<0.001	1.964 (1.738–2.219)	<0.001

ref, reference category; n.s., non significant.

TABLE 5 | Multivariate Cox regression model of early (1-month) mortality prognosis.

Risk Factor	Male (C-index = 0.68)		Female (C-index = 0.67)	
	Coefficient (95% CI)	p-value	Coefficient (95% CI)	p-value
Age of recipient	1.220 (1.074–1.387)	0.002	—	n.s.
MELD	—	n.s.	1.587 (1.103–2.285)	0.010
Number of retransplant transplants				
1	ref	—	Ref	—
2	3.665 (2.834–4.731)	<0.001	2.935 (1.230–7.004)	0.010
≥3	6.048 (2.831–12.923)	<0.001	36.123 (10.986–118.780)	<0.001
Ischemia time	1.226 (1.116–1,345)	<0.001	—	n.s.
Main Disease				
Acute liver failure	5.646 (3.779–8.437)	<0.001	ref	—
Cholestasis	2.661 (1.662–4.263)	<0.001	—	n.s.
HCV positive -Cirrhosis	1.451 (1.124–1.874)	0.004	—	n.s.
HCV negative-Cirrhosis	ref	—	—	n.s.
Liver cancer	0.680 (0.507–0.911)	<0.001	—	n.s.
Other	2.235 (1.380–3.620)	<0.001	—	n.s.

ref, reference category; n.s., non-significant.

TABLE 6 | Mortality and overall causes of death disaggregated by recipient sex.

Feature/Sex	Male (N = 11,914)	Female (N = 4,069)	p-value
Mortality (≤ 1 year)	1,543 (12.31%)	553 (13.71%)	0.023
Mortality (≤3 years)	2,214 (18.75%)	801 (19.86%)	0.127
Mortality (≤ 5 years)	2,692 (22.79%)	943 (23.38%)	0.461
Mortality (≤ 10 years)	2,908 (28.96%)	1,126 (27.91%)	0.212
Mortality (overall)	3,723 (31.52%)	1,241 (30.76%)	0.379
Cause of death (overall)			<0.001
Surgical complications	306 (8.37%)	104 (8.54%)	
Infection	684 (18.70%)	282 (23.15%)	
Rejection	40 (1.09%)	18 (1.48%)	
Non-malignancy recurrence HCV+	455 (12.44%)	252 (20.69%)	
Non-malignancy recurrence HCV-	62 (1.70%)	33 (2.71%)	
De novo liver disease	145 (3.96%)	48 (3.94%)	
Cardiovascular disease	370 (10.12%)	116 (9.52%)	
Malignancy recurrence	459 (12.55%)	88 (7.22%)	
De novo malignancy	561 (15.34%)	101 (8.29%)	
Renal failure	39 (1.07%)	14 (1.15%)	
Other causes	536 (14.66%)	162 (13.30%)	

Significant p values are shown in bold.

Mortality Risk Related to Previous Liver Disease

On the other hand, there are important differences in the etiology of liver diseases (11) that may explain, in part, some of the

differences in mortality. Different liver diseases have different outcomes after LT, but the role of sex in the prognosis of these diseases has not been thoroughly evaluated. Our findings demonstrate that differences exist in this context. For example,

TABLE 7 | Cause of death during follow-up by recipient sex. Data are reported as % over the entire dataset.

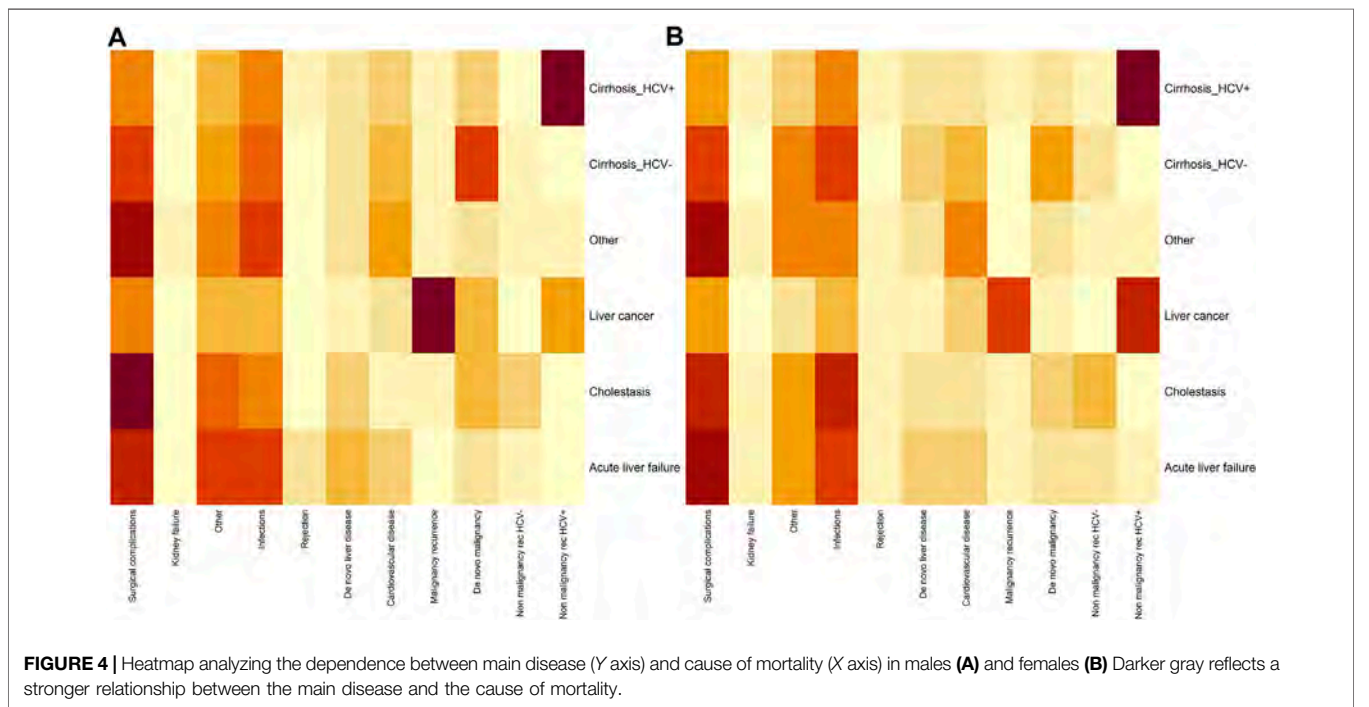
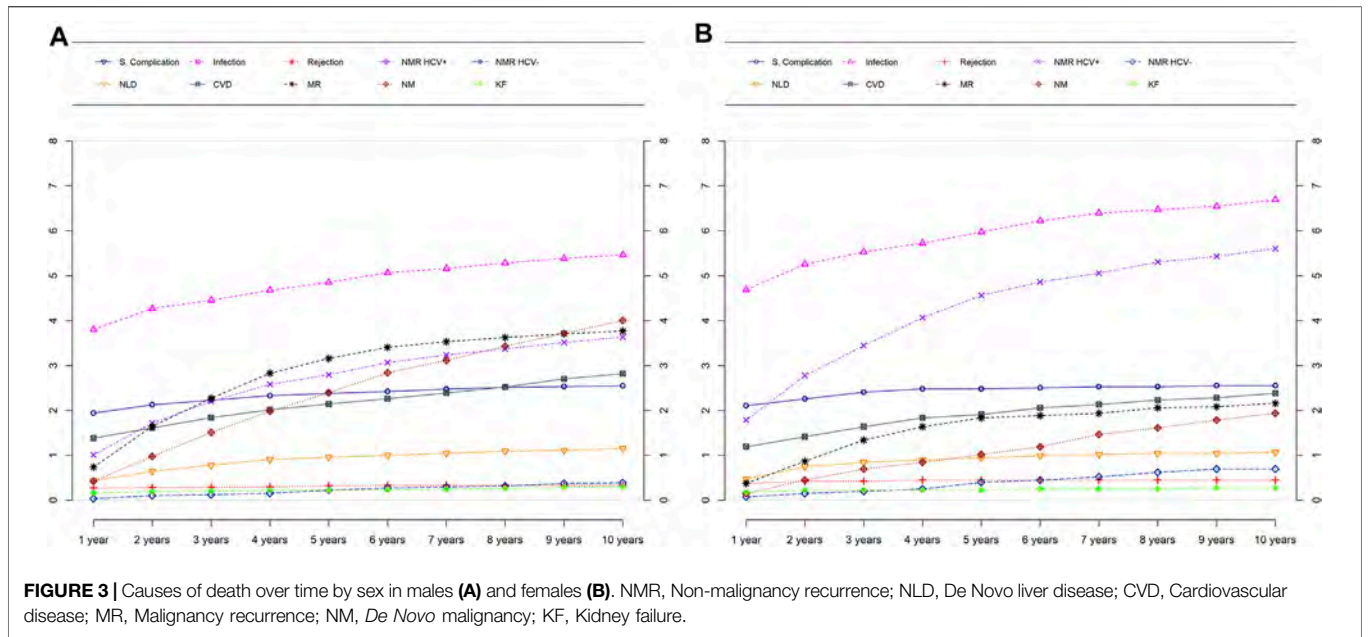
Cause of death/follow-up		1 year	3 years	5 years	10 years	Overall (>10 years)
Surgical complication	O	1.98%	2.27%	2.41%	2.55%	2.59%
	M	1.94%	2.23%	2.38%	2.55%	2.59%
	F	2.11%	2.41%	2.48%	2.55%	2.58%
	<i>p</i> -value	0.956	0.451	0.413	0.728	0.386
Infection	O	4.03%	4.73%	5.14%	5.78%	6.07%
	M	3.80%	4.45%	4.85%	5.46%	5.79%
	F	4.69%	5.53%	5.97%	6.69%	6.99%
	<i>p</i> -value	0.082	0.002	<0.001	<0.001	0.186
Rejection	O	0.30%	0.32%	0.35%	0.36%	0.37%
	M	0.27%	0.29%	0.32%	0.33%	0.34%
	F	0.37%	0.42%	0.45%	0.45%	0.45%
	<i>p</i> -value	0.526	0.233	0.228	0.287	0.205
Non-malignancy recurrence HCV+	O	1.21%	2.52%	3.24%	4.13%	4.46%
	M	1.01%	2.20%	2.79%	3.63%	3.85%
	F	1.79%	3.45%	4.56%	5.60%	6.25%
	<i>p</i> -value	<0.001	<0.001	<0.001	<0.001	<0.001
Non-malignancy recurrence HCV-	O	0.04%	0.14%	0.27%	0.47%	0.60%
	M	0.03%	0.12%	0.22%	0.39%	0.53%
	F	0.07%	0.20%	0.40%	0.69%	0.82%
	<i>p</i> -value	0.593	0.332	0.058	0.012	0.671
<i>De novo</i> liver disease	O	0.44%	0.80%	0.95%	1.13%	1.22%
	M	0.43%	0.78%	0.96%	1.15%	1.23%
	F	0.47%	0.84%	0.94%	1.07%	1.19%
	<i>p</i> -value	1.000	0.703	0.890	0.917	0.417
Cardiovascular disease	O	1.33%	1.79%	2.08%	2.71%	3.07%
	M	1.38%	1.84%	2.14%	2.82%	3.13%
	F	1.19%	1.64%	1.91%	2.38%	2.88%
	<i>p</i> -value	0.2%	0.517%	0.707%	0.295%	1
Malignancy recurrence	O	0.64%	2.03%	2.82%	3.36%	3.45%
	M	0.74%	2.27%	3.16%	3.77%	3.89%
	F	0.37%	1.34%	1.83%	2.16%	2.18%
	<i>p</i> -value	0.007	<0.001	<0.001	<0.001	0.003
<i>De novo</i> malignancy	O	0.35%	1.30%	2.05%	3.48%	4.18%
	M	0.42%	1.51%	2.40%	4.01%	4.75%
	F	0.15%	0.69%	1.02%	1.93%	2.50%
	<i>p</i> -value	0.010	<0.001	<0.001	<0.001	<0.001
Renal failure	O	0.16%	0.21%	0.22%	0.29%	0.34%
	M	0.16%	0.20%	0.22%	0.30%	0.33%
	F	0.17%	0.22%	0.22%	0.27%	0.35%
	<i>p</i> -value	1.000	0.934	1.000	1.000	0.246
Other causes	O	1.97%	2.57%	3.00%	3.95%	4.41%
	M	1.97%	2.56%	2.98%	4.09%	4.54%
	F	1.96%	2.60%	3.05%	3.55%	4.02%
	<i>p</i> -value	0.589	0.793	0.454	0.307	0.506

O, overall population; M, male population; F, female population. Significant *p* values are shown in bold.

males have 50% increased 1-year mortality when LT is performed for acute liver failure and 37% increased overall mortality when it is due to HCV-negative cirrhosis, whereas females have approximately 15% increased overall mortality when the liver disease is HCV-positive cirrhosis. This finding was expected because more severe HCV recurrence and related mortality has been described in women after LT (12–14). However, HCV-related outcomes, including LT, have changed dramatically since the emergence of new antivirals (15). Data collection in our study extended until 2017, so the effect of these drugs on survival could not be observed, but it will undoubtedly be demonstrated in the analysis of subsequent years.

Conversely, outcomes of HCV-negative cirrhosis are worse in male than in female patients. In Spain, the leading etiology in

patients with HCV-negative cirrhosis is alcohol related liver disease (ALD) (16). Tobacco use, sedentary lifestyle, and unhealthy diet are often associated with alcohol consumption, and all of them are risk factors for both cardiovascular and cancer mortality. ALD patients have been shown to have excess all-cause mortality, mainly mortality related to cardiovascular disease and cancer (17), and this excess mortality is higher in males than in females. In a large Danish cohort, Sallhman et al. (18) found a significant excess of different cancers in males with ALD, with an overall standardized incidence ratio of 3.01 in males and 2.33 in females ($p < 0.001$). Other findings, such as higher mortality risk in transplanted males due to acute liver failure, are more unexpected. Although acute liver failure affects females more



than males and it is also associated with lower short-term survival after LT, men have a greater probability of dying when being transplanted because of this indication. Several studies have investigated the outcomes of LT in patients with acute liver failure in Western countries (19,20), but only Nephew et al. analyzed mortality according to recipient sex in the UNOS database (21). They found differences in 1-year mortality, which was no longer significant when recipient age and underlying etiology were added to the model.

Our data combined with prior studies demonstrate that mortality risk after LT related to different liver diseases varies according to sex. This is an important finding that should be considered when designing post-LT survival models.

Causes of Mortality

Though causes of mortality have been described throughout the transplant follow-up, no sex-disaggregated analysis has been published previously. As in the non-transplanted population,

there are important differences in the causes of mortality between men and women. Overall, infections are the most frequent cause of mortality in males and females, though they are significantly higher in females.

In our cohort, the main causes of mortality within the first year after transplantation were infections and surgical complications in both sexes. Although females were more frequently retransplanted, mortality due to surgical complications was similar in both. In contrast, death related to infections was significantly more common in females than in males and was evenly distributed across the different causes of liver disease, except for liver cancer. This may be explained by the clinical situation at the time of LT, crucial in explaining mortality from infections in the short-term (22). Differences in the prevalence and severity of infections between males and females vary depending on type of infection (23). Women have higher mortality in influenza A outbreaks (23), whereas male sex is a risk factor for developing severe SARS Cov-2 infection or sepsis (24,25). It seems that both immunological and hormonal factors play a role in these differences.

More differences were found in short-term mortality. Mortality because of recurrence of HCV infection was significantly higher in females, and mortality due to recurrence of hepatocarcinoma and *de novo* cancer was more frequent in males.

These differences increased with follow-up, so that in the long-term (>10 years), mortality due to infections, including HCV recurrence, was 40% higher in women than in men and mortality due to *de novo* neoplasms was almost twice as high in men as in women. Though the latter accounted for more than 15% of mortality in males, it accounted for only 8.3% in females. When we added mortality because of tumor recurrence, cancer was the leading cause of overall mortality in males, accounting for 27.9% of events and a cumulative relative frequency of 8.6% of patients, but it was the third leading cause of death in women (15.5% of events) and approximately half of the cumulative relative frequency. Hepatocellular carcinoma (HCC) is overrepresented in males, resulting in a higher number of deaths because of HCC recurrence among this population. Nevertheless, higher recurrence risk was also recently described among males. Cullaro et al. found an independent effect of sex on the risk of HCC recurrence post-LT (26). Mortality because of liver cancer recurrence increases in the first 6 years after LT and subsequently stabilizes, whereas mortality due to *de novo* cancer follows an upward trend over time.

Circulatory diseases and kidney disease are important, but not different causes of death after LT in men and women. Approximately 3% of patients globally die from circulatory disease after LT and slightly more than a third of them die in the first year after LT. A careful analysis of cardiovascular risk factors before transplantation is mandatory, as detecting patients at risk of early mortality from circulatory disease is important to avoid futile transplantation.

As expected, we found an association between some causes of mortality and certain liver diseases prior to LT. For women, the strongest association was found between acute liver failure and mortality due to surgical complications. HCV cirrhosis was associated with mortality due to non-tumor recurrence in both men and women. However, when the transplant was due to liver cancer, the strongest association was found between mortality

due to tumor recurrence in men and non-tumor recurrence in women.

Mortality in LT patients is mainly related to immunosuppression. Both infections and cancer, two sides of the same coin, are related to immunosuppressive treatment. However, our data show that they are distributed differently in both sexes. Though infections result in higher mortality among females, neoplasms affect predominantly males. Knowledge of these differences is important to improve the management of patients in both the short- and long-term. In recent years, special immunosuppression protocols and surveillance programs have been proposed for the prevention or early detection of *de novo* cancer (5, 27). These results could be important to designing suitable and more cost-effective protocols according to the sex of the recipient.

Finally, although it was not the objective of our research, the imbalance found between male and female transplant recipients is remarkable. Many end-stage liver diseases affect predominantly males, and sex differences among transplant patients have been increasing over the years. From 2000 to 2016, only 25.5% of LT patients were female. Sex differences in our registry are higher than described in other registries (9,28). These differences could reflect disparities in listing patients or in waiting-list mortality (8, 29,30). Further studies are needed to clarify this. LT is a medical process strongly influenced by sex and gender issues such that disaggregated analyses at all levels of the procedure should be mandatory to avoid disparities.

The limitations of the present study are mainly derived from its retrospective nature. Although the data entered in RETH were standardized and periodically audited, the information, as well as the consistency between sites, cannot be guaranteed. As with most studies using data from record collections, the current study may have been susceptible to practice variations and incompletely reported covariates. In addition, the definitions for causes of death may vary due to different interpretations between different teams. However, the data source is a national registry with a large number of cases that allows robust statistical analyses of a nationally representative dataset. On the other hand, due to the difficulty of national registries to rapidly adapt to changing epidemiological scenarios, we have not been able to analyze the impact of new diseases such as non-alcoholic steatohepatitis (NASH) on post-LT prognosis and causes of death. Thus, sex differences in this increasingly important disease could not be analyzed.

In summary, short- and long-term mortality and their causes are different between male and female liver transplant recipients. The risk of mortality after LT associated with different liver diseases also varies by sex. These findings are important and highlight the need for sex and gender-disaggregated analyses of clinical data.

SPANISH LIVER TRANSPLANT REGISTRY REPRESENTATIVES

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SS, LME and MS. Drafting the article: MTS, SS, LME, MS, SL and LC. Critical revision of the article: MB, CF, GS-A, JN, GR. Final approval of the version to be published: MTS, SS, LME, MB, CF, SL, LC, GS-A, JN, GR and MS.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: Are datasets belonging to Spanish Liver Transplant Society and managed and administered by the National Transplant Organization. Requests to access these datasets should be directed to www.ont.es.

AUTHOR CONTRIBUTIONS

Conception or design of the work: MTS, SS, LME and MS. Data collection: SL and LC. Data analysis and interpretation: MTS,

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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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Postoperative Trapped Lung After Orthotopic Liver Transplantation is a Predictor of Increased Mortality

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Pleural effusions are a common complication of orthotopic liver transplantation (OLT), and chronic post-OLT pleural effusions have been associated with worse outcomes. Furthermore, “trapped lung” (TL), defined as a restrictive fibrous visceral pleural peel preventing lung re-expansion, may have prognostic significance. We performed a retrospective analysis of adult OLT recipients over a 9-year period at UCLA Medical Center. Post-OLT patients with persistent pleural effusions, defined by the presence of pleural fluid requiring drainage one to 12 months after OLT, were included for analysis. Outcomes for patients with and without TL were compared using univariate and multivariate analysis. Of the 1722 patients who underwent OLT, 117 (7%) patients met our criteria for persistent postoperative pleural effusion, and the incidence of TL was 21.4% (25/117). Compared to patients without TL, those with TL required more surgical pleural procedures (OR 59.8, 95%CI 19.7–181.4, $p < 0.001$), spent more days in the hospital (IRR 1.56, 95%CI 1.09–2.23, $p = 0.015$), and had a higher risk of mortality (HR 2.47, 95%CI 1.59–3.82, $p < 0.001$) following transplant. In sum, we found that post-OLT TL was associated with higher morbidity, mortality, and healthcare utilization. Future prospective investigation is warranted to further clarify the risk factors for developing postoperative pleural effusions and TL.

Keywords: trapped lung, hepatic hydrothorax, liver transplantation, pleural effusions, pneumothorax-ex-vacuo

Abbreviations: ESLD, end-stage liver disease; HR, hazard ratio; IPTW, inverse probability of treatment weighting; IRR, incidence rate ratio; LDH, lactate dehydrogenase; MELD, Model for End-Stage Liver Disease; OLT, orthotopic liver transplantation; OR, odds ratio; TL, trapped lung; VATS, video-assisted thoracoscopic surgery.

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INTRODUCTION

Orthotopic liver transplantation (OLT) is the only definitive treatment for end-stage liver disease (ESLD), but even with rigorous patient and donor selection criteria, most patients experience at least one complication post-transplant (1). With improvements in the understanding of pre-transplant risk factors and post-transplant clinical course, 1-year survival rates have steadily improved for liver transplant recipients over the last 3 decades (2, 3).

Pulmonary complications after OLT remain common and are associated with increased morbidity and mortality (4–8). Postoperative pleural effusions are among the most frequently recognized pulmonary complications of OLT, occurring in 39–95% of all patients (4, 6, 9–10). Retrospective chart reviews reveal that most of these pleural effusions are clinically insignificant and resolve quickly after OLT. However, up to 25% persist after transplantation, often in patients with complicated postoperative courses (7–12). None of these small-scale studies, however, have specifically described the etiology, pathogenesis, and clinical impact of persistent post-OLT pleural effusions or identified risk factors for poor outcomes in this population.

Trapped lung (TL) is a complication of persistent pleural effusion defined by chronically atelectatic lung that is unable to expand due to the development of a fibrous visceral pleural peel (10). The diagnosis of TL is made based on radiographic and clinical criteria once drainage of the pleural fluid has been attempted and demonstrates pneumothorax ex-vacuo and thickened visceral pleura on imaging. Manometry, if performed, confirms a sharp decline in pleural pressure with minimal fluid drainage. Exudative effusions resulting from inflammatory and infectious conditions have been identified as a risk factor for the development of fibrinous change in the visceral pleura (13–14). Trapped lung is less commonly associated with ESLD, although a few small studies have shown it to be a complication of hepatic hydrothorax in a small subset of patients (15–17). When it does occur, trapped lung and pleural disease have been shown to be indicative of poor outcomes in liver transplant recipients (15, 18).

To our knowledge, this is the largest study to date to describe the demographic, clinical, biochemical, and radiological characteristics of patients with persistent pleural effusions after OLT. Furthermore, given existing data to suggest an association with adverse outcomes in patients with pre-transplant trapped lung, we also sought to characterize and evaluate clinical outcomes with postoperative trapped lung.

PATIENTS AND METHODS

Study Population

We performed a retrospective chart review of the 1722 patients who received an OLT between January 2006 and October 2015 at Ronald Reagan UCLA Medical Center, a high-volume quaternary liver transplant center performing over 150 transplants annually. Patients were included if they were 18 years or older at the time of transplantation and had a pleural effusion that was present

between one and 12 months postoperatively. To be considered clinically relevant, only effusions assessed by invasive approach such as thoracentesis or surgical intervention qualified for inclusion in the study. Two subgroups in this cohort were defined as those with trapped lung (TL) present and those with only persistent pleural effusions in the first year post-OLT. Data collection was approved by the institution's internal review board (IRB #14-000365) and was performed in accordance with the 2000 Declaration of Helsinki and the Declaration of Istanbul 2008.

Data Collection

Patient data were entered into a secure database. Patient demographics, including age, sex, transplant Model for End-Stage Liver Disease (MELD) score, and clinical characteristics were recorded. Pleural fluid analysis including cell count and differential, microbiology data, lactate dehydrogenase (LDH) and protein concentration, was collected for the most proximate pleural fluid sampling both before (if available) and after transplantation. Corresponding proximate serum LDH and protein were recorded as well. A transudative effusion was defined by Light's criteria, which required that the three following criteria were met: serum to pleural protein ratio less than 0.5, serum to pleural LDH ratio less than 0.6, and pleural LDH less than two thirds of the upper limit of the normal serum LDH assay (19). Effusions were classified as exudative if they failed to meet one or more of the prespecified pleural fluid criteria.

All chest imaging was reviewed. Patients' pre- and post-transplantation pleural effusions were described based on chest imaging. TL was defined by radiological evidence of thickened pleural rind and lack of expansion after drainage (resulting in a pneumothorax ex-vacuo or hydropneumothorax). Additional clinical data, such as a pulmonologist's clinical documentation and assessment, was also reviewed to ensure concordance with imaging findings.

Both preoperative and postoperative pleural interventions, including thoracentesis, chest tube thoracostomy, and video-assisted thoracoscopic surgery (VATS) decortication were also recorded. Ventilator and hemodialysis dependence pre-OLT were defined as requiring mechanical ventilation or dialysis immediately preceding transplant. Ventilator and hemodialysis dependence post-OLT were defined as requiring these interventions for longer than 2 weeks post-OLT.

In addition to descriptive data of the entire cohort, two primary clinical outcomes were identified: mortality and total number of hospital days in the first year following transplantation. Secondary outcomes included mechanical ventilation for a duration of greater than 2 weeks post-transplantation and the need for multiple pleural procedures including thoracentesis, chest tube placement or VATS decortication and/or pleurodesis.

Statistical Analysis

Descriptive statistics were reported for the full study sample. Comparisons between TL and non-TL pleural effusion cases were performed using two-sample t-tests for age and transplant MELD, and using chi-squared or Fisher's exact tests as appropriate for the other qualitative characteristics. Analysis of

TABLE 1 | Baseline characteristics of the study cohort.

	Original Cohort Total	Propensity Score Weighted Cohort		
	(n = 117)	Trapped lung	No trapped lung	p-value
Demographics				
Age, mean ± SD (years)	56 ± 9.4	56 ± 14	56 ± 9	0.742
Female	46/117 (39%)	31%	37%	0.381
Comorbidities				
Diabetes Mellitus	56/114 (49%)	49%	46%	0.697
Hepatopulmonary Syndrome	16/115 (14%)	17%	12%	0.280
Portopulmonary Hypertension ^b	3/115 (3%)	5%	2%	0.298
Etiology of Liver Disease				
Alcoholic Cirrhosis	34/116 (29%)	17%	34%	0.005
HBV Cirrhosis	14/115 (12%)	19%	12%	0.116
HCV Cirrhosis	56/117 (48%)	56%	50%	0.381
HCC	43/114 (38%)	53%	38%	0.023
Preoperative Characteristics				
Transplant MELD, mean ± SD	34 ± 7.8	33 ± 15	34 ± 9	0.573
Pre-OLT Effusion Present	74/117 (63%)	69%	64%	0.481
Pre-OLT Exudate ^a	5/16 (31%)	43%	38%	0.760
Pre-OLT Empyema ^a	8/34 (24%)	24%	28%	0.677
Pre-OLT Thora	26/112 (23%)	25%	24%	0.835
Pre-OLT Chest Tube ^b	10/111 (9%)	20%	3%	<0.001
Pre-OLT Trapped Lung ^b	5/117 (4%)	10%	0%	<0.001
Pre-OLT HD Dependence	57/117 (49%)	46%	45%	0.836
Pre-OLT Vent Dependence	35/117 (30%)	36%	28%	0.253
Admitted from Home	43/117 (37%)	39%	39%	0.985
Intraop Chest Tube Placement ^b	5/116 (4%)	16%	0%	<0.001
Postoperative Characteristics				
Post-OLT Exudate ^a	90/100 (90%)	95%	86%	0.031
Post-OLT Empyema ^a	10/103 (10%)	24%	3%	<0.001
Post-OLT HD Dependence	53/116 (46%)	42%	46%	0.565

Characteristics are compared by subgroup (trapped lung vs. no trapped lung) and shown after inverse probability of treatment weighting. Covariates chosen to create the propensity score model are shown in bold. Variables which could not be included in the model due to data constraints are indicated with superscripts. p-values reaching significance are also bolded. HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HD, hemodialysis; intraop = intraoperative; MELD, Model for End-Stage Liver Disease; OLT, orthotopic liver transplantation; thora = thoracostomy; vent = ventilator.

^aMore than 10 observations missing.

^bLess than 5% event rate observed.

study endpoints was performed using a propensity score approach, based on inverse probability of treatment weighting (IPTW). Age, gender and transplant MELD were pre-specified for inclusion in the propensity score model. Other variables were selected based on a significance threshold of 0.05, having no more than 10 observations missing, and having event rates of at least 5% in both the TL and non-TL cohorts. Propensity scores were estimated using a logistic regression model. After weighting by propensity scores, the cohorts were again compared using the two-sample tests described above.

Analysis of the mortality endpoint was performed using a Cox proportional hazards model, while analysis of number of days hospitalized in the year following transplant was performed using a negative binomial regression model. All other outcomes were analyzed using logistic regression models with Firth's penalized likelihood method. The primary model term was trapped lung (yes versus no). Each analysis was performed once using the unweighted cohort and once using the weighted cohort. Variables which remained or became significant after IPTW, and which had sufficient non-missing observations and event rates, were included as controls in the weighted versions of the models. The two-stage approach was chosen to most accurately model the

effect of TL on outcomes and adjust for pre-treatment differences between TL and non-TL pleural effusion patients in the setting of a potentially large number of confounders and small number of events, which would avoid overfitting the regression models.

Comparisons between TL and non-TL pleural effusion patients were reported in terms of hazard ratios (HR), incidence rate ratios (IRR), and odds ratios (OR) respectively, along with 95% confidence intervals. p-values less than 0.05 were considered statistically significant. A Kaplan-Meier diagram for the mortality endpoint was produced using R v. 3.6.2 (<http://www.r-project.org/>). All other analyses were performed using SAS v. 9.4 (SAS Institute Inc., Cary, NC).

RESULTS

Characteristics of the Study Cohort

Of the 1722 patients who received a transplant during the study period, a total of 117 (7%) adult patients had a persistent pleural effusion requiring invasive management within the first year after OLT. Baseline characteristics of the study cohort are shown in the first column of **Table 1**. The mean age was 56 ± 9.4 years and a

minority of the patients were female (46/117, 39%). Medical comorbidities were common, including diabetes mellitus (56/114, 49%) and hepatopulmonary syndrome (16/115, 14%). Diverse etiologies of liver disease were represented, including alcoholic cirrhosis, hepatitis B, hepatitis C, and hepatocellular carcinoma. Transplant MELD scores indicated severe liver disease with a mean score of 34 ± 7.8 . Effusions were often present by radiographic criteria prior to transplantation (74/117, 63%). Unfortunately, pleural fluid sampling was only performed in 26 of the patients, making analysis of the preoperative pleural fluid limited. Within this limitation, spontaneous bacterial peritonitis was also common pre-operatively (31/105, 30%), although only 9/105 (9%) had documented positive ascites fluid culture and data was not available for 12 patients. A total of 57/117 (49%) patients were dialysis dependent and 35/117 (30%) were ventilator dependent prior to transplant, while 43/117 (37%) were admitted from home. Postoperatively, the majority of effusions were exudative (90/100, 90%) and need for hemodialysis was common (53/116, 46%).

