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Table of contents


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TRANSPLANT TRIAL WATCH
published: 30 January 2025
doi: 10.3389/ti.2025.14323

Transplant Trial Watch

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

[1]*Centre for Evidence in Transplantation, Nuffield Department of Surgical Sciences, University of Oxford, Oxford, United Kingdom,* [2]*University Hospitals Coventry & Warwickshire, Coventry, United Kingdom,* [3]*Oxford Transplant Centre, Churchill Hospital, Oxford, United Kingdom*

Keywords: randomised controlled trial, liver transplantation (LT), hypothermic oxygenated machine perfusion, colorectal liver metastases, chemotherapy

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.

ECONOMIC EVALUATION

Cost-Effectiveness of Dual Hypothermic Oxygenated Machine Perfusion Versus Static Cold Storage in DCD Liver Transplantation.

by Endo, C., et al. Transplantation 2024 [record in progress].

Aims

The aim of this study was to perform an economic evaluation of the DHOPE-DCD trial, which investigated hypothermic oxygenated machine perfusion versus static cold preservation in liver transplant recipients receiving livers from donors after circulatory death.

Interventions

Participants in the original trial were randomised to either receive liver preserved with hypothermic oxygenated machine perfusion following static cold preservation or with static cold preservation alone.

Participants

156 liver transplant recipients that obtained from a donor after circulatory death that were included in the DHOPE-DCD trial.

Outcomes

The main outcomes of interest were costs per healthcare activity, costs for machine perfusion, transplant-related healthcare costs, mean reduction in cost per patient for the 3 cost scenarios, minimal number of procedures needed per year for cost-effectiveness.

Follow-Up

N/A.

CET Conclusion

by Simon Knight

This manuscript reports an economic evaluation from the Dutch centres participating in the DHOPE-DCD randomised controlled trial. The authors looked at 3 scenarios: (1) cost for the

Transplant Trial Watch

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***Correspondence**
John M. O'Callaghan,
ocallaghan.john@gmail.com
Simon R. Knight,
simon.knight@nds.ox.ac.uk

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device and consumables only, (2) costs for device and personnel, (3) costs for device, personnel and depreciation. They found that the use of D-HOPE reduced the cost of medical care in the first year post-transplant, mainly due to a reduction in ITU and intervention costs. D-HOPE achieves cost effectiveness after 30 procedures/year when personnel and depreciation costs were accounted for. This study highlights the importance of considering personnel costs, infrastructure and logistics when evaluating machine perfusion technology. In high-volume DCD transplant centres, the use of D-HOPE with a dedicated perfusion team is likely to be cost-effective, whereas in smaller volume centres it will only prove cost effective if perfusion is managed by existing staff within existing facilities. Further studies will be required to see if these findings will generalise to other healthcare settings.

Trial Registration
ClinicalTrials.gov - NCT02584283.

Funding Source
No funding received.

RANDOMISED CONTROLLED TRIAL

Liver Transplantation Plus Chemotherapy Versus Chemotherapy Alone in Patients With Permanently Unresectable Colorectal Liver Metastases (Transmet): Results From a Multicentre, Open-Label, Prospective, Randomised Controlled Trial.

by Adam, R., et al. Lancet 2024; 404(10458): 1107-1118.

Aims
This study aims to examine effect of liver transplantation combined with chemotherapy on overall survival among patients with permanently unresectable colorectal liver metastases.

Interventions
Participants were randomly assigned to receive either liver transplantation plus chemotherapy or to chemotherapy alone.

Participants
94 adult patients (18–65 years) with permanently unresectable colorectal liver metastases.

Outcomes
The primary outcome was overall survival at 5 years. Secondary outcomes were overall survival at 3 years, progression-free survival and recurrence rate at 3 and 5 years and health-related quality of life.

Follow-Up
5 years.

CET Conclusion
by Simon Knight

This manuscript reports the outcomes from TransMet, a multicentre European open-label RCT comparing a combination of liver transplantation (LT) and chemotherapy to chemotherapy alone in patients with unresectable colorectal liver metastases and no extrahepatic disease. 94 patients were randomised, of whom 20 patients (11 in the LT and 9 in the chemotherapy group) did not receive the randomised treatment. In intent-to-treat analysis, the hazard ratio for overall 5-year survival was 0.37 (95% CI 0.21–0.65) in favour of transplantation. There were no obvious differences in the incidence of adverse events, and quality of life was similar in the two groups during follow-up. These results are impressive and suggest a significant benefit to transplantation in carefully selected patients. Methodology is good and the study is clearly reported. The findings are limited to patients with partial response or stable disease after chemotherapy, and patients with BRAF mutations were excluded. It requires prioritisation of this patient cohort in organ allocation policy to ensure expedited transplant.

Jadad Score
3.

Data Analysis
Strict intention-to-treat analysis.

Allocation Concealment
Yes.

Trial Registration
ClinicalTrials.gov - NCT02597348.

Funding Source
Non-industry funded.

CLINICAL IMPACT SUMMARY

by John O'Callaghan

This exciting paper presents significant findings regarding the management of patients with unresectable colorectal liver metastases (CRLM). The clinical implications of this research are potentially profound.

Less than 30% of patients with CRLM are thought to be resectable. Traditionally, patients with unresectable CRLM face a poor prognosis, typically receiving chemotherapy without curative potential. This study challenges the *status quo* by exploring the role of liver transplantation not merely as a salvage procedure, but as a potential curative approach. With the increasing efficacy of chemotherapy, expertise of transplantation teams, and improvements in immunosuppression a paradigm shift in patient management is possible.

The study was a multicentre, open-label, prospective, randomised controlled trial done in 20 tertiary centres in Europe, including 94 patients randomised 1:1 between control

and study arms, and stratified by centre. The liver transplantation plus chemotherapy group underwent liver transplantation within 2 months of the last chemotherapy cycle. Transplanted patients received a tailored immunosuppression regimen with postoperative chemotherapy. The control arm continued on chemotherapy. In cases of progression while on the transplant waiting list, chemotherapy was restarted, and the patient was temporarily suspended from transplantation until disease control was achieved. The primary endpoint was 5-year survival (presented in intention-to-treat and per-protocol analysis).

Intention to treat analysis showed a clinically significant difference in overall survival at 5 years: 57% for liver transplantation plus chemotherapy versus 13% for chemotherapy alone (HR 0.37 [95% CI 0.21–0.65]; p = 0.0003). The impact of liver transplantation was even greater in per protocol analysis. A similarly high proportion of patients had an adverse event in both groups (80% versus 83%).

The randomised nature of this trial, and the intention-to-treat analysis circumvents the confounding element of prior publications in this field, where patients with better prognosis may have been selected for liver transplantation over chemotherapy alone.

In summary, these findings could significantly impact clinical practice by redefining treatment pathways for patients with unresectable CRLM. This trial highlights the importance of innovative treatment strategies and the need for multidisciplinary approaches in complex cases of liver metastases.

AUTHOR CONTRIBUTIONS

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

CONFLICT OF INTEREST

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

GENERATIVE AI STATEMENT

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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EDITORIAL
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2024: A Year in Review

Thierry Berney[1], Maria Irene Bellini[2], Louise Benning[3], Oriol Bestard[2], Christophe Masset[3], Beat Moeckli[3], Marco Maria Pascale[3], Nina Pilat[2], Mario Sabatino[3] and Stefan Schneeberger[2]*

[1]Editor-in-Chief, Transplant International, [2]Deputy Editor-in-Chief, Transplant International, [3]Editorial Fellow, Transplant International

As we embark on a new year at the helm of Transplant International (TI), the time has come to reflect on our journal's activities and discuss some of the projects we wish to develop for 2025.

Several articles published in TI have aroused considerable attention, addressing various topics in the broad field of organ replacement. Congratulations to the authors who have submitted these papers and contributed to the maintenance of the high-quality standard of the journal (**Table 1**).

Four important special issues were completed in 2024: among them, "Living well after transplantation," a collection focusing on various aspects that make quality of life so much improved after whole organ transplantation [12] and "Current challenges and advances on Infectious Diseases in Solid Organ Transplantation," a timely series of articles as we exit the pandemic times that have had so much impact on our field [13].

Importantly, two of the completed special issues are the product of the tightened relationships between ESOT and TI editorial board:

1) An extensive collection on Diversity, Equity and Inclusion (DEI) in transplantation has been a significant realization of ESOT and TI commitment to the DEI values [14]. The articles published address a variety of issues that require immediate attention, from gender and race equity [15–17] to access to transplantations in underprivileged populations [18–20] or low or middle income countries [21, 22].

2) The long-awaited series of ESOT guidelines [23], written after a rigorous process during the Transplantation Learning Journey meeting held in Prague at the end of 2022 [24], are now fully available and range from oncological issues in liver transplantation [25, 26], to machine perfusion [27, 28] and biomarkers [29–31], and will undoubtedly be of regular utility and use for transplant surgeons and physicians.

All this publishing activity would not have been possible without our team of executive, associate and statistical editors, the staff at TI editorial office and the project manager from the ESOT office, Ms. Ketevan Rukhadze. Above all, the contribution of the reviewers who have spent their time and expertise to assess the papers submitted to our journal is gratefully acknowledged and we thank them for their work and voluntary participation to TI. The list of the reviewers who have contributed to TI in 2024 appears at the end of this editorial (**Appendix A1**).

Transplant International is proud to have started an editorial fellowship program, in which five young investigators, from different countries and professional background, have been closely associated with one of the editors-in-chief, to learn and assist in the editorial activities. In their final report, all have emphasized the educational value of the experience, the better understanding of the mechanisms of scientific publishing they got from it and the career progression it has represented for them. Editorial fellows were also engaged in communication projects for the journal and have been instrumental in creating or developing newsletters for the ESOT website, "tweetorials" and a live and interactive journal club, in which the authors and invited experts discuss selected articles recently published in TI. We have welcomed a new group of editorial fellows this past fall and we are excited to develop new projects with them. Meanwhile, we thank Chiara Becchetti, Saskia Bos, Fabian Eibensteiner, Mehdi Maanaoui and Tudor Moisoiu for their engagement and we wish them the great career in transplantation they deserve.

Editorial

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Correspondence
Thierry Berney,
eic.ti@frontierspartnerships.org

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TABLE 1 | Top 10 2024 highest attention articles (Altmetric scores).

Outcomes of Kidney Transplants From Toxoplasma-Positive Donors: An Organ Procurement and Transplant Network Database Analysis	Butani et al. [1]
Clinical Pig Heart Xenotransplantation—Where Do We Go From Here?	Cooper and Cozzi [2]
Liver Transplantation for Intrahepatic Cholangiocarcinoma After Chemotherapy and Radioembolization: An Intention-To-Treat Study	Maspero et al. [3]
Pre-Transplant Hyperparathyroidism and Graft or Patient Outcomes After Kidney Transplantation	Rodrigues et al. [4]
Paraneoplastic Syndrome After Kidney Transplantation: Frequency, Risk Factors, Differences to Paraneoplastic Occurrence of Glomerulonephritis in the Native Kidney, and Implications on Long-Term Kidney Graft Function	Zacrocka et al. [5]
Simultaneous Heart and Kidney Transplantation: A Systematic Review and Proportional Meta-Analysis of Its Characteristics and Long-Term Variables	Sampaio et al. [6]
Multi-Centre UK Analysis of Simultaneous Pancreas and Kidney (SPK) Transplant in Recipients With Type 2 Diabetes Mellitus	Owen et al. [7]
Public Opinions on Removing Disincentives and Introducing Incentives for Organ Donation: Proposing a European Research Agenda	Ambagtsheer et al. [8]
European Society for Organ Transplantation (ESOT) Consensus Statement on Outcome Measures in Liver Transplantation According to Value-Based Health Care	Carbone et al. [9]
Survival Advantage Comparing Older Living Donor Versus Standard Criteria Donor Kidney Transplants	Patel et al. [10]
Burnout Among Physicians of Specialties Dedicated to Liver Transplantation	Sanchez-Antolin et al. [11]

The biennial ESOT Congress will take place in London in 2025, from June 29 to July 2, and Transplant International will actively participate in the congress activities. Sessions designed and organized by the editorial board and editorial fellows will discuss some of the abstracts presented during the congress as well as a selection of articles recently published in TI and covering each of the five tracks defined by the scientific program committee. A "meet the editors" workshop, aiming at the younger delegates will discuss in an interactive format several aspects of scientific publication that are not always obvious for young investigators. Finally, we will publish toward the end of the year a collection of original articles based on the best communications presented at the congress.

2024 has been a busy and productive year, but more will come the way of TI readership in 2025. A collection on xenotransplantation is nearing completion and will be finalized in early 2025. We are preparing two new special issues on very hot topics in our field, namely, how transplantation practices must evolve to better serve an ageing population and the challenges and opportunities -and potential threats- of artificial intelligence when applied to our field.

From the beginning of our tenure, it has been our stated aim to knit tighter together the links between TI readership and ESOT membership. We hope that an ever-improving journal will contribute to these two communities realizing that they really are, or should be, the same and that we are stronger together in making the field of transplantation progress in Europe and beyond.

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GENERATIVE AI STATEMENT

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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APPENDIX

TABLE A1 | Reviewers for transplant international – 2024.

Davide A. Abate
Krishna Agarwal
Kaisa Ahopelto
Emin Baris Akin
Ayman Al Jurdi
Ammar Al Midani
David Peter Al-Adra
Andreas Albertsen
Carlo Alfieri
Hakim Ali
Marc Antoine Allard
Ian Alwayn
Kristina Andrijauskaite
Madonna Rica Anggelia
Rahul Argula
Olivier Aubert
Federico Aucejo
Fahad Aziz
Daniel Azoulay
Friederike Bachmann
Rafael Badenes
Nicolas Barros Baertl
Richard James Baker
Amit Banga
David A Baran
Louise Barbier
Adam Barlow
Ivan Barone
Yuri Battaglia
Sara Battistella
Sonja Beckmann
Hanne Beeckmans
Alilis Fontana Bellorín
Alberto Benazzo
Giuditta Benincasa
David Bennett
Ilies Benotmane
Stefan Philip Berger
Michiel G.H. Betjes
Abu Bakar Hafeez Bhatti
Luigi Biancone
Christopher Blosser
Georg Böhmig
Cecilia Bonazzetti
Yuri Boteon
Rita Bottino
Dawn Bowles
Ana Cristina Breithaupt-Faloppa
Matteo Breno
Olivier Brugiere
Antoine Buemi
Leonid Bunegin
Patrizia Burra
Kadir Caliskan
Chris Callaghan
Jasper Callemeyn
Luis Camargo
Diego Cantarovich
Javier Carbone
Benno Cardini
Fatma Cebeci
Michael Cecka

(Continued in next column)

TABLE A1 | (*Continued*) Reviewers for transplant international – 2024.