Trapped Lung Cohort Characteristics and Outcomes

The incidence of TL in those with persistent pleural effusion after OLT was 21.4% (25/117). Five (4%) of these patients had evidence of trapped lung prior to transplant. Data are stratified by the presence of TL after OLT and shown after IPTW in **Table 1** (data before and after IPTW are presented in the **Supplementary Table S1**). Covariates chosen for the model include mean age, gender, transplant MELD, presence of an effusion pre-OLT, and preoperative thoracentesis. Mean age, gender, and transplant MELD were balanced in the original cohort and did not differ significantly after IPTW. In comparison to those without TL, patients with TL were more likely to have preoperative effusions (88% vs. 57%, $p = 0.008$) and more likely to have undergone preoperative thoracentesis (54% vs. 15%, $p < 0.001$), although these findings did not retain significance after IPTW (**Supplementary Table S1**).

Other clinical characteristics that were not included in the IPTW model are also stratified by the presence of TL (**Table 1**). None of these differences attained significance after IPTW. Compared to patients without postoperative TL, patients with postoperative TL were more likely to have had preoperative chest tube placement (35% vs. 2%, $p < 0.001$) and carry a preoperative diagnosis of trapped lung (20% vs. 0%, $p < 0.001$) (**Supplementary Table S1**). Additionally, infectious complications were common, and 56/117 (48%) of all patients had radiographic concern for pneumonia post-OLT, although there was no significant difference between those with and without TL (56% vs. 46%, $p = 0.489$). Among those with radiographic concern for pneumonia, only 21/117 (18%) had clinical suspicion of pneumonia, and again there was no difference between TL and non-TL cohorts (16% vs. 18.5%, $p = 1.00$). Lastly, post-OLT sepsis was present in 30/117 (26%) and not significantly different between groups (32% vs. 24%, $p = 0.574$).

Covariates which became unbalanced after IPTW include the presence of alcoholic cirrhosis, hepatocellular carcinoma, and post-OLT exudative effusion, and these were included in regression analyses as covariates. Covariates which were significantly different before and after IPTW and could not be included in the regression analysis due to missing observations and/or low event rates included presence of chest tube or thoracic surgical intervention pre-OLT, pre-OLT trapped lung, and post-OLT empyema.

Postoperative Clinical Outcomes

The overall 1-year survival for patients with persistent pleural effusion was 78% (91/117), and compared to simple pleural effusion, those with TL had a significantly higher risk of mortality (HR 2.47, 95%CI 1.59–3.82, $p < 0.001$) (**Table 2** and **Figure 1**). The number of hospital days in the first year post-transplant was also significantly higher for the TL group (IRR 1.56, 95%CI 1.09–2.23, $p = 0.015$). **Figure 2** displays these differences in hospitalized days between the two groups as a histogram. These differences were significant both before and after adjustment for differences in baseline characteristics between the groups (**Supplementary Table S2**).

The TL group also experienced more thoracic surgical interventions (OR 59.8, 95%CI 19.7–181.4, $p < 0.001$) and a higher rate of pleural procedures overall post-OLT (OR 26.8, 95%CI 6.7–107.6, $p < 0.001$). Both of these outcomes were significant before and after adjustment for baseline characteristics as shown in **Table 2** and **Supplementary Table S2**. Ventilator dependence did not differ between the two groups (OR 1.01, 95%CI 0.54–1.89, $p = 0.966$).

DISCUSSION

Pulmonary complications following OLT are frequent and are associated with increased morbidity and mortality (4–6, 8, 11). While preoperative and early postoperative pleural effusions represent well-described and frequent complications of end-stage liver disease, published evidence about the clinical relevance and outcomes in patients with persistent pleural effusions after an OLT is scant (7,8, 12, 16, 17, 20). In this retrospective analysis, we investigated the clinical relevance of persistent pleural effusions with a specific focus on the risk factors for and complications related to TL, which we found is a common complication of persistent postoperative pleural effusions.

Prior work suggests that most pleural effusions following liver transplantation resolve within 1–3 months (8, 20), but the significance of the remaining persistent effusions is less clear. Further, effusions complicated by TL may portend a different prognosis in comparison to those without TL. For example, in a report of Shirali et al., pleural complications represented the major indication for post-OLT thoracic surgery interventions and were associated with poor outcomes. In the same report, the majority of patients who required thoracic intervention had trapped lung, which was found to be a significant predictor of overall mortality with a 1-year survival of less than 40% (18). In patients with persistent pleural effusion after OLT who undergo

TABLE 2 | Clinical outcomes of the study cohort.

	Cohort after IPTW	
	Estimate (95% CI)	p-value
Mortality (HR)	2.47 (1.59, 3.82)	<0.001
Number of Hospitalized Days in 1st Year Post-Transplant (IRR)	1.56 (1.09, 2.23)	0.015
Thoracic Surgical Interventions (OR)	59.8 (19.7, 181.4)	<0.001
Multiple Pleural Procedures Post-OLT (OR)	26.8 (6.7, 107.6)	<0.001
Ventilator Dependence >2 Weeks (OR)	1.01 (0.54, 1.89)	0.966

Clinical outcomes of the study cohort stratified by the presence of trapped lung after orthotopic liver transplantation and shown after inverse probability of treatment weighting. p-values reaching significance are bolded.

HR, hazard ratio; IRR, incident rate ratio; OR, odds ratio.

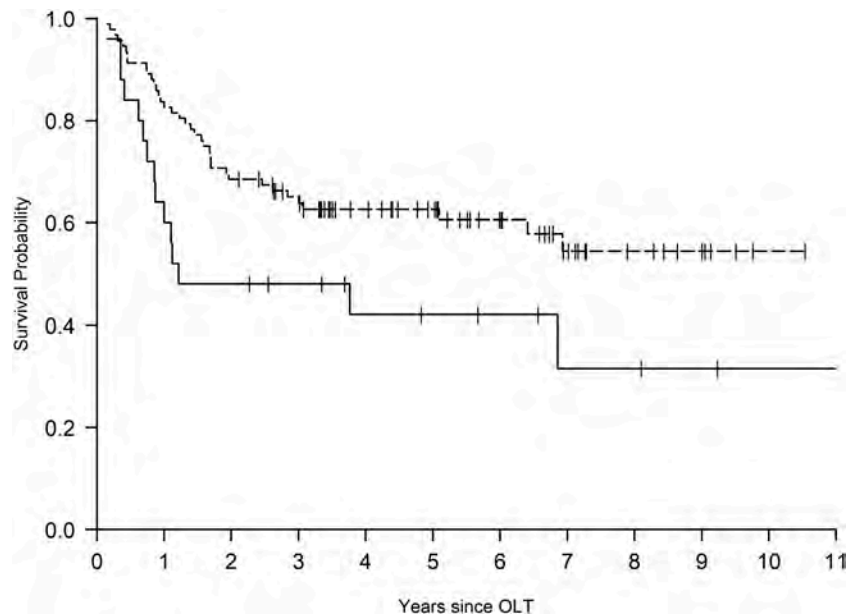


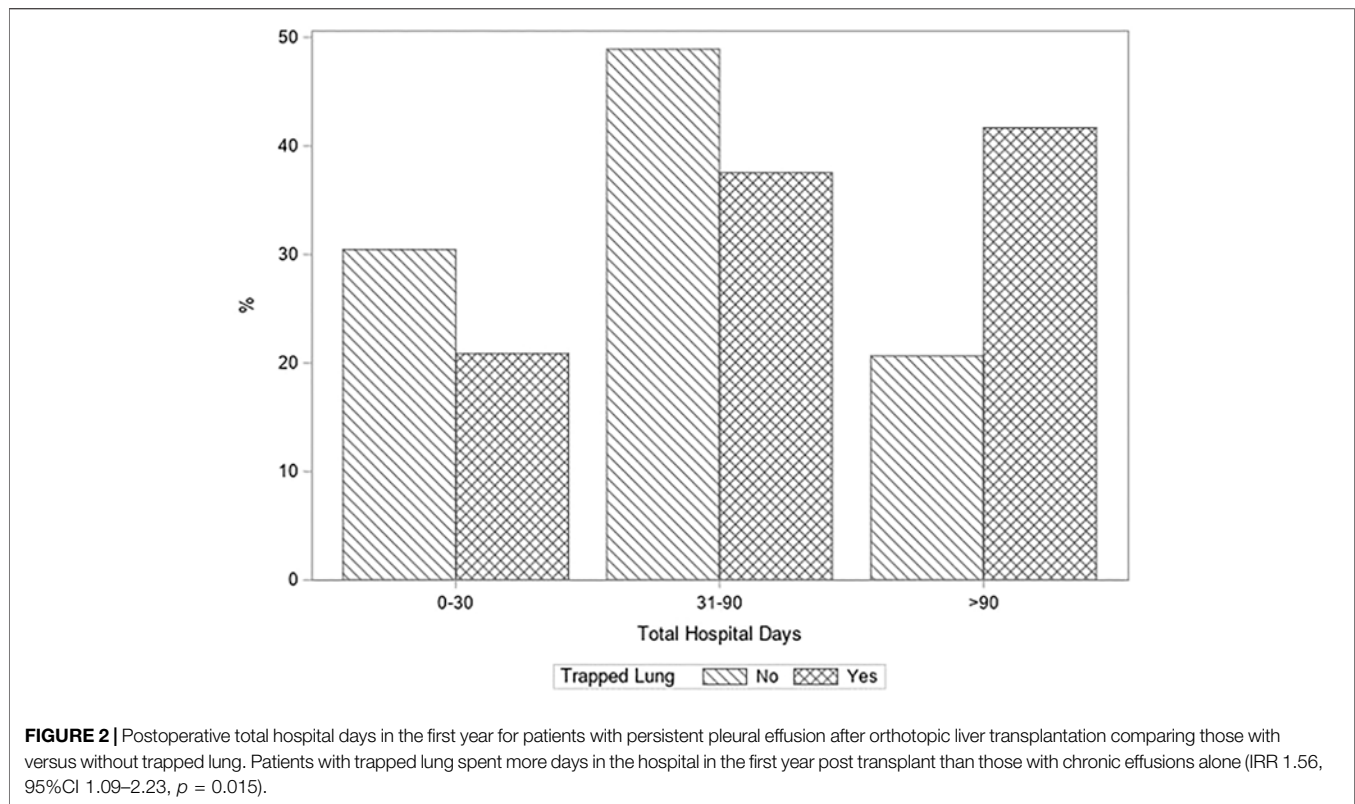
FIGURE 1 | Survival probability of patients with persistent pleural effusion after orthotopic liver transplantation with versus without trapped lung. Kaplan-Meier survival curves demonstrating survival probability based on the presence or absence of postoperative trapped lung. Patients with trapped lung (solid line) have decreased probability of survival compared to patients with chronic postoperative effusion alone (dashed line) (HR 2.47, 95%CI 1.59–3.82, $p < 0.001$).

diagnostic thoracentesis, the presence of trapped lung may lead to a diagnosis of post-procedural pneumothorax ex-vacuo. This radiographic finding is often interpreted as a procedural complication caused by direct lung injury, rather than an intrinsic lack of lung re-expansion due to a pleural rind. This misinterpretation can lead to further pleural interventions, including tube thoracostomy and surgical intervention, which themselves carry additional risk (17).

In our cohort, we showed that compared to effusions without TL, the presence of TL postoperatively was associated with increased mortality and morbidity, as evidenced by total hospital days, surgical intervention, and pleural procedures within the first postoperative year. To understand why these patients had worse outcomes, we first examined the risk factors for development of trapped lung.

We found that patients with persistent effusions were often diabetic, dialysis-dependent, and had pleural effusion prior to

transplant, and qualitatively it appears that spontaneous bacterial peritonitis was also common, together suggesting that there may be some level of chronic systemic illness or inflammatory process not captured by the MELD score that may increase the risk for postoperative effusion. Moreover, trapped lung has been typically associated with inflammatory pleural conditions, such as complex parapneumonic effusions, empyema, or malignant effusions (14). Hepatic hydrothorax is typically thought of as a bland, transudative fluid, resulting from the changes in the hydrostatic and oncotic pressure gradients that occur commonly with portal hypertension and cirrhosis (21–23). However, trapped lung has been described as a rare complication in patients with ESLD and hepatic hydrothorax as well (15–17). Our data reveal that pleural effusions among the TL cohort were nearly all exudative by Light's criteria and one quarter were due to empyema. Also, although some of our data is limited, there is a suggestion that there was a notable prevalence



of associated pneumonia and sepsis further supporting the role of inflammation in the pathophysiology and development of pleural effusions and TL after OLT.

Additionally, those with TL more often had pleural effusions and pleural interventions prior to transplant, suggesting a preexisting complex pleural space even before transplant. In fact, one fifth of patients also had evidence of TL prior to transplant. We suspect that ongoing repeated pleural interventions can introduce trauma and consequent pleural inflammation, allowing for formation of a thick pleural rind. The consistent presence of the pleural effusion then keeps the lung in the atelectatic position, with the resultant fibrous rind restricting lung expansion on subsequent drainage procedures.

Taken together, this suggests that there are two possible contributors to poor outcomes in postoperative TL. At the local level, mechanical and functional impairment of the pleura, as evidenced by the presence of an exudative pleural effusion, leads to an increased risk of development of trapped lung. The TL itself or the presence of pleural infection results in the need for additional surgical interventions, which is associated with significant risk in this patient population (18). Additionally, TL likely also represents the consequence of systemic inflammatory pathophysiologic processes that lead to exudative effusion, empyema, and systemic illness, all of which put the patient at added risk for poor outcomes.

The optimal management of trapped lung, when found, is not clear. However, taken together with the known pathophysiologic origins of TL, our results allow us to propose some potential strategies for management. First, thorough investigation for

underlying reversible systemic infection or inflammatory process should be undertaken. Our study was not designed for nor large enough to comment on implications for the liver transplant candidacy selection process. Within these limitations, we posit that it is possible that optimization of chronic conditions such as diabetes and renal failure pre-transplant serve as modifiable risk factors for TL. Additionally, those with trapped lung are much more likely to have multiple pleural procedures and require thoracic surgical intervention, which is associated with significant morbidity and mortality in the postoperative period after OLT (18). In the population of patients with ESLD, asymptomatic patients with pneumothorax ex-vacuo who have suspected trapped lung may benefit from observation and conservative management, and limited surgical interventions only when absolutely necessary (17). In fact, small studies have suggested that some cases of TL may spontaneously resolve on their own (17). This prior work and our data demonstrate that avoiding procedural pleural space intervention may be the most appropriate approach in post-OLT patients with TL or those at high risk for developing TL. Concurrently, when diagnostic thoracentesis is performed, pleural manometry and evaluation of pleural elastance may help confirm the diagnosis of TL and clarify the risk of additional procedures (24).

Our study is limited by its retrospective nature that limits full causal understanding of our findings. The size of our cohort was not adequate to determine if procedural intervention was a necessary treatment of the trapped lung, or rather if the pleural interventions increased the risk of development of TL

or the risk of poor outcomes after TL had formed. Although several important preclinical factors were chosen and IPTW was performed, many other unmeasured preclinical factors could have affected outcomes and were not measured or were incomplete. Some clinical characteristics were unbalanced in the original cohort and were not included in the model, and thus could have contributed to residual selection bias. Due to a low event rate and high correlation with trapped lung, pre-operative chest tube placement was a potential confounder. Further study would be required to disentangle the effect of pre-OLT chest tube placement from trapped lung. The retrospective nature of our cohort also limited the depth of the data we otherwise would have wanted to collect. We would have liked to follow all patients with persistent pleural effusion with serial imaging post-operatively. Similarly, our study would have benefited from additional pre-operative pleural fluid analysis and infectious studies. However, since we are a quaternary referral center, many of the patients were referred from outside facilities and systemic assessment of pre-operative characteristics was not available, thus limiting thorough determination of pre-OLT risk factors. Lastly, our data represent the findings of a single high volume, high acuity liver transplant center. TL may not be seen, at least in this frequency, at other institutions who transplant at lower MELD scores or transplant patients with fewer comorbidities.

Several strengths of our analysis, however, merit emphasis. This is, to our knowledge, the largest cohort of OLT recipients with persistent pleural effusions and diagnosed trapped TL. Despite the retrospective nature of the study, all patients were longitudinally followed over at least 2 years with well-defined pre- and postoperative imaging, clinical characteristics, and adequately reported outcomes. While previous data suggests that atelectasis is a common complication after OLT, focus on TL lung as a chronic and more clinically significant form of atelectasis addresses an underreported problem and one of the most frequent reasons for thoracic surgery interventions.

CONCLUSION

In patients with clinically significant, persistent pleural effusions after OLT, trapped lung is a frequent complication and is associated with increased morbidity, mortality, and health care utilization in the post-OLT period. Awareness of the risk factors for postoperative trapped lung, such as preoperative trapped lung, may be helpful with evaluation and determination of transplant risk stratification.

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Moving forward, a targeted evaluation of persistent pleural effusions in patients who have undergone OLT should be performed to further characterize these patients and their postoperative clinical outcomes. A better understanding would inform strategies to reduce the incidence of trapped lung, assess risk for transplant candidacy, and optimize the management of this common complication after orthotopic liver 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 UCLA Office of Human Research Protection Program. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

NC and KM: performed research, collected data, analyzed data and wrote paper. SV: analyzed data. RS, WM, SS, DD and ZA: performed research and collected data. TW, VA, JD, DF, JY and FK: analyzed data and wrote paper. IB: designed study, performed research, analyzed data and wrote 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.2022.10387/full#supplementary-material>

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Incidence and Outcomes of Early Cancers After Kidney Transplantation

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Outcomes of early cancers after kidney transplantation are not well-understood. We included recipients of first live and deceased donor kidney transplants who developed *de novo* cancers in Australia and New Zealand between 1980–2016. We compared the frequency and stage of specific cancer types that developed early (≤ 12 -months) and late (> 12 -months) post-transplantation. Risk factors for death were evaluated using multivariable Cox regression analyses. Of 2,759 recipients who developed *de novo* cancer, followed-up for 40,035 person-years, 243 (8.8%) patients were diagnosed with early cancer. Post-transplant lymphoproliferative disease, urinary cancers and melanoma were the most common cancer types (26%, 18%, and 12%) and the majority were either *in-situ* or locally invasive lesions (55%, 84%, and 86%). Tumors arising early from the gastrointestinal and respiratory systems were uncommon but aggressive, with 40% presenting with metastatic disease at time of diagnosis. Overall, 32% of patients with early cancers died within a median of 4.7 months (IQR:0.6–16) post-diagnosis and 91% were cancer-related deaths. Older recipient and donor age were associated with an increased risk of all-cause death. Early cancers, though infrequent in kidney transplant recipients, are associated with poor outcomes, as nearly 1 in 3 died from cancer-related death; with majority of deaths occurring within 12-months of cancer diagnosis.

Keywords: kidney transplantation, early cancer, cancer, ANZDATA, registry, cancer outcome, cancer death

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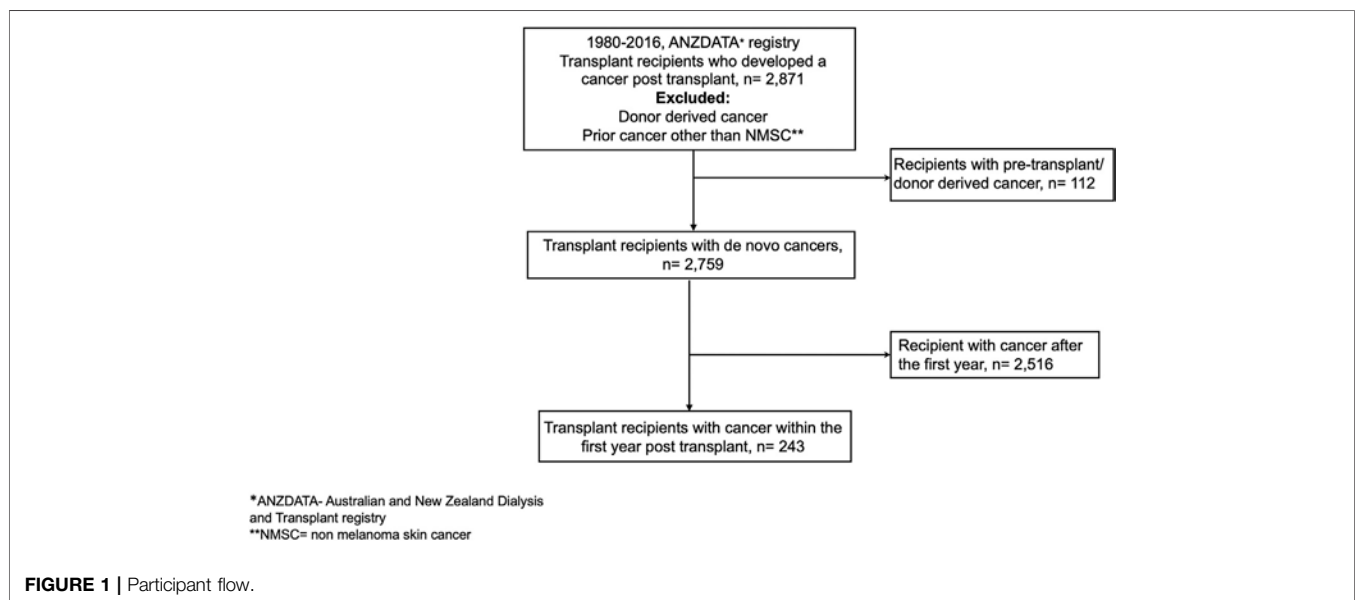
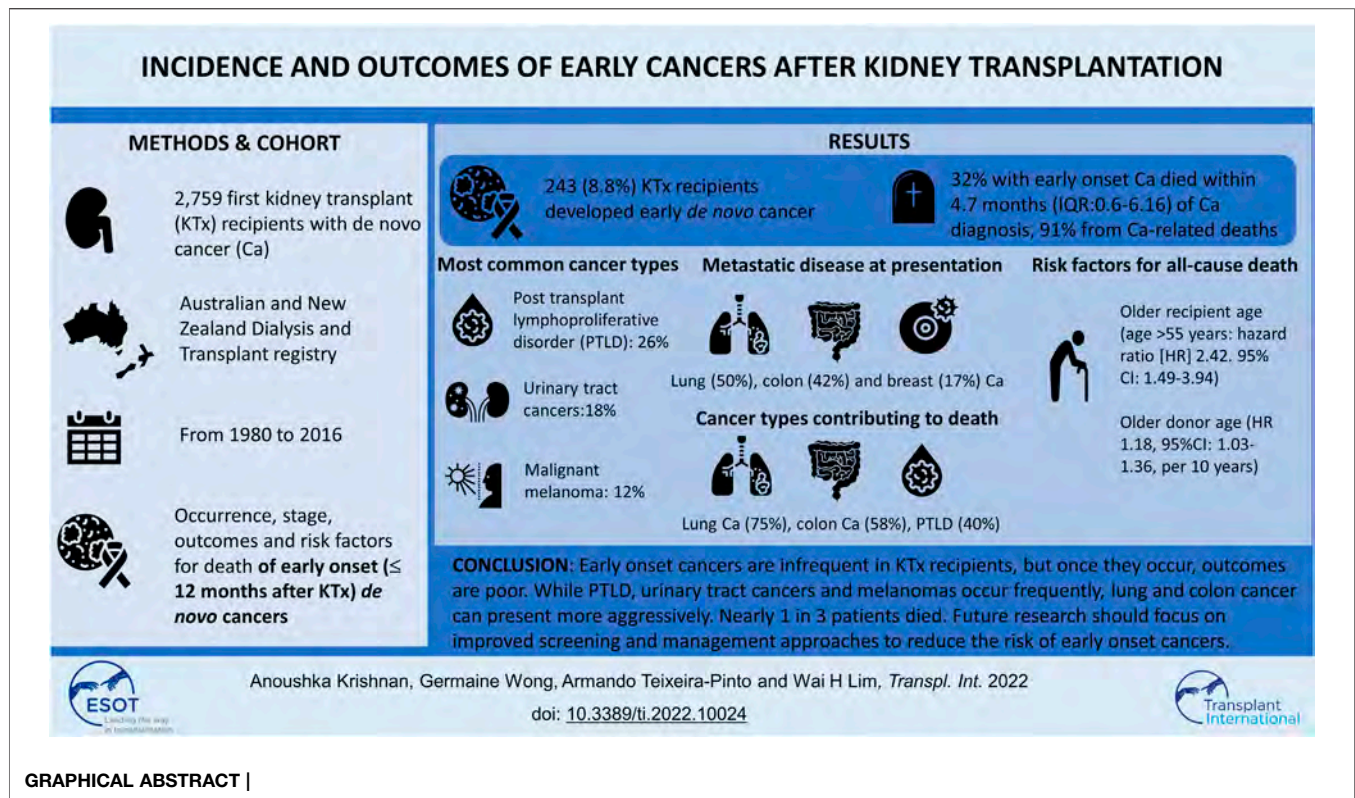
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INTRODUCTION

Cancer is a leading cause of death for many patients after kidney transplantation (1, 2). Compared to age and sex matched general population, cancer incidence and mortality rates are 2-3 times higher among transplant recipients (3). Epidemiological data have reported the mean time from transplantation to cancer diagnoses is approximately 6 years, suggesting that intensity of immunosuppression and cumulative drug exposure play key roles in cancer development (4, 5). However for some early cancers, such as post-transplant lymphoproliferative disease (PTLD) that commonly occur within a short timeframe after transplantation, the mechanistic pathways for cancer

Abbreviations: ANZDATA, Australian and New Zealand dialysis and transplant; ANOVA, analysis of variance; EBV, epstein-barr virus; ESKD, end stage kidney disease; HLA, human leukocyte antigen; ICD, international classification of disease; IQR, inter-quartile range; NMSC, non-melanoma skin cancer; PTLT, post-transplant lymphoproliferative disease; SD, standard deviation; SMR, standardized mortality rates.



development may be different to those that occur later (5). Patients on dialysis are also at risk of certain cancers such as urinary tract cancer (6). Clinical practice guidelines recommend age-specific screening for potential transplant candidates and some guidelines suggest additional screening for kidney cancers in patients on dialysis (7). However, the sensitivity of these screening tests is imperfect (8) and may therefore, miss

occult malignancies. Under the influence of immunosuppression, occult cancers may grow rapidly through deficiencies in tumor surveillance, and manifest early after transplantation.

Prior research has not quantified the burden and outcomes of these early cancers after transplantation. Knowledge of the epidemiology of these cancers and their risk factors for adverse outcomes will help to identify complex and high-risk

TABLE 1 | Baseline characteristics of patients who developed early (within 12 months) and late (after 12 months) *de novo* cancer post-transplant ($n = 2,759$).

	Early cancers ($n = 243, n, \%$)	Late cancers ($n = 2,516, n, \%$)	p-values
Donor characteristics			
Age (years, mean [SD])	42.3 (17.1)	35.7 (18.6)	<0.001
Female gender ($n, \%$)	118 (48.6)	1004 (39.9)	0.001
Type			0.03
Deceased	179 (73.7)	1927 (76.6)	
Live	64 (26.3)	589 (23.4)	
Recipient characteristics			
Age (years, mean [SD])	50.8 (15.4)	45.3 (14.2)	<0.001
Female gender ($n, \%$)	98 (40.3)	1051 (41.8)	0.66
Race ($n, \%$)			0.61
Caucasian	208 (85.5)	2224 (88.4)	
Aboriginals/Maori	11 (4.6)	87 (3.4)	
Others	24 (9.9)	205 (8.2)	
Diabetes ($n, \%$)	40 (16.5)	265 (10.5)	0.002
Coronary artery disease ($n, \%$)	30 (12.3)	152 (6.0)	<0.001
Peripheral vascular disease ($n, \%$)	10 (4.1)	80 (3.2)	0.09
Cerebrovascular disease ($n, \%$)	9 (3.7)	41 (1.6)	0.04
Smoker ($n, \%$)			0.19
Non- smokers	116 (57.4)	1008 (52.4)	
Former smokers	67 (33.2)	655 (34.0)	
Current smokers	19 (9.4)	262 (13.6)	
Cause of ESKD ($n, \%$)			0.03
Glomerulonephritis	104 (42.8)	1186 (47.1)	
Cystic	32 (13.2)	374 (14.9)	
Diabetes	30 (12.3)	201 (8.0)	
Vascular	16 (6.6)	96 (3.8)	
Analgesic nephropathy	11 (4.5)	107 (4.3)	
Others	50 (20.6)	552 (21.9)	
Viral serology			
CMV			<0.001
Negative	65 (26.7)	565 (22.5)	
Positive	138 (56.8)	1143 (45.4)	
Unknown	40 (16.5)	808 (32.2)	
EBV			<0.001
Negative	43 (17.7)	237 (9.4)	
Positive	134 (55.1)	1061 (42.1)	
Unknown	66 (27.2)	1218 (48.5)	
Immunology/transplant			
Waiting time (days, mean [SD])	868 (777)	774 (768)	0.07
Ischemic time (hours, mean [SD])	11.1 (7.4)	12.5 (7.9)	0.01
Transplant era ($n, \%$)			<0.001
1980-1989	30 (12.3)	667 (26.5)	
1990-1999	58 (23.9)	998 (39.7)	
After 2000	155 (63.8)	851 (33.8)	
Induction immunosuppression			
None	131 (54)	1917 (76)	<0.001
Interleukin-2 receptor therapy	102 (42)	446 (18)	
T-cell depleting therapy	10 (4)	153 (6)	
Maintenance immunosuppression			
Steroids (Prednisolone)	226 (93)	2245 (89)	0.06
Calcineurin inhibitors			<0.001
None	16 (7)	297 (12)	
Cyclosporine	145 (60)	1833 (73)	
Tacrolimus	82 (33)	386 (15)	
Anti-metabolites			<0.001
None	27 (11)	321 (13)	
Azathioprine	60 (25)	1246 (49)	
Mycophenolate mofetil/sodium	156 (64)	949 (38)	

patients and facilitate appropriate interventions such as targeted cancer screening in this at-risk population. In this study we aimed to compare the frequency, types, sites and stage of cancers that

occurred early compared to those that occurred later after transplantation. We also compared the risk of cancer-related and all-cause death between recipients with early and late cancers

TABLE 2 | Distribution of cancer types amongst patients who developed early cancer compared to those who developed late cancer.

Cancer type (n, %)	Cancer within 12 months (n = 243)	Cancer after 12 months (n = 2,516)	p-value
Post-transplant lymphoproliferative disease	62 (25.5)	351 (14)	<0.001
Urinary tract cancer ^a	44 (18.1)	342 (13.6)	0.05
Melanoma	29 (11.9)	266 (10.6)	0.51
Other GI tract ^b	17 (7.0)	152 (6.0)	0.35
Genital ^c	15 (6.2)	262 (10.4)	0.04
Colorectal	12 (4.9)	197 (7.8)	0.10
Breast	12 (4.9)	143 (5.7)	0.23
Prostate	9 (3.7)	172 (6.8)	0.06
Lung	8 (3.3)	182 (7.2)	0.02
Thyroid	7 (2.9)	54 (2.1)	0.45
Brain	3 (1.2)	24 (1.0)	0.18
Lip	1 (0.4)	30 (1.2)	0.27
Unknown origin	2 (0.8)	70 (2.8)	0.006
Others	22 (9.1)	271 (10.8)	0.68

^aCancers of the kidney, ureters and bladder.

^bOther gastrointestinal tract cancers (including gall bladder, small intestine, bile duct, pancreas, liver, stomach, esophagus).

^cCervix, ovaries, uterus, penile.

and defined the risk factors for deaths in patients with early cancer.

MATERIALS AND METHODS

Study Population

Using data from the Australia and New Zealand Dialysis and Transplant (ANZDATA) registry, kidney failure patients who have received a first deceased and living donor kidney transplant in Australia and New Zealand between 1980 and 2016 and had developed “*de novo*” cancer after transplantation were included in the analyses. Recipients with a prior history of cancer (other than a history of non-melanoma skin cancer- NMSC) and known “donor-transmitted” and “donor-derived” cancers were excluded (Figure 1). *De novo* cancer was defined as a cancer that occurred in a kidney transplant recipient with no prior history of cancer before transplantation and included all cancer types except NMSC. Donor-transmitted cancers are those cancers which are present in the donated organ and tissue at transplantation, whereas donor-derived cancers are those that are of donor origin but developed *de novo* in the allograft after transplantation. Details of both donor-derived and donor-transmitted cancers are provided to the ANZDATA registry by the individual units. However, the ANZDATA registry does not verify whether the cancer cells were of donor origin.

The clinical and research activities being reported are consistent with the Principles of the Declaration of Istanbul as outlined in the “Declaration of Istanbul on Organ Trafficking and Transplant Tourism.” Ethics approval was obtained from the Human Research Ethics Committee of Western Australia, Australia. Written informed consents were sought from patients with kidney failure at time of entry into the registry, including the utilization of aggregate data for future research.

Exposure

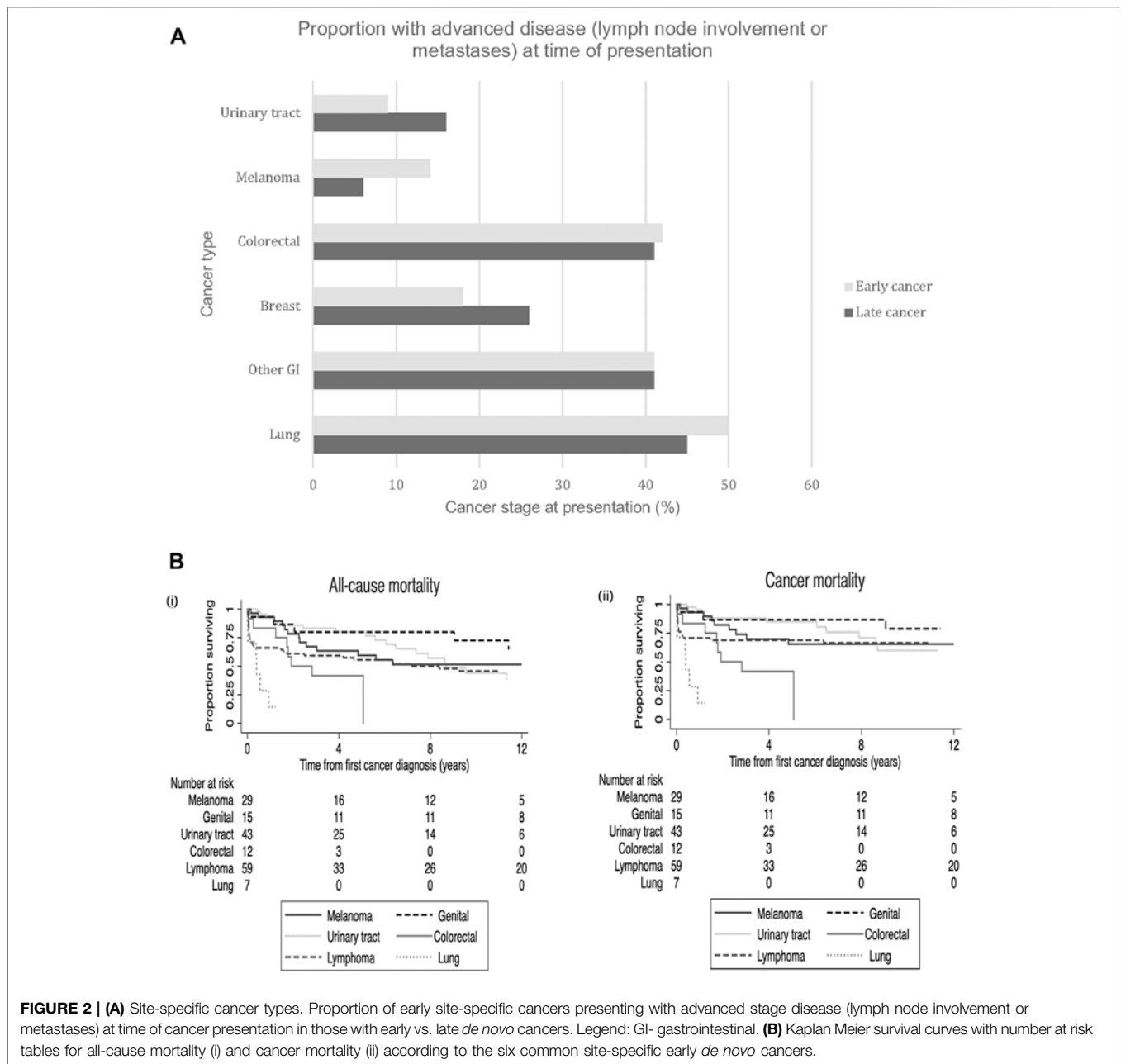
Recipients were categorized according to whether they had developed early cancer or late cancer post kidney transplant. Early-onset cancers were defined as those cancers occurring within the first 12-months post transplantation, whereas late-onset cancers were defined as those occurring 12-months after transplantation.

Data Collection

Baseline characteristics recorded by the ANZDATA registry included donor factors of age, type and sex; recipient characteristics of age, sex, ethnicity, body mass index, waiting time prior to transplantation, comorbid conditions at time of transplantation (presence or absence of diabetes, coronary artery disease, cerebrovascular disease and peripheral vascular disease), primary causes of kidney failure; and transplant-related factors including the number of human leukocyte antigen (HLA) mismatches, total ischemic time (in hours), induction (none, interleukin-2 receptor therapy and T-cell depleting therapy) and initial immunosuppressive therapies (prednisolone, calcineurin-inhibitor and anti-metabolite therapies) and transplant era (categorized into 1980–1989, 1990–1999 and 2000–2016 transplant periods).

Ascertainment of *De Novo* Cancers

De novo cancers occurring post-kidney transplantation were reported to the ANZDATA registry. The registry does not verify the histology of the *de novo* cancers, but the cancer records within the ANZDATA registry are accurate with a high concordance rate compared to those reported to the New South Wales Cancer Registry (9), a mandatory requirement for cancer reporting in New South Wales. *De novo* cancers are recorded according to cancer sites and cell types according to the International Classification of Disease for Oncology, edition 3, first revision (ICD-O-3.1) (10).



Clinical Outcomes

The primary outcomes included the frequency, types, sites, stage (including presence of lymph node involvement and distant metastatic disease) and occurrence of cancer recurrence. Other outcomes included treatment of the *de novo* cancers in recipients with early cancers and comparison of the risk of cancer-related and all-cause death between recipients with early cancer and those with late cancer. We also defined the risk factors for all-cause deaths in recipients with early cancers.

Statistical Analyses

Data were expressed as number (proportion), mean and standard deviation (SD) and median and interquartile range (IQR) where

appropriate, with comparisons between groups by chi-square test, analysis of variance (ANOVA) and Kruskal–Wallis test, respectively. We compared the frequency, cancer types, stage and outcomes of patients who developed early cancers with those who developed cancers 12 months after transplantation. The treatment patterns, responses to treatment and outcome of early-onset cancers were also described. Kaplan Meier survival curves were constructed for all-cause and cancer-specific mortality in recipients with early cancers and stratified by site-specific cancer types. The log-rank test was used to test the trend of all-cause and cancer-specific survival functions across the cancer types. Survival time was censored at the date of the clinical outcome or on 31 December 2017. The cumulative survivals (and 95%CI) from the

TABLE 3 | Outcomes of early cancer.

	Multiple incident cancers (n, %)	Number of cancers (n)	First cancer causing allograft failure (n, %)	First cancer causing Death (n, %)
Cancer first 12 months	39 (16.0)	243	3 (1.2)	77 (31.7)
Melanoma		29	0	7 (24.1)
Urinary tract ^a		44	1 (2.3)	7 (15.9)
Lymphoproliferative disease		62	1 (1.6)	25 (40.3)
Colorectal		12	0	7 (58.3)
Other GI ^b		17	0	14 (82.4)
Lung		8	0	6 (75.0)
Brain		3	0	1 (33.3)
Genital ^c		15	0	1 (6.7)
Prostate		9	0	1 (11.1)
Breast		12	0	0
Thyroid		7	0	0
Lip		1	0	0
Unknown origin		2	0	2 (100.0)
Others		22	1 (4.5)	6 (27.3)

Cancer occurring >12 months post-transplant: 13.4% multiple cancers, 1.2% first cancer causing allograft failure and 31.7% first cancer causing death.

^aCancers of the kidney, ureters and bladder.

^bOther gastrointestinal tract cancers (including gall bladder, small intestine, bile duct, pancreas, liver, stomach, esophagus).

^cCervix, ovaries, uterus, penile.

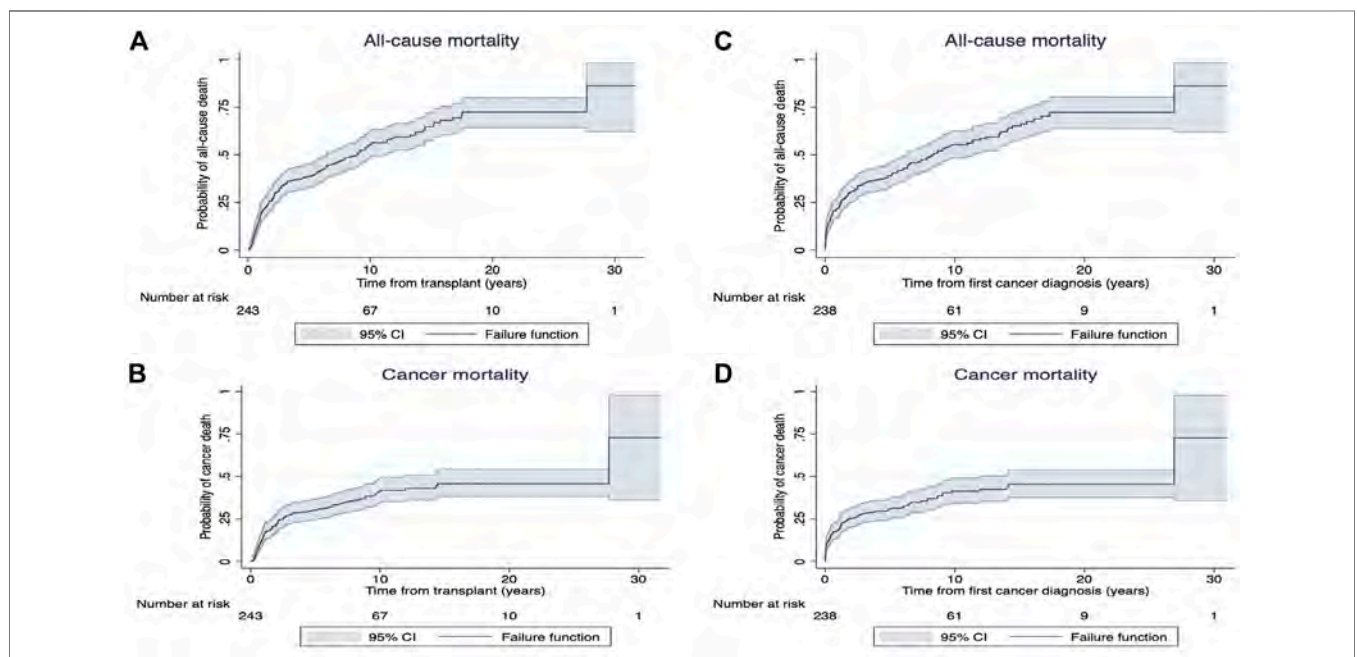
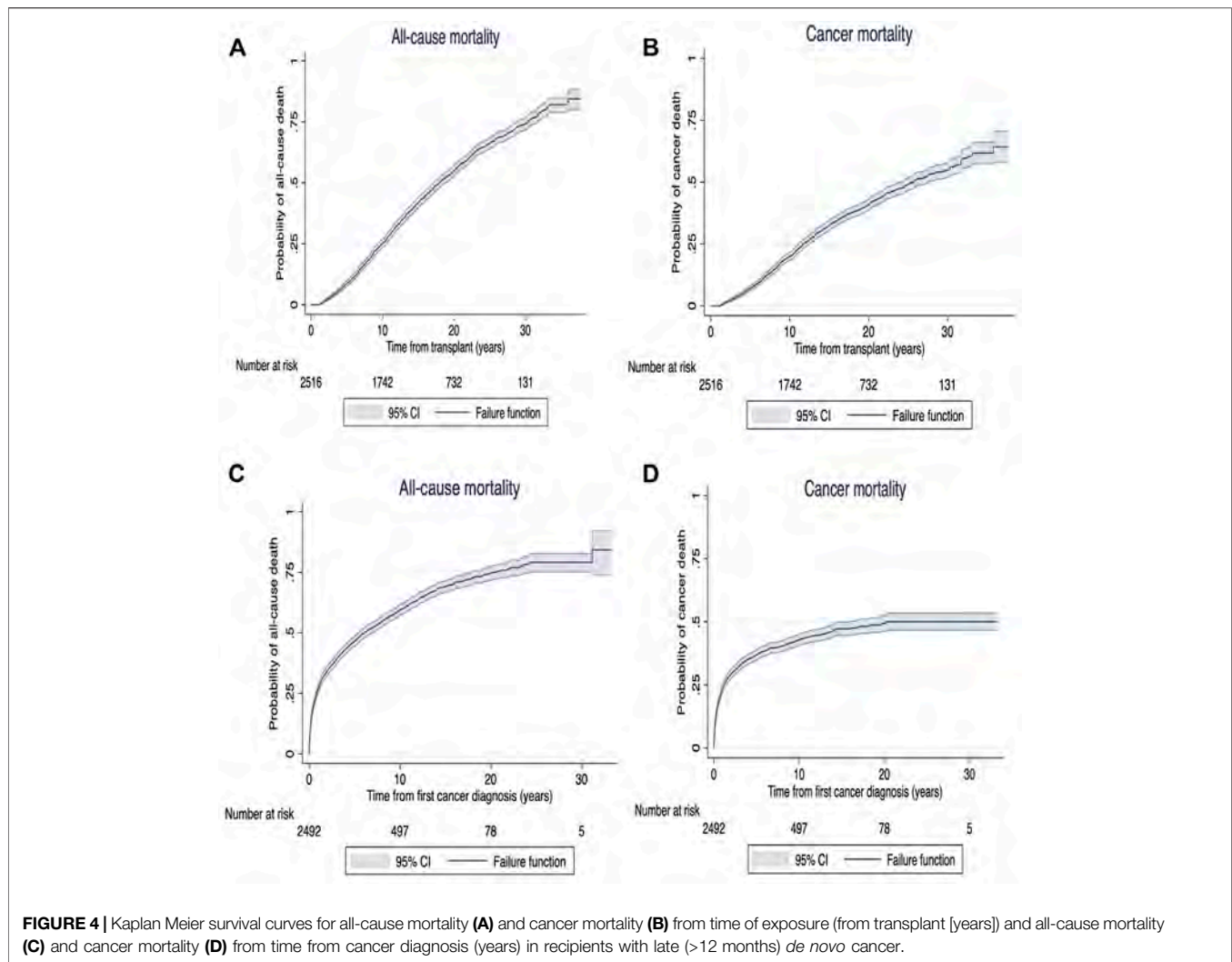


FIGURE 3 | Kaplan Meier survival curves for all-cause mortality (A) and cancer mortality (B) from time of exposure (from transplant [years]) and all-cause mortality (C) and cancer mortality (D) from time from first cancer diagnosis (years) in recipients with early *de novo* cancer.

time of cancer diagnosis till the time of death were calculated for patients with early and late-onset cancers. Adjusted multivariable cox regression models were used to evaluate the risk factors for all-cause mortality in patients with early-onset cancers. Covariates with p-values of <0.25 in the unadjusted association for all-cause mortality were included in the multivariable analyses. Proportional hazard assumptions were checked, and two-way interactions were tested. The final model retained the covariates that remained

significant after adjustment using a backward stepwise strategy. Variables included in the final multi-variable model included donor age, recipient age (stratified as <35, 35–55 and over 55 years), sex, race (Indigenous Australians, Maori and other), smoking status (stratified as current smoker, ex-smoker or non-smoker), induction immunosuppression, initial anti-metabolite therapy (none, azathioprine and mycophenolic acid) and transplant era.



Analyses were undertaken using SPSS V10 statistical software program (SPSS Inc., North Sydney, Australia), R (version 3.6) and STATA (version 11 StataCorp LP, College Station, TX). P-values of <0.05 were considered statistically significant.

RESULTS

Study Population

Between 1980 and 2016, a total of 21,844 patients received a first kidney transplant. Of these, 2,871 kidney transplant recipients developed cancer(s) post-transplantation, with 2,759 (96.1%) recipients developing *de novo* cancers post-transplant and 112 (3.9%) with either pre-transplant or donor derived cancers, respectively. Of the 2,759 recipients with *de novo* cancers, 243 (8.8%) developed *de novo* cancers within the first 12 months post-transplantation (Figure 1). The median (IQR) patient-follow up time for all recipients was 13.4 years (7.84–20.44) resulting in 40,006 patient-years of follow up with shorter median (IQR) follow-up periods for those who developed early cancer (4.8 [1.6–10.9]) years with 1,699 patient-years of follow-up.

Baseline characteristics of the study population with early and late onset *de novo* cancers are shown in Table 1. Recipients who developed early cancer were older (mean [SD] age: 50.8 [15.4] vs. 45.3 [14.2] years, $p < 0.001$), more likely to have pre-transplant diabetes (16.5% vs. 10.5%, $p = 0.002$) and coronary artery disease (12.3% vs. 6%, $p < 0.001$) and received kidneys from older donors (mean [SD] age: 42.3 [17.1] vs. 35.7 [18.6] years, $p < 0.001$) compared to those who developed late cancers. Additionally, a higher proportion of early *de novo* cancers developed in later transplant era (after the year 2000) [63.8% vs. 33.8%, $p < 0.001$]. The incidence rate of early onset cancer was 0.01 (95%CI: 0.007, 0.013) per 1000-person-days between 1980–1989, 0.02 (95%CI: 0.013, 0.022) per 1000-person-days between 1990–1999 and 0.09 (95%CI: 0.08, 0.10) per 1000-person-days after the year 2000.

Recipients who developed early cancer in the latter era (after 2000) were older compared to those who developed cancer in the earlier eras ($p \leq 0.01$, Supplementary Table S1). The proportion of incident kidney transplant recipients with early-onset cancers was similar across the three eras of 1980–1989 (0.8%), 1990–1999 (1.2%) and 2000–2016 (1.2%) ($p = 0.36$, Supplementary Figure S1).

TABLE 4 | Characteristics of early cancers contributing to death.

Cancer type, Sex distribution and median age (IQR)	Initial cancer location, type and number	Stage at presentation/number/time to advanced disease	Treatment
Melanoma ($n = 7$), M3, F4, Median age 61 (54–62)	Skin-7	Metastatic lesion- 6 (2 metastasized at initial presentation - 4 metastasized later; Median 15 months [12–27])	Local excision- 6 Chemotherapy and reduction IS- 1 No treatment- 1
Urinary tract ($n = 7$) ^a , M6, F1, Median age 56 (49–60)	Urinary bladder- 5 (3x TCC, 2x adenocarcinoma)—4 invasive presentation) Native kidney-2 (adenocarcinoma)	Metastatic lesions- 3 - Kidney: 1- at presentation - Bladder:2- at 7 and 10 months	Bladder excision-2 Chemotherapy- 1 Kidney mass excision- 1
Lymphoproliferative disease ($n = 25$), M16, F9, Median age 48 (28–56)	Lymph nodes/blood/bone marrow- 10 Liver- 2 Small intestine- 2 Brain- 3 Colon- 2 Kidney- 2 Lung- 2 Unknown primary- 2	<i>In situ</i> lesion- 4 Invasive lesion- 7 Metastatic lesion- 12 (all at presentation) Local lymph nodes- 1 Unknown- 1	Reduced IS- 10 Chemotherapy- 5 Radiotherapy- 5 Local excision (small intestine- 1) No treatment- 8 Unknown- 1
Colorectal ($n = 7$), M4, F3, Median age: 59 (49–69)	Colon- 5 Recto-sigmoid- 2 (adenocarcinoma- 1, squamous cell carcinoma- 1)	Invasive- 3 Metastatic lesion- 4 at presentation All 3 invasive metastasized at 8, 13 and 55m post-diagnosis	Local excision- 3 Radiotherapy-1 Chemotherapy- 3 No treatment- 1
Other GI ($n = 14$) ^b , M 11, F3, Median age: 59 (35–66)	Pancreas- 5 (adenocarcinoma) Stomach- 2 (adenocarcinoma) Oesophageal- 2, (adenocarcinoma) Ampulla of Vater- 2 Hepatocellular carcinoma- 1 Small intestine- 1 (adenocarcinoma) Oropharynx- 1	Metastatic lesions- 8 (6 at presentation: pancreas- 3, oesophagus- 2, small bowel- 1, stomach- 1) Invasive- 4	Pancreas: none- 2, reduction in IS- 2, chemotherapy- 1 Stomach: none- 1, chemotherapy- 1 Oesophageal: Chemo-radiotherapy- 2 Ampulla of Vater: None- 2 Small intestine: Excision- 1 Oropharynx: Excision, reduction IS and radiotherapy- 1 HCC: excision- 1
Lung ($n = 6$), M3, F3, Median age: 56 (50–62)	Adenocarcinoma- 2 Small cell cancer- 2 Large cell cancer- 1 Unknown- 1	Metastatic lesion- 4 (3 at presentation, 1 at 4 months) Invasive- 2	Radiotherapy- 2 Chemotherapy- 1 Multiple- 2 (chemo-radiotherapy and reduction IS- 1) None- 1

M, males, F, females.

^aCancers of the kidney, ureters and bladder

^bOther gastrointestinal tract cancers (including gall bladder, small intestine, bile duct, pancreas, liver, stomach, esophagus).

Cancer Types of Early-Onset and Late-Onset *De Novo* Cancers

The median (IQR) time to cancer onset was 205 days (107–298) in those with early-onset cancer compared to 2,083 days (1,675–4,914) in those with late-onset cancer. The three most common types of cancers in those who developed early *de novo* cancers were PTLD (25%), urinary tract cancers (18%) and malignant melanoma (12%). For late-onset cancers, the three most common types of *de novo* cancers were PTLD (14%), urinary tract cancers (13.6%) and melanomas (10.6%) (Table 2). Supplementary Table S2 demonstrates differences between recipients of living and deceased donor kidneys who developed early cancers. The distribution of the three most frequently occurring cancers (PTLD, urinary cancers and melanomas) were similar between the two groups.

Cancer Stage and Outcomes of Early-Onset and Late-Onset *De Novo* Cancers

Of recipients who had early-onset cancers, 25% ($n = 61$) developed or presented with advanced stage disease (lymph node involvement or distant metastases). At the time of presentation, 50% of lung, 42% of colorectal and 17% of breast cancers had evidence of advanced disease (Figure 2A). For late-onset cancers, 45% of lung, 41% of colorectal and 26% of breast cancers had evidence of advanced disease at the time of diagnosis. In contrast, 9% and 14% of early kidney cancers and melanomas and 16% and 6% of these late cancers respectively, presented with evidence of advanced disease. Kaplan-Meier survival curves for all-cause mortality and cancer mortality according to the most common site-specific cancer types are shown in Figure 2B.

Among recipients who developed *de novo* early-onset cancers, 77 (32%) died with 70 (91%) deaths attributed to cancer related deaths (Table 3). The median (IQR) time from cancer diagnosis to cancer-specific and all-cause deaths was 145 days (IQR: 20–464) and 144 days (20–505), respectively. For recipients with late-onset cancers, 1,473 (59%) recipients died with 977 (39%) attributed to cancer-related deaths. The median time from cancer diagnosis to cancer-specific and all-cause deaths were 229 days (53–781) and 427 days (80–1,623), respectively. Figures 3, 4 shows the Kaplan Meier curves of cancer-related deaths and all-cause deaths for recipients with early and late-onset *de novo* cancer; both from time of transplant and from time of cancer diagnosis, with majority of deaths being related to cancer in those who developed early *de novo* cancer.

Following cancer diagnosis, the overall patient survivals at 1, 5 and 10 years for recipients who developed early *de novo* cancer were 77% (95%CI: 70.7, 81.4), 46% (95%CI: 39.7, 52.2) and 25% (95%CI: 19.8, 30.7). In recipients who developed late *de novo* cancers, the overall patient survivals at 1, 5 and 10 years were 73% (95%CI: 70.7, 74.2), 41% (95%CI: 38.6, 42.3) and 20% (95%CI: 18.2, 21.3) (Supplementary Figure S1).

Early-Onset Cancers Outcomes-Deaths, Recurrent and Second Cancers

Most of the early cancer related deaths were associated with lung (75%) and colorectal (58.3%) cancers (Table 3).

Of those who developed lung cancer, 75% ($n = 6$) died, with the median (IQR) time to death of 142 days (6–236). The median (IQR) age at diagnosis was 56 years (50–62) with 50% being males. 50% ($n = 3$) of the patients presented with advanced stage disease at the time of presentation.

Of those who developed early colorectal cancer, 58% ($n = 7$) died, with the median (IQR) time to death of 651 days (96–924). The median (IQR) age at diagnosis was 59 years (49–69) with 57% being males. 57% ($n = 4$) of the recipients presented with advanced stage disease at the time of presentation.

Of the more common cancer types, 25 (40%) recipients died from PTLTD (median [IQR] age at diagnosis was 48 [28–56] years) while 7 (16%) died from urinary tract cancers (median [IQR] age at diagnosis was 56 [49–60] years), with nearly 50% of those dying from the latter presenting with advanced stage disease. A detailed description of all the early onset *de novo* cancers that had contributed to premature mortality is shown in Table 3.

Of all recipients with early-onset cancers ($n = 243$), onset of a second (new) cancer or recurrence of *de novo* cancer occurred in 39 recipients (16%), with a median (IQR) time to cancer occurrence of 1,165 (71–2,309) days. The most common cancers were those that involved the urinary tract, lung and the gastrointestinal tracts (15% each). Seven (18%) of these second cancers occurred within 1 year of the primary malignancy.

In this cohort, 77% ($n = 33$) recipients developed a second new malignancy at a different site within a median (IQR) of 1,414 (435–2,423) days, of which, the most common cancer sites were lung cancer ($n = 6$, 18%) closely followed by cancer of the urinary tract ($n = 5$, 15%). Additionally, 15% ($n = 6$) recipients had recurrence of the *de novo* primary malignancy within a median (IQR) of 5 (0–159) days, of which 33% ($n = 2$) had recurrence of

melanomas and 33% ($n = 2$) had recurrence of transitional cell cancer of the urinary tract.

Treatments of cancers that resulted in death were diverse and included various combinations of surgical resection, chemotherapy, radiotherapy and reduction of immunosuppressive medications (Table 4).

Factors Associated With All-Cause Mortality in Early-Onset Cancers

Risk factors associated with all-cause death among those with early cancers were older recipient age [>55 years: 2.42 (1.49–3.94), ref: 35–55 years] and older donor age [1.18 (1.03–1.36), per 10-years].

DISCUSSION

In this large contemporaneous cohort of kidney transplant recipients with *de novo* cancers spanning over 3 decades, we have shown that almost 1 in 10 of these cancers occurred within the first 12 months post-transplantation. The most common cancer types were PTLTD, malignant melanoma and cancers of the urinary tract, and typically, most of these cancers were of early stage at the time of presentation. On the contrary, recipients with other cancer types such as cancers of the digestive and respiratory systems tend to present with advanced stage disease. Overall, 32% of patients with early cancers died within a median of 4.7 months (IQR: 0.6–16) post-diagnosis and 91% were cancer-related deaths. Characteristics associated with an increased risk of death in recipients with early-onset cancer included increasing donor and recipient age.

Early cancers after transplantation are devastating events with a high burden of morbidity and mortality. Additionally, treatment strategies lack robust trial-based evidence and usually consist of surgical resection, radiotherapy and judicious reduction in immunosuppression with regular monitoring for cancer progression and allograft function. Certain strategies such as cancer screening may reduce the incidence of late-stage cancer through early detection, allowing interventions to be instigated early and before transplantation when the disease is still at a precancerous stage. Most clinical practice guidelines recommended routine age and sex-specific population-based cancer screening prior to listing (7). These include biennial bowel screening using either fecal immunochemical testing, or 5-years flexible sigmoidoscopy, biennial mammography for breast cancer, low-dose computer-tomography for lung cancer screening, and routine cervical screening using human papillomavirus test (HPV) for oncogenic cervical genotypes and pre-cancerous cervical lesions prior to transplantation (7). Despite these recommendations, uptake for screening in general among our candidates with chronic kidney disease is likely to be low and may potentially explain the late presentation of certain cancer types such as lung and gastrointestinal cancers within the early months after transplantation. While we do not routinely collect screening data in our transplant candidates, our prior work has indicated that the uptake of certain cancer screenings such as breast and cervical cancer are quite low amongst patients with kidney disease (11). Patients with kidney disease and kidney transplants undergo significant changes to

their overall physical and psycho-social health and tend to focus on their current kidney health and are less inclined to prioritize cancer screening over imminent health problems.

Other guidelines suggest routine ultrasonographic screening (either annual or biennially) for renal cell cancers. However, evidence to support these recommendations are limited. For instance, the accuracy of ultrasonography in detecting malignant lesions in those with kidney failure is uncertain. Ultrasonography is largely operator-dependent and test performance varies with patient habitus, the kidneys and the size of the lesion (12). In the general population, test sensitivity and specificity are lower in detecting tumours <3 cm in size. In patients with kidney failure, who have scarred native kidneys with acquired cystic disease, the accuracy of detecting small renal cell cancers is ambiguous. Moreover, prior Markov modelling studies have suggested that routine surveillance for renal cancers may not be cost effective in the low to moderate risk population (13). Screening is not without harm as uncertain lesions may lead to further investigations or treatments, and therefore undue delays for transplant waitlisting. Currently, there is no clear consensus on screening for post-transplant renal cell cancers as data are limited (14). There are similar concerns regarding the risk-benefit ratio of screening high risk population for lung cancers with annual low-dose computed tomography even in the general population (15) and this modality has not been validated in the transplant population.

Viral linked cancers such as lymphoma or post-transplant lymphoproliferative disorders (PTLD) have a higher incidence in the transplant population, compared to the general population, with standardized mortality rates (SMRs) being as high as 10.7 for PTLD (3, 6, 16). A quarter of our cohort with early cancers developed PTLD within the first year of transplant with a younger median age of 48 years compared to other cancers and nearly 40% died. This is consistent with previous findings of a bimodal distribution in the incidence of PTLD development after transplantation (17). PTLD most commonly occurs within the first year of transplant affecting younger (<25 years) or older (>60 years) patients (18) and has a high mortality rate of ~50% (19, 20). Primary Epstein-Barr virus (EBV) infection and pre-transplant EBV sero-negativity are risk factors for early onset PTLD, especially in younger transplant recipients, while late B-cell PTLD involves EBV-negative lesions (in 40–50%) (17). Once PTLD occurs, the risk of death is high (>14 fold higher than in recipients without PTLD) with median time of 6 months from diagnosis to death (21).