Leonardo Centonze
Miriam Cortés Cerisuelo
Laurens J. Ceulemans
Ernest G Chan
Xavier Charmetant
Julien Charpentier
Xingxing Cheng
Toyofumi Chen-Yoshikawa
Aravind Cherukuri
Chi Yuen Simon Cheung
Wisit Cheungpasitporn
Umberto Cillo
Arielle Cimeno
Marc Clancy
Maarten Coemans
Luc Colas
Thomas Combriat
Laura Cosmai
Andrew Courtwright
Lionel Couzi
Peter Cowan
Emanuele Cozzi
Kristopher Croome
Elena Cuadrado-Payán
David Cucchiari
Madison Cuffy
Zoltan Czigany
John Dark
David Ross Darley
Marieke T. De Boer
Riccardo De Carlis
J. L. Campo-Cañaveral De La Cruz
Marc De Perrot
Luca Salvatore De Santo
Nicola De Stefano
Aiko P. J. De Vries
Caroline Den Hoed
Fungai Dengu
Olivier Detry
Sebastien Dharancy
Carlos Diaz
Fabienne Dobbels
Daniele Dondossola
Victoria Gomez Dos Santos
Pedro Augusto Reck Dos Santos
Jane Duffy
Michael Dunn
Magdalena Durlik
Antoine Durrbach
Philipp Dutkowski
Michael Eder
Per Ederoth
Verner Eerola
Jim Egan
Gunilla Einecke
Burcin Ekser
Kathrin Eller
Juliet Emamaullee
Marten Engelse
Eric Epailly
Michiel Elardus Erasmus
Dilmurodjon Eshmuminov
Dong Eun Lee
Patrick Evrard
Hana Fakhoury

(Continued on following page)

TABLE A1 | (*Continued*) Reviewers for transplant international – 2024.

Mario Fernández-Ruiz
Alberto Ferrarese
Sylvie Ferrari-Lacraz
Joana Ferrer-Fàbrega
Gottfried Fischer
Alan Wayne Flake
John Forsythe
Kevin Fowler
Jacopo Fumagalli
Vasiliki Galani
Zita Galvin
Ilaria Gandolfini
Wei Gao
Eduardo Miñambres Garcia
Dale Gardiner
Jeffrey J Gaynor
Laurette Geldenhuys
Anand Ghanekar
Luiza Ghila
Davide Ghinolfi
Pierre Gianello
Ian Gibson
Nicholas Gilbo
Patricia Ging
Emmanouil Giorgakis
Magali Giral
Peter Girman
Laurent Godinas
Hilary Goldberg
Masafumi Goto
Claire Goumard
Andrea Gramegna
Veronika Grau
John R Greenland
Sharlene Greenwood
Mark Greer
Paolo Antonio Grossi
Markus Guba
Benoit Guery
Luiz Felipe Guimarães
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ORIGINAL RESEARCH
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Multi-Center Outcome Analysis of 16 Face Transplantations – A Retrospective OPTN Study

Leonard Knoedler[1], Thomas Schaschinger[1], Tobias Niederegger[1], Gabriel Hundeshagen[2,3], Adriana C. Panayi[1], Curtis L. Cetrulo Jr.[4], Maxime Jeljeli[4], Elena Hofmann[1], Max Heiland[1], Steffen Koerdt[1] and Alexandre G. Lellouch[4,5,6]*

[1]Department of Oral and Maxillofacial Surgery, Charité - Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany, [2]Department of Hand, Plastic and Reconstructive Surgery, Burn Center, BG Trauma Hospital Ludwigshafen, University of Heidelberg, Ludwigshafen, Germany, [3]Department of Plastic and Hand Surgery, Burn Center, BG Trauma Hospital Ludwigshafen, University of Heidelberg, Ludwigshafen, Germany, [4]Vascularized Composite Allotransplantation Laboratory, Massachusetts General Hospital, Harvard Medical School, Boston, MA, United States, [5]Shriners Children's Boston, Boston, MA, United States, [6]Cedars-Sinai Medical Center, Los Angeles, CA, United States

Facial Vascularized Composite Allotransplantation (fVCA) restores form and function for patients with severe facial disfigurements, yet multi-center outcome data remain scarce. We accessed the Organ Procurement and Transplantation Network (OPTN) database from 2008 to 2024 to identify all full- or partial-face fVCA recipients, excluding patients under 18 years and those with physiologically impossible BMIs. Of 25 identified patients, 16 (64%) met inclusion criteria (69% male; mean age 43 ± 14 years). Recipients experienced a median of 5 [IQR 0.0–10] acute rejection episodes, which correlated with inotrope use during donor procurement ($p = 0.033$). On average, patients were hospitalized 2.4 ± 1.8 times, with arginine vasopressin (AVP) administration linked to fewer hospitalizations ($p = 0.035$). Seven recipients (44%) experienced complications, and extended-criteria donor (ECD) status was associated with higher complication rates ($p = 0.049$). These findings underscore the promise of fVCA to address complex facial defects while identifying key risk factors—particularly inotrope use and ECD status, while AVP administration may mitigate hospital stays. Further studies with larger cohorts are warranted to refine perioperative strategies, improve outcomes, and expand the clinical utility of fVCA.

Keywords: face transplantation, facial transplantation, vascularized composite allotransplantation, VCA, OPTN

***Correspondence**
Leonard Knoedler,
leonard.knoedler@charite.de

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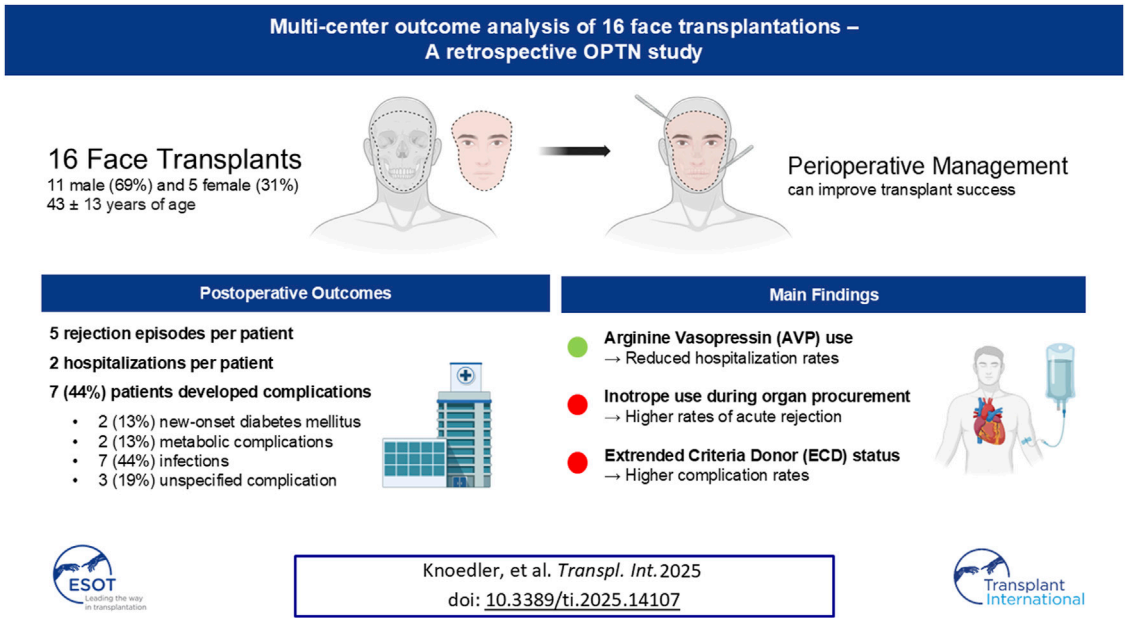
INTRODUCTION

Facial Vascularized Composite Allotransplantation (fVCA) has expanded the reconstructive ladder, providing a novel strategy for patients with extended facial defects. Such defects include severe burns or traumatic accidents. Conventional reconstructive techniques (e.g., full-thickness skin grafts, local flap surgery) may provide insufficient wound coverage and healing in these scenarios [1–3]. First performed in 2005, fVCA has since evolved from an experimental procedure to a robust reconstructive route, offering a lifeline to patients with severe facial disfigurements. Besides improving basic functions (e.g., breathing, eating, speaking), fVCA can provide a renewed sense of personal identity and social integrity [4].



GRAPHICAL ABSTRACT

Currently, more than 50 fVCA have been performed worldwide [5]. The main barriers to increasing the number of fVCA procedures and broadening the access to fVCA care include the need for life-long immunosuppression, immune rejection, which has led to two cases of re-transplantation, and the paucity of multi-center and multi-surgeon fVCA outcome research [6]. Over the past decade, promising pathways to induce immunotolerance and reduce/taper immunosuppressive drugs have been proposed [7, 8]. Similarly, recent research has focused on defining acute and chronic rejections after fVCA surgery, as well as investigating novel diagnostic techniques to promptly detect rejection episodes [9].

Despite such advancements, fVCA outcomes can vary widely [10–12]. This variability is multifactorial, and includes patient health, extent of fVCA, and comorbid conditions. Therefore, understanding risk factors predisposing to poorer surgical outcomes and transplant survival is crucial for tailoring preoperative counseling, perioperative protocols, and postoperative monitoring. However, to date, there is a scarcity of comprehensive studies investigating the outcomes and risk factors of fVCA surgery. While these studies may provide valuable insights into single-surgeon and/or single-center center experiences they often lack the generalizability needed to inform decision-making in a broader context.

In contrast, the use of multi-institutional and multi-surgeon databases such as the Organ Procurement and Transplantation Network (OPTN) database can overcome these limitations. The OPTN database provides a dataset that records a wide range of information across the transplantation process. Capturing more than 100,000 candidates for solid organ transplantation, the OPTN dataset also records information on VCA surgery. This multi-faceted patient population may provide a more generalizable reflection of real-world clinical practices. To date, the OPTN database has only been accessed for descriptive research work on VCA surgery [13].

In this study, we queried the OPTN database to investigate outcomes after fVCA surgery and identify risk factors for adverse events. For fVCA providers, these insights can help advance the preoperative patient screening and perioperative treatment algorithms. Identifying high-risk patients may reduce postoperative morbidity and prolong graft survival. On the other hand, these lines of research may empower patients with knowledge about potential risks and benefits following fVCA surgery. Thus, patients may participate more actively in their care decisions, leading to more personalized and satisfactory healthcare experiences. Ultimately, the herein presented data can navigate the development of best-practice guidelines and protocols and improve the quality and safety of surgical fVCA care.

PATIENTS AND METHODS

Data Source and Patient Selection

Data were obtained from the OPTN database, which was developed by the United States Organ Donation and Transplant System (UNOS). This comprehensive database contains detailed records of every organ donation and transplant event in the United States. A search of the OPTN database was conducted to identify all transplant cases involving fVCAs dating back to 10 December 2008. From an initial cohort of 172 entries, 25 cases involving either isolated or combined

TABLE 1 | Recipient demographics, medical, and immunological information. Reported as n (%), unless otherwise stated.

Demographics	Recipients (n = 16)	
Gender		
Female	5	(31)
Male	11	(68)
Age [years], mean ± SD		
At transplantation	43	±14
At listing	43	±16
Ethnicity		
White, Non-Hispanic	7	(44)
Black, Non-Hispanic	1	(6.2)
Unknown	8	(50)
Primary diagnosis		
Trauma	9	(56)
Burn/explosion	6	(38)
Unknown	1	(6)
Survival time [days], mean ± SD	1,764	±1,226
BMI [kg/m^2], mean ± SD		
At transplantation	26	±7.5
At registration	25	±8.4
Weight [kg], mean ± SD		
At transplantation	78	±30
At listing	73	±29
At registration	73	±29
Height [cm], mean ± SD		
At transplantation	171	±10
At registration	152	±56
CPRA score [%], median (IQR)		
At transplantation	23	(0–30)
At listing	30	(20–38)
Laboratory values		
Serum creatinine [mg/dL], mean ± SD		
Pre-transplant	0.84	±0.25
Discharge	0.86	±0.23
Hemoglobin A1c (%), median (IQR)		
Pre-transplant	0.0	(0.0–5.0)
Discharge	0.0	(0.0–6.0)
Immunological Characteristics		
AB0		
0	5	(31)
A	4	(25)
Unknown	7	(44)
Donor-recipient ABO match level		
1	8	(50)
2	1	(6.2)
Unknown	7	(44)
HLA mismatch level		
1	1	(6.2)
4	3	(19)
5	2	(13)
6	2	(13)
Unknown	8	(50)
A locus mismatch level		
0	2	(13)
1	3	(19)
2	3	(19)
Unknown	8	(50)
B locus mismatch level		
0	1	(6.2)
2	7	(44)
Unknown	8	(50)
DR locus mismatch level		
1	4	(25)
2	4	(25)
Unknown	8	(50)

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TABLE 1 | (*Continued*) Recipient demographics, medical, and immunological information. Reported as n (%), unless otherwise stated.

Demographics	Recipients (n = 16)	
Computed donor antigens, median (IQR)		
A1	2	(1.0–5.0)
A2	28	(3.0–31)
B1	11	(7.8–39)
B2	45	(25–61)
DR1	7	(4.0–8.5)
DR2	15	(13–17)
HLA A1 antigen		
1	3	(19)
2	3	(19)
11	1	(6.2)
32	1	(6.2)
Unknown	8	(50)
HLA A2 antigen		
2	1	(6.2)
3	1	(6.2)
11	2	(13)
24	1	(6.2)
30	1	(6.2)
31	1	(6.2)
Unknown	9	(56)
HLA B1 antigen		
7	2	(13)
8	1	(6.2)
35	1	(6.2)
51	1	(6.2)
53	1	(6.2)
60	1	(6.2)
63	1	(6.2)
Unknown	8	(50)
HLA B2 antigen		
8	1	(6.2)
27	1	(6.2)
41	2	(13)
44	1	(6.2)
57	1	(6.2)
58	1	(6.2)
Unknown	9	(56)
HLA DR1 antigen		
4	2	(13)
7	1	(6.2)
11	1	(6.2)
13	2	(13)
15	1	(6.2)
17	1	(6.2)
Unknown	8	(50)
HLA DR2 antigen		
4	2	(13)
11	1	(6.2)
13	1	(6.2)
15	1	(6.2)
17	2	(13)
Unknown	9	(56)
HBV core antibody		
Positive	1	(6.2)
Negative	13	(81)
Unknown	2	(13)
HBV surface antigen		
Positive	1	(6.2)
Negative	13	(81)
Unknown	2	(13)
HCV serostatus		
Positive	1	(6.2)
Negative	14	(88)

(Continued on following page)

TABLE 1 | (*Continued*) Recipient demographics, medical, and immunological information. Reported as n (%), unless otherwise stated.

Demographics	Recipients (n = 16)	
Unknown	1	(6.2)
EBV serostatus		
Positive	14	(88)
Negative	1	(6.2)
Unknown	1	(6.2)
CMV status		
Positive	9	(56)
Negative	6	(38)
Unknown	1	(6.2)
Evaluated Scores		
Physical Functioning (PF) score [0–100 scale], mean ± SD	70	±35
Role-Physical (RP) score [0–100 scale], mean ± SD	57	±39
Bodily Pain (BP) score [0–100 scale], mean ± SD	67	±31
General Health (GH) score [0–100 scale], mean ± SD	85	±11
Vitality (VT) score [0–100 scale], mean ± SD	67	±27
Social Functioning (SF) score [0–100 scale], mean ± SD	57	±39
Role-Emotional (RE) score [0–100 scale], mean ± SD	87	±17
Mental Health (MH) score [0–100 scale], mean ± SD	87	±14

CPRA score, Calculated Panel Reactive Antibody score.

fVCA transplants were identified. Nine patients were excluded due to missing follow-up data on outcome measurements, resulting in a final cohort of 16 fVCA transplant cases eligible for outcome analysis.

Variable Extraction

fVCA recipient and donor demographics, transplant and operative data were extracted for analysis. Transplant recipient data were evaluated as follows: (a) recipient demographics [gender, age, ethnicity, primary diagnosis, body mass index (BMI), weight, height, Calculated Panel Reactive Antibody (CPRA) score], (b) laboratory values (serum creatinine, hemoglobin A1c), (c) immunological characteristics [AB0 classification, donor-recipient AB0 mismatch level, Human Leukocyte Antigen (HLA) mismatch level, A locus mismatch level, B locus mismatch level, DR locus mismatch level, computed donor antigens, HLA A1 antigen, HLA A2 antigen, HLA B1 antigen, HLA B2 antigen, HLA DR1 antigen, HLA DR2 antigen, HBV core antibody, HBV surface antigen, HCV serostatus, EBV serostatus, CMV status], and (d) evaluated scores [Physical Functioning (PF) score, Role-Physical (RP) score, Bodily Pain (BP) score, General Health (GH) score, Vitality (VT) score, Social Functioning (SF) score, Role-Emotional (RE) score, Mental Health (MH) score].

We investigated the following donor data: (a) donor demographics [gender, age, ethnicity, BMI, weight, height, type, Expanded Criteria Donor (ECD) status], and (b) immunological characteristics [AB0 classification, HBV core antibody, HBV surface antigen, HBV NAT test result, HCV antibody, HCV NAT test result, EBV (VCA) (IgG) status, EBV (VCA) (IgM) status, risk for blood-borne disease transmission].

With regards to transplant characteristics and perioperative data, we evaluated previous transplants of the same organ, instances of multiple VCA transplantations, use of additional allografts, warm ischemia time, cold ischemia time, distance of donor hospital to transplant center, use of inotropic medication during donor organ procurement, donor administration of arginine vasopressin (AVP) within 24 h pre-cross clamp, donor administration of insulin within 24 h pre-cross clamp, protein in donor urine, recipient pre-transplant blood transfusion, recipient coagulopathies, recipient pre-transplant life support, recipient other risk factors, recipient use of tolerance induction technique, skin type, UNOS transplant region, UNOS listing region, transplant allocation type, and year of transplant.

The postoperative outcomes investigated included the number of acute rejection episodes, the number of hospitalizations, and the occurrence of any complication. The number of acute rejection episodes was evaluated for each patient by checking for the occurrence at each follow-up stamp. Any complication was defined as the occurrence of at least one of the following events within the entire follow-up period: new-onset diabetes mellitus, metabolic complication, infectious complication, or other complication.

Statistical Analysis

Data were collected and securely stored using an electronic laboratory notebook (LabArchives, LLC, San Marcos, CA, United States). Analyses were conducted using GraphPad Prism (V10 for MacOS, GraphPad Software, La Jolla, CA, United States) and Python within the Google Colaboratory environment (Google Colab). Spearman's rank correlation was employed to assess relationships between continuous variables, such as age and BMI. For categorical variables, including recipient AB0 blood group and UNOS transplant region, the Kruskal-Wallis test was applied. In instances where statistical significance was found, post-hoc pairwise comparisons were carried out using Dunn's test with Bonferroni correction to determine specific group differences. A significance threshold of $p < 0.05$ was used for all tests.

TABLE 2 | Donor demographics and immunological information. Reported as n (%), unless otherwise stated.

Demographics	Donors (n = 16)	
Gender		
Female	5	(31)
Male	11	(69)
Age [years], mean ± SD	38	±14
Ethnicity		
White, Non-Hispanic	15	(94)
Black, Non-Hispanic	1	(6.3)
BMI [kg/m^2], mean ± SD	27	±4.0
Weight [kg], mean ± SD	78	±16
Height [cm], mean ± SD	171	±10
ECD		
Yes	4	(25)
No	12	(75)
Immunological Characteristics		
Donor AB0		
0	9	(56)
A	2	(13)
A1	5	(31)
HBV core antibody		
Negative	16	(100)
HBV surface antigen		
Negative	16	(100)
HBV NAT test result		
Negative	6	(38)
Unknown	10	(62)
HCV antibody		
Unknown	16	(100)
HCV NAT test result		
Negative	6	(38)
Unknown	10	(62)
HIV NAT test result		
Negative	6	(38)
Unknown	10	(62)
EBV (VCA) (IgG) status		
Positive	14	(88)
Negative	2	(13)
EBV (VCA) (IgM) status		
Negative	16	(100)
Risk for blood-borne disease transmission		
Negative	16	(100)

RESULTS

Transplant Recipient Demographics

The studied cohort consisted of 16 patients who underwent fVCA surgery. The average age at the time of transplantation was 43 ± 14 years. Most patients were male (n = 11; 69%) and of white ethnicity (n = 7; 44%). At the time of surgery, the mean BMI was 26 ± 7.5 kg/m^2. The primary cause for fVCA was trauma (n = 9; 56%; **Table 1**).

Serum creatinine levels rose from a pre-transplant average of 0.84 ± 0.25 mg/dL to 0.86 ± 0.23 mg/dL at discharge. The mean postoperative survival duration was 1,764 ± 1,226 days. Performance on the GH score resulted in an average of 85 ± 11 points on an 100 points scale (**Table 1**).

Transplant Donor Demographics

The 16 donors were mostly male (n = 11; 69%) and of white ethnicity (n = 15; 94%) with a mean age of 38 ± 14 years and a BMI of 27 ± 4.0 kg/m^2. Most donors (n = 12; 75%) were not classified as ECDs. The most common AB0 group was type 0 (n = 9; 56%; **Table 2**).

Surgical and Transplant Characteristics

Some donors received AVP (n = 9; 56%) within 24 h before cross-clamping (i.e., clamping of a major vessel to stop blood-flow towards the harvested organ) or inotropic medications during organ procurement (n = 11; 69%; **Table 3**).

Outcomes in fVCA Transplant Recipients

Transplant recipients experienced a median of 5 (IQR 0.0–10) acute rejection episodes. The longer the recipient survived (p = 0.006), the more acute rejections were observed. Furthermore, A1 donor blood group (p = 0.047), and the use of inotropic medication during organ procurement (p = 0.033) correlated with increased frequency of acute rejection episodes. On the other hand, higher pre-transplant serum creatinine levels (p = 0.020), elevated hemoglobin A1c at discharge (p = 0.049), and better GH scores (p = 0.016) were linked to fewer acute rejection episodes (**Tables 4–7**).

On average, patients were hospitalized 2.4 ± 1.8 times during the follow-up period. The longer the recipient survived (p = 0.001), the more hospitalizations were observed. Conversely, higher serum creatinine levels at discharge (p = 0.033), and donor administration of AVP before cross-clamping (p = 0.035) were associated with fewer hospitalizations post-transplant (**Tables 4–6**).

Complications occurred in seven patients (44%), with specific complications including new-onset diabetes mellitus (n = 2; 13%), metabolic issues (n = 2; 13%), infectious complications (n = 7; 44%), and other types of complications (n = 3; 19%). Positive ECD status was associated with higher complication rates (p = 0.049), while a higher GH score was linked to fewer occurrences of complications (p = 0.017; **Tables 4–6**).

DISCUSSION

Big databases present a tool for tracking outcomes, identifying associated factors, and improving patient care. fVCA patients are particularly vulnerable due to multiple factors, such as severity of initial trauma, surgical complexity, and the need for life-long immunosuppression. Thus, we analyzed 16 cases of fVCA from the OPTN database to identify potential risk factors correlating with postoperative complications.

Acute rejection is one of the most common complications in VCA transplantation occurring more frequently than in solid organ transplants (SOT) [14]. In our study, we found a positive correlation between the use of inotropic medication during donor organ procurement and the frequency of postoperative acute rejection episodes.

From a broader perspective, our results align with studies in SOT. For example, Nixon et al. and D'Ancona et al. showed that high-dose inotrope donor support had a higher tendency for early post-transplant complications and was the major determinant for primary graft failure after heart transplantation [15, 16]. While

TABLE 3 | Surgical and transplant characteristics. Reported as n (%), unless otherwise stated.

Surgical and transplant characteristics	Patients (n = 16)	
Previous transplant of same organ type		
Yes	1	(6.3)
No	15	(94)
Multiple-VCA-transplant		
Yes	1	(6.3)
No	15	(94)
Extra allograft used		
Yes	8	(50)
No	6	(38)
Unknown	2	(13)
Warm ischemia time [min], median (IQR)	2.0	(0.0–75)
Cold ischemia time [min], median (IQR)	150	(90–195)
Distance donor-hospital to transplant center [nM], median (IQR)	19	(0.0–79)
Donor procurement with inotropic medication		
Yes	11	(69)
No	5	(31)
Donor pre-cross clamp administration of arginine vasopressin		
Yes	9	(56)
No	7	(44)
Donor pre-cross clamp administration of insulin		
Yes	6	(38)
No	10	(63)
Donor protein in urine		
Yes	6	(38)
No	10	(63)
Recipient pre-transplant blood transfusion		
Yes	12	(75)
No	2	(13)
Unknown	2	(13)
Recipient Coagulopathies		
Yes	1	(6.3)
No	13	(81)
Unknown	2	(13)
Recipient pre-transplant life support		
No	15	(94)
Unknown	1	(6.3)
Recipient Other risk factors		
Yes	4	(25)
No	10	(63)
Unknown	2	(13)
Recipient tolerance induction technique		
No	14	(88)
Unknown	2	(13)
Skin Type		
Type I (scores 0–6) Pale white; blond/red hair; blue eyes; freckles; always burns, never tans	1	(6.3)
Type II (scores 7–13) White; fair; blond/red hair; blue/green/hazel eyes; usually burns, tans minimally	2	(13)
Type III (scores 14–20) Cream white; fair, any hair/eye color; quite common; sometimes mild burn, tans uniformly	4	(25)
Type V (scores 28–34) Dark brown; Middle Eastern skin types; Very rarely burns, tans very easily	1	(6.3)
Unknown	8	(50)
UNOS transplant region		
Region 1	10	(63)
Region 2	1	(6.3)
Region 7	1	(6.3)
Region 9	1	(6.3)
Region 10	3	(19)
UNOS listing region		
Region 1	4	(25)
Region 7	1	(6.3)
Region 9	1	(6.3)
Region 10	2	(13)
Unknown	8	(50)
Allocation type		
Local	14	(88)
Regional	1	(6.3)

(Continued on following page)

TABLE 3 | (*Continued*) Surgical and transplant characteristics. Reported as n (%), unless otherwise stated.

Surgical and transplant characteristics	Patients (n = 16)	
National	1	(6.3)
Year of transplant		
2008	1	(6.3)
2009	1	(6.3)
2011	3	(19)
2012	1	(6.3)
2013	1	(6.3)
2014	3	(19)
2015	1	(6.3)
2016	1	(6.3)
2017	1	(6.3)
2018	1	(6.3)
2019	1	(6.3)
2020	1	(6.3)

TABLE 4 | Postoperative outcomes in facial VCA transplant recipients. Reported as n (%), unless otherwise stated.

Outcomes	Patients (n = 16)	
Number of acute rejection episodes, median (IQR)	5	(0.0–10)
Number of hospitalizations, mean ± SD	2.4	±1.8
Any complication	7	(44)
New-onset diabetes mellitus	2	(13)
Metabolic complication	2	(13)
Infectious complication	7	(44)
Other complication	3	(19)

Blitzer et al. concluded that donor inotropic medication did not impact short-term heart transplant recipient survival, administration of even one inotrope was associated with increased 1-year mortality by 14% [17]. This lends support to the hypothesis that donor hemodynamic maintenance with inotropes influences outcomes of organ transplantation, such as postoperative acute rejection episodes. This might be due to the wide-spread ischemic consequences of vasoconstriction [18, 19]. To overcome this obstacle, Westphal et al. suggested the additional use of hormone replacement therapy (i.e., vasopressin, thyroid hormones, and corticosteroids) to improve the hemodynamics of deceased donors, ultimately decreasing the need for inotropic medication.

Overall, reducing the need for inotrope administration in potential donors during organ procurement might improve post-transplant outcomes of fVCA, and decrease rates of acute rejection episodes. However, future research is warranted to determine the optimal drug regime for hemodynamic management in potential donors.

In our study, we also found a direct correlation between donor administration of AVP during organ procurement and decreased frequency of postoperative hospitalizations after fVCA.

Broadly speaking, these findings echo previous studies that report improved medical outcomes after donor administration of AVP during solid organ procurement [20–22]. For instance, in their analysis of lung transplants using the OPTN database, Callahan et al. observed a notable rise in the number of successfully procured organs and enhanced preservation of transplanted lung function when donors were administered AVP. They propose that AVP exerts a catecholamine-sparing effect, reducing the need for inotropes in cases of brain-death-induced cardiovascular collapse, minimizing inflammatory mediator release, and decreasing reliance on crystalloid supportive therapy [21].

Additional evidence highlights the beneficial effects of AVP on blood pressure, vascular tone, and the need for inotropic medication. Pennefather et al. demonstrated that administering AVP to brain-dead donors significantly reduced plasma hyperosmolality and inotrope requirements, while improving blood pressure and hemodynamic stability. They noted that low-dose AVP infusion allows for a reduction in inotropic support without causing adverse hemodynamic effects, thereby mitigating the detrimental impact of catecholamines on transplant outcomes. Furthermore, AVP administration was associated with a decrease in postoperative hospitalizations [23].

Nakagawa et al. further reported that AVP contributed to maintaining hemodynamic stability and fluid homeostasis in deceased organ donors, ultimately improving both the quality and quantity of transplanted organs and enhancing post-transplant organ function [24]. However, Pennefather et al. also cautioned that insufficient AVP dosing carries a significant risk of cardiovascular overstimulation, potentially leading to organ damage or a decline in organ quality [23].

Our findings align with previous research demonstrating that donor administration of AVP during organ procurement is associated with improved postoperative outcomes, including reduced hospitalizations, enhanced hemodynamic stability, and better organ preservation. These results further support the role of AVP in optimizing donor management strategies to improve transplant success.

The concept of ECD encompasses organ donors who possess one or more characteristics that may adversely affect transplant outcomes—such as advanced age, a history of smoking, or pre-existing comorbidities like diabetes—yet are utilized in order to address the persistent organ shortage in SOT [25, 26]. While multiple studies have reported mixed findings regarding the

TABLE 5 | Numerical risk-associated factors for complications.