Older recipient age and donor age were both associated with an increased risk of cancer-related death. Over the past decades, there has been a changing demographic of transplant recipients. We are increasingly transplanting older patients with higher comorbidity burden and this in turn may have implications on screening procedures, cancer monitoring and degree of immunosuppression.

This study has several limitations. ANZDATA registry does not collect information on the uptake, adherence, type and timing of cancer screening for each transplant recipient. We lacked information on histological cancer data and treatment specific data, EBV data, relevant habits such as tobacco or alcohol use, therapeutic drug levels of immunosuppressive drugs, patients who were listed for kidney transplantation or were subsequently delisted (including those who may have developed incident cancer on the

waiting list), quality of life measures and the severity of comorbid disease. There is a likelihood of selection bias due to systematic differences in the management of recipients who developed cancer.

CONCLUSION

In conclusion, early cancer is an infrequent complication after kidney transplantation but once it occurs, outcomes are generally poor. Clinicians should be more cognizant of the development of early cancers especially in the older population. Examination of granular data and the development of screening and management approaches to decrease post-transplant cancers without increasing the risk of allograft failure, with clear considerations of patient preferences and values may improve outcomes in this population.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: Data was obtained from the Australian and New Zealand Dialysis and Transplant registry. Requests to access these datasets should be directed to <https://www.anzdata.org.au>.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Research Ethics Committee of Western Australia, Australia. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AK and WL participated in the research design, data analysis, performance of research and in writing of the paper. GW participated in research design and in writing of the paper. AT-P participated in data analysis and review 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.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10024/full#supplementary-material>

Supplementary Figure S1 | Proportion of recipients who developed a *de novo* cancer post-transplant stratified by transplant era.

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Clinical Significances of Anti-Collagen Type I and Type III Antibodies in Antibody-Mediated Rejection

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It is important to determine the clinical significance of non-human leukocyte antigen (HLA) antibodies and their association with antibody-mediated rejection (ABMR) of kidney allografts. We collected post-transplant sera from 68 ABMR patients, 67 T-cell mediated rejection (TCMR) patients, and 83 control subjects without rejection, and determined the titers of 39 non-HLA antibodies including antibodies for angiotensin II receptor type I and MICA. We compared all these non-HLA antibody titers among the study groups. Then, we investigated their association with the risk of death-censored graft failure in ABMR cases. Among the antibodies evaluated, anti-collagen type I ($p = 0.001$) and type III ($p < 0.001$) antibody titers were significantly higher in ABMR cases than in both TCMR cases and no-rejection controls. Both anti-collagen type I [per 1 standard deviation (SD), adjusted odds ratio (OR), 11.72 (2.73–76.30)] and type III [per 1 SD, adjusted OR, 6.22 (1.91–31.75)] antibodies were significantly associated with the presence of ABMR. Among ABMR cases, a higher level of anti-collagen type I [per 1 SD, adjusted hazard ratio (HR), 1.90 (1.32–2.75)] or type III per 1 SD, [adjusted HR, 1.57 (1.15–2.16)] antibody was associated with a higher risk of death-censored graft failure. In conclusion, post-transplant anti-collagen type I and type III antibodies may be novel non-HLA antibodies related to ABMR of kidney allografts.

Keywords: kidney transplantation, kidney, non-HLA antibody, antibody-mediated rejection, graft failure

Abbreviations: ABMR, antibody-mediated rejection; DSA, donor specific antibody; HLA, human leukocyte antigen; TCMR, T-cell mediated rejection.

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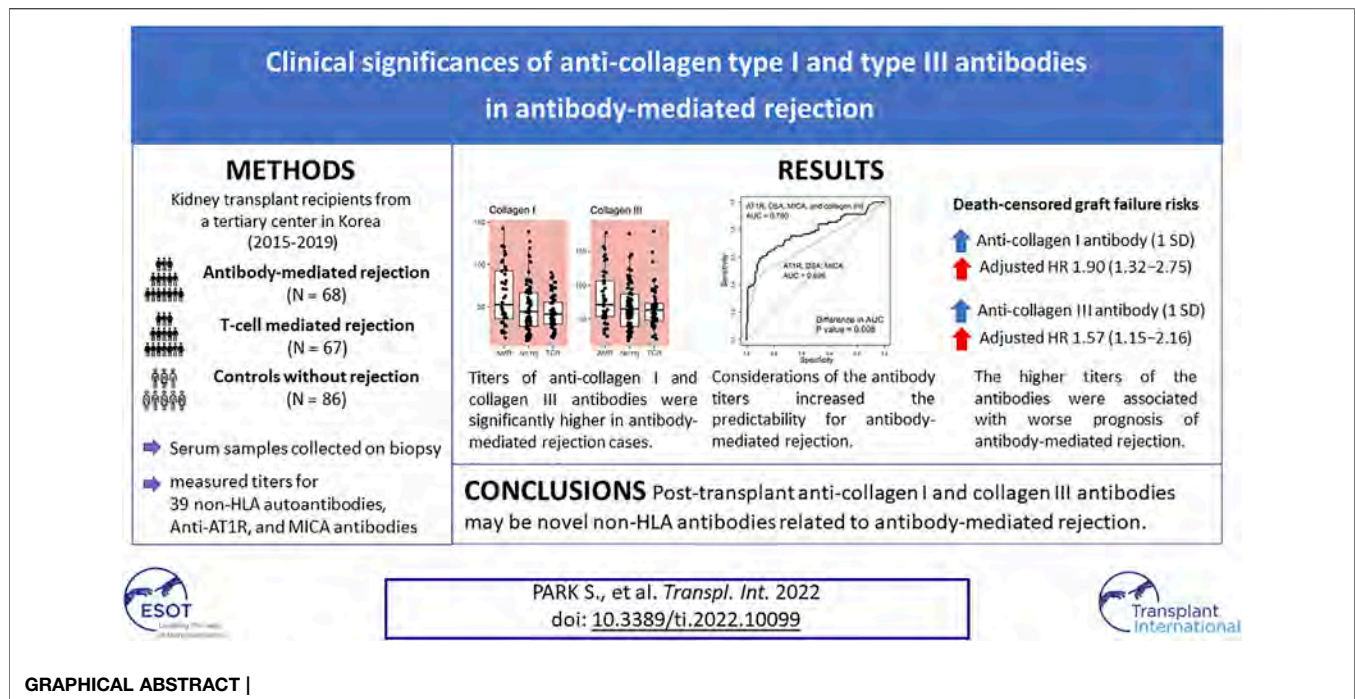
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INTRODUCTION

Kidney transplantation is the best treatment strategy for end-stage kidney disease. Although the overall prognosis of kidney transplantation has improved with the advances in potent immunosuppressive treatment strategies, the risk of graft loss in later periods after transplantation remains substantial. The majority of late graft failure cases are due to antibody-mediated rejection (ABMR), which has historically been described as chronic allograft nephropathy or transplant glomerulopathy (1, 2).

Currently, ABMR cases are diagnosed based on the presence of donor-specific antibodies (DSAs) against human leukocyte antigen (HLA) or non-HLA antigens and morphologic evidence of allograft injury represented by capillary injury, or glomerular inflammation (3). With the advances in anti-HLA antibody measurement methods, early detection and the management of preformed or *de-novo* DSAs has become possible. However, recent studies have revealed that a non-negligible portion of patients have histologic ABMR in the absence of HLA-DSAs (4).

In such HLA-DSA-negative histologic ABMR cases, the importance of non-HLA antibodies has been emphasized. Initially, anti-endothelial cell antibody was suggested to be formed during ischemia-reperfusion injury during organ transplantation, which accelerated ABMR even in the absence of HLA-DSA (5, 6). Later studies reported that angiotensin receptor I (AT1R) was a target antigen for non-HLA antibody in steroid-refractory vascular rejection cases with malignant hypertension (7). Autoantibodies against major histocompatibility complex class I chain-related antigens (MICA) have also been reported to have clinical significance for ABMR cases (8). Nevertheless, non-HLA antibodies remain understudied for their clinical significance in the

kidney transplantation field (9). Further investigation is needed to identify novel non-HLA antibodies related to ABMR as there remain ABMR cases without detectable causal autoantibodies. Moreover, whether the presence of such non-HLA antibodies affects the prognosis of patients with ABMR needs to be assessed. Such evidence would enable clinicians to monitor and treat patients with ABMR in the early phases, which may reduce the risk of late graft failure in kidney transplant recipients.

In this study, we measured and compared the levels of 39 non-HLA antibodies in transplant recipients with ABMR, T-cell-mediated rejection (TCMR) cases, and control subjects without any evidence of rejection with the aim to identify a non-HLA antibody that can serve as a biomarker of ABMR and/or a predictor of prognosis. We hypothesized that by using an unsupervised approach, a novel non-HLA antibody with clinical significance in ABMR could be identified.

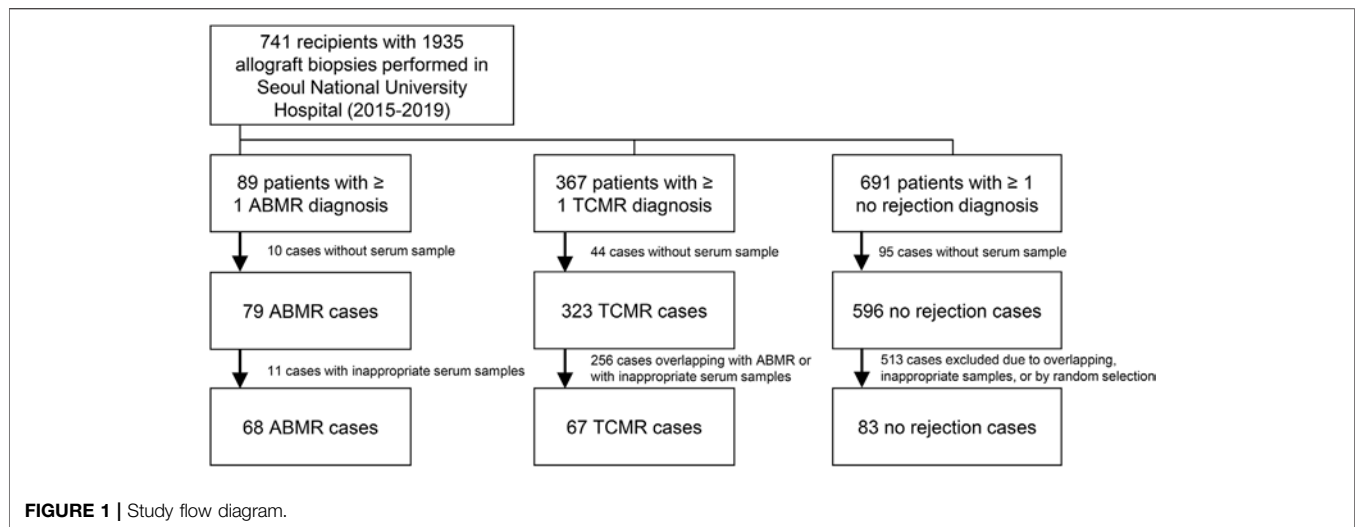
MATERIALS AND METHODS

Ethical Considerations

This study was conducted in compliance with the Declaration of Helsinki and the Declaration of Istanbul. The institutional review board of Seoul National University Hospital, Seoul, Korea (H-1808-181-970) approved the study. All clinical characteristics and bio-specimens were prospectively collected with the approval of the study subjects.

Study Cases

The Seoul National University Hospital operates a human biobank for kidney transplant recipients and donors. In the biobank, serial samples (including serum, plasma, urine, and



stool) collected (after acquiring informed consent from the patient) before transplantation, 2 weeks and 3 months after transplantation, and annually thereafter, if available, are stored. In addition, allograft biopsies were sampled from kidney transplant recipients. In the hospital, kidney biopsies were mostly performed based on the following clinical criteria: a progressive decline in renal function, persistent hematuria, or significant proteinuria of more than 1.0 g/day, and we collected pathologically confirmed ABMR cases as the main study group. There have been certain number of biopsy cases without any rejection, and such cases were mostly collected from protocol-based biopsies which were performed within short period from transplantation. In addition to ABMR and control cases without rejection, TCMR cases were collected as a rejection control group to identify non-HLA antibodies that are specifically associated with ABMR. We collected serum samples from non-overlapping 68 ABMR patients, 67 TCMR patients without ABMR, and 83 control subjects without any rejection confirmed by allograft biopsies between 2015 and 2019 at Seoul National University Hospital (**Figure 1**). ABMR or TCMR was distinguished based on the Banff classification, and 2013 and 2017 criteria were applied according to the time-periods (10–12). The samples were routinely reviewed by two kidney pathology specialists. Criteria for the selection of the ABMR cases were the availability of informed consent and of serum samples stored in the Seoul National University Hospital Biobank; no selection based on other clinical criteria was applied. The numbers of TCMR and control cases were determined to construct control groups of similar numbers based on random selection. We initially collected ABMR cases regardless of coexisting pathology (e.g., TCMR or calcineurin inhibitor toxicity) to maximize the number of cases.

Antibody Screening Methods and Data Collection

We measured 39 non-HLA antibodies by the Luminex method using a commercial kit (LABScreen Autoantibody, One Lambda,

CA, United States, URL: <https://www.onelambda.com/en/product/labscreen-autoantibody-new.html>, last accessed 2020-09-22) that reports mean fluorescence intensity (MFI) values. The targeted non-HLA antibodies in the kit were selected by the manufacturer based on a review of the literature in the transplantation field. The MFI values were calculated by subtracting the sample-specific fluorescence value for negative control beads from the sample-specific fluorescence value for non-HLA antigen beads. We additionally measured anti-MICA antibody levels (Luminex method, LABScreen Mixed, One Lambda) and anti-AT1R antibody levels (enzyme immunoassay, EIA-AT1RX, One Lambda) in the serum samples to determine the clinical significance of identified non-HLA antibodies independent from non-HLA antibodies that are known in the kidney transplantation field. The anti-MICA (normalized MFI ratio ≥ 2.7 -fold) and anti-AT1R (≥ 10 U/mL) antibody test results were stratified as positive/negative according to the manufacturer's recommended cutoff values. The other clinical data were collected through electronic medical record review, which are presented in **Supplementary Methods**. Serum samples and data of the ABMR or TCMR cases were collected at the timing of kidney biopsy which was performed to diagnosis the rejections.

Internal Validation Study

We collected cases with available serum samples for internal validation by ELISA, including 26 ABMR cases and 28 controls (22 no rejection controls and 6 TCMR cases). The correlation between the anti-collagen I IgG antibody titers (unit/ml) measured by ELISA and the MFI values measured by the Luminex method was assessed by the Pearson's correlation test, and the average or categorical values were compared between the two groups by t-test and the chi-squared test.

Statistical Analysis

Non-HLA antibodies of which the levels significantly differed in the ABMR cases were selected as potential target biomarkers by the Mann-Whitney U test. We compared the characteristics

TABLE 1 | Clinical characteristics of the study cases.

	ABMR group (N = 68)	No rejection control (N = 83)	TCMR group (N = 67)
Age at diagnosis (years)	49.0 [39.5;58.5]	51.0 [39.5;58.0]	49.0 [34.5;55.0]
Sex			
Female	31 (45.6%)	35 (42.2%)	24 (35.8%)
Male	37 (54.4%)	48 (57.8%)	43 (64.2%)
Duration from transplantation to diagnosis (days)	73.5 [9.0;1002.5]	9.0 [9.0;11.0]	9.0 [9.0;11.5]
Duration from diagnosis to serum acquisition (days)	0.0 [0.0; 0.0]	0.0 [0.0; 0.0]	0.0 [0.0; 0.0]
Relation			
Living related	17 (25.0%)	42 (50.6%)	26 (38.8%)
Living unrelated	24 (35.3%)	20 (24.1%)	21 (31.3%)
Cadaveric	27 (39.7%)	21 (25.3%)	20 (29.9%)
Immunologic risk status			
ABO incompatibility	14 (21.2%)	19 (23.5%)	7 (10.4%)
HLA incompatibility at transplantation			
Crossmatch (+), DSA (+)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Crossmatch (-), DSA (+)	9 (17.3%)	0 (0.0%)	0 (0.0%)
DSA on time of kidney biopsy	31 (45.6%)	5 (6.0%)	6 (9.0%)
Type			
Class I	11 (16.2%)	0 (0.0%)	0 (0.0%)
Class II	17 (25.5%)	0 (0.0%)	0 (0.0%)
Class I and class II	3 (4.4%)	5 (6.0%)	6 (9.0%)
Total MFI of those with DSA	7178 [736;21502]	2317 [961;2452]	1521 [1446;1584]
Peak MFI of those with DSA	6559 [736;20758]	1425 [961;2452]	1475 [878;1539]
Number of HLA mismatch	4 [3; 5]	3 [1; 4]	3 [2; 5]
Number of mismatches in HLA-A			
1	40 (65.6%)	41 (52.6%)	31 (47.7%)
2	12 (19.7%)	8 (10.3%)	13 (20.0%)
Number of mismatches in HLA-B			
1	19 (31.1%)	34 (43.6%)	26 (40.0%)
2	39 (63.9%)	24 (30.8%)	27 (41.5%)
Number of mismatches in HLA-DR			
1	27 (44.3%)	44 (56.5%)	31 (47.7%)
2	25 (41.0%)	9 (11.5%)	22 (33.8%)
Immunosuppressive treatment			
Desensitization	20 (29.4%)	22 (27.2%)	5 (7.5%)
Induction therapy			
Anti-thymocyte globulin	6 (25.0%)	8 (9.8%)	7 (10.4%)
IL-2 receptor subunit α inhibitor	50 (78.1%)	73 (88.0%)	67 (100.0%)
Maintenance immunosuppression			
Calcineurin inhibitors	65 (96.6%)	83 (100.0%)	65 (97.0%)
Tacrolimus	57 (83.8%)	83 (100.0%)	65 (97.0%)
Cyclosporine	8 (11.8%)	0 (0.0%)	0 (0.0%)
Mycophenolic acid	46 (67.6%)	77 (92.8%)	61 (91.0%)
Steroid	67 (98.5%)	83 (100.0%)	65 (97.0%)
Laboratory findings			
Serum albumin (g/dL)	3.7 [3.2; 4.0]	3.5 [3.3; 3.9]	3.6 [3.3; 3.8]
BUN (mg/dL)	31.0 [20.0;44.5]	19.0 [15.0;23.0]	22.5 [16.0;27.0]
Creatinine (mg/dL)	1.7 [1.1; 2.7]	1.0 [0.8; 1.4]	1.2 [0.9; 1.6]
Proteinuria (g/g or g/day)	0.8 [0.4; 1.7]	1.0 [0.4; 1.4]	1.0 [0.5; 1.6]
Blood pressures			
Systolic BP (mmHg)	135.0 [122.0;141.0]	128.0 [118.0;138.0]	127.5 [117.0;139.0]
Diastolic BP (mmHg)	85.0 [75.5;90.0]	82.0 [73.5;89.5]	82.0 [76.0;93.0]

(Continued on following page)

TABLE 1 | (Continued) Clinical characteristics of the study cases.

	ABMR group (N = 68)	No rejection control (N = 83)	TCMR group (N = 67)
Peri-transplant findings			
Delayed graft function	8 (13.1%)	3 (3.7%)	4 (6.0%)
Cold ischemic time (minutes)	54.0 [33.0;103.0]	3.0 [2.0; 3.0]	3.0 [2.0; 3.0]
Warm ischemic time (minutes)	41.0 [35.5;47.0]	79.0 [67.0;94.0]	73.5 [57.5;94.0]

Continuous values are presented as median [interquartile ranges] and categorical variables are presented as number (%). ABMR, antibody-mediated rejection; TCMR, T-cell mediated rejection; HLA, human leukocyte antigen; DSA, donor specific-antibody; PRA, panel reactive antibody; BP, blood pressure.

between the subgroups and evaluated predictability by receiver-operating-characteristics (ROC) curves. The prognosis analysis was performed by Cox regression. Statistical significance was determined using a Bonferroni-corrected p -value ($p < 0.05/39$), and the 75th percentile values for ABMR, TCMR, and no-rejection cases were determined as cut-offs to determine a high non-HLA antibody titer. In other analyses, conventional two-sided p -values < 0.05 were considered significant. All statistical analyses were conducted using R (version 4.0.2, the R foundation). The other details of the statistical analysis are presented in **Supplementary Methods**.

RESULTS

Study Population

The median duration from transplantation to biopsy was longer in the ABMR cases than in TCMR and no-rejection cases (**Table 1**). No-rejection cases were mostly from living, related donors. All cases had negative crossmatch results. Regarding lab findings, the ABMR group had the highest median serum creatinine values. The ABMR cases had a higher proportion of ABO-incompatible transplantation than the no-rejection group, but the proportion was smaller than that in the TCMR cases. Delayed graft function, which occurred in more than 10%, was more prevalent, and cold ischemic time was longer in the ABMR cases than in the other groups. Among the ABMR cases, biopsy specimens of 52 (76%) cases were positively stained for C4d, including 1+ (19 cases), 2+ (20 cases), and 3+ (13 cases) results, respectively. Among the TCMR cases, 48 cases did not have any v-lesions but t- and c-lesions (grade I TCMR). Of the remaining 19 cases with v-lesions, 12 TCMR cases were identified to have isolated v-lesions as they had minimal-to-no interstitial or microvascular inflammation and negative C4d staining results. There were 2 calcineurin inhibitor toxicity and 2 acute tubular necrosis cases diagnosed among the no rejection controls, otherwise, there were no pathologic diagnosis among the samples.

Levels of Non-HLA Antibodies

Anti-AT1R antibodies were more frequently observed in the ABMR cases than in the TCMR or no-rejection control group. However, the proportion of patients testing positive for anti-MICA antibody was not significantly different among the three groups (**Supplementary Table S1**).

The antibody screening results are shown in **Figure 2**, which shows the comparison between the study groups, and **Figure 3**,

which shows a heatmap presenting the relative levels in each sample, and **Supplementary Table S2**, which shows the statistical test results for differences in median values. We found that anti-collagen type I and anti-collagen type III antibodies were significantly higher in the ABMR cases than in the TCMR and no-rejection cases ($p < 0.05/39$). Differences in other antibodies did not reach the significance level when compared with the control groups. Moreover, when we stratified the antibody levels according to upper quartile (≥ 75 th percentile) cut-offs among the ABMR, TCMR, and no-rejection cases, the proportions of recipients who had upper quartile ranges for anti-collagen type I and III antibodies were significantly higher among the ABMR patients than in the controls (**Supplementary Table S3**). Thus, anti-collagen type I and type III antibodies were selected for further analysis as target antibodies that may have clinical significance for ABMR. The antibody levels of anti-collagen type I and type III were strongly correlated (**Supplementary Figure S1**).

Factors Associated With the Target Antibody Levels

Within the ABMR cases, those with high anti-collagen type I or type III levels had higher proportion of cadaveric transplantation cases and, thus, lower proportion of ABO incompatible cases (**Supplementary Tables S4, S5**). Otherwise, the transplantation characteristics were generally similar according to presence of high anti-collagen type I or type III levels, except for that those with high anti-collagen type III titers were more sensitized according to the results from PRA class II screenings.

We found that anti-collagen type I and type III antibody levels did not differ depending on the presence of HLA-DSAs, chronic-active lesions or coexisting TCMR or calcineurin inhibitor toxicity (**Supplementary Tables S6–S8**). The cold ischemic time showed a significant association with the anti-collagen type I antibody level, but was marginally associated with the anti-collagen type III antibody level (**Supplementary Table S9**). As for the relation with pathologic parameters (**Supplementary Table S10**), we found patients with a higher anti-collagen type I or III level more commonly had higher scores for peritubular capillaritis (ptc). Further, patients with a higher anti-collagen type I level more commonly had positive findings for interstitial inflammation (i). When we investigated the correlation between anti-collagen type I or type III antibody titer and the Banff lesions, again, ptc was identified to be significantly correlated with the titers (**Supplementary Figure S3**). Finally,

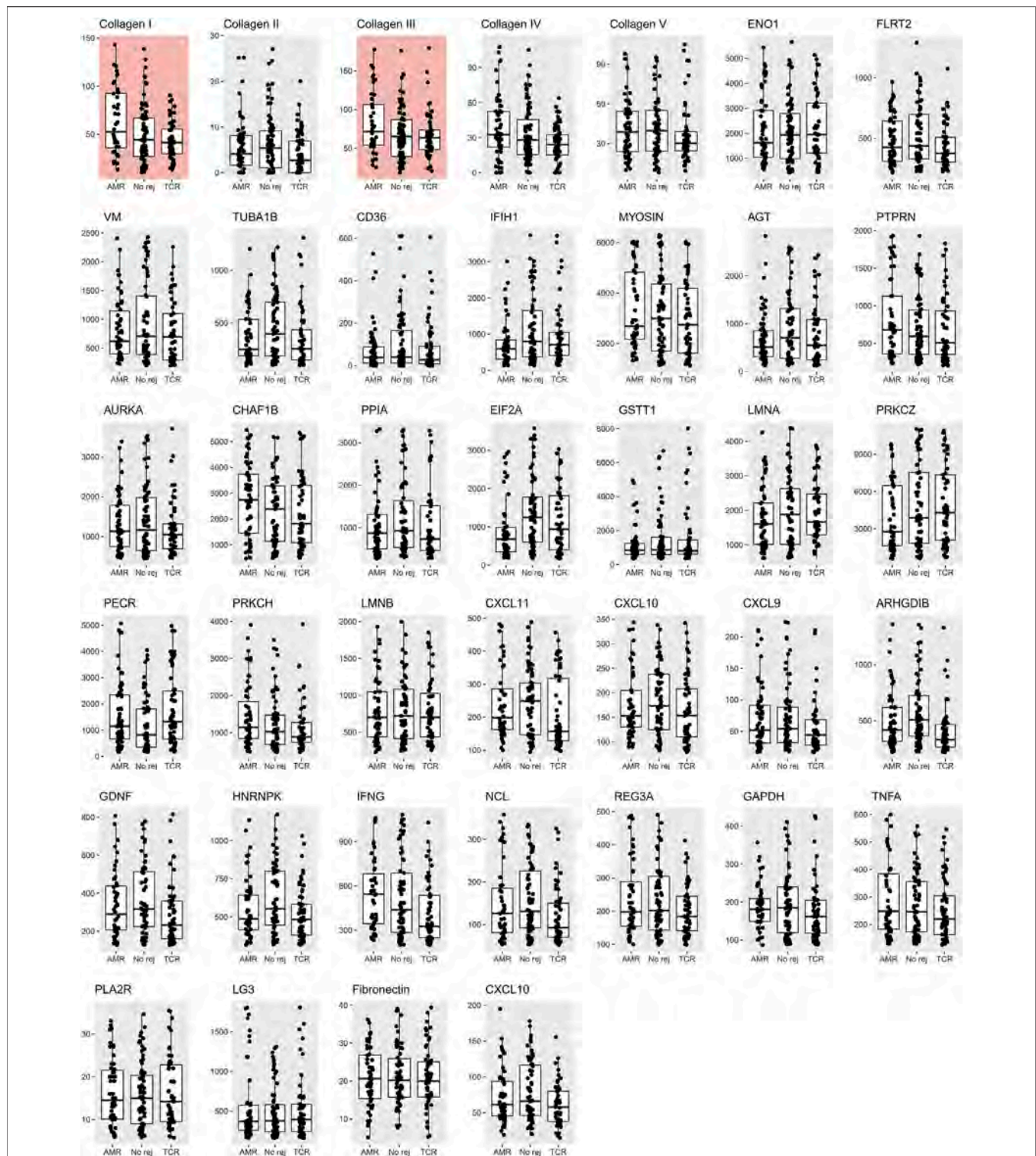
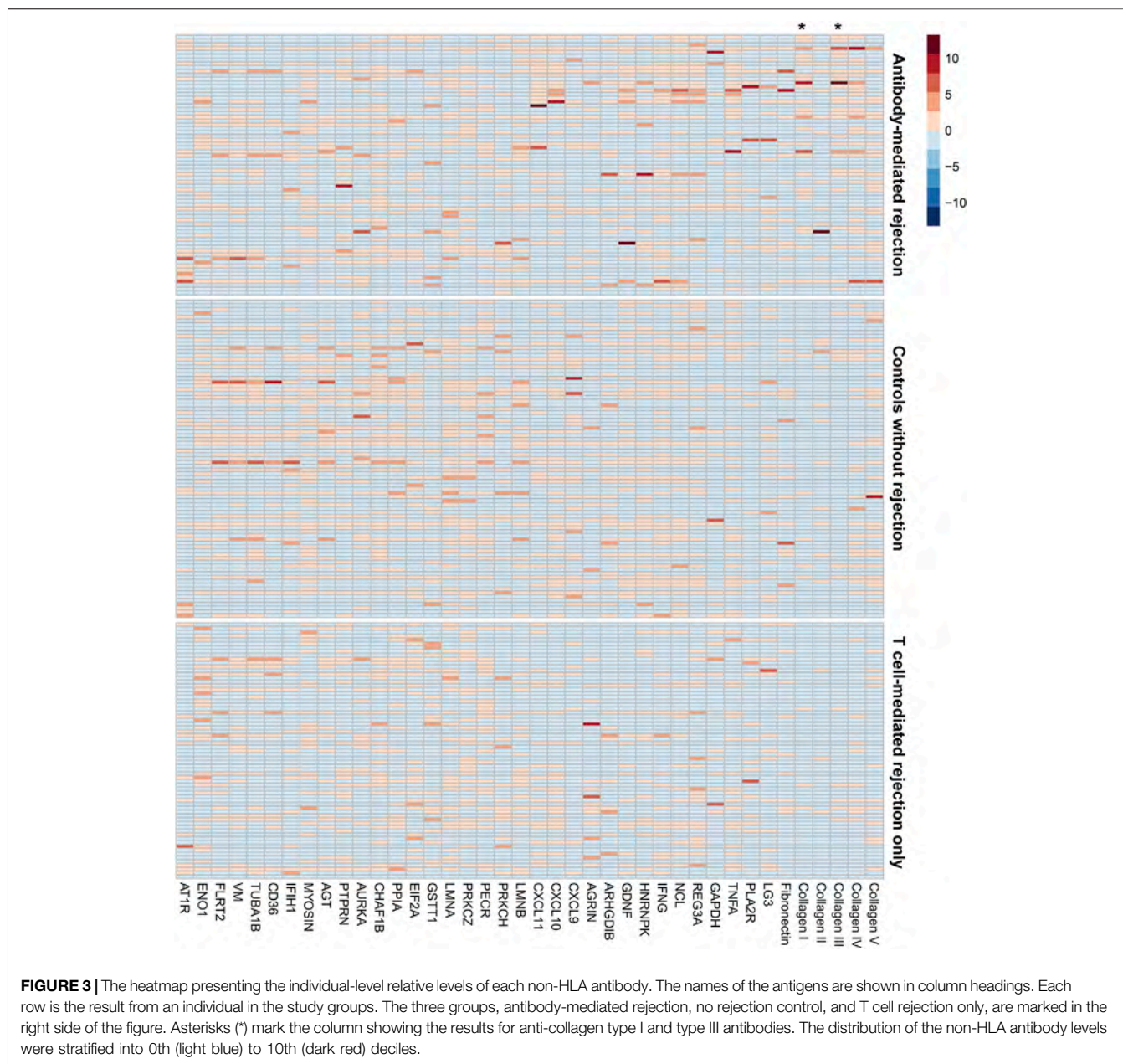


FIGURE 2 | Measured non-HLA antibody levels among the antibody-mediated rejection, T-cell mediated rejection, and no rejection control groups. The median and interquartile values are presented by box and horizontal lines. The dots represent each level of a patient. The red background boxes for collagen I and III indicate that anti-collagen type I and type III antibody levels were significantly higher in the antibody-mediated rejection cases when compared to the T-cell mediated rejection and no rejection controls.



when we compared anti-collagen type I and type III antibody levels of the 47 ABMR cases with the levels before transplantation measured in those with available samples, we found no significant differences in the median values or proportion of patients with high levels for anti-collagen type I and type III antibodies (**Supplementary Table S11**).