	Number of acute rejection episodes		Number of hospitalizations		Any complication	
	Spearman's Coefficient	**p-value**	**Spearman's Coefficient**	**p-value**	**Spearman's Coefficient**	**p-value**
Recipient characteristics						
Age						
At transplantation	–0.27	0.31	–0.26	0.34	–0.42	0.10
At listing	–0.57	0.14	–0.65	0.081	–0.62	0.10
BMI						
At transplantation	0.034	0.90	0.24	0.39	–0.16	0.58
At registration	0.63	0.13	0.71	0.074	0.72	0.067
Weight						
At transplantation	–0.032	0.91	–0.018	0.95	–0.22	0.44
At listing	0.56	0.15	0.38	0.35	0.45	0.26
At registration	0.56	0.15	0.38	0.35	0.45	0.26
Height						
At transplantation	0.14	0.61	–0.44	0.10	0.0	>0.99
At registration	0.54	0.17	0.32	0.45	0.40	0.33
CPRA score						
At transplantation	0.28	0.32	0.10	0.72	–0.07	0.82
At listing	–0.14	0.74	–0.23	0.58	–0.17	0.69
Days on liver waiting list	–0.17	0.70	–0.18	0.66	–0.17	0.69
Survival time	0.68	**0.006**	0.82	**<0.001**	0.50	0.061
Laboratory values						
Serum creatinine						
Pre-transplant	–0.59	**0.020**	–0.49	0.065	–0.37	0.17
Discharge	–0.34	0.23	–0.57	**0.033**	–0.12	0.67
Hemoglobin A1c						
Pre-transplant	–0.62	0.075	0.0	>0.99	0.19	0.62
Discharge	–0.71	**0.049**	–0.43	0.29	–0.13	0.76
Warm ischemia time	–0.53	0.14	–0.16	0.68	–0.23	0.56
Cold ischemia time	–0.015	0.96	–0.013	0.97	–0.31	0.30
Donor characteristics						
Age	0.087	0.75	0.14	0.59	–0.26	0.33
BMI	0.21	0.43	0.41	0.11	0.31	0.24
Weight	–0.033	0.90	0.077	0.78	0.12	0.65
Height	–0.32	0.22	–0.39	0.13	–0.082	0.76
Surgical characteristics						
Distance donor-hospital to transplant center	–0.15	0.58	–0.16	0.55	–0.014	0.96
Computed donor antigens						
DA1	0.14	0.61	0.11	0.70	0.39	0.13
DA2	0.43	0.10	0.025	0.93	0.30	0.25
DB1	0.32	0.23	0.082	0.76	0.13	0.65
DB2	0.40	0.12	0.45	0.080	0.12	0.65
DDR1	0.26	0.33	–0.24	0.38	0.0	>0.99
DDR2	–0.24	0.36	–0.016	0.95	–0.14	0.60
Evaluated Scores						
Physical Functioning (PF) score	–0.58	0.23	0.088	0.87	–0.29	0.57
Role-Physical (RP) score	–0.64	0.17	–0.12	0.82	–0.49	0.33
Bodily Pain (BP) score	0.37	0.47	0.81	0.053	0.59	0.21
General Health (GH) score	–0.89	**0.016**	–0.76	0.079	–0.89	**0.017**
Vitality (VT) score	–0.33	0.52	0.0	>0.99	–0.29	0.57
Social Functioning (SF)	–0.091	0.86	0.24	0.65	0.10	0.85
Role-Emotional (RE)	0.48	0.33	0.28	0.59	0.31	0.55
Mental Health (MH) score	–0.46	0.44	–0.76	0.13	–0.59	0.29

Statistically significant p-values are highlighted in bold.

impact of ECD on transplant complications and clinical outcomes, their use is steadily increasing due to the urgent need for grafts. In our study, we found that the use of ECD donor organs in fVCA correlated with higher rates of postoperative complications, aligning with previously documented trends in liver and kidney transplantation.

For instance, ECD liver grafts have been associated with an increased incidence of severe surgical complications (60% vs. 45%), graft loss (14% vs. 8%), and mortality (14% vs. 4%), as identified by Pagano et al. [27] In addition, ECD liver recipients are at higher risk for primary nonfunction, biliary complications, and graft-related fatalities (approximately 10%), with a further 10% of patients

TABLE 6 | Categorical risk-associated factors for complications.

	Number of acute rejection episodes		Number of hospitalizations		Any complication	
	H-statistic	p-value	H-statistic	p-value	H-statistic	p-value
Recipient characteristics						
Gender	0.75	0.39	2.4	0.12	0.73	0.39
Primary diagnosis	0.014	0.91	0.0040	0.95	0.67	0.41
AB0	0.41	0.52	0.57	0.45	0.80	0.37
A1 antigen	0.067	0.80	0.051	0.82	0.0	>0.99
DR1 antigen	1.0	0.32	1.5	0.22	1.0	0.32
DR2 antigen	2.7	0.10	2.4	0.12	3.0	0.083
CMV status	0.17	0.68	1.3	0.25	1.5	0.22
Pre-transplant hospitalisation within 90 days	0.029	0.86	0.0070	0.93	0.010	0.92
Pre-transplant transfusion	3.4	0.064	2.2	0.14	2.2	0.14
Donor characteristics						
Gender	0.75	0.39	2.4	0.12	0.73	0.39
AB0	6.1	**0.047**	4.8	0.090	4.3	0.12
EBV (VCA) (IgG) status	1.7	0.20	0.059	0.81	0.034	0.85
Tattoos	1.3	0.25	0.21	0.64	0.039	0.84
Protein in donor urine	0.51	0.47	0.0	>0.99	1.9	0.17
ECD	0.0040	0.95	0.034	0.85	3.9	**0.049**
General immunological characteristics						
HLA mismatch level	2.39	0.30	0.13	0.94	0.17	0.92
A locus mismatch level	2.02	0.37	3.22	0.20	2.02	0.36
DR locus mismatch level	2.86	0.09	0.00	1.00	0.47	0.50
Surgical characteristics						
Year of transplant	0.20	0.66	2.4	0.12	2.5	0.11
UNOS transplant region	2.4	0.13	4.0	**0.047**	4.2	**0.040**
UNOS listing region	2.2	0.14	3.9	**0.049**	5.0	**0.025**
Use of extra allograft	2.5	0.12	2.5	0.11	1.1	0.30
Skin type	0.0	>0.99	3.5	0.060	2.5	0.11
Donor pre-transplant administration of arginine vasopressin	2.4	0.12	4.4	**0.035**	0.85	0.36
Donor pre-transplant administration of insulin	0.20	0.66	1.9	0.17	0.14	0.71
Donor procurement with inotropic medication	4.5	**0.033**	0.16	0.69	1.6	0.21

Statistically significant p-values are highlighted in bold.

requiring re-listing for a second transplant within 1 year of their initial procedure [28–30]. However, ECD kidney transplants present a more nuanced picture: although Fellmann et al. observed no significant increase in recipient postoperative complication rates *per se*, they did identify a heightened risk of delayed graft function and diminished graft survival—factors attributable to ischemia-reperfusion injury—while overall recipient survival remained unaffected [31, 32]. Importantly, they argued that many observed complications in SOT recipients derive more from pre-existing comorbidities of the recipient such as diabetes, cardiovascular disease, and the necessity for anticoagulation therapy than from ECD status alone.

Together, these findings underscore the importance of meticulous patient selection, thorough preoperative risk stratification, and comprehensive informed consent—particularly when considering the use of ECD grafts in VCA. Although employing ECDs may bolster the donor pool and mitigate organ shortages, vigilance is warranted to balance the potential for increased complication rates with the life-enhancing benefits that transplantation can provide.

LIMITATIONS

To the best of our knowledge, this study is the first to analyze acute complication and hospitalization rates, as well as the occurrence of complications in fVCA cases using multi-center data collected over more than a decade. However, it is essential to interpret these findings in light of the study's limitations.

First, the statistical analyses used in this study revealed correlations rather than causal relationships, meaning that the underlying causal mechanisms remain unclear. Additionally, our data was extracted from the OPTN database, which provided only 16 fVCA cases with follow-ups, thereby limiting the sample size.

The retrospective nature of the study also introduces the potential risk of bias and confounding factors. Inconsistencies in data collection across centers, due to the varying expertise and subjectivity of database contributors, present a challenge for intra- and interinstitutional data comparisons. This may impair the of the dataset [33].

Furthermore, because the OPTN is a national U.S. database, the study is inherently limited by its focus on the U.S. healthcare system. As a result, ethical and racial disparities in VCA donation and transplantation may not be fully addressed, limiting the generalizability of the findings [34]. The lack of standardized criteria for diagnosing and validating episodes of acute rejection in the OPTN database further complicates the interpretation of rejection-related outcomes. More specifically, the OPTN database only records the date of an acute rejection episode but lacks details regarding the diagnostic methods or validation process.

TABLE 7 | Categorical results and post-hoc Dunn's test with Bonferroni correction of significant categorical risk-associated factors.

	p-value	Category	Median
Number of acute rejection episodes			
Donor procurement with inotropic medication			
Yes vs. No	**0.033**	Yes	8.0
		No	0.0
Donor ABO			
A vs. A1	0.368*	A	4.0
A vs. 0	1.0*	A1	16.0
A1 vs. 0	**0.047***	0	3.0
Number of hospitalizations			
Donor pre-transplant administration of arginine vasopressin			
Yes vs. No	**0.035**	Yes	1.0
		No	4.0
UNOS transplant region			
1 vs. 10	**0.047**	1	2.5
		10	5.0
UNOS listing region			
1 vs. 10	**0.049**	1	0.0
		10	4.0
Any complication			
Donor ECD			
Yes vs. No	**0.049**	Yes	1.0
		No	0.0
UNOS transplant region			
1 vs. 10	**0.040**	1	0.0
		10	1.0
UNOS listing region			
1 vs. 10	**0.025**	1	0.0
		10	1.0

**corrected p-values.*
Statistically significant p-values are highlighted in bold.

Despite these limitations, we believe that this study makes a valuable contribution to the field by offering insights into potential risk factors and new strategies for fVCA transplantation. The findings provide a foundation for future research, which may expand on these results to clarify underlying mechanisms and improve patient outcomes.

CONCLUSION

In summary, this study identified novel factors that influence postoperative outcomes in fVCA. We found that inotropic medication use during donor procurement was correlated with higher rates of acute rejection, consistent with trends seen in SOT. In contrast, the use of AVP was associated with fewer postoperative hospitalizations, which may improve donor stability and transplant outcomes. Our findings also highlighted increased complications in ECD-based fVCA, reinforcing the need for careful patient selection and preoperative evaluation. While the study has limitations, including small sample size and retrospective design, this line of research may unlock untapped potential for improving fVCA management. Future prospective studies are needed to confirm these findings and optimize the perioperative donor and recipient management for improved transplant success.

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 studies involving humans were approved by United States Organ Donation and Transplant System (UNOS). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

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

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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.

GENERATIVE AI STATEMENT

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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Impact of Organ Donor Pretreatment With Anti-Thymocyte Globulin in a Murine Model of Allogenic Kidney Transplantation

An He[1,2], *Yiren Yang*[1], *Katja Kotsch*[1*†] *and Arne Sattler*[1*†]

[1]*Charité–Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Department for General and Visceral Surgery, Berlin, Germany,* [2]*Department of Urology, The Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, Zhejiang, China*

Kidney transplantation is the treatment of choice for end-stage organ failure. To improve transplantation outcomes, particularly of "marginal" organs from extended criteria donors (ECD), attempts have been made to therapeutically modulate donor or graft pre-transplantation. Anti-thymocyte globulin (ATG) has a history as lymphocyte-depleting, immunosuppressive drug for treating rejection episodes post transplantation. In this study, however, we aimed to comprehensively analyze the effects of ATG donor pre-conditioning in a mouse model of kidney transplantation. ATG pre-treatment of potential donors led to a broad depletion of T- and NK cells in peripheral blood, non-lymphoid (including kidney) and lymphoid organs within 48 h, whereas myeloid cells were spared. ATG was also effectively depleting renal innate lymphoid type 1 and 2 cells. Importantly, transplantation of kidneys from ATG pre-treated donors into fully mismatched recipients showed only mild effects on leukocyte re-composition post transplantation. In line with this, serum creatinine and urea levels were similar in animals receiving kidneys from ATG treated donors or controls, demonstrating that donor treatment had no effect on allograft function in the early post-transplantation phase. In summary, our findings are suggestive of a more cell-type-specific depletion strategy in concert with an experimental model better reflecting aspects of clinical transplantation.

Keywords: anti-thymocyte globulin, kidney transplantation, donor pre-treatment, passenger leukocytes, kidney function

***Correspondence**
Katja Kotsch,
katja.kotsch@charite.de
Arne Sattler,
arne-sattler@gmx.de

†These authors have contributed equally to this work

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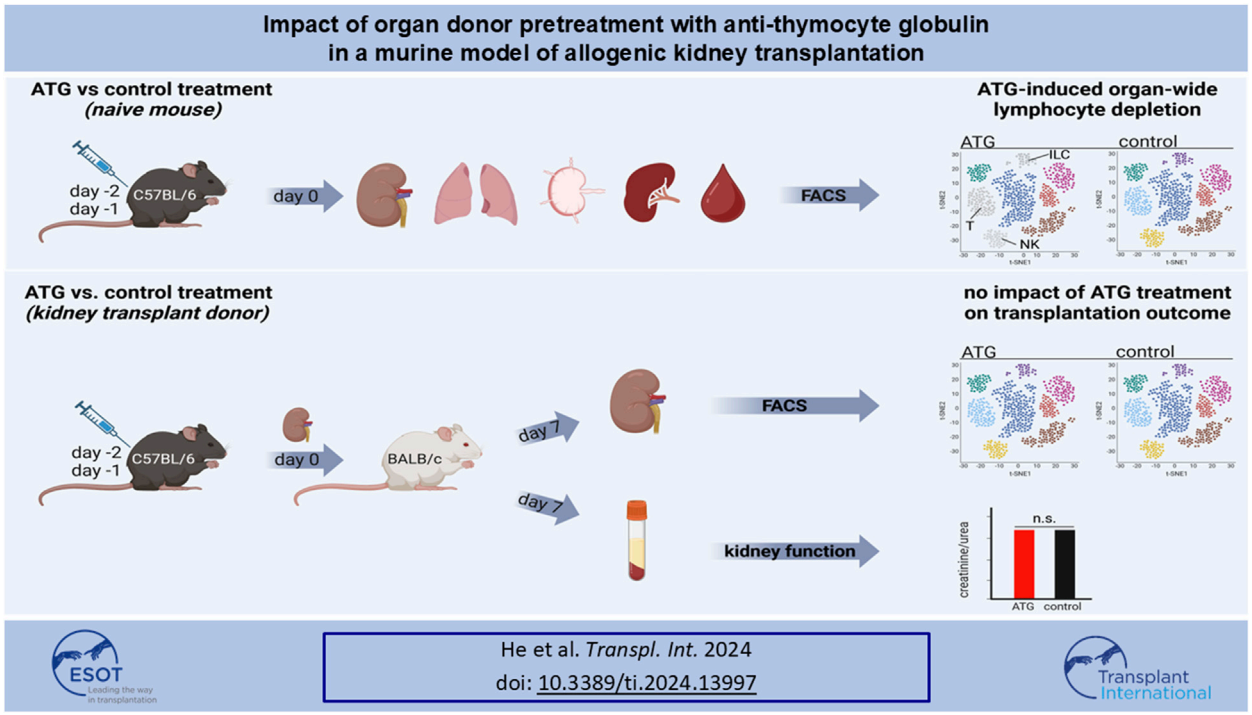
INTRODUCTION

The increasing demand for organ transplantation and the shortage of available organs limit the success of transplantation programs. Consequently, acceptance of expanded criteria donor (ECD) organs, being associated with a higher risk of unfavorable transplantation outcome, has become an increasing reality [1–3]. Among the most prominent characteristics distinguishing ECD from Standard Criteria Donors (SCD) are risk factors such as brain death, prolonged cold ischemic time, increased donor age, hypertension or diabetes. Altogether, these conditions could impact the intra-renal milieu towards a higher inflammatory burden [4], thereby compromising long-term graft function [3]. On the cellular level, age-related changes in donor organ composition have been shown to include functional programming of resident lymphocytes towards a more pro-inflammatory phenotype [5]. Furthermore, it has been meanwhile revealed in a number of studies that such donor



GRAPHICAL ABSTRACT

cells, being transferred to the organ recipient as "passenger leukocytes" [6], could impact transplantation outcomes in a celltype-specific manner [7–9].