Predictability of Antibody-Mediated Rejection

The anti-collagen type I and type III antibody levels showed a positive association with the probability of ABMR occurrence in the studied patients (**Supplementary Figure S2**). A one standard

deviation increase in the levels of both antibodies was associated with approximately 10-fold higher odds for ABMR in the univariable analysis (**Table 2**). In the multivariable analysis, anti-collagen type I or type III antibody levels and odds for ABMR were again significantly correlated. Female sex, presence of HLA-DSAs, longer duration from transplantation to biopsy, and higher serum creatinine values were other significant factors associated with a higher probability of ABMR occurrence. Further, addition of the anti-collagen type I antibody level to the ROC model including presence of HLA-DSA, anti-AT1R antibody, and anti-MICA antibody significantly improved the AUC values (0.781 vs. 0.696, $p = 0.007$) (**Figure 4**). Similarly, addition of the anti-collagen type III antibody level (0.783 vs.

TABLE 2 | Regression analysis to investigate the association between HLA or non-HLA antibody levels and odds for ABMR.

Exposure	Univariable model		Multivariable model ^a	
	OR for ABMR (95% CI)	P	Adjusted OR for ABMR (95% CI)	P
Anti-collagen type I antibody ^b				
continuous (1 SD increase)	12.92 (3.82–59.58)	<0.001	11.72 (2.73–76.30)	0.003
categorical (≥ 75 percentile)	5.64 (2.94–11.08)	<0.001	5.43 (2.24–13.70)	<0.001
Anti-collagen type III antibody ^b				
continuous (1 SD increase)	9.58 (3.33–36.11)	<0.001	6.22 (1.91–31.75)	0.01
categorical (≥ 75 percentile)	5.33 (2.80–10.39)	<0.001	1.53 (0.71–3.56)	0.28
HLA-DSA (yes vs. no)	9.28 (4.41–20.85)	<0.001	6.88 (2.69–18.60)	<0.001
Anti-AT1R antibody (yes vs. no)	3.58 (1.53–8.69)	0.004	2.46 (0.57–10.07)	0.21
Anti-MICA antibody (yes vs. no)	1.00 (0.44–2.15)	0.996	0.74 (0.18–2.51)	0.65

OR, odds ratio; ABMR, antibody-mediated rejection; CI, confidence interval; SD, standard deviation.

The effect sizes for HLA-DSA, anti-AT1R antibody, and anti-MICA antibody were from the model which was adjusted with the anti-collagen type I antibody levels (continuous).

The effect sizes of the other variables that reached significance level in the multivariable model was male sex [adjusted OR 0.31 (0.12–0.75), $p = 0.01$], serum creatinine [1 mg/dl increase, adjusted OR 2.56 (1.75–4.00), $p < 0.001$], and duration from transplantation to biopsy [30 days increase, adjusted OR 1.02 (1.002–1.03), $p = 0.045$]. The variables reaching statistically significant level was the same when the anti-collagen type III antibody level was included in the multivariable model.

^aMultivariable model included age, sex, presence of HLA-DSA, anti-AT1R antibody, and anti-MICA, antibody at the time of allograft biopsy, duration from transplantation to allograft biopsy, donor types (living or deceased), serum creatinine levels, and anti-collagen type I or type III, antibody (each).

^bThe analysis was separately performed including continuous levels and categorically defined high levels as the exposures.

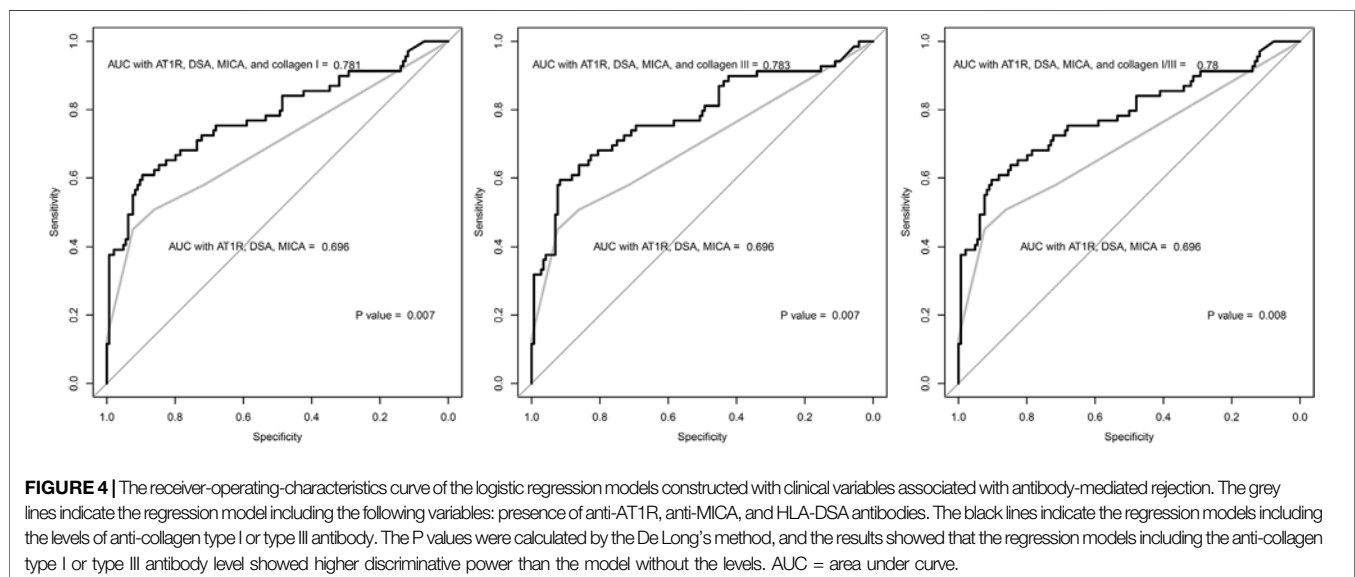


FIGURE 4 | The receiver-operating-characteristics curve of the logistic regression models constructed with clinical variables associated with antibody-mediated rejection. The grey lines indicate the regression model including the following variables: presence of anti-AT1R, anti-MICA, and HLA-DSA antibodies. The black lines indicate the regression models including the levels of anti-collagen type I or type III antibody. The P values were calculated by the De Long's method, and the results showed that the regression models including the anti-collagen type I or type III antibody level showed higher discriminative power than the model without the levels. AUC = area under curve.

0.696, $p = 0.007$) or of both anti-collagen type I and III antibody levels (0.780 vs. 0.696, $p = 0.008$) significantly improved the AUC values.

Prognosis Analysis

In the ABMR group, 13 patients progressed to death-censored graft failure during a median of 2.1 [1.3–3.1] years of follow-up (Figure 5). There were two cases of death-with-graft function for which the follow-up was censored with the event. In the no-rejection controls, only one patient progressed to death-censored graft failure. In the TCMR group, there were three events of death-censored graft failure. Both patients with ABMR with

HLA-DSA and those with ABMR without HLA-DSA had a significantly worse prognosis than the no-rejection controls (Supplementary Table S12). This significant difference remained when we used the TCMR cases as the reference group; ABMR cases, regardless of the presence of HLA-DSA, showed a significantly higher (>4-fold) hazard for death-censored graft failure than the TCMR patients.

When we evaluated the prognostic significance of the target antibodies among the ABMR cases, a one standard deviation increase in the anti-collagen type I or type III antibody level was associated with a significantly higher risk of death-censored graft failure (Table 3). The significance remained after multivariable

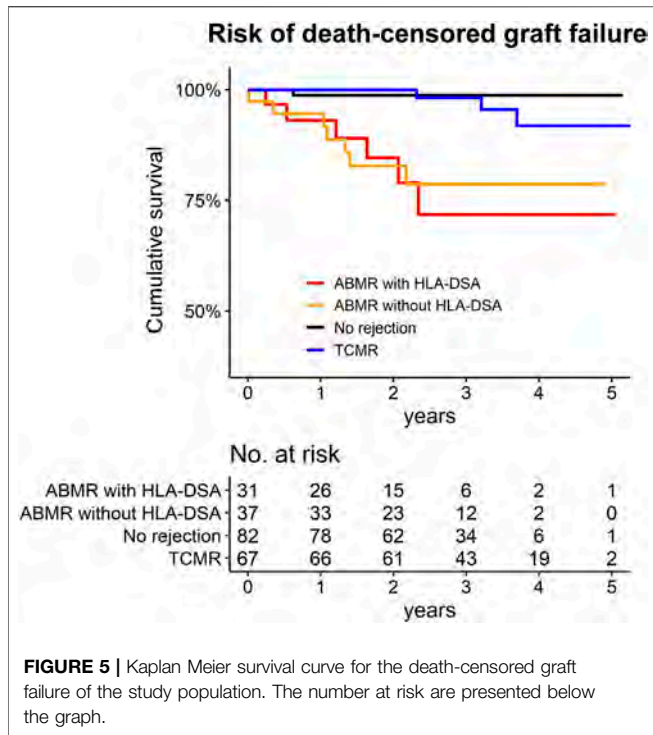


FIGURE 5 | Kaplan Meier survival curve for the death-censored graft failure of the study population. The number at risk are presented below the graph.

adjustment for age, serum creatinine, presence of a mixed TCMR, and presence of HLA-DSA at the time of ABMR diagnosis, and a one standard deviation increase in the antibody level was associated with more than 50% higher risk of death-censored graft failure. In subgroups stratified by a high anti-AT1R antibody level (≥ 10 U/mL) or anti-collagen antibody levels, both antibodies showed potential prognostic significance, with higher levels being

associated with a higher hazard of death-censored graft failure. Although statistical significance was not observed in the univariable model, after adjusting the baseline variables, patients with high levels of both anti-AT1R and anti-collagen type I and type III antibody showed a significantly higher risk of death-censored graft failure.

Internal Validation

The OD-based anti-collagen I IgG antibody values measured by ELISA and the MFI titers measured by the Luminex method showed significant ($p < 0.001$) correlation with each other (Pearson $R = 0.580$, **Supplementary Figure S4**). In addition, the ABMR group showed average 44.9 ± 57.5 unit/ml of anti-collagen I IgG antibody measured by ELISA, which was significantly ($p = 0.040$) higher than that of the controls (20.4 ± 21.6 unit/ml). Those with high (>75 percentile) anti-collagen I antibody titers measured by the Luminex method were significantly associated with higher values (>75 percentile) measured by ELISA (8/15, 53.3%), while those with the lower ranges of titers by the Luminex method also frequently showed low values by ELISA (33/39, 84.6%) ($p = 0.003$).

DISCUSSION

In this study, we measured serum levels of 39 non-HLA antibodies in patients with biopsy-confirmed ABMR, TCMR, and absence of rejection, which revealed that anti-collagen type I and type III antibody levels were significantly elevated particularly in the ABMR patients. The addition of the anti-collagen type I or III antibody level significantly improved the predictability of models for ABMR including the presence of HLA-DSA or other previously reported non-HLA antibodies. Further, patients with a high anti-collagen type I or III antibody level showed a worse prognosis.

TABLE 3 | Risk of death-censored graft failure of the ABMR group according to the levels of anti-collagen type I or III antibody.

Exposure	Univariable model		Multivariable model ^a	
	HR (95% CI)	P	Adjusted HR (95% CI)	P
Anti-collagen type I antibody level				
1 standard deviation increase	1.65 (1.24–2.19)	<0.001	1.90 (1.32–2.75)	<0.001
>1st tertile value within ABMR cases	5.00 (1.89–13.25)	0.001	5.99 (1.16–30.91)	0.03
Subgroup divided by anti-AT1R antibody and anti-collagen type I antibody level ^b				
Low anti-AT1R antibody level and low anti-collagen type I antibody level	Reference		Reference	
Low anti-AT1R antibody level and high anti-collagen type I antibody level	1.47 (0.33–6.57)	0.62	1.77 (0.32–9.38)	0.51
High anti-AT1R antibody level and low anti-collagen type I antibody level	4.21 (0.85–20.86)	0.08	4.44 (0.86–22.75)	0.07
High anti-AT1R antibody level and high anti-collagen type I antibody level	4.18 (0.84–20.74)	0.08	8.48 (1.08–66.40)	0.04
Anti-collagen type III antibody level				
1 standard deviation increase	1.44 (1.10–1.88)	0.007	1.57 (1.15–2.16)	0.005
>1st tertile value within ABMR cases	1.23 (0.41–3.65)	0.713	1.76 (0.44–6.99)	0.42
Subgroup divided by anti-AT1R antibody and anti-collagen type III antibody level ^b				
Low anti-AT1R antibody level and low anti-collagen type III antibody level	Reference		Reference	
Low anti-AT1R antibody level and high anti-collagen type III antibody level	1.11 (0.25–4.98)	0.89	1.44 (0.28–7.52)	0.66
High anti-AT1R antibody level and low anti-collagen type III antibody level	3.60 (0.60–21.56)	0.16	3.74 (0.59–23.69)	0.16
High anti-AT1R antibody level and high anti-collagen type III antibody level	3.65 (0.82–16.37)	0.09	6.70 (1.05–43.03)	0.04

HR, hazard ratio; CI, confidence interval; SD, standard deviation.

^aMultivariable model was adjusted for age and serum creatinine values, presence of a mixed T-cell mediated rejection, and presence of any HLA-DSA at the time of ABMR diagnosis.

^bHigh level of anti-AT1R, antibody was determined as ≥ 10 U/mL. Anti-collagen type I or type III antibody level was recategorized as > 1st tertile value. The alternate threshold for anti-collagen type I or type III antibody was applied because a Cox regression model was not constructed for certain analysis when 75 percentile value was applied because there was a subgroup with zero outcome.

Thus, anti-collagen type I or III antibody may be a biomarker with diagnostic and prognostic value for ABMR.

ABMR currently is a major cause of late graft failure in the transplantation field. Although the identification of the significance of HLA-DSA has enabled the development of therapeutic strategies for the prevention, monitoring, and treatment of a portion of ABMR patients, a non-negligible portion of ABMR patients is HLA-DSA-negative (1, 4). Further, previous studies on ABMR pathology or using allograft transcriptome profiling could not clearly distinguish HLA-DSA-negative ABMR from HLA-DSA-positive cases (4, 13), thus, additional serologic biomarkers may be helpful to diagnose ABMR. Considering the urgent need for biomarkers to aid the diagnosis of ABMR, the recent 2017 Banff classification included C4d deposition, intrarenal transcriptomic findings associated with ABMR, and circulating non-HLA antibodies as surrogate markers for ABMR (10). Nevertheless, non-HLA antibodies show wide ranges, and specific non-HLA antibodies associated with ABMR needs to be further clarified (9). One strength of our study was that we determined the levels of various non-HLA antibodies in a relatively large number of ABMR cases and compared them with those in both pure TCMR and no-rejection control cases. The aim of this approach was to identify a non-HLA antibody that is associated with the presence of ABMR in allograft biopsy. Indeed, we successfully identified anti-collagen type I and type III antibodies as being related to ABMR independent of the presence of HLA-DSA, anti-AT1R, or anti-MICA antibody. In addition, the HLA-DSA-negative ABMR patients showed significantly worse prognosis than TCMR patients or no-rejection controls, which was different from findings in previous reports (4, 13). This further highlights the necessity of additional biomarkers for ABMR. Even the prognosis of ABMR was different according to the antibody levels; a higher level of anti-collagen type I or type III antibody was associated with a higher risk of death-censored graft failure. Thus, our findings suggest the potential diagnostic and prognostic value of anti-collagen type I and type III antibody levels for kidney transplant recipients with a suspected risk of rejection.

A high collagen turnover has been suggested as a marker for certain kidney pathologies (14). Enzyme-degraded collagen molecules have been associated with ischemia-reperfusion injury, which has been suggested to be the cause of anti-endothelial cell antibody production in ABMR (15). Clinically, high collagen turnover, detected from urine, has been reported in immunoglobulin A nephropathy (14), interstitial fibrosis of kidney transplant recipients (16), or kidney fibrosis in chronic kidney disease (17, 18). In lung transplantation, collagen type V present in airway epithelial cells is the antigen of non-HLA antibody associated with pulmonary graft injury (19). Collagen type V is important for cardiovascular organs, and anti-collagen type V has been reported to be related to ABMR in heart transplantation (20). Further, the fact that collagen type I and type III molecules are the abundant collagen types in kidneys supports the relevance of our findings regarding ABMR in kidney allografts (21). We observed that anti-collagen type I or type III antibody titer was

particularly associated with peritubular capillaritis (ptc) and the collagen molecules are present in kidney microvascular structure. Thus, anti-collagen type I antibody may be a measurable marker of extracellular matrix remodeling or endothelial damage, which occurs in ABMR (22). As anti-collagen type III level was not associated with such relevant findings, anti-collagen type I antibody may be prioritized for further investigation for the significance in ABMR.

Our study could not confirm whether the high anti-collagen type I and type III antibody levels have a causal effect to ABMR. The finding that the anti-collagen type I antibody level was higher in ABMR cases than in the donor controls and was associated with longer cold ischemic time may support that the formation of the antibodies during transplant surgery might have caused ABMR. Considering that collagen I or III molecule would be present in kidney microvascular structure, the exposure of neoepitope during transplant surgery by ischemic-reperfusion injury may cause formation of anti-collagen autoantibodies, further contributing to the development of ABMR. Or, preformed anti-collagen type I and type III antibodies may bind to the transplanted graft and cause ABMR. However, the cold ischemic time information was available in the limited portion of patients and the timing of serum collection was heterogeneous, so confirmation of the hypothesis was hardly possible. Among the available samples, pre-transplant levels of anti-collagen type I or type III were not different from post-transplant levels, thus, it is possible that these antibodies may simply be a surrogate biomarker reflecting the fibrotic change in the ABMR pathology. Additional study is warranted to investigate whether these antibodies may cause ABMR and the mechanistical background.

Our study has several limitations. First, whether the levels of anti-collagen type I and type III antibodies change after treatment strategies for ABMR (e.g., plasmapheresis or high-dose immunosuppression) along with improvement in allograft function or a serial sample investigation for kinetics of autoantibody development was not studied. Such information would support that anti-collagen type I or type III antibody may be a novel causal non-HLA antibody that facilitates ABMR. Second, further experimental validation is necessary to determine the direct effects of anti-collagen type I and type III antibodies on the allograft. Third, the study was performed in a single center, implying a possibility of selection bias, although we randomly selected cases with available serum specimens. Additional validation in an independent cohort is warranted to confirm the clinical significance of anti-collagen type I and type III antibodies. Fourth, because of the selection bias and a modest sample size, clinical significance of other non-HLA antibodies might not have been observed due to false negative bias. Therefore, the null findings of our study may not preclude the possibility that other non-HLA antibodies may be related to development of ABMR. Lastly, the study patients were of Asian ethnicities, which have distinct peritubular characteristics from other ethnicities; thus, our study findings cannot be generalized.

In conclusion, among measured 39 non-HLA antibodies, anti-collagen type I and type III antibody levels were significantly higher in ABMR cases. Higher levels of these two antibodies were associated with a higher risk of death-censored-graft failure in ABMR. The

mechanisms of action of anti-collagen type I and type III antibodies on kidney allograft need to be investigated in future studies.

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 Seoul National University Hospital Institutional Review Boards. Written informed consent for participation was collected from the study subjects. All clinical characteristics and bio-specimens were prospectively collected with the approval.

AUTHOR CONTRIBUTIONS

SP, KM, ES, and HL contributed to the conception and design of the study. SP, S-HY, JK, and SC collected the clinical data. JY, S-IM, JH, CJ, YCK, DK, K-HO, and HL advised on statistical aspects and interpreted the data. SP, S-HY, JK, SB, ES, and HL performed the experiments. JY, S-IM, JH, CJ, YCK, DK, K-HO, KJ, YSK, KM, ES, and HL offered advisement regarding the data

interpretation. SP, KM, ES, and HL obtained funding and supervised the overall project. All of the authors participated in drafting the manuscript. All of the authors reviewed the manuscript and approved the final version to be published.

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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.2022.10099/full#supplementary-material>

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The MUC5B Promoter Polymorphism is Not Associated With Non-ILD Chronic Respiratory Diseases or Post-transplant Outcome

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The MUC5B promoter polymorphism (rs35705950) has been associated with interstitial lung disease (ILD) and with prolonged pre-transplant survival in idiopathic pulmonary fibrosis (IPF), but no information is available regarding its prevalence in other respiratory diseases and its influence on post-transplant outcome. We included the Leuven lung transplantation cohort between 1991 and 2015 ($n = 801$). We assessed the minor allele frequency (MAF) of the MUC5B variant in the entire study cohort and investigated the influence of recipient MUC5B promoter polymorphism on post-transplant outcome in patients who were transplanted after 2004. MUC5B was successfully genotyped in 746 patients. The MAF was significantly higher in ILD (17.6%) compared to chronic obstructive pulmonary disease (COPD)/emphysema (9.3%), cystic fibrosis (CF)/bronchiectasis (BRECT) (7.5%) and pulmonary hypertension (PHT) (7.4%) ($p < 0.001$). No association was observed between rs35705950 and chronic lung allograft dysfunction (CLAD)/graft loss in the ILD population [CLAD: HR 1.37 95% CI (0.70–2.68); graft loss: HR 1.02 95% CI (0.55–1.89)], nor the entire study cohort [CLAD: HR 0.96 95% CI (0.69–1.34); graft loss: HR 0.97 95% CI (0.70–1.35)]. The MUC5B promoter polymorphism is a very specific predictive factor for the presence of pulmonary fibrosis

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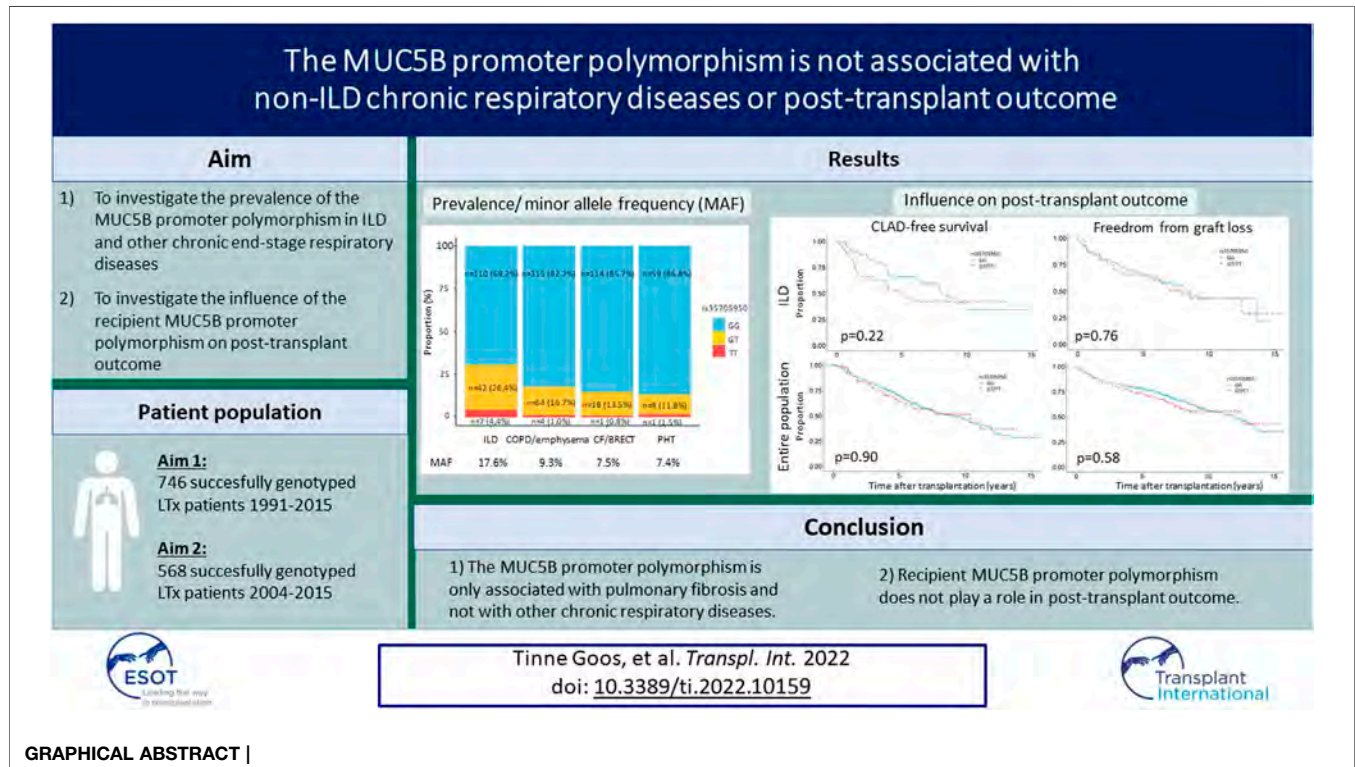
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Abbreviations: AR, acute rejection; AZA, azathioprine; BRECT, bronchiectasis; CF, cystic fibrosis; cHP, chronic hypersensitivity pneumonitis; CI, confidence interval; CLAD, chronic lung allograft dysfunction; CMV, cytomegalovirus; COPD, chronic obstructive pulmonary disease; CS, corticosteroids; CTD-ILD, connective tissue disease-associated ILD; FEV1, forced expiratory volume in one second; HL, heart-lung transplantation; HR, hazard ratio; ILD, interstitial lung disease; iNSIP, idiopathic non-specific interstitial pneumonia; IPAF, interstitial pneumonia with autoimmune features; IPF, idiopathic pulmonary fibrosis; LTx, lung transplantation; LB, lymphocytic bronchiolitis; MAF, minor allele frequency; MCTD, mixed-connective tissue disease-associated ILD; MMF, mycophenolate mofetil; PHT, pulmonary hypertension; PM-ILD, polymyositis-associated ILD; RA-ILD, rheumatoid arthritis associated-interstitial lung disease; SL, single lung transplantation; SLE-ILD, systemic lupus erythematosus-associated ILD; SSc-ILD, systemic sclerosis associated-interstitial lung disease; SSL, sequential single lung transplantation.

as it is only associated with pulmonary fibrosis and not with other chronic respiratory diseases. While the MUC5B promoter variant is associated with better pre-transplant survival among IPF patients, recipient MUC5B promoter variant does not play a role in post-transplant outcome.

Keywords: lung transplantation, MUC5B, genetics, interstitial lung diseases, respiratory diseases



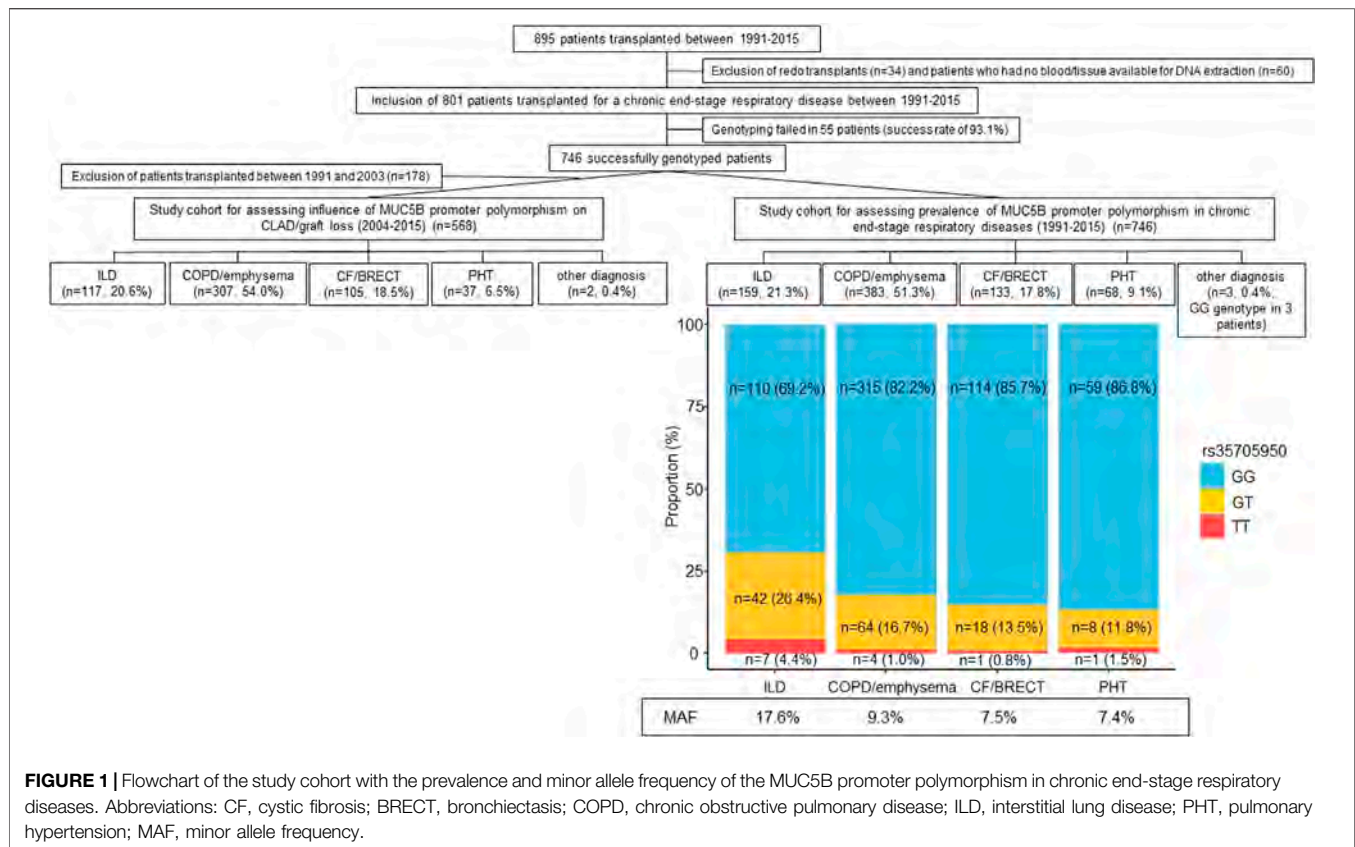
INTRODUCTION

Family clustering of pulmonary fibrosis first suggested important roles for genomics in the underlying pathophysiology. In the last decade, several studies have identified rare and common genetic variants that are associated with pulmonary fibrosis. In 2011, Seibold et al. identified a common variant (rs35705950) in the promoter region of the mucin 5b (MUC5B) gene, which was associated with familial pulmonary fibrosis, sporadic idiopathic pulmonary fibrosis (IPF) and increased expression of mucin 5B in the lung (1). This identification suggested a potential role for the distal airways and mucus overproduction in the pathogenesis of pulmonary fibrosis. Since 2011, a significantly higher frequency of the minor T allele of the MUC5B promoter polymorphism has also been demonstrated in patients with idiopathic non-specific interstitial pneumonia (iNSIP), rheumatoid arthritis associated-interstitial lung disease (RA-ILD), chronic hypersensitivity pneumonitis (cHP), asbestosis and interstitial lung abnormalities, but not

in patients with systemic sclerosis associated-ILD (SSc-ILD), myositis-associated ILD, antisynthetase syndrome and sarcoidosis (1–9). The prevalence of the minor T allele in other non-ILD chronic respiratory diseases such as chronic obstructive pulmonary disease (COPD)/emphysema, cystic fibrosis (CF)/bronchiectasis (BRECT) and pulmonary hypertension (PHT) is still unknown.

Although the MUC5B minor T allele and its increased expression of mucin 5B in the lung has been associated with IPF, IPF patients carrying the minor allele have reduced pre-transplant mortality compared to IPF patients without the minor allele (10). In contrast, rare pathogenic variants in telomere-related genes (e.g., TERT, TERC, PARN, and RTEL1) have been associated with increased mortality and poor post-transplant outcome (2,11–14). The influence of the recipient MUC5B polymorphism on post-transplant outcome is still unknown.

In this study, we assessed the prevalence of the MUC5B minor T allele in patients with ILD and according to ILD subtype, COPD/emphysema, CF/BRECT and PHT who underwent lung transplantation (LTx) at our center between 1991 and 2015. We



compared the prevalence of the MUC5B promoter polymorphism between ILD and other chronic end-stage respiratory diseases. Furthermore, we investigated the influence of recipient MUC5B polymorphism on post-transplant incidence of chronic lung allograft dysfunction (CLAD) and graft loss in the ILD population and the entire population of patients who underwent lung transplantation for a chronic end-stage respiratory disease between 2004 and 2015.

METHODS

Study Cohort

Between 1991 and 2015, 895 patients were transplanted at the University Hospitals Leuven. Redo transplants ($n = 34$) and patients who had no blood or tissue available for DNA extraction were excluded ($n = 60$). The study cohort therefore encompassed 801 patients who were transplanted for a chronic end-stage respiratory disease between 1991 and 2015. Genotyping of MUC5B polymorphism (rs35705950) was performed in this cohort. Genotyping failed in 55 patients (success rate of 93.1%). The prevalence of the MUC5B promoter polymorphism was therefore assessed in 746 successfully genotyped patients: 159 patients had any form of ILD, 383 COPD/emphysema, 133 CF/BRECT, 68 PHT and 3 another diagnosis. As since 2004 all patients are uniformly treated with azithromycin and more electronic clinical data are available, the influence of recipient MUC5B polymorphism on post-transplant incidence

of CLAD and graft loss has only been studied in patients who were transplanted after 2004. Therefore, for this analysis, 568 patients transplanted between 2004 and 2015 were included: 117 patients had any form of ILD, 307 COPD/emphysema, 105 CF/BRECT, 37 PHT and 2 another diagnosis. Study design is presented in **Figure 1**. Clinical information was retrospectively extracted from the electronic medical records. Patient follow-up was recorded until the October 17, 2019, resulting in a minimal follow-up of at least 4 years post-transplantation. This study was approved by our local Ethics Committee and all patients gave written informed consent to access their clinical and biobank data for research (S51577/S54739/ML5629).

Genotyping

Recipient DNA was extracted from peripheral blood or from tissue of explanted lungs when no blood was available. A part of the samples has been previously used by Ruttens et al. (15). DNA from blood samples was extracted using the QIAamp DNA Blood Midi kit (Qiagen, Hilden, Germany) and from lung tissue by using Qiagen DNeasy Blood & Tissuekit. The Nanodrop-1000 (NanoDrop Technologies, Wilmington, DE, United States) was used for the control of DNA purity according to standard guidelines (260/280 ratio ~ 1.7 – 1.9 and 260/230 ratio ~ 2.0 – 2.2). DNA (5 ng/ μ L) was aliquoted into 384-well plates and genotyped at the Vesalius Research Center (Leuven).

Genotyping for MUC5B polymorphism (rs35705950) was performed using iPLEX technology on a MassARRAY

TABLE 1 | Patient characteristics of the LTx cohort transplanted for ILD (2004–2015) according to genotype.

	GG (N = 79; 67.5%)	GT + TT (N = 38 (GT N = 33, TT N = 5); 32.5%)	p-value
Age at LTx (years)	50.9 ± 10.5	56.3 ± 7.9	0.008
Gender (female)	37 (46.8%)	9 (23.7%)	0.03
Date of LTx			0.16
2004–2007	24 (30.4%)	13 (34.2%)	
2008–2011	25 (31.6%)	17 (44.7%)	
2012–2015	30 (38.0%)	8 (21.1%)	
Type of LTx (SSL/HL vs. SL)	64-15 (81.0%–19.0%)	26-12 (68.4%–31.6%)	0.20
Treatment			
AZA-MMF—unknown	41-36-2 (51.9-45.6-2.5%)	23-15-0 (60.5-39.5-0.0%)	0.59
Tacrolimus-cyclosporine—unknown	49-28-2 (62.0-35.4-2.5%)	17-21-0 (44.7-55.3-0%)	0.08
CMV disease	15 (19.0%)	16 (42.1%)	0.02
CMV status			0.52
D+/R +	8 (10.1%)	6 (15.8%)	
D-/R-	31 (39.2%)	10 (26.3%)	
D+/R-	22 (27.8%)	10 (26.3%)	
D-/R+	15 (18.9%)	9 (23.7%)	
unknown	3 (3.8%)	3 (7.9%)	
Preoperative use of CS	54 (68.4%)	24 (63.2%)	0.73
Average dose of CS	6.6 ± 7.0	7.4 ± 9.4	0.92
Ischemic time (min)			
First lung	314 ± 112	271 ± 50	0.04
Second lung	482 ± 138	437 ± 86	0.15
Average	380 ± 125	323 ± 74	0.01
Acute rejection history			
Any AR	38 (48.1%)	21 (55.3%)	0.60
Severe AR (≥B2)	14 (17.7%)	9 (23.7%)	0.61
Any LB	24 (30.4%)	19 (50.0%)	0.06
Severe LB (≥B2)	17 (21.5%)	6 (15.8%)	0.63
CLAD	31 (39.2%)	19 (50.0%)	0.37
Graft loss	39 (49.4%)	21 (55.3%)	0.70

LTx, lung transplantation; SL, single lung transplantation; SSL, sequential single lung transplantation; HL, heart-lung transplantation; AZA, azathioprine; MMF, mycophenolate mofetil; CMV, cytomegalovirus; D, donor; R, receptor; CS, corticosteroids; AR, acute rejection; LB, lymphocytic bronchiolitis; CLAD, chronic lung allograft dysfunction.

Compact Analyzer (Sequenom Inc., San Diego, CA, United States) (16). This method is based on distinguishing allele-specific primer extension products by mass spectrometry (matrix-assisted laser desorption/ionization time-of-flight - MALDI-TOF). The MassARRAY RTTM software was used for generating automated genotyping calls followed by validation through manual review of the raw mass spectra. Results were paralleled with clinical patient data.

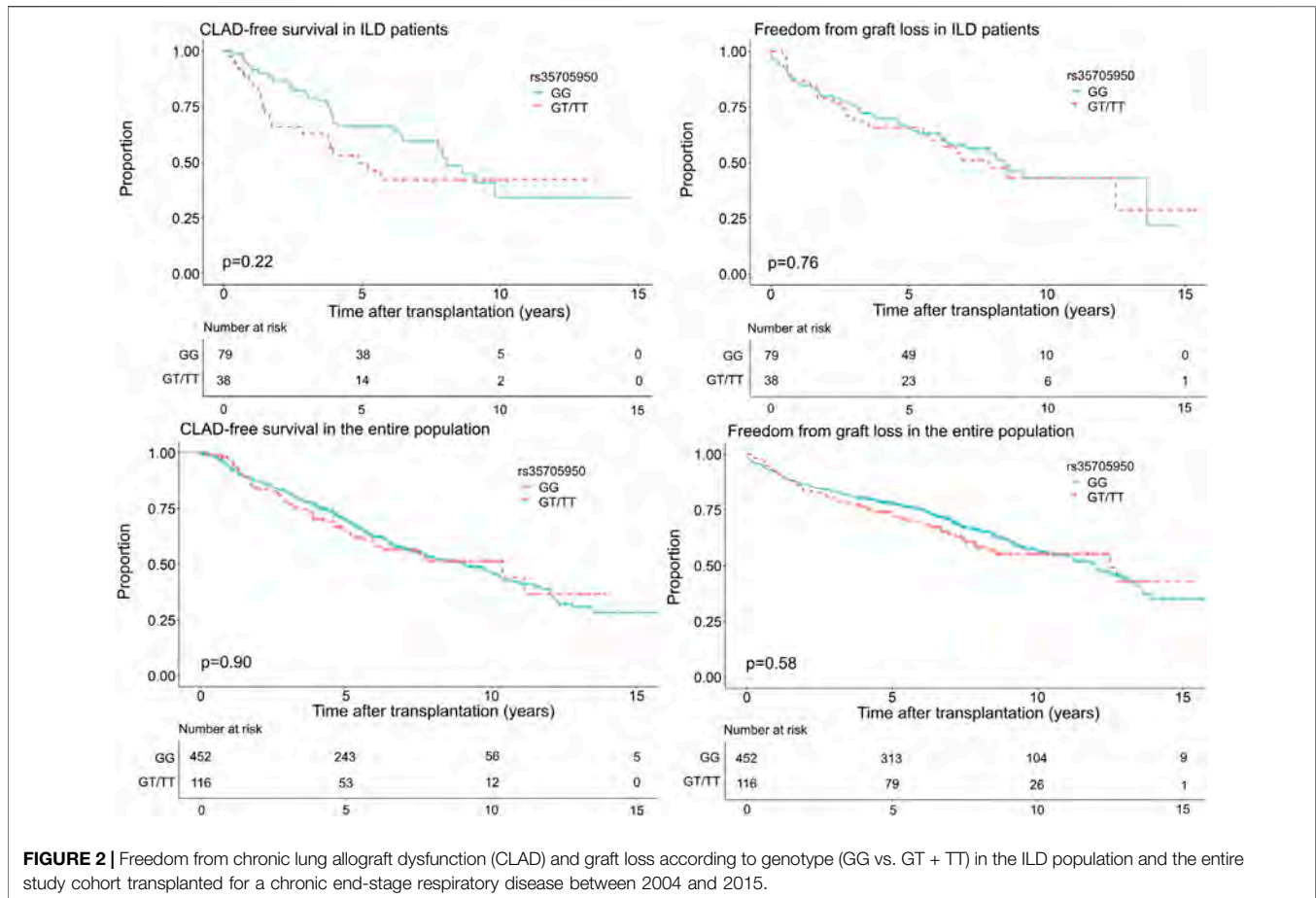
Patient Characteristics and Outcome

Patient characteristics included age at time of LTx, gender, date of LTx, type of LTx [single lung transplantation (SL), sequential single lung transplantation (SSL) or heart-lung transplantation (HL)] and underlying disease. Clinical follow-up data were collected for ILD patients and included ischemic time, immunosuppressive treatment at time of discharge from intensive care unit, corticosteroid use and dose at time of pre-transplant screening, cytomegalovirus (CMV) status (donor/recipient) and

CMV disease as determined by the CMV Drug Development Forum (17). Acute rejection (AR) and lymphocytic bronchiolitis (LB) were defined on histopathology as perivascular or peribronchiolar infiltrates as described by the International Society for Heart and Lung transplantation (ISHLT) guidelines (18). AR/LB was analyzed as a binary variable by comparing at least one AR/LB event during follow-up versus no single event of AR/LB. Severe grades of AR/LB (≥A2/B2) were analyzed separately. CLAD was defined consistent with the ISHLT guidelines as a persistent decline in forced expiratory volume in one second (FEV1) less than 80% of baseline (average of two best FEV1 values after LTx) in the absence of other confounding conditions (19). Graft loss was characterized as death or redo transplantation.

Statistical Analysis

Results are presented in numbers (percentage) for binary variables or by the mean (± standard deviation) for continuous variables. The minor allele frequency (MAF) was calculated by dividing the



number of times the minor T allele was observed by the total number of copies of all the alleles at the genetic locus of interest in the cohort. The chi-square test of independence was used to compare the count of T and G alleles between underlying diseases. The chi-square test of independence, Fisher exact test, *t*-test and Wilcoxon rank sum test were used where appropriate to compare clinical characteristics between patients without the MUC5B promoter polymorphism and patients who were homozygous or heterozygous for the MUC5B promoter polymorphism (GG vs. GT/TT).

Kaplan-Meier survival analysis was performed to compare graft loss and CLAD-free survival between patients without the MUC5B promoter polymorphism and patients who were homozygous or heterozygous for the MUC5B promoter polymorphism (GG vs. GT/TT). Observations were censored when the endpoint was not observed before October 17, 2019 and in the analysis of CLAD when the patient died without evidence of CLAD. Cox proportional-hazards model was used in multivariate analysis to investigate the association between rs35705950 (GG vs. GT/TT) and CLAD and graft loss, while controlling for age, gender, date of LTx, type of LTx in the ILD population and entire study cohort. Multivariate analysis by cox proportional-hazards model was additionally adjusted for the presence of at least one AR and LB event in the ILD population

and for underlying disease in the entire study cohort. All variables were determined *a priori*.

To assess the influence of the MUC5B polymorphism (GG vs. GT/TT) on age at LTx, linear regression was used while adjusting for underlying ILD entity.

To investigate the association between MUC5B minor T allele (GG vs. GT/TT) and CMV disease, logistic regression was performed while adjusting for CMV status, age, gender, date of LTx and type of LTx.

No imputation of missing data was performed. All analyses were performed in R (version 4.0.3, R Project for Statistical Computing, Vienna, Austria). $p < 0.05$ was considered to represent statistical significance for all analyses.

RESULTS

Minor Allele Frequency of MUC5B Polymorphism in LTx Cohort (1991–2015)

The study cohort encompassed 746 successfully genotyped patients: 159 (21.3%) patients had any form of ILD, 383 (51.3%) COPD/emphysema, 133 (17.8%) CF/BRECT, 68 (9.1%) PHT and 3 (0.4%) another diagnosis. The GG genotype was found in 601 patients (80.6%), the GT genotype in 132

TABLE 2 | Univariate and multivariate analysis by cox-proportional hazards model of CLAD and graft loss in LTx cohort transplanted for ILD (2004–2015) and the entire LTx cohort transplanted for a chronic end-stage respiratory disease (2004–2015).

	HR [95% CI]	p-value
ILD population		
Univariate analysis		
CLAD	1.43 [0.81–2.44]	0.22
Graft loss	1.09 [0.64–1.85]	0.76
Multivariate analysis ^a		
CLAD	1.37 [0.70–2.68] ^a	0.35
Graft loss	1.02 [0.55–1.89] ^a	0.96
Total population		
Univariate analysis		
CLAD	1.02 [0.74–1.41]	0.90
Graft loss	1.10 [0.70–1.50]	0.58
Multivariate analysis ^b		
CLAD	0.96 [0.69–1.34] ^b	0.81
Graft loss	0.97 [0.70–1.35] ^b	0.87

^aAdjusted for age at LTx, gender, date of LTx, type of LTx, and presence of AR, and LB.

^bAdjusted for age at LTx, gender, date of LTx, type of LTx, and underlying disease. CLAD, chronic lung allograft dysfunction; HR, hazard ratio; CI, confidence interval; ILD, interstitial lung disease.

patients (17.7%) and the TT genotype in 13 patients (1.7%). The MAF of the MUC5B variant was higher in ILD (17.6%) compared to COPD/emphysema (9.3%), CF/BRECT (7.5%), and PHT (7.4%) ($p < 0.001$) (Figure 1).

Influence of MUC5B Polymorphism on CLAD/Graft Loss in LTx Cohort Transplanted for ILD (2004–2015)

To investigate the influence of the MUC5B promoter polymorphism on CLAD/graft loss, 117 ILD patients who were transplanted between 2004 and 2015 were included. The GG genotype was observed in 79 patients (67.5%), the GT genotype in 33 (28.2%) and TT genotype in 5 patients (4.3%). Among the 117 ILD patients, IPF was the most common subtype (29.9%; MAF of MUC5B promoter polymorphism 27.1%), followed by connective tissue disease associated-ILD (23.1%; MAF 22.2%), hypersensitivity pneumonitis (13.7%; MAF 12.5%), sarcoidosis (9.4%; MAF 9.1%), lymphangioleiomyomatosis (5.1%; MAF 8.3%), iNSIP (4.2%; MAF 20.0%) and exposure-induced ILD (4.2%; MAF 20.0%). Other diagnoses (10.3%; MAF 4.2%) included unclassifiable ILD, combined pulmonary fibrosis and emphysema, smoking-related ILD, histiocytosis X and eosinophilic pneumonitis. Native lung fibrosis phenotypes according to genotype and with corresponding MAF are shown in Supplementary Table S1. Patient characteristics stratified according to GG and GT + TT genotype are summarized in Table 1. The ILD subgroups did not significantly differ in year of LTx ($p = 0.16$), type of LTx ($p = 0.20$), immunosuppressive treatment (comparing azathioprine vs. mycophenolate mofetil: $p = 0.59$; and tacrolimus vs. cyclosporine: $p = 0.08$), CMV status ($p = 0.52$), preoperative use and dose of corticosteroids ($p = 0.73$ and $p = 0.92$), AR/LB history ($p = 0.60$ and $p = 0.06$) and AR/LB severe grades ($\geq A2$:

$p = 0.61$; $\geq B2$: $p = 0.63$). Patients with the MUC5B minor T allele were significantly older at time of transplantation ($p = 0.01$), more frequently male ($p = 0.03$) and had a shorter lung ischemic time (average time $p = 0.01$). More CMV infections were observed in patients with at least one minor allele ($p = 0.02$). To investigate the association between age at LTx and the MUC5B promoter polymorphism, linear regression adjusting for underlying entity was performed. Age at LTx was significantly associated with the underlying ILD entity, but not with the MUC5B polymorphism in multivariate analysis [coefficient: 2.83 95% CI (–0.61–6.28) $p = 0.11$]. To investigate the association between the MUC5B variant and CMV disease, logistic regression was performed adjusting for CMV status, age, gender, date of LTx and type of LTx. The MUC5B variant remained not significantly associated with CMV disease in this multivariate analysis [OR 1.15 95% CI (0.98–1.36) $p = 0.09$].

CLAD was diagnosed in 31 patients (39.2%) with the GG genotype and in 19 patients (50.0%) with the GT or TT genotype ($p = 0.37$). Graft loss was observed in 39 patients (49.4%) without a minor allele and in 21 patients (55.3%) with at least one minor allele ($p = 0.70$). Kaplan-Meier analysis showed no significant association between MUC5B promoter polymorphism and CLAD [HR 1.43 95% CI (0.81–2.44) $p = 0.22$] or between the MUC5B promoter polymorphism and graft loss [HR 1.09 95% CI (0.64–1.85) $p = 0.76$] in univariate analysis. Multivariate analysis by cox-proportional hazards model including the MUC5B variant, age at LTx, gender, date of LTx, type of LTx and presence of AR or LB, demonstrated no association between recipient MUC5B polymorphism and CLAD [HR 1.37 95% CI (0.70–2.68) $p = 0.35$], or between recipient MUC5B polymorphism and graft loss [HR 1.02 95% CI (0.55–1.89) $p = 0.96$] (Figure 2 and Table 2). Both multivariate models are presented in Supplementary Table S2.

Influence of MUC5B Polymorphism on CLAD/Graft Loss in LTx Cohort Transplanted for a Chronic End-Stage Respiratory Disease (2004–2015)

To investigate the influence of the MUC5B promoter polymorphism on CLAD/graft loss, 568 patients transplanted for a chronic end-stage respiratory disease between 2004 and 2015 were included: 117 (20.6%) patients had any form of ILD, 307 (54.0%) COPD/emphysema, 105 (18.5%) CF/BRECT, 37 (6.5%) PHT and 2 (0.4%) another diagnosis. The GG genotype was found in 452 patients (79.6%), the GT genotype in 107 patients (18.8%) and the TT genotype in 9 patients (1.6%). Patient characteristics stratified according to GG and GT + TT genotype are summarized in Table 3. The subgroups differed significantly in age at LTx ($p < 0.01$), gender ($p = 0.03$), year of LTx ($p = 0.01$) and underlying disease ($p = 0.01$), but showed no difference in type of LTx ($p = 0.05$). CLAD and graft loss was observed in 193 patients (42.7%) and in 179 patients (39.6%) with a GG genotype compared to 46 patients (39.7%) and 49 patients (42.2%) with a GT or TT genotype ($p = 0.63$ and $p = 0.68$), respectively. No significant association between the MUC5B promoter polymorphism and CLAD [HR 1.02 95% CI (0.74–1.41) $p =$

TABLE 3 | Patient characteristics of LTx cohort transplanted for a chronic end-stage respiratory disease (2004–2015) according to genotype.

	GG [N = 452 (79.6%)]	GT + TT [(N = 116; GT N = 107; TT N = 9) (20.4%)]	p-value
Age at LTx (years)	49.4 ± 13.7	53.5 ± 11.6	0.004
Gender (female)	236 (52.2%)	47 (40.5%)	0.03
Type of LTx (SLL/HL vs. SL)	409 (90.5%)–43 (9.5%)	97 (83.6%)–19 (16.4%)	0.05
Date of LTx			0.007
2004–2007	139 (30.8%)	31 (26.7%)	
2008–2011	138 (30.5%)	53 (45.7%)	
2012–2015	175 (38.7%)	32 (27.6%)	
Indication for LTx			0.007
ILD	79 (17.5%)	38 (32.8%)	
COPD/emphysema	249 (55.1%)	58 (50.0%)	
CF/BRECT	90 (19.9%)	15 (13.0%)	
PHT	32 (7.1%)	5 (4.3%)	
Other diagnosis	2 (0.4%)	0 (0.0%)	
CLAD	193 (42.7%)	46 (39.7%)	0.63
Graft loss	179 (39.6%)	49 (42.2%)	0.68

LTx, lung transplantation; SL, single lung transplantation; SLL, sequential single lung transplantation; HL, heart-lung transplantation; ILD, interstitial lung disease; COPD, chronic obstructive pulmonary disease; CF, cystic fibrosis; BRECT, bronchiectasis; PHT, pulmonary hypertension; CLAD, chronic lung allograft dysfunction.

0.90], or between the MUC5B promoter polymorphism and graft loss [HR 1.10 95% CI (0.70–1.50) $p = 0.58$] was observed in univariate analysis. In multivariate analysis including the MUC5B variant, age at LTx, gender, date of LTx, type of LTx and underlying disease, the MUC5B variant was neither associated with CLAD [HR 0.96 95% CI (0.69–1.34) $p = 0.81$] nor with graft loss [HR 0.97 95% CI (0.70–1.35) $p = 0.87$] (Table 2 and Figure 2). Both multivariate models are presented in Supplementary Table S3.

DISCUSSION

This is the first study reporting on the prevalence of the MUC5B minor T allele in chronic end-stage respiratory diseases and the association between this variant and post-transplant outcome. In the present study, MAF of the MUC5B promoter polymorphism was significantly higher in ILD than in other end-stage respiratory diseases. No association between recipient MUC5B polymorphism and post-transplant outcome was observed.

As expected, we found a higher prevalence of the MUC5B polymorphism in end-stage ILD with a MAF of 17.6%. This was lower than the previous reported MAF of 34–38% in IPF, 33% in RA-ILD, 24–32% in cHP and 29% in asbestosis, but in the present study, subgroups with a reported prevalence of the MUC5B variant equal to the normal population (i.e., sarcoidosis, SSc-ILD, myositis-associated ILD and antisynthetase syndrome) were also included (1–5,7–9). Indeed, our data on the prevalence of the MUC5B minor allele in the different ILD subgroups suggest a higher MAF in some ILD entities such as IPF (27.1%). The MUC5B promoter polymorphism was only associated with ILD as the MAF of the MUC5B polymorphism in the non-ILD end-stage respiratory

diseases was comparable to the reported 9% in the normal Caucasian population (1). Although association between genetic variants and disease is not the same as causation, this finding suggests a specific role for the MUC5B promoter polymorphism and its associated increased mucin5b production in pulmonary fibrosis. How MUC5B exactly is involved in ILD susceptibility is still an unanswered question, but it highlights a potential role for the distal airways and mucus overproduction in the pathogenesis of pulmonary fibrosis (20,21).

The MUC5B minor T allele has been related to reduced mortality in IPF patients (10). Newton et al. confirmed this relationship in IPF patients but observed a worse transplant-free survival in interstitial pneumonia with autoimmune features (IPAF) patients and a trend toward worse transplant-free survival in CTD-ILD patients (22). Similarly, the presence of rs35705950 was of borderline statistical significance with worse survival in a cohort of cHP patients (2). Rare variants in telomere-related genes and short telomere lengths have been associated with progressive disease and worse survival across different ILD entities and with worse post-transplant outcome, even in the absence of notable syndromic clinical features of telomeropathies (2, 11–14, 22). Suggested mechanisms for this worse post-transplant outcome include defects in adaptive immunity and intolerance of the hematological stress of transplant-related myelosuppressive medications (12,14). For the MUC5B minor allele, no association with CLAD-free and graft survival was observed in both the ILD population and the total patient population. This could be explained by the fact that the MUC5B polymorphism only influences mucin5b producing cells, while telomere-related gene variants affect every organ system with increased cell turnover such as the bone marrow and immune system. Indeed, proposed mechanisms for reduced

pre-transplant mortalities in IPF patients with a MUC5B minor T allele such as an enhanced host defense as a result of increased mucin production involve only the lung as organ system (23). After LTx, there is evidence for chimerism in a small percentage of epithelial cells in bronchial and alveolar tissue, but the majority of the cells originate from the donor (24). Regarding MAF of 9% in the normal Caucasian population, donor cells are more likely to express the major allele. It would therefore be very interesting to investigate the influence of donor MUC5B polymorphism on post-transplant outcome, but it was not possible to determine this within the scope of this study.

Although this is a very large cohort of lung transplant patients genotyped for rs35705950, there are several limitations to this study. First, this forms a single-center, retrospective cohort study and not all variables were present for each patient and genotyping could not be performed in all included patients. Second, the ILD population was relatively small and it was therefore not possible to draw conclusions about the MAF in the ILD subgroups or the influence of MUC5B polymorphism on post-transplant outcome in the specific ILD entities. Furthermore, while we investigated the influence of the recipient MUC5B polymorphism on post-transplant outcome, we were not able to do the same for donor MUC5B polymorphism. Lastly, some patients were only referred to our center for LTx and diagnostic work-up was performed in another center. The diagnostic certainty of the underlying lung disease in these cases is limited.

In conclusion, the prevalence of rs35705950 in chronic end-stage respiratory diseases in the context of COPD/emphysema, CF/BRECT and PHT was similar to the normal Caucasian population, while we confirmed the higher MAF in end-stage ILD. Therefore, the MUC5B promoter polymorphism is a very specific predictive factor for the presence of pulmonary fibrosis as it is only associated with pulmonary fibrosis and not with other chronic respiratory diseases. We found no association between recipient MUC5B polymorphism and post-transplant incidence of CLAD and graft loss in the ILD population and in the entire study cohort. While the MUC5B promoter variant is associated with better pre-transplant survival among IPF patients, recipient MUC5B promoter variant does not play a role in post-transplant outcome.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because of ethical issues. Requests to access the datasets should be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the University Hospitals Leuven. The patients/

participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

TG, SEV, LD, AVH, AS, AV, JK, VG, CA, DR, DL, SV, LC, DV, LG, JY, BV, GV, RV and WW contributed substantially to the study design, data analysis and interpretation and writing 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.2022.10159/full#supplementary-material>

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Role of the Intramural Vascular Network of the Extrahepatic Bile Duct for the Blood Circulation in the Recipient Extrahepatic Bile Duct Used for Duct-to-Duct-Biliary-Anastomosis in Living Donor Liver Transplantation

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A duct-to-duct-biliary-anastomosis is the preferred biliary reconstruction technique in liver transplantation; biliary complications remain the major concerns for the technique. We examined the significance of the intramural vascular network of the extrahepatic bile duct (EBD) and its relevant vessels. We microscopically examined the axial sections of the EBD with 5 mm intervals of 10 formalin-fixed deceased livers. The luminal-areas of the 3 and 9 o'clock arteries correlated significantly and positively with the distance from the bifurcation of the right and left hepatic ducts (the 3 o'clock artery, $r = 0.42$, $p < 0.001$; the 9 o'clock artery, $r = 0.39$, $p < 0.001$); the ratios of the numbers of the intramural vessels to the areas of the corresponding sections of the EBD significantly correlated positively with the distance from the bifurcation of the right and left hepatic ducts (total vessels, $r = 0.78$, $p < 0.001$; arterioles, $r = 0.52$, $p < 0.001$; venules, $r = 0.45$, $p < 0.001$). This study demonstrated that there is a significant locoregional distributional heterogeneity of the intramural vessels among the EBD. The hepatic arteries neighboring the EBD primarily supply the blood flow to the EBD; thus, when the broader isolation of the EBD from the neighboring arteries is necessary, this locoregional distributional heterogeneity of the intramural vessels may render the EBD likely to suffer ischemia of the anastomotic site.

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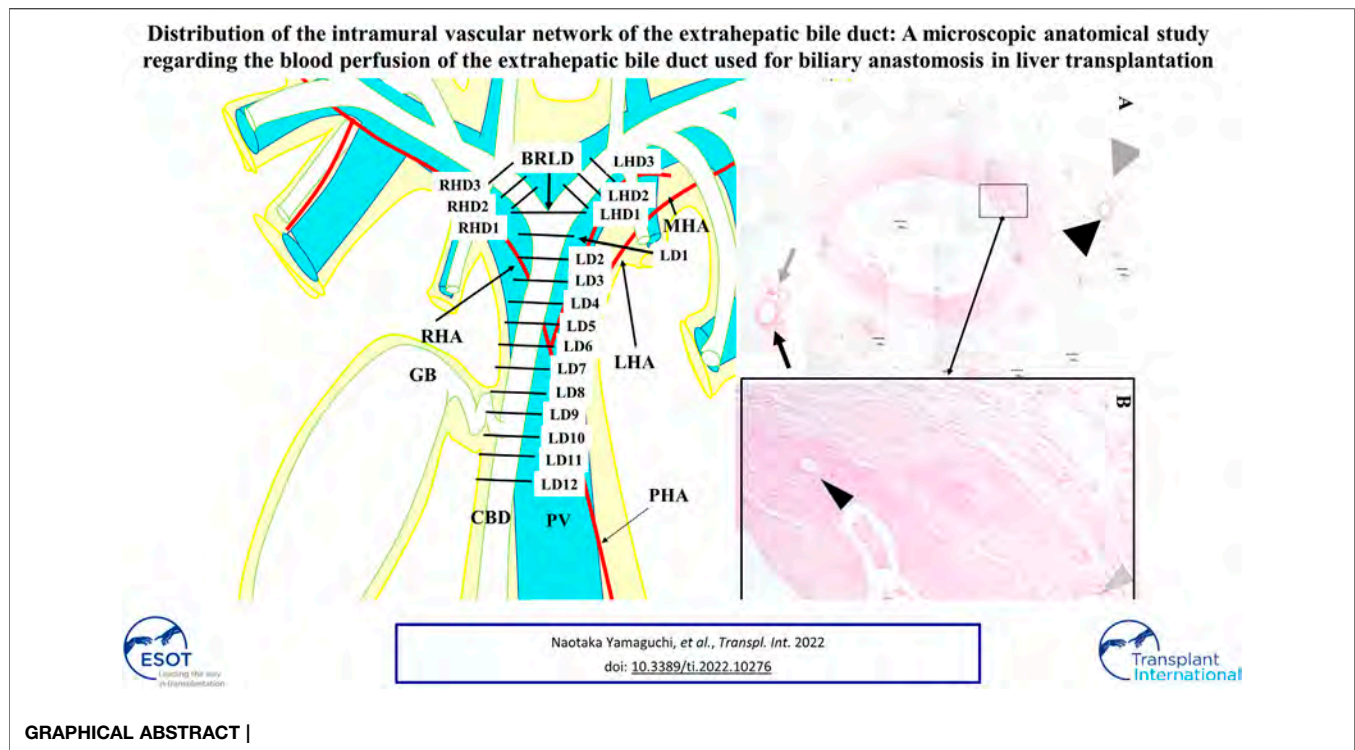
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Keywords: liver transplantation, duct-to-duct anastomosis, biliary anastomotic stricture, blood perfusion of the extrahepatic bile duct, peribiliary vascular plexus, the 3 and 9 o'clock arteries

INTRODUCTION

A duct-to-duct-biliary-anastomosis (DDBA) is the preferred biliary reconstruction technique in liver transplantation (LT); biliary-complications (BCs) remain the major concern with this technique (1–5). The blood perfusion of the extrahepatic bile duct (EBD) of the graft liver or the recipient is believed to affect the BCs strongly. The EBD is perfused by the peribiliary vascular plexus that consists of the following three layers: the inner-, intermediate-, and outer-layers (6).