Current experimental and clinical research therefore aims at therapeutically modulating grafts pre-transplantation to improve organ function. Principally, strategies focusing on systemic (deceased) donor pre-treatment or therapeutic targeting of the explanted graft have been proposed (summarized in [10, 11]). Amongst post-explantation procedures, we could demonstrate that perfusion of human kidneys with rabbit anti-human ATG results in improved short-term graft function [12]. Such peri-operative window of intervention is short by nature of the surgical procedure; furthermore, at low temperatures, therapeutics targeting biological processes might not unfold their full potential. Particularly the latter aspect also applies to machine perfusion as a novel framework for graft modification: although meanwhile a standard organ preservation method e.g., in the Netherlands [13], machine perfusion is routinely conducted hypothermically, thereby likely limiting drug metabolism.

On that background, pre-treatment of deceased, brain dead donors, being kept for hours near body temperature pre-explantation, might constitute an alternative option for graft modification. In clinical liver transplantation, methylprednisolone treatment not only reduced systemic inflammation associated with brain death in hepatic donors, but also ameliorated ischemia/reperfusion injury post-transplantation in concert with reduced acute rejection episodes [14]. In line with this, donor treatment with low dose dopamine improved renal function after transplantation in a large randomized controlled trial [10]. So far, anti-thymocyte globulin for pre-conditioning of the donor has been mainly studied in animal models of ischemia/reperfusion injury [15–17]. Its precise effects on leukocyte depletion across organs after donor pre-treatment and ensuing consequences for experimental kidney transplantation remained to be determined.

We therefore comprehensively studied quantitative changes within lymphoid and myeloid cell lineages in multiple murine lymphoid and non-lymphoid organs after intra-peritoneal anti-murine ATG administration. Furthermore, we analyzed its impact on cellular infiltration and renal function in a fully mismatched model of murine kidney transplantation.

MATERIALS AND METHODS

Animals

8–12-week-old male wildtype BALB/c, C57BL/6, and B6.SJL-Ptprca Pepcb/BoyJ (CD45.1$^+$ for donor/recipient discrimination) mice were purchased from Charles River Laboratories (Charles River, Cologne, Germany) and kept under standard laboratory animal conditions, receiving human care in compliance with the "Principles of Laboratory Animal Care" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH Publication No. 86–23, revised 1985). All animal experiments were approved by the Landesamt für Gesundheit und Soziales Berlin, Germany (G 0089/16).

ATG Treatment

Anti-mouse ATG was prepared by purifying the IgG fraction from serum of rabbits immunized with pooled thymocytes prepared from NOD, C3H/He, DBA/2, and C57BL/6 mice (Sanofi). ATG was administered to C57BL/6 mice intraperitoneally at 25 mg/kg bodyweight at day −2 and −1. Control animals received purified IgG from unimmunized rabbits. Animals were sacrificed on day 0 for direct cellular analysis or kidney procurement for transplantation.

Murine Kidney Transplantation

Allogeneic renal transplantations were performed as previously described [5, 18, 19]. Briefly, the left donor kidney was flushed with saline containing heparin (100 U/mL, Panpharma, Trittau, Germany) and procured. End-to-side anastomoses between the renal donor vessels and the recipient's abdominal aorta and inferior vena cava were performed following a knotless technique. For urinary tract reconstruction, the ureter was directly anastomosed into the bladder. The duration of cold and warm ischemia of allografts was maintained at 30 min, respectively. Animals were sacrificed on day 7 without receiving immunosuppression.

Assessment of Kidney Function

Serum samples were stored at −20°C until creatinine and urea were measured using the CREP2 Creatinine Plus version 2 and Urea/BUN assays, respectively, on a Roche/Hitachi Cobas C 701/702 system (Roche, Basel, Switzerland).

Isolation of Mononuclear Cells

For isolating renal mononuclear cells (MNCs), kidney tissue was mechanically dissociated and digested in RPMI medium (Corning, Manassas, VA, United States) supplemented with collagenases II and IV (Gibco/Invitrogen, Worthington) and DNase I (Roche Diagnostics) for 45 min at 37°C. Afterwards, leukocytes were enriched using CD45 Microbeads over MACS LS columns (both Miltenyi Biotec, Bergisch Gladbach, Germany). Mechanically dissociated lung tissue was digested with collagenase II and DNAse I; leukocytes were retrived after red blood cell lysis using ACK buffer. MNCs from spleen, lymph nodes and peripheral blood were isolated by density gradient centrifugation.

Flow Cytometric Analysis

Typically, 1×10^6 cells were surface stained with the respective antibodies listed in **Supplementary Figures S1, S3**. For intracellular staining, cells were fixed, permeabilized (FoxP3/transcription factor staining buffer set; Thermo Fisher, Darmstadt, Germany) and stained with the respective anti-transcription factor antibodies (**Supplementary Figure S3**). Data was acquired on a FACS Fortessa X20 (BD Biosciences, Heidelberg, Germany) and analyzed using FlowJo software 10 (BD Biosciences). A gating strategy for identification of lymphoid and myeloid cells is depicted in **Supplementary Figure S2**. As the predominant population, $CD11b^+F4/80^+$ macrophages were quantified in kidney, whereas $Ly6C^+MHC\text{-}II^-$ monocytes were analyzed in all other organs. Innate lymphoid cells were identified as illustrated in **Supplementary Figure S4**. For generation of t-Distributed Stochastic Neighbor Embedding (t-SNE) plots, data from samples of interest were concatenated, followed by t-SNE analysis in FlowJo. Graphical illustrations were designed with Biorender.

Statistics

Statistical analysis was performed using GraphPad Prism 8.4.3 (GraphPad, Boston, MA, United States). Distribution of values was assessed by Kolmogorov-Smirnov normality test. Depending on distribution, comparisons were conducted using either T- or Mann–Whitney U test. Statistical significance was considered for p values ≤ 0.05.

RESULTS

Treatment of the Naïve Organ Donor With Anti-Mouse ATG

First, we aimed to address how application of ATG to a potential solid organ donor impacts the lymphocyte composition in various organs. Naïve C57/BL6 mice were therefore treated with ATG before organ harvesting as summarized in **Figure 1A**. Control Ig treated mice of the same age and sex served as controls. For assessing all major lymphoid and myeloid cell subsets, mononuclear cells isolated from kidney, lung, spleen, lymph nodes and peripheral blood were surface stained using a FACS panel covering major cell lineage markers as depicted in **Supplementary Figure S1**, followed by acquisition on an BD Fortessa X20 flow cytometer.

To identify major differences induced by treatment, we first followed an unbiased analysis approach employing the t-SNE (t-Distributed Stochastic Neighbor Embedding) algorithm embedded in FlowJo. For that, FACS data derived from organs of controls and ATG treated animals were tagged according to sample type, followed by concatenation into one single data file. Thereafter, t-SNE analysis was conducted, allowing unbiased assessment of dominant differences. Overlay of t-SNE plots for each organ type consistently identified clusters present in controls, but absent in treated animals (**Figure 1B**, left column). For identification of cell types driving this different clustering, expression levels of all markers used for t-SNE were overlaid, pointing to a reduction of $CD4^+$ and $CD8^+$ T cells in all organs, as exemplarily depicted in **Figure 2B** (right columns).

We further analyzed all datasets after manual gating (depicted in **Supplementary Figure S2A**), enabling quantification and statistical examination of differences as illustrated in **Figure 2A**, where frequencies of the indicated cell types are presented as percentage of the total $CD45^+$ leukocyte population across all organs. Overall, lymphocytes were strongly diminished by ATG treatment, whereas this effect was not observed for myeloid cell subsets. In detail, $CD4^+$ and $CD8^+$ T cells were significantly reduced in lymphoid organs (lymph nodes and spleen) and almost completely depleted in blood, lung and kidney. With respect to the latter, raw data presented in **Supplementary Figure S2B** allows a more direct

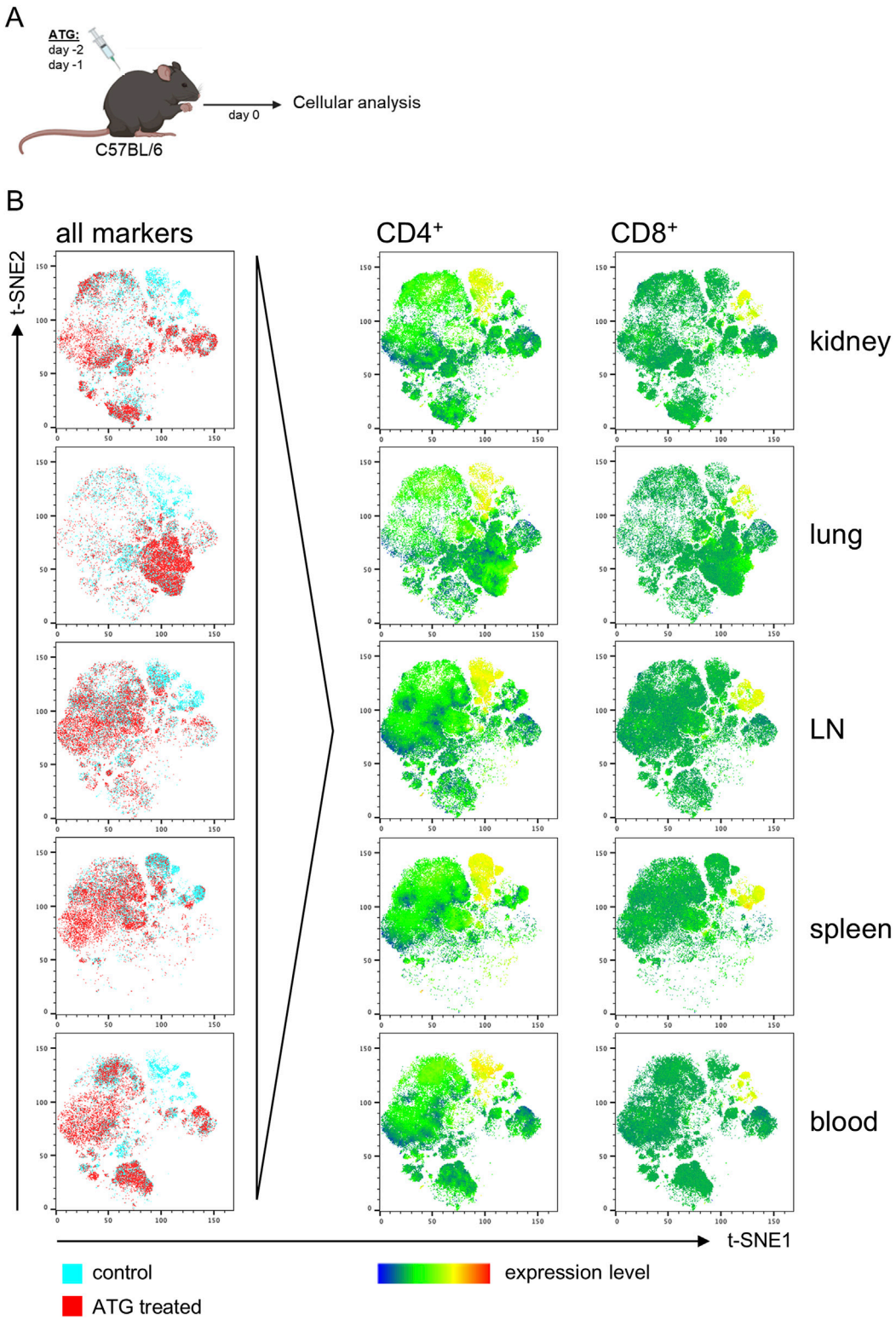


FIGURE 1 | ATG-induced T cell depletion across organs in naive mice. **(A)** Intra-peritoneal ATG treatment scheme of naïve C57BL/6 mice. **(B)** t-SNE plots of flow cytometric data illustrate separate clustering of cells from the indicated organs derived from control vs. ATG-treated animals. For t-SNE analysis, cells were pre-gated on live CD45$^+$ leukocytes; thereafter, datasets from n = 4–7 animals/group were concatenated. All remaining markers listed in **Supplementary Figure S1** were used for clustering. Data from control (blue) and ATG-treated (red) animals were overlaid to identify differences in cluster composition. For prominently different clusters, cellular identity was determined based on expression of the respective lineage markers, thereby e.g., identifying CD4$^+$ and CD8$^+$ T cell depletion in ATG-treated animals.

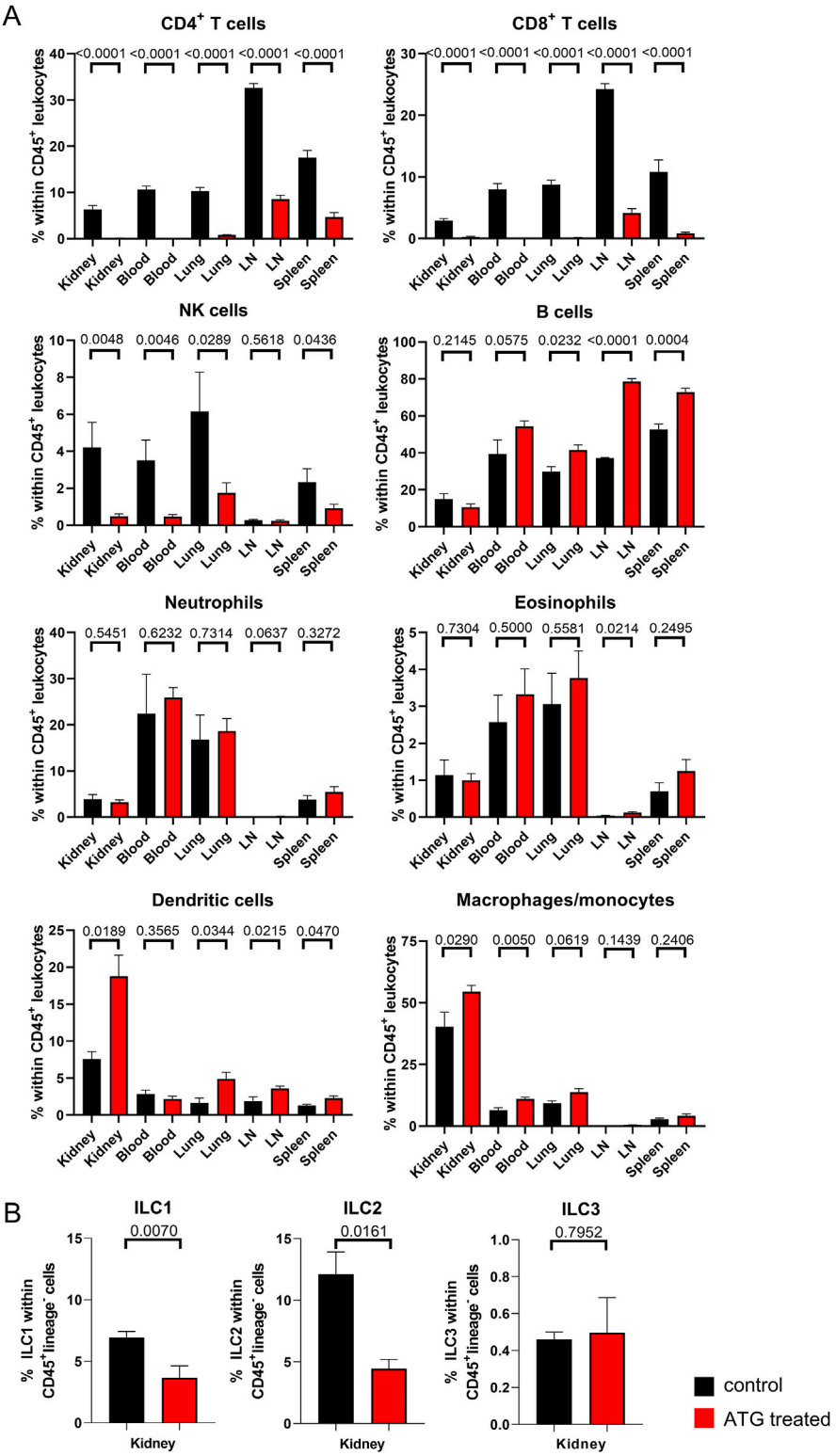


FIGURE 2 | Lymphocyte depletion in naive mice after ATG treatment. **(A)** The indicated leukocyte lineages were quantified by FACS in kidney, blood, lung, lymph node (LN) and spleen in control (con) and ATG-treated (ATG) C57BL/6 animals. **(B)** Renal composition of type 1, 2 and 3 innate lymphoid cells in control and ATG treated animals. In all experiments, data from both groups were examined for normal distribution. Differences were statistically analyzed by unpaired t- or Mann-Whitney-test, depending on distribution. Bar graphs show the respective means ± SEM. n = 4–8 animals/group, respectively.

estimation of renal T cell numbers remaining after ATG administration.