Among these layers, the outer-layer corresponds to the connective tissue sheath surrounding the EBD; this sheath includes the abundant vascular network (6, 7). The outer-layer has been considered



to act as the primary resource of the blood perfusion of the EBD; the arteries neighboring the EBD provide the blood inflow via the numerous thin macroscopically invisible arterioles into the EBD through the outer-layer (6–9).

Meanwhile, the intermediate-layer is regarded as the EBD itself. The EBD itself only has scarce intramural vessels; thus, the intramural vessels are considered insignificant for the blood perfusion of the EBD (6).

The unfavorable blood perfusion of the EBD of the graft liver or the recipient has been considered as the primary cause of the BCs; the significance of the outer-layer and the neighboring arteries has been vigorously discussed (1–5). This discussion argued that the hepatic arteries neighboring the EBD should be left as attached to the EBD as possible and the outer-layer should be as preserved as possible. However, the neighboring arteries often have to be isolated from the EBD to some extent to enable tension-free secure arterial and biliary anastomoses; this isolation leaves the outer-layer as the sole blood perfusion resource to the anastomotic site of the EBD other than the intramural-vessels. The incidence rate of the BCs remains high, at 20–50% (1–5). These findings may suggest that the preservation of the outer-layer alone cannot guarantee the favorable blood perfusion of the EBD of either the graft liver or the recipient (3–5); some additional insight into the blood circulation of the EBD may be necessary to reduce the incidence of the BCs.

Obtaining the overview of the intramural vessels of the EBD may lead to additional insight into the blood circulation of the EBD of the graft liver or the LT recipient. In this study, we investigated the intramural vascular network of the EBD using 10 deceased Japanese bodies.

MATERIALS AND METHODS

This study used 10 formalin-fixed adult Japanese deceased livers that had neither a history of previous hepatobiliary surgery nor any hepatobiliary diseases. The Institutional Review Board (IRB) of Yokohama City University approved and regarded this study to be compliant with the Hospital and the Declaration of Istanbul 2008 (IRB approve No. was #B19-1003045).

Existence of the 3 and 9 O’Clock Arteries and Veins and Their Luminal Areas

We investigated the EBD above the upper margin of the pancreas in this study: the suprapancreatic duct, the left hepatic duct (LHD), and the right hepatic duct (RHD) (**Figure 1A**). First, the connective tissue was carefully dissected so as to preserve the peribiliary vascular plexus; this careful dissection enabled subsequent microscopical investigation of the 3 and 9 o’clock vessels. Then, axial sections of the EBD with 5 mm intervals were prepared. Each section was termed as shown in **Figure 1B**. Several 3- μ m slices were excised from each surface of the sections. These slices were histologically examined after hematoxylin eosin staining.

Subsequently, we examined whether the 3 and 9 o’clock arteries and veins existed in the outer-layer of the peribiliary vascular plexus in each section (**Figure 2A**). If existed, the luminal areas of the 3 and 9 o’clock arteries and veins were calculated. In this study, the area microscopically evaluated was determined as follows. First, a microscopic field was projected onto a digitizing board; then, an object, of which we attempted to calculate the

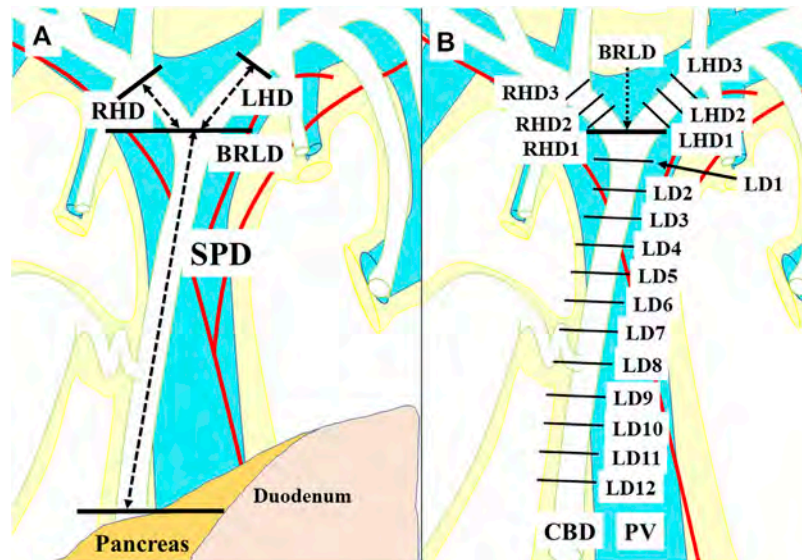


FIGURE 1 | A term for each section for the histological examinations. The suprapancreatic duct was defined as the portion between the upper border of the pancreas and the bifurcation of the right and left hepatic ducts (BRLD) (A). The ducts above the BRLD were the right hepatic duct (RHD) and the left hepatic duct (LHD) (A). Axial sections with 5 mm intervals of the extrahepatic bile duct were prepared. Each section above the BRLD was termed LHD1, LHD2, and LHD3 along with the LHD, and RHD1, RHD2, and RHD3 from distal to proximal. The sections below the CRLD were termed CRLD, LD1, LD2, LD3, LD4, LD5, LD6, LD7, LD8, LD9, LD10, LD11, and LD12 from proximal to distal, respectively (B).

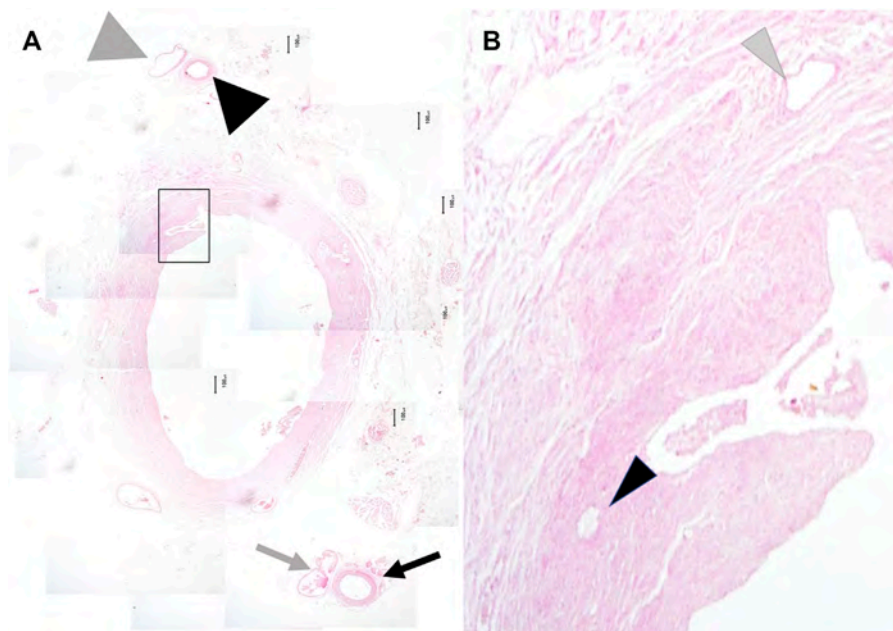


FIGURE 2 | Microscopic findings of the extrahepatic bile duct. The 3 o'clock artery (black arrow) and vein (gray arrow) and the 9 o'clock artery (big black arrowhead) and vein (big gray arrowhead) were observed in all cases. The former was located immediately inside the right lateral border of the outer layer of the peribiliary vascular plexus. The latter was located immediately inside the right lateral border of the peribiliary vascular plexus (A) (original magnification, x40). Numbers of the arterioles (small black arrowhead) and venules (small gray arrowhead) were counted under the high-power field (B) (original magnification, x200).

area, was outlined by a computerized delineation. Subsequently, the area of the object was calculated using Image J software (National Institute of Health, Bethesda, MD, United States) (8, 9).

Then, these areas were compared among the sections; furthermore, their correlations with the distance from the bifurcation of the right and left hepatic ducts were investigated.

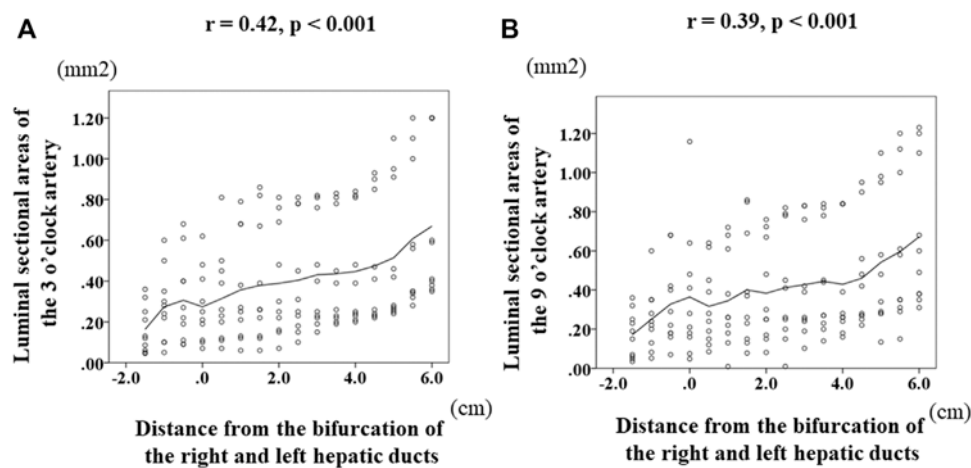


FIGURE 3 | Transition of the luminal areas of the 3 and 9 o'clock arteries according to the distance from the bifurcation of the right and left hepatic ducts. Zero on the horizontal axis indicates the section at the bifurcation of the right and left ducts (BRLD). Luminal-area (mm^2) of the 3 o'clock artery was 0.125 (0.045–0.36) at LHD3, 0.26 (0.05–0.6) at LHD2, 0.25 (0.09–0.68) at LHD1, 0.23 (0.07–0.62) at BRLD, 0.25 (0.07–0.81) at LD1, 0.26 (0.06–0.79) at LD2, 0.26 (0.06–0.86) at LD3, 0.8 (0.07–0.81) at LD4, 0.28 (0.10–0.81) at LD5, 0.33 (0.15–0.82) at LD6, 0.33 (0.20–0.84) at LD7, 0.32 (0.22–0.93) at LD8, 0.34 (0.24–1.10) at LD9, 0.35 (0.24–1.10) at LD10, 0.46 (0.28–1.20) at LD11, and 0.5 (0.35–1.20). A statistically significant difference was observed among the various combinations of these areas. Of note, the consecutive luminal areas of the 3 o'clock artery significantly correlated positively with the distance from the BRLD (**A**). Regarding the 9 o'clock artery, the luminal areas were 0.17 (0.04–0.36) at RHD3, 0.23 (0.05–0.60) at RHD2, 0.26 (0.07–0.68) at RHD1, 0.25 (0.05–1.158) at CRLD, 0.26 (0.09–0.64) at LD1, 0.26 (0.01–0.72) at LD2, 0.32 (0.08–0.86) at LD3, 0.28 (0.08–0.76) at LD4, 0.34 (0.01–0.82) at LD5, 0.41 (0.10–2.25) at LD6, 0.36 (0.14–0.84) at LD7, 0.27 (0.16–0.84) at LD8, 0.35 (0.22–0.95) at LD9, 0.41 (0.13–1.10) at LD10, 0.47 (0.15–1.20) at LD11, and 0.55 (0.31–1.23) at LD12. A statistically significant difference was observed among the various combinations of these areas. Moreover, the consecutive luminal areas of the 9 o'clock artery correlated significantly and positively with the distance from the BRLD (**B**).

Numbers of the Intramural Vessels of the Extrahepatic Bile Duct

At each section, the numbers of total intramural vessels, arterioles, and venules of the EBD were counted under high-power field of the microscopy ($\times 200$ magnification) (**Figure 2B**). Moreover, the area of the EBD wall in each section was determined; then, the ratios of the number of the intramural vessels to the areas of the corresponding sections of the EBD wall ($/\text{mm}^2$) were calculated. These ratios reflect the enrichment of the intramural vessels. These ratios were compared among the sections; their correlations with the distance from the bifurcation of the right and left hepatic ducts were investigated.

Statistical Analysis

Numerical variables were expressed as median (range) and compared using the Wilcoxon rank sum test for paired variables. Post-hoc analyses were performed by the Holm-Bonferroni method. Correlation coefficient (r) was assessed with the Spearman rank correlation coefficient. Two-tailed $p < 0.05$ was accepted as significant. All statistical analyses were carried out using the SPSS commercial statistic software version 23 (IBM, Armonk, NY, United States).

RESULTS

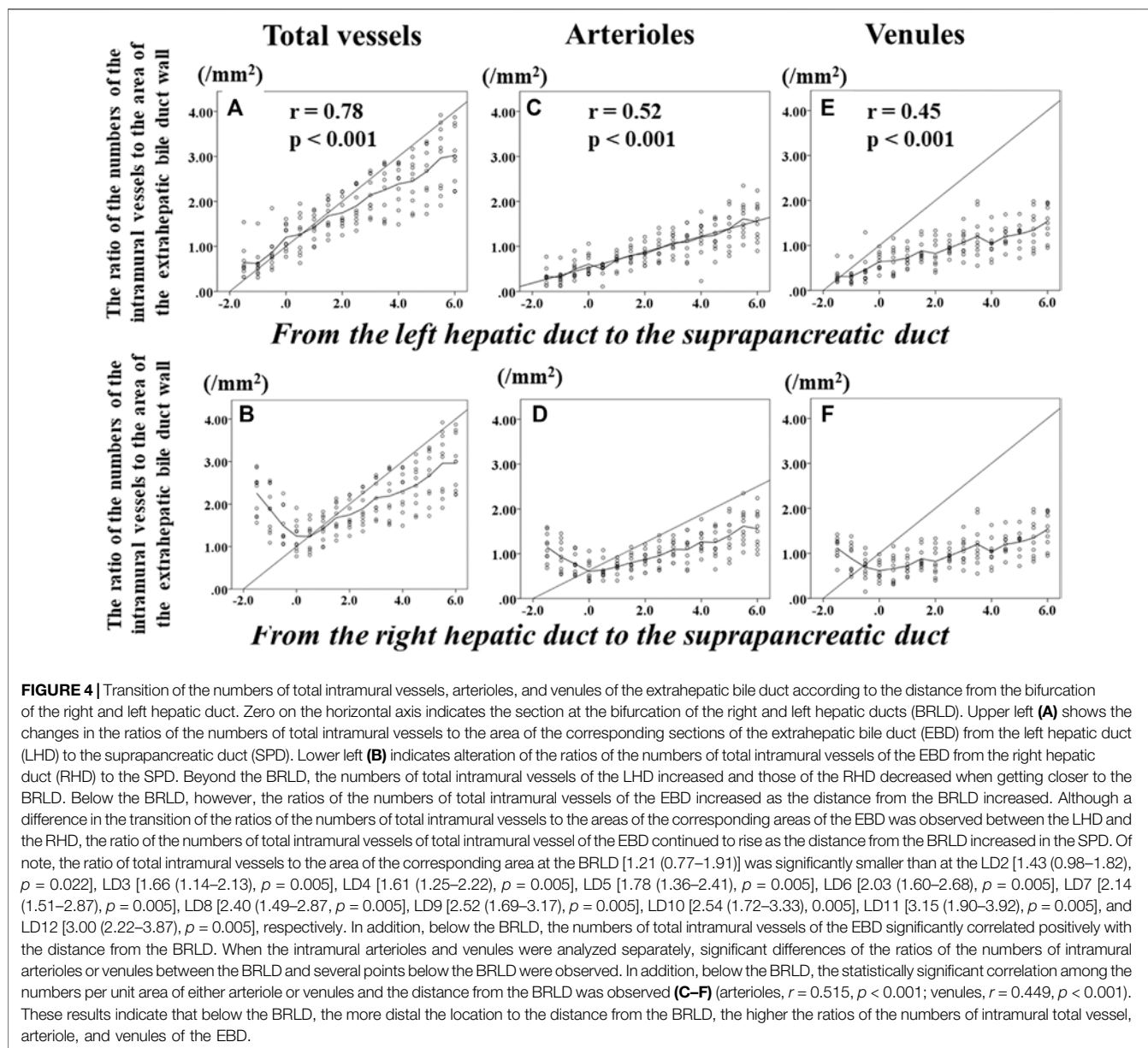
Existence of the 3 and 9 O'Clock Arteries and Veins

The outer-layer included the 3 and 9 o'clock arteries and veins in all cases. The 3 o'clock and 9 o'clock vessels ascended along the

left and right lateral border of the outer-layer, respectively; at the confluence of the right and left ducts, the 3 o'clock vessels ascended along the left lateral border of the LHD. The 9 o'clock vessels ascended along the RHD. In other words, both the 3 and 9 o'clock vessels accompanied the suprapancreatic duct; however, the LHD or RHD had only one. Instead, a mesh-like-structure composed of thin arterial branches connecting with the LHD and/or RHD that arose from the RHA, LHA, and MHA existed above the bifurcation of the left and right hepatic ducts (**Figures 2A, B**).

Luminal-Areas of the 3 and 9 O'Clock Arteries

Change of the luminal-area (mm^2) of the 3 o'clock artery according to the sections was demonstrated in **Figure 3A**. A statistically significant difference was observed among the various combinations of the sections; the luminal area was smallest at the level of the bifurcation of the right and left hepatic ducts among these sections. Of note, the consecutive luminal areas of the corresponding sections of the 3 o'clock artery correlated significantly and positively with the distance from the bifurcation of the right and left hepatic ducts ($r = 0.42$, $p < 0.001$). Regarding the 9 o'clock artery, the change of the luminal areas was shown in **Figure 3B**. A statistically significant difference was observed among the various combinations of these sections. Moreover, the consecutive luminal areas of the corresponding sections of the 9 o'clock artery significantly correlated positively with the distance from



the bifurcation of the right and left hepatic ducts ($r = 0.39$, $p < 0.001$).

The Ratios of the Numbers of the Intramural Vessels to the Areas of the Corresponding Sections of the Extrahepatic Bile Duct

Figure 4 shows the changes in the ratio of the numbers of total intramural vessels to the areas of the corresponding sections of the EBD according to the distance from the confluence of the right and left hepatic ducts (0 of the horizontal axis label indicates the level of the confluence of the right and left hepatic ducts); this indicates the changes in the ratios from the LHD to the suprapancreatic duct. Figure 4B indicates the alteration of the ratios from the RHD to the suprapancreatic duct. Beyond the

bifurcation of the right and left hepatic ducts, the numbers of total intramural vessels of the LHD (Figure 4A) increased and those of the RHD (Figure 4B) decreased as the corresponding sections were getting closer to the bifurcation of the right and left hepatic ducts. Beneath the bifurcation of the right and left hepatic ducts, however, the higher the ratios of the numbers of total intramural vessels to the areas of corresponding sections of the EBD, the further the distance from the bifurcation of the right and left hepatic ducts (Figures 4A,B). Above the bifurcation of the right and left hepatic ducts, there was no correlation between the ratios of the numbers of vessels to the areas of the corresponding section of the LHD or RHD and the distance from the bifurcation of the right and left hepatic ducts; the ratios of the number of the intramural -vessels to the areas of the corresponding sections of the suprapancreatic duct continued to rise as the distance from

the bifurcation of the right and left hepatic ducts increased. Of note, the ratio of the numbers of total intramural vessels to the area of the EBD wall at the bifurcation of the right and left hepatic ducts was fewest among the sections. In addition, below the bifurcation of the right and left hepatic ducts, the ratio of the number of total intramural vessels to the areas of the corresponding sections significantly correlated positively with the distance from the bifurcation of the right and left hepatic ducts ($r = 0.78$, $p < 0.001$) (Figures 4A,B).

When the arterioles and venules were analyzed separately, the significant differences of the ratios of the number of each vessel to the area of the corresponding sections were observed between the bifurcation of the right and left hepatic ducts and several points beneath the bifurcation of the right and left hepatic ducts (Figures 4C–F). In addition, the ratios of the numbers of the intramural arterioles and venules to the areas of the corresponding sections correlated significantly and positively with the distance from the bifurcation of the right and left hepatic ducts in the suprapancreatic duct (arterioles, $r = 0.52$, $p < 0.001$; venules, $r = 0.45$, $p < 0.001$).

DISCUSSION

The most important of our findings is that there is a significant locoregional heterogeneity of the numbers and luminal broadness of the intramural vessels among the EBD. If the neighboring arteries are left attached to the EBD, this heterogeneity may be not significant because of the numerous bridging arterioles connecting the neighboring arteries to the EBD. A few institutions argued that the dissection between the neighboring arteries and the EBD must be minimal to retain the sufficient blood flow to the EBD and this procedure can almost always be performed (3–5). However, the hepatic arteries and the EBD often need to be isolated from each other for elongation that enables the tension-free secure reconstruction (1, 2, 10, 11); this isolation requires the dissection of the hepatic arteries from the EBD that unavoidably divide the connecting arterioles between the hepatic arteries and the EBD. On such occasion, the locoregional heterogeneity of the intramural vessels may impair the perfusion of the EBD. This is especially true in the graft liver EBD of the deceased donor LT (DDLT), where the blood flow goes through the proximal EBD to reach the distal end of the EBD; namely, blood flow passes through the thinner and fewer intramural vessels and reaches the thicker and more abundant intramural vessels. This means that the amount of blood flow decreases for the sizes of the intramural vessels as it goes further; thus, this is likely to cause the ischemia of the anastomotic site of the graft liver EBD. The ischemia may deteriorate as the length of the EBD isolated from the hepatic arteries grows. The deterioration of the ischemia easily causes the BCs, including the anastomotic leakage, anastomotic stricture, and the ischemic cholangiopathy (1–5, 10, 11). In the recipient of the living donor LT (LDLT) using DDBA, the vicinity of the bifurcation of the right and left hepatic ducts of the EBD is usually used for the anastomotic site. Our present findings showed that the intramural vessels are thinnest and fewest in the bifurcation of

the right and left hepatic ducts; this suggests that the scarcity of the intramural vessels may cause the blood flow paucity at the anastomotic site of the recipient EBD, especially when the EBD is isolated extensively from the neighboring hepatic arteries. With regard to the DDLT recipient, the distributional imbalance of the intramural vessels between the proximal and distal parts of the EBD is likely to become less severe compared to the LDLT recipient because the EBD is divided more distally in the DDLT than in the LDLT. However, the location of the graft EBD to anastomose will be strongly associated with the decision of the anastomotic site of the recipient EBD; if the graft EBD is divided at an unusually proximal site, it requires further isolation of the EBD from the neighboring arteries to elongate the recipient EBD to achieve the tension-free secure biliary anastomosis. As a result, the anastomotic site of the recipient EBD becomes likely to suffer the ischemia.

As such, the anastomotic site of both the graft and recipient EBD is likely to suffer the ischemia because of the locoregional distributional heterogeneity of the intramural vessels. We consider that it requires some technical ingenuity to avoid this ischemia which is inevitable on some occasions. One solution may be a more distal implantation of the graft livers compared to the orthotopic implantation (10, 11); this enables the tension-free biliary anastomosis even in occasions where both the graft and the recipient EBDs are short for the tension-free anastomosis in the orthotopic implantation.

There are several limitations in this study. First, we used the livers of deceased bodies; this study cannot assess the actual hemodynamics. To confirm the significance of our present findings in a clinical setting, we are currently performing a study that can assess the actual hemodynamics of the EBD by the near-infrared imaging using indocyanine green (12). Second is the exclusion of the donors who had a history of previous hepatobiliary surgery and/or any hepatobiliary diseases. The LT recipients have severe hepatobiliary diseases and a certain population of them received some hepatobiliary surgery; these pathologic conditions undoubtedly affect the blood-circulation of the recipient EBD. The exclusion of the donors without such pathologic conditions rendered this study unsuitable for obtaining an insight into the recipient EBD. However, the inclusion of the donors who had such conditions leads to the enormous heterogeneity of the study samples; this heterogeneity requires an extremely large sample size to neutralize. Unfortunately, we had a limited number of the donors. Thus, we had to limit the donors who had neither a history of previous hepatobiliary surgery and/or any hepatobiliary diseases to this study sample to counteract the heterogeneity as much as possible. Meanwhile, the present study was quite suitable for gaining insight into the perfusion of the EBD of the graft liver, especially the DDLT graft. Third, the anatomy of the biliary system and surrounding vascular network vary markedly (6–9). Therefore, our sample size of 10 deceased Japanese bodies may be too small to draw any conclusion. However, this study focused specifically on the intramural vascular network of the suprapancreatic duct; the anatomy, its feeding arteries, and its drainage veins of this part were reported to be less various compared to the other part (6, 7). Thus, a sample size of 10 may be sufficient for analyzing the intramural

vascular network of the suprapancreatic duct; in fact, the variables we examined were quite less diverse. However, we realize that the results of the present study were debatable because of the probable bias due to the small sample size. Besides, we recognize that the hepatobiliary diseases from which the liver transplant recipients suffer easily cause the structural and/or functional alterations of the EBD; these alterations have to be taken into consideration. Therefore, we will again examine the issues discussed in the present study in future studies using larger samples that include donors who have a history of previous hepatobiliary surgery and/or hepatobiliary diseases. Despite these limitations, we believe that this study is valuable because this is the first report focusing on the intramural vascular network of the EBD relevant to the DDBA in either the DDLT or the LDLT.

In conclusion, our present findings demonstrated that there is a significant distributional imbalance of the intramural vessels between the proximal and distal parts of the EBD; this could have a significant negative impact on the perfusion of the EBD, especially when the EBD has to be broadly isolated from the neighboring arteries. When the broader isolation of the EBD from the neighboring arteries is necessary, it requires some technical ingenuity for the DDBA to avoid the ischemia of the anastomotic site of the EBD; this needs to take the locoregional heterogeneity of the intramural vessels into consideration.

DATA AVAILABILITY STATEMENT

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

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ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board of Yokohama City University Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

HS, IE, YK, and RM participated in research design. NY, YK, YY, YH, and TK participated in the performance of the research. HS, IE, KT, and DM contributed an analytic tool. NY, SS, DM, RM, IE, and HS participated in data-analysis. NY, SS, KT, and DM participated on writing 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.

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Increased Tacrolimus Exposure in Kidney Transplant Recipients With COVID-19: Inflammation-Driven Downregulation of Metabolism as a Potential Mechanism

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Kidney transplant recipients (KTRs) are at increased risk of severe COVID-19 disease compared to the general population. This is partly driven by their use of immunosuppressive therapy, which influences inflammatory responses and viral loads. Current guidelines suggest to withdraw mycophenolate while calcineurin inhibitors are often continued during a COVID-19 infection. However, clinical signs of calcineurin toxicity have been described in multiple COVID-19 positive KTRs. In this report we describe the course of tacrolimus exposure prior to, during, and post COVID-19 in observations from three clinical cases as well as four KTRs from a controlled trial population. We postulate inflammation driven downregulation of the CYP3A metabolism as a potential mechanism for higher tacrolimus exposure. To mitigate the risk of tacrolimus overexposure and toxicity therapeutic drug monitoring is recommended in KTRs with COVID-19 both in the in-, out-patient and home monitoring setting.

Keywords: COVID-19, kidney transplant, Tacrolimus, metabolism, CYP3A, phenoconversion

INTRODUCTION

Kidney transplant recipients (KTRs) with severe acute respiratory syndrome due to coronavirus-2 (SARS-CoV-2) are at threefold increased risk of a severe course of coronavirus disease-2019 (COVID-19) (1). Immunosuppressive therapy to prevent transplant rejection may diminish antiviral immunity potentially causing higher viral loads and a longer time-to-negativity for SARS-CoV-2 nucleic acid testing in nasopharyngeal swabs although it may also protect against an overshooting immune response (2, 3). Most KTRs are on a triple immunosuppressive maintenance regime consisting of the antimetabolite mycophenolate mofetil (MMF), a calcineurin inhibitor (CNI) and prednisolone (4). In SARS-CoV-2 positive KTRs, consensus guidelines recommend to withdraw MMF in low immunological risk patients, partly based on experience with the influenza H1N1 pandemic (5, 6). By contrast, CNIs are usually continued as these can be more closely titrated via therapeutic drug monitoring (TDM). Moreover, CNIs may have *in-vitro* activity against SARS-CoV2 (7). During the COVID-19 pandemic in Europe thousands of KTRs have been diagnosed with

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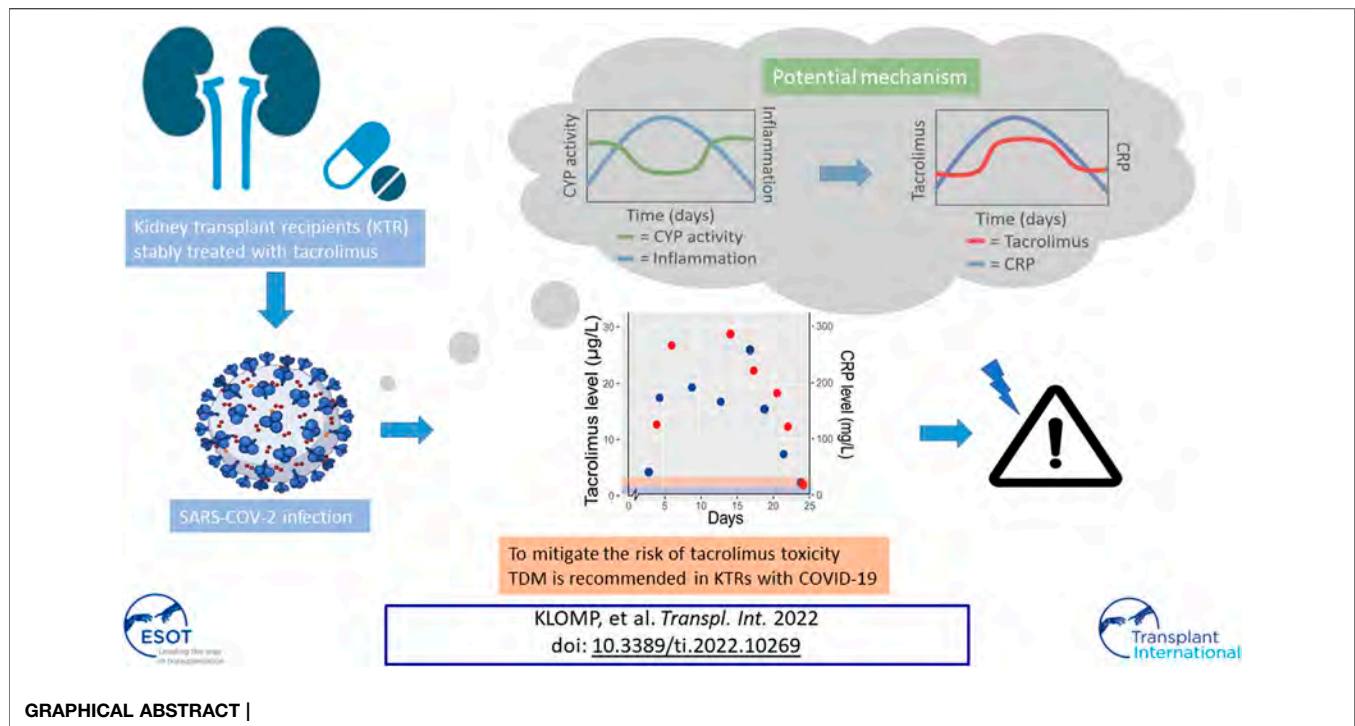
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COVID-19 (8). At the Leiden University Medical Center all KTRs with mild COVID-19 symptoms were home-monitored with a “COVID-box” which included a blood pressure monitor, a thermometer, and a pulse oximeter to combine subjective and objective parameters for adequate monitoring (9).