NK cells showed a similar pattern with the exception of lymph nodes where NK cells were already rare in controls. Interestingly, B cells, identified according to B220 expression, showed a relative increase within the $CD45^+$ leukocyte population in both lymphoid organs. Within the myeloid cell compartment, we observed only few changes following ATG injection. Of note, most prominent alterations were confined to the kidney and encompassed a moderate relative increase in frequencies of $CD11c^+MHC\text{-}II^+$ dendritic cells and $CD11b^+F4/80^+$ macrophages. In all other organs, $Ly6C^+MHC\text{-}II^-$ monocytes instead of macrophages were quantified as the dominating population that was only modestly, but significantly increased in blood of ATG treated animals.

Provided that lymphocytes represented the cell population most strongly affected by ATG treatment, we further aimed at analyzing innate lymphoid cells (ILCs), a multifaceted group of lymphocytes that does not express characteristic T-, B- or dendritic cell associated lineage markers [20]. Their transcriptional hallmarks broadly mirror the discrimination among T helper (Th) cell type 1, 2 and 17 subsets; accordingly, group 1 ILC, that include conventional NK cells, express T-bet, group 2 ILC are GATA-3^+, whereas group 3 members are RORγt$^+$. ILC type 2 cells have already been demonstrated to protect murine kidneys from glomerulosclerosis [21] and renal ischemia reperfusion injury [22]. Given the potential importance of ILCs in transplantation, we therefore particularly focused on the kidney for ILC analysis. The flow cytometric marker panel for ILC subset identification is depicted in **Supplementary Figure S3** with the gating strategy being summarized in **Supplementary Figure S4**. Of note, ILC frequencies were calculated within $CD45^+lineage^-$ cells; therefore, they could not directly be compared to the leukocyte percentages depicted in **Figure 2A**. Following ATG treatment, we detected a significant reduction in ILC type 1 and 2 subsets compared to controls, whereas portions of the ILC3 subpopulation were not affected (**Figure 2B**).

As a summary, all major lymphocyte subpopulations with the exception of B cells and ILC3 were significantly diminished as a consequence of ATG treatment in both non-lymphoid (kidney, lung) and lymphoid (lymph node, spleen) organs as well as in peripheral blood.

Impact of Donor ATG Pre-Treatment on Allogeneic Kidney Transplantation Outcome

The persistence of donor-derived, intra-graft leukocytes after transplantation has been already shown to impact transplant survival and -function in clinical studies as well as in experimental models [8, 23]. We therefore addressed whether depletion of donor cells by ATG affects allogenic kidney transplantation outcome both on the cellular and functional level. As depicted in **Figure 3A**, kidneys from control or treated C57BL/6 mice were transplanted into BALB/c mice, representing a fully MHC-mismatched donor:recipient combination. Cellular and functional readout was performed on day 7, representing a typical timepoint in this acute rejection model, as recently published by us [5].

In an identical approach as for the analysis of ATG-induced alterations in naïve mice, we employed t-SNE for unbiased identification of pre-treatment effects on kidney transplantation outcome. For that, MNCs from the kidney graft were surface stained as depicted in **Supplementary Figure S1** and data was acquired by FACS. Overlay of t-SNE plots indicated only few dominant differences in cellular composition (**Figure 3B**, left column). For identification of cell types driving slightly different clustering, expression levels of candidate markers were overlaid, pointing to a moderate reduction of graft $CD4^+$ T and NK cells and an increase in neutrophils (**Figure 3B**, right columns).

We further analyzed all datasets after manual gating (**Supplementary Figure S2**), enabling quantification and statistical examination of differences as illustrated in **Figure 4A**. Of note, we did not detect additional intra-graft differences than those identified by t-SNE that did only reach statistical significance for neutrophils and a trend for $CD4^+$ T cells. Analysis of MNCs from other organs showed a slight, but significant decrease in lung B cells in case of ATG donor-pre-treatment, whereas neutrophils were increased in spleen and showed a trend in lung.

Based on the finding that donor ATG-pretreatment resulted in moderately diminished frequencies of intra-graft $CD4^+$ T cells, we conducted subanalyses, assessing quantities of effector-memory (T_{em}) and central-memory- (T_{cm}) type T cells identified according to CD44 and CD62L expression (**Figure 4B**). Interestingly, we noted a trend towards reduced portions of intra-graft $CD4^+$ T_{cm} after ATG pre-treatment, whereas T_{em} remained unchanged (**Figure 4C**). The same applied to the $CD8^+$ T cell compartment where the drop in T_{cm} frequencies reached significance (**Figure 4D**).

Importantly, as already demonstrated earlier using mice expressing the congenic markers CD45.1 and CD45.2 for donor/recipient discrimination [5], $CD45^+$ donor leukocytes were gradually depleted in untreated animals until day 7 in our fully mismatched transplantation model, highlighting that more than 99% of analyzed cells at this timepoint were recipient-derived (**Supplementary Figure S5A**). Subset analysis revealed that a considerable number of renal TCRβ$^+$ donor T cells is still detectable at day 3 post transplantation (**Supplementary Figure S5B**).

For deciphering the consequences of donor ATG-pretreatment on renal function, creatinine and urea levels were determined in serum on day 7 after transplantation. Importantly, we did not detect significant differences between graft recipients of control or ATG pre-treated donor organs (**Figure 5**).

In summary, ATG pre-treatment of the organ donor did only result in a minor cellular re-composition of the allograft in our transplantation model. In line with the aforementioned, we did not note relevant changes in graft function in association with this type of therapeutical intervention.

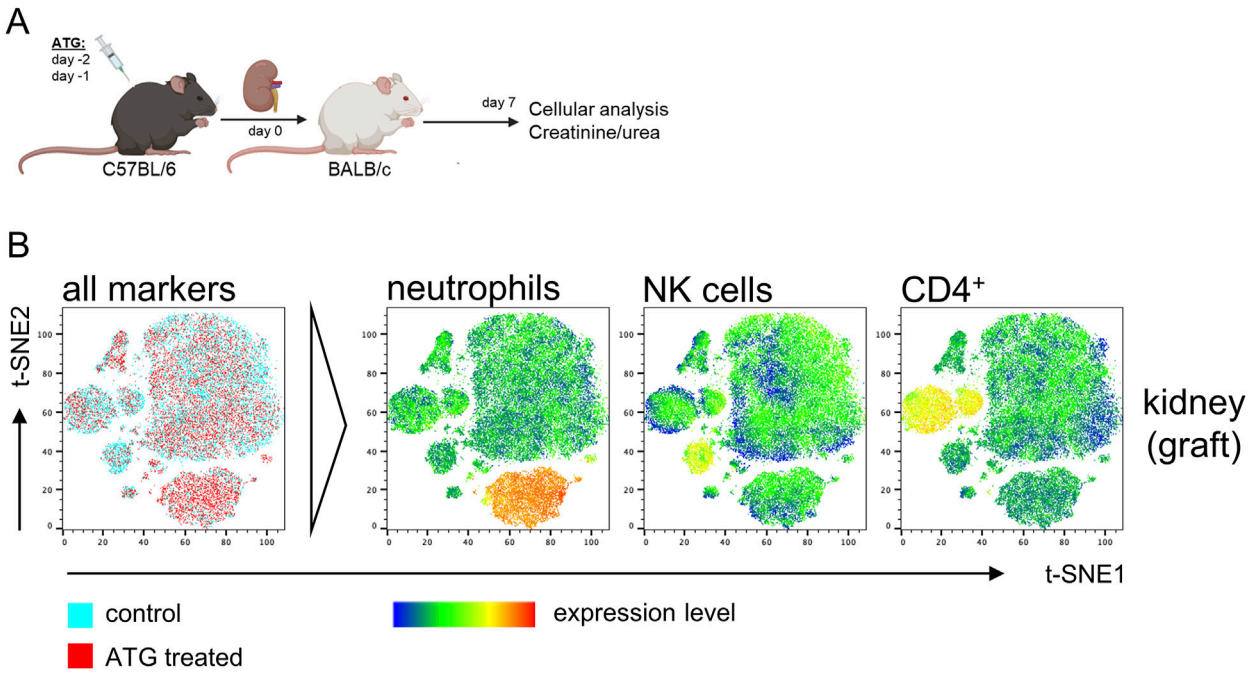


FIGURE 3 | Impact of ATG donor-pretreatment on leukocyte composition in allografts after experimental kidney transplantation. **(A)** Experimental design and strain combination for murine kidney transplantation in context with intraperitoneal ATG pretreatment of the donor. **(B)** Exemplary t-SNE plots of flow cytometric data derived from kidney graft samples using the antibody panel shown in **Supplementary Figure S1**. For t-SNE analysis, renal cells were pre-gated on live CD45$^+$ leukocytes; thereafter, datasets from n = 4–7 animals/group were concatenated. All remaining markers listed in **Supplementary Figure S1** were used for clustering. Data from control (blue) and ATG-treated (red) animals were overlaid to identify differences in cluster composition. For prominently different clusters, cellular identity was determined based on expression levels of the respective lineage markers, thereby e.g., identifying increased neutrophil and decreased CD4$^+$ T cell frequencies in kidneys from ATG-treated animals.

DISCUSSION

In this study, we experimentally followed the hypothesis that pre-treatment of a potential murine renal organ donor with ATG leads to changes in the immunological composition of the kidney, thereby potentially impacting the recipient´s allogenic immune response once the modified organ is transplanted. Such approach might be of particular interest in a setting where inferior long-term transplantation outcomes are to be expected, e.g., due to marginal organ usage from donors with advanced age or certain comorbidities (summarized in [10, 11]).

So far, it remained largely obscure how systemic administration of ATG particularly affects tissue-residing leukocytes in solid organs that might critically impact transplantation outcomes [24]. We chose a comparably high ATG dose of 25 mg/kg that had been previously demonstrated to reduce e.g., splenic T cells in naïve mice by up to 95% [25, 26]. Of note, a similar dosing (30 mg/kg) is clinically applied for immune ablation in severe autoimmunity before autologous stem cell transplantation [27]. For prevention of organ rejection in clinical kidney transplantation, our dosage exceeds commonly applied drug levels (typically 1.5 mg/kg Thymoglobuline® [28]) where immunosuppression in the recipient has to be balanced with preservation of protective immunity. The latter considerations, however, do not apply in our organ donor-centered approach aiming at robust leukocyte depletion.

In our hands, as presented here, i.p. ATG treatment of naïve mice resulted in a strong and significant depletion of T-, NK- and innate lymphoid cells in both lymphoid and non-lymphoid organs, including the kidney. Of note, in accordance with *in vitro* data on anti-mouse ATG binding affinities to potential murine target cell populations [25], treatment spared B cells and all myeloid subsets. Particularly the latter aspect is of interest in the transplantation context, provided that donor-derived myeloid cells could contribute to allo-immunity by different mechanisms. These might not only involve direct presentation of donor antigens to recipient T cells (summarized in [29]), but also include the recently discovered priming of recipient by donor myeloid cells via CD47/SIRPalpha-polymorphisms [30]. Although these mechanisms argue in favor of myeloid cell depletion strategies in the donor, pointing to a potential limitation of our approach, their relevance needs to be critically examined within the time frame of the renal persistence of donor leukocytes, which we determined to fade until day 7 post transplantation in our model.

Despite efficient depletion of donor lymphocytes, we did not observe a pronouncedly altered cellular infiltration into the kidney after transplantation, nor an improvement of graft function.

In conclusion, the broad donor lymphocyte depletion approach chosen herein obviously does not substantially impact the course of alloimmune inflammation in the acute

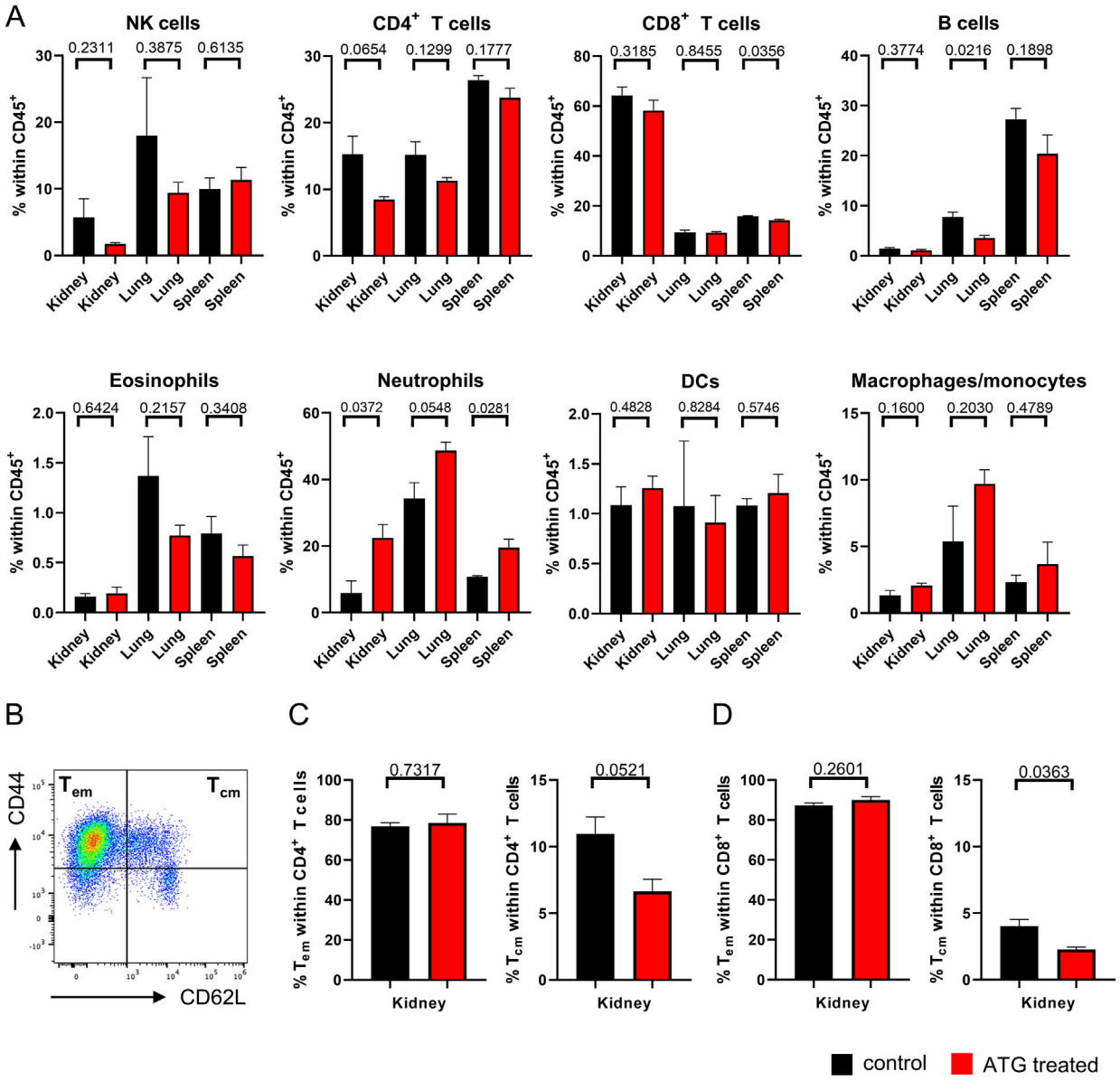


FIGURE 4 | Organ-specific leukocyte composition after kidney transplantation. **(A)** The indicated leukocyte lineages were quantified by FACS in kidney, lung and spleen in mice receiving allogenic kidney grafts from either control or ATG-pretreated donors. **(B)** Definition of T_{em} and T_{cm} subsets based on the indicated marker combinations. Subset composition in renal **(C)** $CD4^+$ and **(D)** $CD8^+$ T cells from mice receiving allogenic kidney grafts of either control and ATG-pretreated donors. In all experiments, data from both groups were examined for normal distribution. Differences were statistically analyzed by unpaired t-oder Mann-Whitney-test, depending on distribution. Bar graphs show the respective means ± SEM. n = 4–7 animals/group.

transplantation model. One explanation might be linked to our finding that donor-derived leukocytes are anyway rapidly depleted within 7 days after transplantation in such complete MHC mismatch setting [5] in the absence of immunosuppressive therapy. Therefore, an impact of so called "passenger leukocytes" might be little, given the specific framework of our experimental model.

The idea that donor-derived passenger leukocytes, being transferred to the transplant recipient, in turn influence anti-donor immunity dates back to the 1950ies [6]. In the meantime, several pre-clinical and clinical observations support a concept that certain transplant derived immune cell populations persist in the recipient and might, depending on the type of cells, contribute to desired or undesired outcomes. In that context, passenger donor $CD4^+$ T cells have been shown to augment early antibody-mediated allo-immunity in a murine heart transplantation model by activating recipient B cells [8, 31]. Since this mechanism boosted allograft vasculopathy and early graft failure, it was concluded that "...passenger donor lymphocytes may therefore ... represent a therapeutic target in solid organ

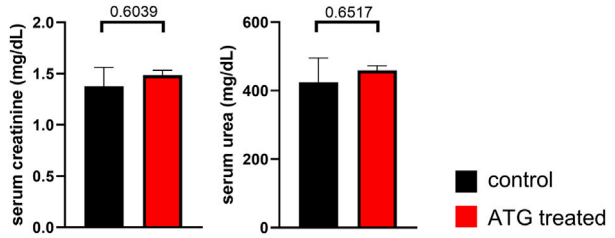


FIGURE 5 | Impact of ATG treatment on allograft function. Serum creatinine and urea levels were determined on day 7 after allogenic kidney transplantation in mice receiving grafts of either control or ATG-pretreated donors. Data from both groups were examined for normal distribution. Differences were statistically analyzed by unpaired t-oder Mann-Whitney-test, depending on distribution. Bar graphs show the respective means ± SEM. n = 4–7 animals/group.

transplantation" [8]. Importantly, the report elegantly revealed at the same time that the impact of donor lymphocytes on transplantation outcome is critically dependent on the degree of donor:recipient mismatch; in a fully mismatched heart model, recipients' NK cells are rapidly depleting donor T cells, thereby preventing an effect on alloimmunity [8]. Whether and how such mechanism of donor T cell help to recipient B cell activation might contribute to donor specific antibody (DSA) production in the murine kidney transplantation model is a complex question that is out of the scope of our small study. At least with respect to donor T cell persistence in the first days after transplantation (according to our data, at least until day 3), we cannot principally exclude it, also considering that we isolated comparable numbers of donor T cells from kidney grafts as those recovered from hearts in the report by Charmetant [31]. Furthermore, DSA have been detected in rat and murine kidney transplantation models from day 5–7 post transplantation, supporting a critical role for very early T:B cell interactions [32, 33].

Conversely, it cannot be excluded that certain T cell subsets being transferred with the graft might serve desired (that is, anti-inflammatory) functions. In this regard, cardiac graft survival was found to be positively associated with the presence of natural $CD4^+$ regulatory T cells, provided that their depletion pre-transplantation augmented alloimmunity [9]. Similar associations were demonstrated in clinical and murine lung transplantation, where tissue-resident Tregs control humoral rejection [7]. In humans, a subset of donor-derived T lymphocytes, termed tissue-resident memory T cells (T_{rm}), being present in transplanted lungs, has recently been found associated with beneficial clinical outcomes. The fact that these cells were predominantly and long-term detectable in bronchoalveolar fluid, but not in peripheral blood, underscores the need for analyzing alloimmunity directly in target tissues [23].

In conclusion, the aforementioned reports point to a main limitation of our approach in that depletion of donor leukocytes pre-transplantation might be a double-edged sword and that a more celltype-selective strategy, e.g., by sparing graft-resident, donor-derived regulatory or resident memory-type T cells, should be envisioned. This might also be accomplished by titrating ATG dosage, provided that reduced drug levels could have slightly different effects on T cell subsets, as demonstrated in human kidney transplant recipients [34]. Major limitations also include that we did not particularly assess alloimmunity after transplantation; it cannot be excluded that subtile ATG treatment effects are only detectable at the antigen-specific level, suggesting that future studies should include examination of allo-specific cellular and/or humoral responses. Indeed, with respect to the latter, the report by Charmetant et al. [31] highlighted that a few thousand donor-derived T helper cells might suffice to prime donor-specific antibody production in the heart model.

Furthermore, the translational potential of our findings might be limited due to differences between targets of anti-murine ATG and the respective human drug(s): Whereas we did not document altered B cell frequencies in naïve mice after ATG treatment, anti-human Thymoglobuline (Sanofi) has been demonstrated to deplete $CD19^+$ B cells in a humanized mouse model [35]. The fact that this observation could not be reproduced after ATG administration to kidney transplant recipients [36] highlights that the study design should be critically scrutinized in view of its clinical transferability. In that context, human kidney transplantation usually involves selection of the donor organ according to MHC-matching and requires life-long administration of immunosuppressive drugs. Both aspects likely shape maintainance and functionality of passenger leukocytes in the clinical setting. The future challenge will be to better mirror these features in an adapted experimental model that will also consider the impact of passenger leukocytes in the chronic phase of anti-donor immunity.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The animal study was approved by the Landesamt für Gesundheit und Soziales Berlin, Germany (G 0089/16). The study was conducted in accordance with the local legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

AH and YY designed the study, conducted experiments and analyzed data. KK designed the study and reviewed the manuscript. AS supervised experiments, analyzed data, designed and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

GENERATIVE AI STATEMENT

The author(s) declare that no Generative AI was used in the creation of this manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2024.13997/full#supplementary-material

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Discrimination of Anti-Donor Response in Allogeneic Transplantation Using an Alloreactive T-Cell Detection Assay

Ryosuke Arata, Naoki Tanimine, Akhmet Seidakhmetov, Kentaro Ide, Yuka Tanaka and Hideki Ohdan*

Department of Gastroenterological and Transplant Surgery, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan

Understanding donor-reactive T-cell behavior post-transplantation is challenging owing to the rarity and diversity of these cells. Here, we aimed to evaluate the relevance of an assay for rapidly detecting alloreactive T cells in a mouse transplantation model. After 18 h of one-way mixed lymphocyte reaction (MLR) culture with pre-activated donor-derived stimulators, $CD4^+$ and $CD8^+$ donor-reactive T cells were identified by CD154 and CD137 expression, respectively. Using full MHC mismatched mouse skin transplant models, we observed an increased donor-reactive T-cell proportion by direct presentation with elevated interferon gamma and granzyme B production 7 days post-transplantation, before graft rejection. Immunosuppression with CTLA-4 IgG and anti-CD154 antibody varied depending on donor-recipient strain combinations. On day 7, donor-reactive $CD8^+$ T-cell proportions were lower in the tolerance model (BALB/c to C3H/HeJ) than in the rejection model (BALB/c to C57BL/6); conventional proliferation readout after 4 days of MLR could not distinguish these responses. Overall, although the conventional readout for evaluating T-cell proliferation following an MLR quantifies the precursor frequency of alloreactive T cells, the assay reported herein assesses T-cell activation markers after a short-term MLR to characterize immediate immune status. These findings offer a promising tool to elucidate immune responses post-transplantation.

Keywords: immune monitoring, allogeneic transplantation, alloreactive T-cell, rejection, tolerance

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Correspondence
Naoki Tanimine,
tanimine@hiroshima-u.ac.jp

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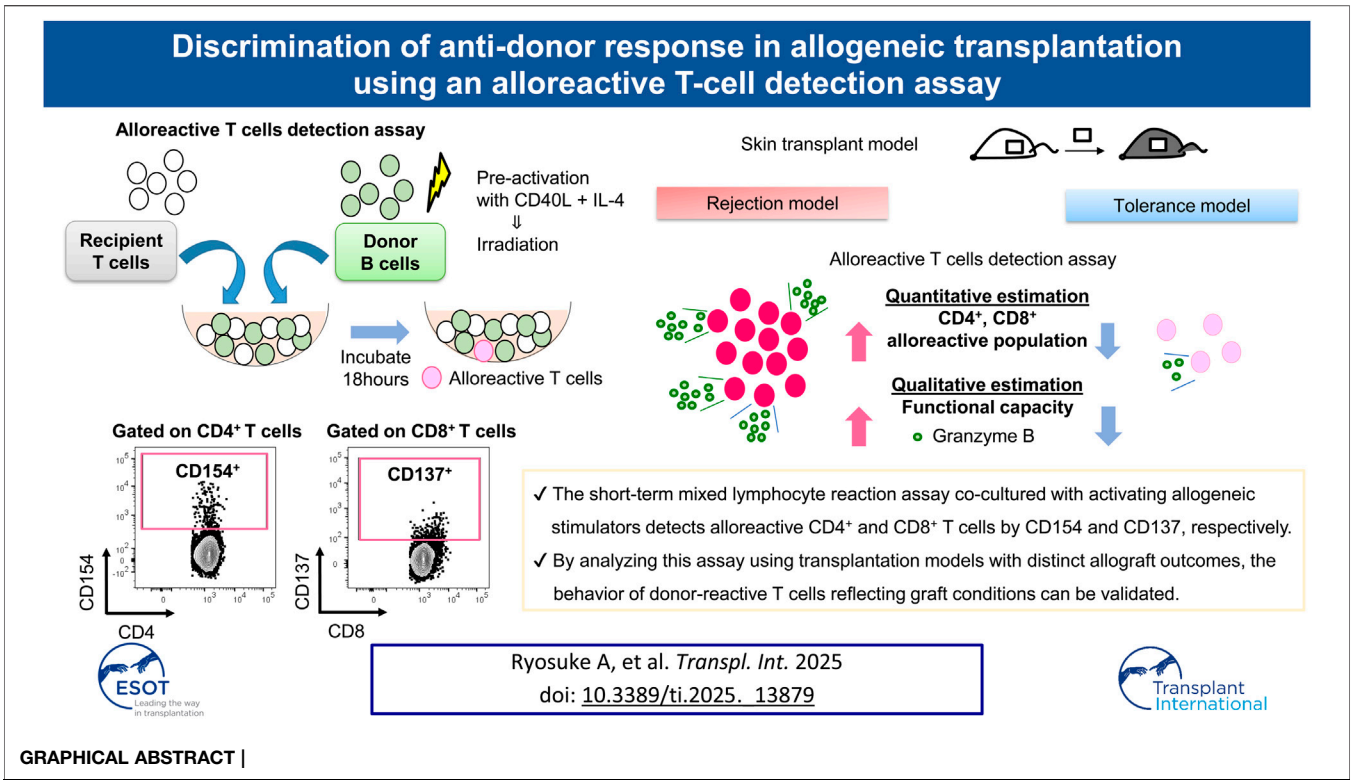
INTRODUCTION

T cells play pivotal roles in orchestrating immune responses after solid organ transplantation [1]. Through their unique T-cell receptors (TCRs), these cells recognize antigens presented on the peptide-major histocompatibility complex (pMHC) on antigen-presenting cells (APCs) [2]. After transplantation, alloreactive T cells can enhance and mediate immune responses, resulting in organ damage and memory formation [3]. Donor-reactive T cells, which are quantitatively rare, reflect the anti-donor immune status,

Abbreviations: APC, antigen-presenting cell; cATD, comprehensive alloreactive T-cell detection; CFSE, carboxy fluorescein succinimidyl ester; GZMB, granzyme B; IFN-γ, interferon gamma; mAb, monoclonal antibody; MHC, major histocompatibility complex; MLR, mixed lymphocyte reaction; TCR, T-cell receptor.



GRAPHICAL ABSTRACT |

which may elucidate the hidden mechanisms underlying complex interactions in T-cell activation and regulation during the immune response [4]. Next-generation sequencing is a robust tool for comprehensive and high-throughput TCR profiling and facilitates the determination of the entire T-cell repertoire profile and tracing of antigen-specific T cells [5]. Although the MHC multimer is also an excellent marker for detecting antigen-specific T-cell clones in the total pool [6], it is challenging to identify alloreactive T cells in the clinical context owing to alloantigen diversity and variability [7].

Mixed lymphocyte reaction (MLR) is a classical and reliable method for estimating T-cell response in allogeneic transplantation and is useful for detecting clones against heterogenous allo-antigens. Previously, a novel comprehensive alloreactive T-cell detection (cATD) assay was developed using the MLR platform with activating markers (CD137 and CD154) [8]. In the present study, we aimed to investigate the relevance of alloreactive T cells via a direct pathway detected using this assay in a transplantation model. Specifically, we monitored alloreactive T cells in a mouse skin transplant model to clarify the importance of detected alloreactive T cells for rejection. In addition, we investigated whether this method could be useful to estimate the immune tolerance status.

MATERIALS AND METHODS

Flow Cytometry

The following antibodies were used: anti-AF700-CD8a (53-6.7), anti-APC-CD154 (MR1), anti-APCCy7-CD8a (53-6.7), anti-PE-CD137 (17B5), anti-PE-CD4 (GK1.5), anti-PerCPcy5.5-CD3 (17A2), anti-BV421-CD62L (MEL-14), anti-BV421-granzyme B (GZMB; QA18A28), anti-BV605-CD4 (RM4-5), anti-BV711-CD44 (IM7), and anti-BV711-interferon gamma (IFN-γ; XMG1.2), purchased from BioLegend (San Diego, CA, United States). Anti-APCCy7-CD19 (1D3) and anti-PE Cy7-FoxP3 (FJK-16s) were purchased from BD Biosciences (San Jose, CA, United States). Nonspecific FcγR binding of labeled monoclonal antibodies (mAbs) was blocked using anti-mouse CD16/32 (2.4G2; BD Pharmingen, Hamburg, Germany). Dead cells were excluded from analysis using the forward Zombie Aqua Fixable Viability Kit (BioLegend), the Zombie NIR Fixable Viability Kit (BioLegend), or 7-aminoactinomycin D (7-AAD; BD Biosciences) staining. For intracellular staining, cells were fixed and permeabilized using the FoxP3/Transcription Factor Staining Buffer Set (BD Biosciences), according to the manufacturer's instructions. To assess cytokine production, the cells were stimulated using monensin (BD Biosciences) in a culture medium at 37°C in a 5% CO_2 incubator for 4 h prior to staining. The data were collected using LSRFortessa X-20, FACS Canto II, or FACS Celesta (BD Biosciences) and were analyzed using FlowJo v. 10 (Tree Star, Ashland, OR, United States).