Observations from our clinical practice raised the suspicion that KTRs, who contracted COVID-19, developed signs of tacrolimus toxicity including complaints of tremors, hypertension, and headaches. This has also been described in other non-controlled cohorts (10, 11).

With this study, we aimed to describe dynamics in tacrolimus exposure in KTRs that contracted COVID-19. Data on inflammatory response and tacrolimus exposure prior to, during, and after COVID-19 have been analysed for three clinical cases. Subsequently we decided to analyse these changes in tacrolimus exposure in a controlled trial population with less severe disease to confirm both our findings and those in other beforementioned cohorts (10, 11).

METHODS

Patient Selection and Data Collection

During the different waves of the pandemic, our general clinical impression was that a majority of our KTRs had increased tacrolimus levels with clinical symptoms of toxicity. For a first exploration on the relationship between tacrolimus exposure and COVID-19 infection, we describe three clinical cases that developed a toxic tacrolimus trough concentration (C_{trough}) above 20 µg/L (target range 5–7 µg/L > 6 weeks posttransplant) at the time of hospitalisation or hospital visit for respiratory

insufficiency caused by COVID-19. To explore a possible correlation with inflammation status, C-reactive protein (CRP) was used as biomarker. Case selection was based on the availability of patient consent, availability of tacrolimus C_{trough} and CRP levels before, during and after COVID-19 infection, and lack of a clear explanation for the tacrolimus concentration increase including drug-drug interactions (DDIs) and/or recent dose adjustments. Tacrolimus C_{trough} obtained during COVID-19 infection were compared to recent pre-COVID tacrolimus C_{trough} of the cases in combination with CRP concentrations.

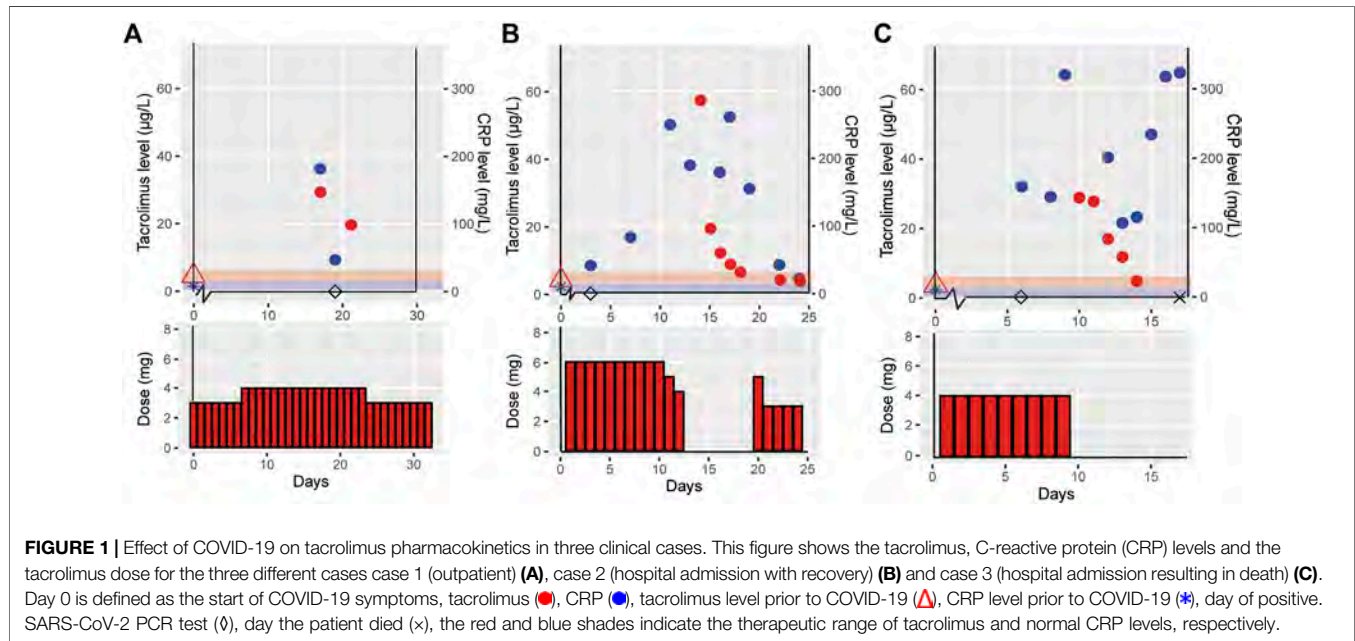
As this clinical case selection inevitably leads to selection bias (e.g., towards most critically ill KTRs), we also investigated data from a randomized clinical trial (RCT) population (VOCOVID trial, NCT04701528) to assess the impact of COVID-19 on tacrolimus exposure. In this prospective open label trial, KTRs on maintenance immunosuppression with tacrolimus (+ mycophenolic acid and/or prednisolone and/or everolimus) with COVID-19 (confirmed by NAT) were randomized between continuation of tacrolimus and prednisolone (as standard of care during a COVID-19 infection) or replacement of tacrolimus by voclosporine (because of possible favourable anti-viral properties). Patients randomized to voclosporine are outside the scope of this article due to absence of voclosporine measurements prior to COVID-19.

Within this RCT, KTRs were followed up on a regular basis with C_{trough} measurements on day 4, 8, and 28, and AUC measurements on day 8 and day 28 using a dried-blood-spot TDM technique (12) which provides extensive insight in pharmacokinetics during and post COVID-19. During these visits multiple laboratory measurements have been performed including CRP concentrations.

TABLE 1 | Demographics and tacrolimus dose and exposure prior to, during and post COVID-19 infection of three clinical cases.

Case	Age (y), sex	Status during COVID-19 & disease Severity [^]	CYP3A5 Genotype	Clinical Symptoms of TAC Toxicity	Immunosuppressive therapy prior to COVID-19 Onset	Day of Positive NAT-test (first Symptoms Started at D0)	Parameter	Prior to COVID-19	During COVID-19	Post COVID-19
1	57, male	Outpatient, Severe disease	*3/*3	Tremors and acute kidney injury (30% increase in baseline creatinine)	Tac bid 1.5 mg Pred qd 5 mg MPA bid 500 mg (day 1–6)	20	Disease day CRP (mg/L) Tac day dose (mg) C _{trough} (µg/L) C _{trough} /dose(µg/L*mg)	3 4 5.8 1.9	17 181.7 4 29.4 7.4	21 46.8 4 19.7 4.9
2	67, male	Hospital admission, Severe disease	*3/*3	Headache, tremors and acute kidney injury (45% increase in baseline creatinine)	Tac bid 3 mg Pred qd 5 mg	3	Disease day CRP (mg/L) Tac day dose (mg) C _{trough} (µg/L) C _{trough} /dose(µg/L*mg)	6 0 7.2 1.2	14 189.9 0 57.2 ∞	24 22.2 3 3.6 1.2
3	56, female	Hospital admission followed by death due to respiratory insufficiency, Severe disease	*3/*3	Tremors and acute kidney injury (60% increase in baseline creatinine)	Tac qd 4 mg Pred qd 5 mg	6	Disease day CRP (mg/L) Tac day dose (mg) C _{trough} (µg/L) C _{trough} /dose(µg/L*mg)	4 4 3.9 0.98	10 515 4 28.6 7.15	14 115 0 4.6 ∞

CRP, C-reactive protein; NAT, nucleic acid-test; NA, not applicable; MPA, methylphenolic acid; Tac, tacrolimus; Pred, prednisolone. Disease day starts at the day of symptom onset. C_{trough}/Dose = the dose-corrected C_{trough} calculated as C_{trough} divided by the total daily dose. Prior to COVID-19, is defined as the most recent available exposure in the previous year. After COVID-19 is defined as an exposure after a negative NAT. The total daily dose is calculated by adding the morning and evening dose in case of BID dosing. ∞ indicates dividing by zero, due to tacrolimus discontinuation. [^]according to WHO, classification.



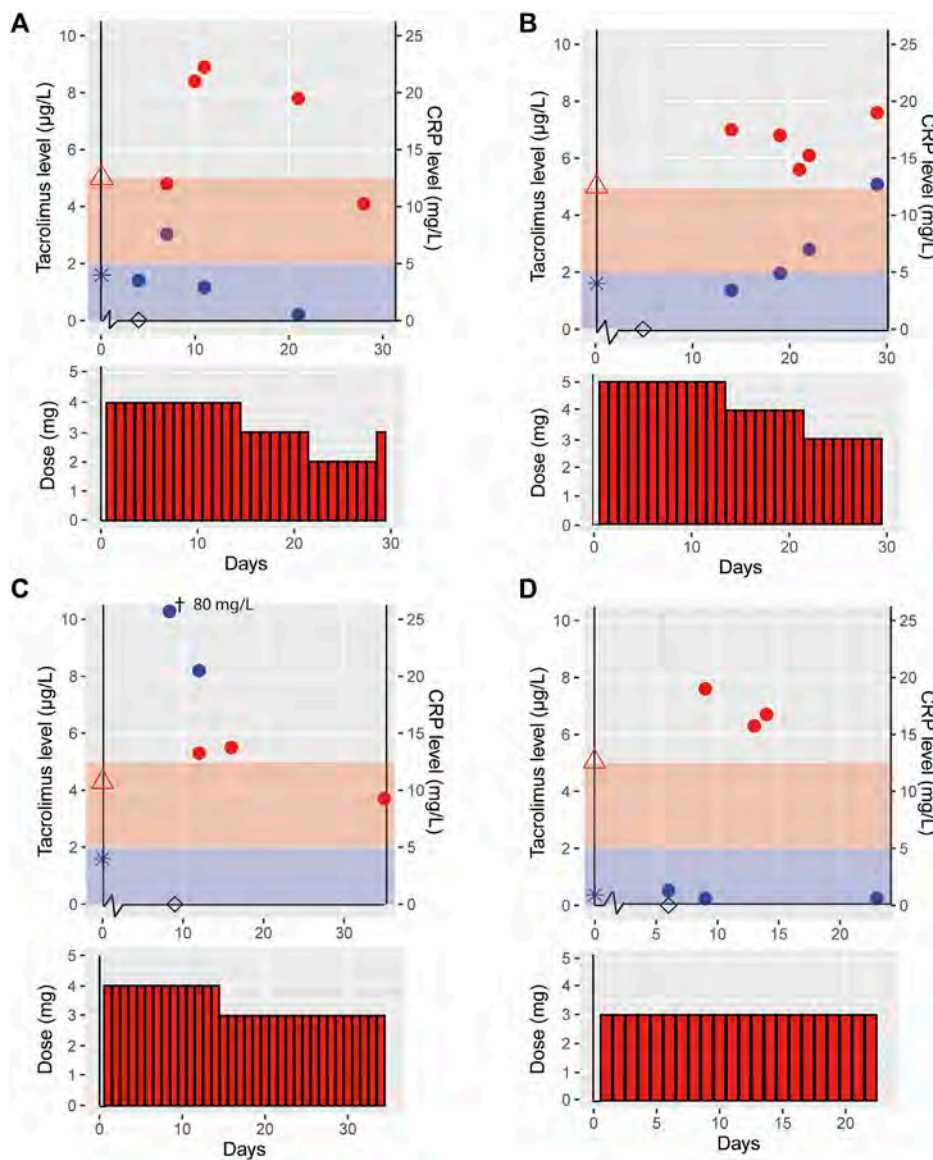


FIGURE 2 | Effect of COVID-19 on tacrolimus pharmacokinetics in patients participating in a clinical trial. This figure shows the tacrolimus, C-reactive protein (CRP) levels and the tacrolimus dose for the cases participating in the control arm of the VOCCOVID trial, case A (A), B (B), C (C) and D (D). Day 0 is defined as the start of COVID-19 symptoms, tacrolimus (●), CRP (●), tacrolimus level prior to COVID-19 (△), CRP level prior to COVID-19 (*), day of positive SARS-CoV-2 PCR test (◇), the red and blue shades indicate the therapeutic range of tacrolimus and normal CRP levels, respectively. †This measurement is out of axis reach, the value is 80 mg/L.

For all cases demographic, clinical, pharmacologic (tacrolimus daily dose and immunosuppressive regimen), and laboratory measurements (CRP, CYP3A5 genotype, tacrolimus C_{trough} and AUC, and NAT) data were retrospectively collected from electronic health records.

Definitions and Statistical Analysis

For the clinical cases, post-COVID-19 status was defined as the period following the first negative nucleic acid test (NAT) after a confirmed COVID-19 infection in combination with resolution of

symptoms. For the RCT cases, post COVID-19 was defined as resolution of symptoms in combination with high cycle threshold-values (>34) in NAT for nasopharyngeal swabs. CRP concentrations, tacrolimus C_{trough} , and AUCs obtained during COVID-19 infection were dose corrected and compared to pre-COVID-19 values. Descriptive statistics and graphical representation were used to summarize each patient’s course. Categorical data was presented using frequencies and percentages, while continuous data was presented as means and ranges. Due to the small sample size of seven patients, no formal statistical analyses were performed.

TABLE 2 | Demographics and tacrolimus dose and exposure prior to, during and post COVID-19 infection of four cases within the VOCOVID trial.

Case	Age (y), sex	Status during COVID-19 & disease Severity [^]	CYP3A5 Genotype	Clinical Symptoms of TAC Toxicity	Immunosuppressive therapy prior to COVID-19 Onset	Day of Positive NAT-test (first Symptoms Started at D0)	Parameter	Prior to COVID-19	During COVID-19 [§]	Post COVID-19
A	47, male	Outpatient, Mild disease	*3/*3	Hypertension, tremors and acute kidney injury (20% increase in baseline creatinine)	Tac qd 4 mg Pred qd 5 mg	4	Disease day		10	30
							CRP (mg/L)		7.6	1.0
							Tac day dose (mg)	4	4	3
							AUC (µg*h/L) #	180	236	114
							AUC/dose(µg*h/L*mg)	45	59	38
B	47, female	Outpatient, Mild disease	NA	Hypertension and tremors	Tac qd 5 mg Pred qd 5 mg Evl bid 4 mg (discontinued on D13)	5	Disease day		21	42
							CRP (mg/L)		7.0	3.8
							Tac day dose (mg)	5	4	2.5
							AUC (µg*h/L)	149	164	103
							AUC/dose(µg*h/L*mg)	30	41	41
C	49, female	Outpatient, Moderate disease	*1/*3	None	Tac bid (2–3) mg Pred qd 5 mg	9	Disease day		16	35
							CRP (mg/L)		20.5	NA
							Tac day dose (mg)	5	3	3
							AUC (µg*h/L)	80*	94	64
							AUC/dose(µg*h/L*mg)	16	31	21
D	57, male	Outpatient, Mild disease	NA	None	Tac bid 1.5 mg Pred qd 5 mg	2	Disease day		13	34
							CRP (mg/L)		0.6	0.8
							Tac day dose (mg)	3	3	3
							AUC (µg*h/L)	77	111	69
							AUC/dose(µg*h/L*mg)	26	37	23

CRP, C-reactive protein; NAT, nucleic acid-test; NA, not applicable; AUC, area under the curve; Tac, tacrolimus; Pred, prednisolone; Evl, everolimus.

Disease day starts at the day of symptom onset. AUC/Dose = the dose-corrected AUC calculated as AUC, divided by the total daily dose. Prior to COVID-19, is defined as the most recent available exposure in the previous year. After COVID-19 is defined as an exposure after a negative NAT. The total daily dose is calculated by adding the morning and evening dose in case of BID dosing.

*This historic value was from 2012.

§This measurement is after the positive NAT.

^According to WHO, classification.

#The AUC, is calculated from C_{through} levels, C₀, C₂ and C₃ and for dried-blood-spot from C₀, C₁, C₂ and C₃.

RESULTS

Cases From Clinical Practice

For the three selected clinical cases, disease status, CYP3A5 genotype, tacrolimus treatment dose and exposure prior to infection, during and post COVID-19 infection are shown in **Table 1**. **Figure 1** depicts tacrolimus C_{through} concentrations prior to and during COVID-19 infection and available CRP concentrations.

Case 1, a 57 years old male, reported COVID-19 symptoms including fever, cough, dyspnea, tremors and an acute kidney injury with 30% increase from baseline creatinine. There were no signs of liver dysfunction (AST 36 U/L and ALT 28 U/L). He visited the clinic two times during his disease course, but was not admitted to the hospital. Pre-COVID-19 he was treated with 3 mg tacrolimus (1.5 mg BID) and during COVID-19 the daily dose was 4 mg tacrolimus. He received no medication that is known to cause any DDI with tacrolimus. During COVID-19 a toxic

tacrolimus C_{trough} level of 29.4 $\mu\text{g/L}$ was measured, resulting in a 289% increased tacrolimus dose-corrected C_{trough} ($C_{\text{trough}}/\text{Dose}$) during COVID-19 infection as compared to pre-COVID-19 (tacrolimus C_{trough} of 5.8 $\mu\text{g/L}$) (**Table 1** and **Figure 1A**). Post COVID-19, the patient recovered well, all laboratory measurements returned back to within normal ranges and the COVID-19 symptoms disappeared.

Case 2, a 67 years old male, presented with COVID-19 symptoms of fever, headache, dyspnea, tiredness, and chest pains. At COVID-19 disease day 11 he was admitted to the hospital, because of respiratory insufficiency requiring additional oxygen supplementation, and with a CRP level of 249.9 mg/L. His liver enzymes were elevated, AST and ALT rose to 117 and 122 U/L, respectively. He was on maintenance immunosuppression with 3 mg tacrolimus BID (6 mg/day). Therapy with hydrochloroquine was initiated on disease day 11 and stopped at day 16. Two weeks after disease onset, he had a tacrolimus C_{trough} of 57.2 $\mu\text{g/L}$ and a CRP level of 189.9 mg/L (**Table 1** and **Figure 1B**). Signs of tacrolimus intoxication included a headache and tremor. Tacrolimus was temporarily interrupted for 6 days, during which C_{trough} declined to 6.3 $\mu\text{g/L}$. Tacrolimus was reinitiated at a conservative dose of 1.5 mg BID, half of the original dose. At disease day 25 he was discharged.

Case 3, a 56 years old female, experienced COVID-19 symptoms including, fever, nausea, cough and dyspnea. At day 8 post-positive COVID-19 PCR she was admitted to the intensive care unit (ICU) due to respiratory insufficiency requiring mechanical ventilation. She had elevated liver enzymes, AST and ALT levels of 105 and 76 U/L, respectively. She was on maintenance immunosuppression with 4 mg tacrolimus qd. Hydroxychloroquine was initiated on disease day 6 and stopped at day 8. At day 10, a tacrolimus C_{trough} of 28.6 $\mu\text{g/L}$ was measured with a CRP level of 515 mg/L, a 630% higher dose-corrected C_{trough} during COVID-19 compared to pre-COVID-19 (**Table 1** and **Figure 1C**). At that point, tacrolimus was withdrawn due to tacrolimus intoxication diagnosis at ICU. The patient died from COVID-19 related complications on disease day 17.

Case Series From the RCT VOCOVID

From November 2020 until February 2021 eight KTRs were included in the VOCOVID trial, of which five were randomized to continue tacrolimus and three switched to voclosporine. One of the five tacrolimus continuers was excluded from our analysis due to lack of available AUC measurements, since the patient died early from COVID-19, leaving a total of 4 cases. The association between tacrolimus C_{trough} and CRP of this fifth case is, nonetheless, depicted in **Supplementary Figure S1**.

Figure 2 illustrates tacrolimus exposure for VOCOVID trial cases (Case A, B, C and D) both prior to and during COVID-19, and available CRP concentrations.

These four cases had an age range of 47–57 years and two were male. Case C was a heterozygous CYP3A5 expressor (CYP3A5*1/*3, resulting in increased tacrolimus metabolism thus requiring a higher daily dose at baseline), case A a non-expressor (CYP3A5*3/*3) and cases B and D were not genotyped

(**Table 2**). All trial cases were more than 3 years post transplantation and on a stable tacrolimus dose prior to contracting COVID-19. Tacrolimus dose and exposure prior to infection, during and post COVID-19 infection are shown in **Table 2**. Case A reported minor and temporary short-lasting minor, self-limiting gastro-intestinal complaints with two loose stools per day without vomiting. Other symptoms were all related to the SARS-CoV-2 infection. All trial cases had complaints of fever, dyspnea and coughing. None of the trial cases required hospitalization for additional oxygen supplementation. Case B had slightly increased liver enzymes; AST was 38 U/L and ALT 73 U/L. All other trial cases had liver enzymes laboratory values with the normal reference range.

The VOCOVID cases displayed on average a 51% (range 31%–94%) higher tacrolimus dose-corrected AUC (AUC/Dose) during COVID-19 as compared to pre-COVID-19. Post-COVID-19 there was a 26% (range 0%–38%) decline in AUC/Dose compared to the situation during disease. At the latest available time point, approximately 1 month after COVID-19, AUC/Dose for cases A, C and D showed a return to baseline, suggesting a correlation between the increased exposure and COVID-19. AUC/Dose at the latest available time point for case B was similar compared to AUC/Dose during infection. For this case, the AUC measurement during COVID-19 was obtained later in the disease course compared to the other trial cases (day 21 vs. day 10–16).

DISCUSSION

In this report, we could confirm observations of tacrolimus toxicity in both clinically admitted KTRs and outpatient managed KTRs who underwent protocolized TDM as part of a clinical trial. COVID-19 infection was associated in with higher tacrolimus levels (range 4%–794%) in our cases, which has previously only been described in hospitalized (and thus more ill) KTRs (10, 11). These data underpin the need of frequent TDM in all COVID-19 KTRs to prevent tacrolimus overexposure, regardless of time after transplantation and treatment status, which has important clinical ramifications, specifically for patients with mild COVID-19 related symptoms that do not require hospitalization. Tacrolimus is notorious for its highly variable PK and narrow therapeutic window in which toxic levels can result in result in life-threatening complications including renal failure, hypertension/thrombotic micro-angiopathy and/or neurotoxicity. Via AUC measurements we managed to get more insight in the potential cause of overexposure in KTRs with COVID-19.

The observed increase in tacrolimus concentrations during COVID-19 infection could potentially result from changes in absorption, metabolism, and excretion, possibly via DDIs.

First of all, tacrolimus absorption shows substantial inter- and intra-patient variability. Intra-patient variability can result from diarrhea, possibly explained by changed drug solubility and intestinal permeability (13, 14). In our study one case reported minor and temporary gastro-intestinal complaints with two loose stools per day, not expected to substantially change absorption

(15), albeit we cannot exclude an effect from SARS-COV-2 on luminal cells (16). Notably, KTRs on cyclosporin also showed higher cyclosporin trough levels during COVID-19 infection, despite its characteristic of reduced absorption in diarrhea (13). Unfortunately, available cyclosporin data were too sparse to allow further analysis. In addition, a nil per os (nothing by mouth) status could affect tacrolimus absorption. With the exception of ICU case 3 who was intubated at the ICU, all cases were able to eat and drink by mouth. At the time of intubation, case 3 was no longer receiving tacrolimus.

Another potential cause of increased tacrolimus exposure is altered hepatic function or mucosal drug metabolism (13, 14) since tacrolimus is metabolized by CYP3A4 and CYP3A5 (17). All cases described within this case series did not have significant elevation of liver enzymes or bilirubin rendering hepatic dysfunction less probable. Furthermore, all RCT-cases were from the outpatient setting and remained outpatient during the whole study period, rendering (multi-) organ failure or significant liver dysfunction as possible explanation for tacrolimus toxicity unlikely.

Furthermore, there was no use of concomitant CYP3A4 inhibitors in our cases, which could also have explained higher tacrolimus exposure resulting from a DDI. Two of the clinical cases were shortly treated with hydroxychloroquine which, to our knowledge, does not inhibit CYP3A4 (unlike chloroquine). The initiation of hydroxychloroquine theoretically could have led to a slight elevation in tacrolimus C_{trough} (18) however the actual elevation observed (>700%) was much higher than what would be expected based on literature (18) (**Figure 1B**). Besides, the other five cases did not use hydroxychloroquine and nonetheless showed increased tacrolimus exposure; which suggests that any influence from DDIs is either small or absent in our population.

It is also unlikely that the observed increase in tacrolimus concentrations are resulting from intra-patient variability. The variability of tacrolimus clearance in our center is known to be <20% [unpublished data] in accordance with the literature where the intra-patient variability is reported to be 17% between measurements for patients 6–12 months after renal transplantation (19, 20). The dose corrected tacrolimus concentrations show little variability during the COVID-19 episode in the clinical and trial cases (range 0.20–0.73 $\mu\text{g/L/mg}$ **Figures 1, 2**), indicating low day to day intra-patient variability. Moreover, all cases showed a similar pattern of tacrolimus levels prior to, during and post COVID-19 indicating an effect of disease course (**Figures 1, 2**).

Contrarily, two of the clinical cases (case 2 and 3) temporarily interrupted tacrolimus treatment and showed a slow clearance [for example, the observed half-life for case 3 was >48 h, where normally this would be in the range of 12–15 h for tacrolimus (21)] (**Figure 1**). The slow decrease in tacrolimus concentration after cessation of tacrolimus points towards an impaired metabolism, since excretion of unmetabolized tacrolimus via feces only contributes to <1% of tacrolimus clearance (21). In addition, liver function enzymes ALT and AST did not indicate clinically relevant liver dysfunction further supporting our hypothesis of an alteration in CYP3A activity.

We observed that increase in dose corrected tacrolimus levels align with increase in CRP levels, especially in the cases admitted to hospital (**Figures 1, 2**). It has been shown that upregulation of interleukin (IL)-6 results in increased CRP levels in patients with acute inflammation (22), and CYP3A activity can be down-regulated by pro-inflammatory cytokines (22, 23), including tumor necrosis factor (TNF)- α , IL-1 β , IL-6, IL-2, and interferon (IFN)- γ . Since COVID-19 has been found to elevate these pro-inflammatory cytokines this could lead to so called inflammatory based phenoconversion (24–26). Indeed, COVID-19 induced phenoconversion has previously been reported for a COVID-19 patient with clozapine toxicity (27). It was reported that inflammation potentially induced downregulation of CYP1A2. Similarly, increased tacrolimus levels can be the result of CYP3A downregulation via inflammatory based phenoconversion (28–30).

Of note, CRP is an imperfect marker for IL-6 or other inflammatory mediators. IL-6 levels were however unavailable and are not part of routine patient care. This would explain why increased tacrolimus exposure is not observed in transplant patients with comparable CRP levels for, e.g., septicemia, where IL-6 or other inflammatory mediators are not expected to be elevated (21).

We, therefore, postulate that inflammation resulting from COVID-19 infection results in CYP3A phenoconversion in KTRs. COVID-19 may thus lead to unwanted higher exposure of tacrolimus, most likely caused by downregulated CYP3A metabolism by pro-inflammatory cytokines.

Our hypotheses may also account for the low rates of rejections during COVID-19 observed in our patients, despite reduction of immunosuppressive treatments. However, this needs to be investigated further in studies specifically designed for this purpose.

If our hypotheses of inflammation driven phenoconversion holds true, this may also be relevant for IL-6-inhibitors including tocilizumab and clazakizumab that are currently being introduced into the KTR population both in COVID-19 and chronic antibody-mediated rejection. Moreover, phenoconversion may also play a role during other infections associated with upregulation of proinflammatory cytokines, but this needs to be studied more before conclusions can be drawn.

In conclusion, tacrolimus exposure should be carefully monitored during COVID-19 to potentially prevent tacrolimus toxicity and a negative impact on cellular immunity and viral load in patients. A plausible cause for toxicity seems inflammation induced phenoconversion of CYP3A activity which needs to be confirmed in future studies.

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

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SK, SM, MV, JS, and AV participated in research design, SK, SM, MV, DM, EA, YT, JS, and AV participated in the writing of the paper, SK, SM, MV, DM, EA, YT, JS, and AV participated in the performance of the research, SK, SM, MV, JS, and AV participated in 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.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10269/full#supplementary-material>

Supplementary Figure S1 | Effect of COVID-19 on tacrolimus pharmacokinetics. This figure shows the tacrolimus, C-reactive protein (CRP) levels and the tacrolimus dose for the tacrolimus trial patient, admitted to the ICU. Day 0 is defined as the start of COVID-19 symptoms, tacrolimus (●) C-reactive protein (CRP) (●), tacrolimus level prior to COVID-19 (▲), CRP level prior to COVID-19 (★), day of positive SARS-CoV-2 PCR test (○), day the patient died (x), the red and blue shades indicate the therapeutic range of tacrolimus and normal CRP levels, respectively.

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In Memoriam: Professor Paolo Muiesan (1961–2022)

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Keywords: DCD, transplant, liver, split, ELITA

The transplant and surgical communities remain shocked and saddened by the recent passing of Professor Paolo Muiesan (1961–2022), renowned hepatopancreatobiliary and transplant surgeon and beloved father and friend.

Professor Muiesan was internationally recognized for his incredible technical skill as well as his diligent focus on research, advancing the evidence and pushing the limits of what could be done in liver transplant and HPB surgery. For many of us, he was also “Paolo”—dashing, affable, and above all kind. He talked to everyone and did not mind being called upon by anyone for help, to give a brilliant talk, to offer his thoughts on a difficult case, or just to discuss the ups and downs of life.

In a field of many dedicated professionals, it is hard to imagine anyone who worked harder than Paolo Muiesan. He performed highly complex surgical procedures skillfully and efficiently, and his hands were integral in saving the lives of thousands of adult and pediatric patients. He was generous and trained many individuals who are now surgeon leaders in their own right. He talked at countless meetings, organized conferences, and led the field by chairing numerous professional groups and societies. He played a particularly important role in the European Liver and Intestine Transplant Association (ELITA), joining the Council in 2007, serving as Secretary from 2008–2012, and ultimately rising to the role of Chair from 2012–2015. He also provided the voice and representation of the European Society for Organ Transplantation (ESOT) in the European Union of Medical Specialists (UEMS). Most recently, he was elected to the Council of the International Liver Transplantation Society (ILTS).

While Paolo Muiesan distinguished himself in many areas related to liver transplant and HPB surgery, perhaps his greatest and most consistent contribution was as a “founding father” of donation after circulatory death (DCD) liver transplantation. Where few dared to venture, Paolo persevered; without his careful, consistent effort, the panorama of DCD liver transplant and organ transplantation in general would be nowhere close to where it is today.

Paolo Muiesan performed his medical school and surgical training in Milan and Brescia. He then left Italy for many years, first establishing himself at Kings College Hospital in London and subsequently moving to Birmingham at the Queen Elizabeth Hospital and Birmingham Children’s Hospital, where he also held a personal chair at the University of Birmingham. He had returned Italy in 2018, working in Florence and most recently at Policlinico in Milan since Fall 2021. Anyone who talked to him in the past year knows how excited he was to finally be home, to embark on new challenges and share his incredible knowledge and skill base in his “terra natia.” The irony of his passing at the pinnacle of his professional career and in the moment of his homecoming carries a particularly biting sting.

We are all saddened by his loss, but the impact of Paolo’s passing is greatest felt by those that were closest to him in life. Professional accomplishments aside, Paolo was first and foremost a father to two beautiful boys, now both young men. Paolo was proud of his sons, Andrea and Matteo, and talked about them frequently. The thoughts and support of our entire community remain with them at this difficult time.

Paolo was an unstoppable “forza della natura” until he left us; his life force now lives on in all that he leaves behind. Paolo’s legacy lies in the hundreds of journal articles and books he published as well

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as the numerous colleagues with whom we worked and helped train. Above all, Paolo's legacy is the two lives he created and the countless others he worked to save.

Ciao, Paolo, you are gone far too soon, but you will never be forgotten.



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AUTHOR CONTRIBUTIONS

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