Mice

C57BL/6 (H-2Db), BALB/c (H-2Dd), and C3H/HeJ (H-2Dk) mice were purchased from CLEA (Osaka, Japan) and maintained in a pathogen-free animal facility of Hiroshima University, Hiroshima, Japan. Female mouse were used at an age of 10–12 weeks. When indicated, the mice were euthanized through cervical dislocation after isoflurane inhalation. All efforts were made to minimize animal suffering [9]. This study was

performed in strict accordance with the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health. All mice received humane care in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of the Graduate School of Biomedical Sciences, Hiroshima University (Permit Number: A23-17). A part of this work was performed at the Research Facilities for Laboratory Animal Science, Natural Science Center for Basic Research and Development (N-BARD), Hiroshima University.

Skin Transplantation

Full-thickness skin grafts were transplanted onto the left lateral dorsum of a recipient. Briefly, donor skin tissues were removed from the tails and trimmed into 10 mm × 10 mm strips. Recipient mice were anesthetized using intraperitoneal injection of xylazine (5 mg/kg body weight) and ketamine (100 mg/kg body weight). Skin tissues of the same size were removed from the recipients' backs and replaced with donor grafts. The skin grafts were covered with bandages for 5 days, and graft survival was evaluated through daily visual inspection. Rejection was defined as destruction of >95% of the skin transplant [10]. An MHC full-mismatch BALB/c into C57BL/6 combination was employed as a rejection model. A BALB/c into C57BL/6 or C3H/HeJ combination previously reported as a tolerance induction model treated with CTLA-4 IgG (abatacept, 200 μg; Bristol-Myers Squibb, Braine-l'Alleud, Belgium) on days 0, 2, 4, and 6, and anti-CD154 antibody (MR1, 250 μg; BioLegend, San Diego, CA, United States) on days 0, 2, and 4 [11] was used for monitoring peripheral tolerance induction.

cATD Assay

We prepared mononuclear cell suspensions of BALB/c mouse spleens and purified the B cells via positive selection using CD19 MicroBeads (Miltenyi Biotec, San Diego, CA, United States) in an autoMACS Pro Separator (Miltenyi Biotec), according to the manufacturer's instructions [9]. The purity of the sorted cells was consistently >95%. Using a cocktail of recombinant mouse CD40L multimer (100 ng/mL; AdipoGen, San Diego, CA, United States) and recombinant mouse IL-4 (10 ng/mL; R&D Systems, Minneapolis, MN, United States), activated B cells were generated by culturing 0.2×10^6 cells/mL at 37°C under 5% CO_2 for 24 h. All cell cultures were performed in complete medium [RPMI 1640 medium (Nacalai Tesque, Kyoto, Japan) supplemented with 5% fetal bovine serum (SERANA, Pessin, Germany), 100 mM sodium pyruvate (Thermo Fisher Scientific, Waltham, MA), 100 U/mL penicillin–streptomycin (Thermo Fisher Scientific), 1% HEPES buffer (Thermo Fisher Scientific), and 50 μM 2-ME] in a 48-well flat-bottom plate. Using activated B cells as stimulators, MLR culture was performed, after which alloreactive T cells were identified. Prior to culturing, the stimulators were irradiated with 40 Gy. Responder T cells were purified from recipient splenocytes via negative selection, using a Pan T-Cell isolation kit (Miltenyi Biotec) in the autoMACS Pro Separator (Miltenyi Biotec), according to the manufacturer's instructions. The purity of the sorted cells was consistently >95%. Responders and stimulators were co-cultured at a 1:1 ratio (10^6 cells each) in 96-well U-bottom plates, with 200 μL complete medium containing APC-conjugated anti-CD154-labeled mAbs (MR1, 1 μL; BioLegend) for 18 h. Protein transport inhibitor (monensin, 2 μL; BD Biosciences) was added to the culture medium for the last 4 h of incubation. Alloreactive $CD4^+$ and $CD8^+$ T cells were identified as $CD3^+CD4^+CD154^+$ and $CD3^+CD8^+CD137^+$ responders, respectively. We collected at least 100,000 counts during flow cytometry acquisition for detecting 0.1% population to keep the coefficient of validation up to 10%.

Proliferation Assay

Recipient splenocytes were labeled with 5 μM carboxy fluorescein succinimidyl ester (CFSE; Molecular Probes) for 5 min prior to culturing. The activated B-cell stimulators were prepared as described in *cATD Assay*. Responders and stimulators were co-cultured at a 1:1 ratio (2×10^5 cells each) for 4 days in 96-well U-bottom plates with 200 μL medium. Attenuation of CFSE fluorescence intensity was evaluated as proliferating activity gated on $CD4^+$ and $CD8^+$ T cells. Mitotic index (MI) was calculated as previously described [12, 13].

Statistical Analysis

Statistical analyses were performed using JMP 16 (SAS Institute, Cary, NC, United States). Chi-square or Fisher's exact test was used to compare categorical variables, and Student's *t*-test or Mann–Whitney U-test was used for continuous variables. Comparisons between groups were made using the one-way analysis of variance (ANOVA), and significant differences were examined using Tukey–Kramer's multiple-comparison post-hoc test. Differences with $p < 0.05$ were considered statistically significant.

RESULTS

cATD Assay Detected Sensitization Leading to Acute Rejection in the Mouse Skin Transplantation Model

Skin allografts were rejected from 7 to 15 days in the full MHC mismatched rejection model (BALB/c into C57BL/6) (MST 11 days, **Supplementary Figure S1**). We did not observe a sensitized reaction in peripheral $CD4^+$ and $CD8^+$ T cells at 3 days after transplantation, as determined using a proliferation assay (syngeneic vs. rejection model, median MI; $CD4^+$ 0.18 vs. 0.08, $p = 0.53$ (upper) and $CD8^+$ 0.20 vs. 0.10, $p = 0.80$ (lower), **Figure 1**). Seven days after transplantation, we observed a higher proliferation response of both $CD4^+/CD8^+$ T cells in the rejection model than in the syngeneic model (median MI; $CD4^+$ 0.18 vs. 0.46, $p < 0.05$ (upper) and $CD8^+$ 0.57 vs. 1.28, $p < 0.05$ (lower), **Figure 1**). The cATD assay revealed a sensitized immune response after skin transplantation at the same time point as the proliferation assay, showing a higher proportion of donor-reactive

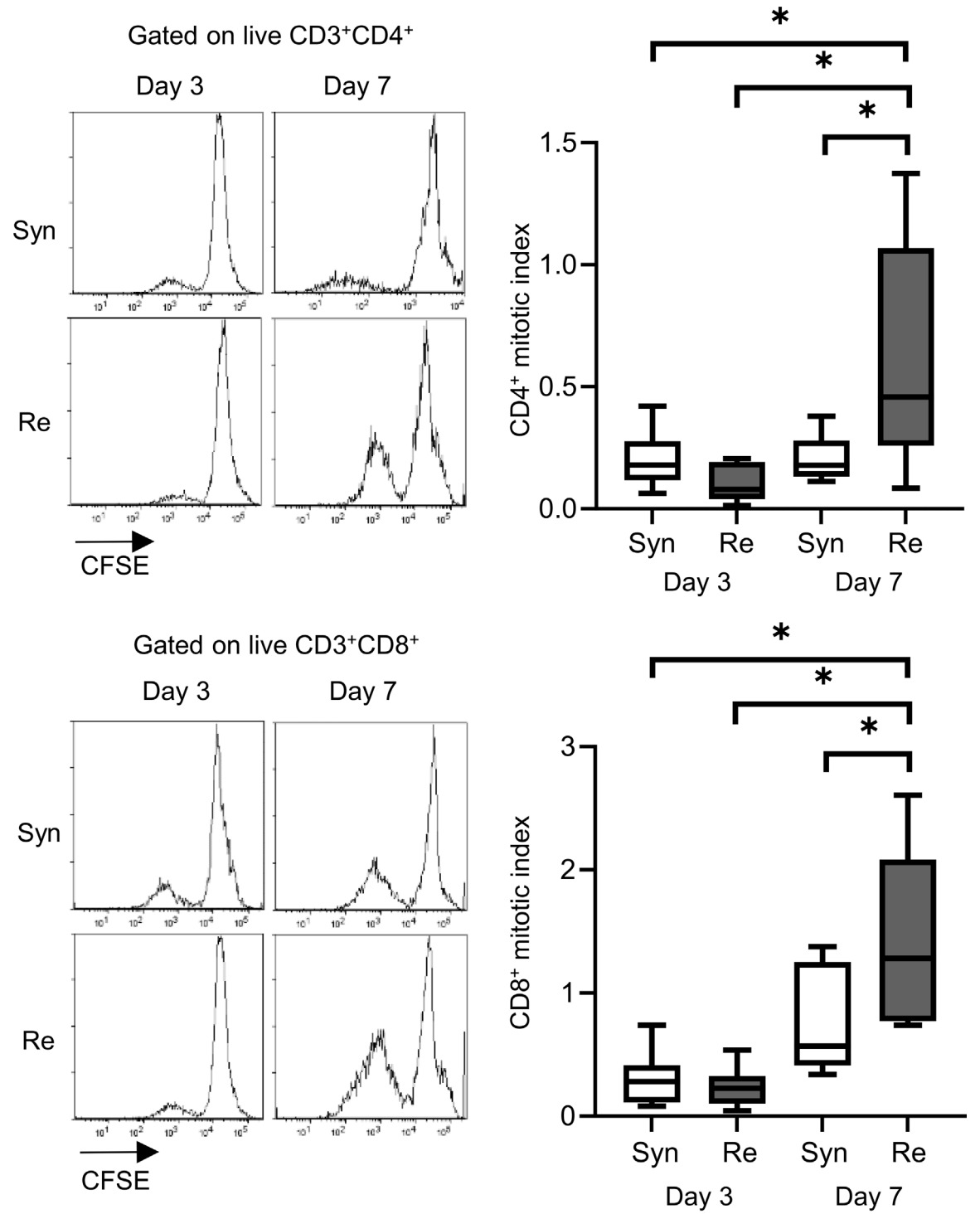


FIGURE 1 | Proliferation assay after mouse skin transplantation. The representative flow plots and box-and-whisker plots of the mitotic index show the proliferation capacity of $CD4^+$ (upper) and $CD8^+$ (lower) T cells from recipients of the syngeneic model (Syn, C57BL/6 into C57BL/6) and rejection model (Re, BALB/c into C57BL/6) at 3 and 7 days after transplantation. *$p < 0.05$. The data were generated from four independent experiments (n = 6). One-way ANOVA and Tukey's multiple-comparison test were employed for statistical analysis.

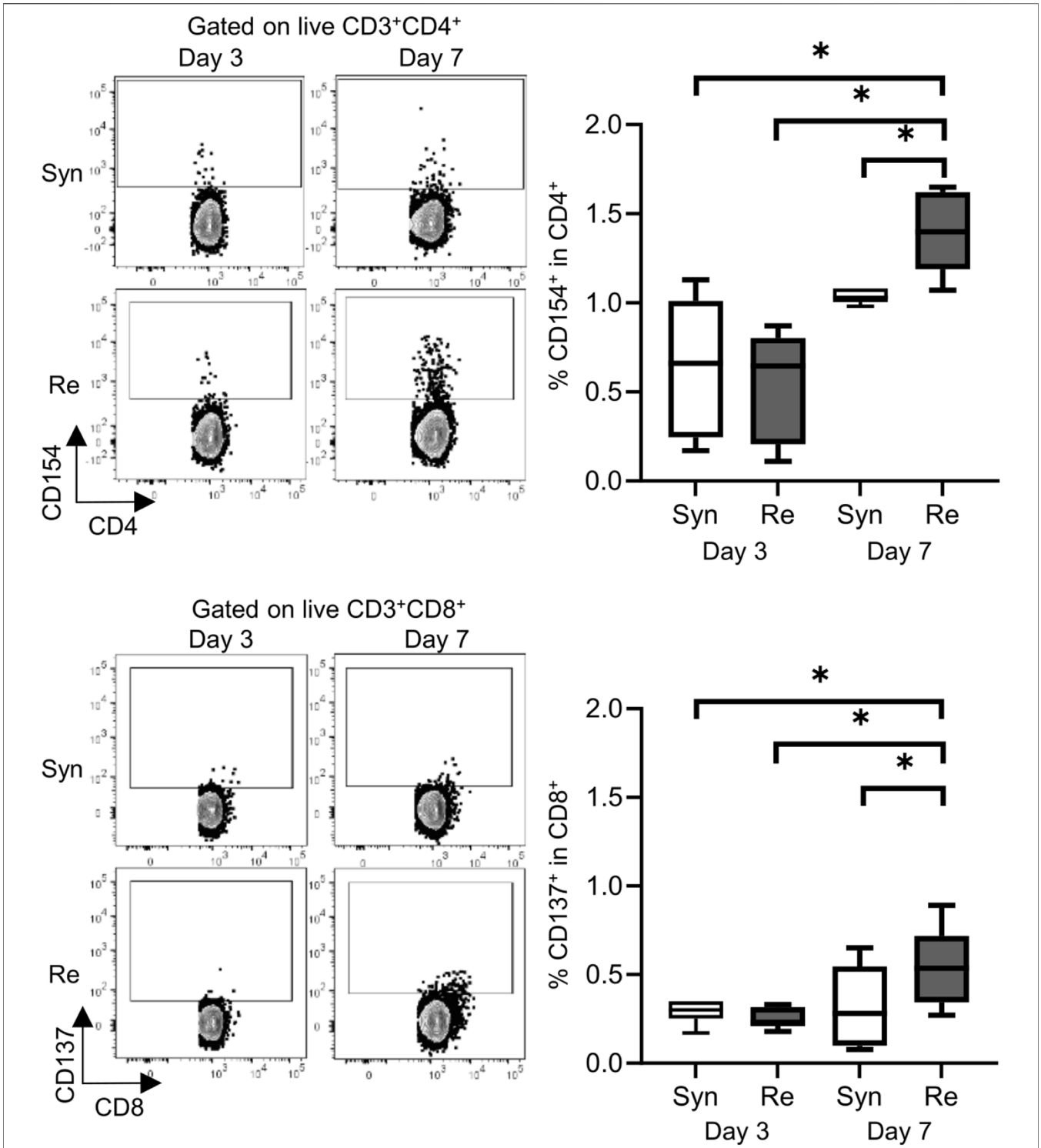


FIGURE 2 | Alloreactive T-cell detection assay revealed donor-reactive T cells in the mouse skin transplantation model. The representative flow plots show the alloreactive population defined by $CD154^+$ in $CD4^+$ T cells (upper) and $CD137^+$ in $CD8^+$ T cells (lower) from recipients of the syngeneic model (Syn, C57BL/6 into C57BL/6) and rejection model (Re, BALB/c into C57BL/6). The box-and-whisker plots show the proportion of donor-reactive T cells at 3 and 7 days after transplantation. *$p < 0.05$. The data were generated from four independent experiments (n = 6). One-way ANOVA and Tukey's multiple-comparison test were employed for statistical analysis.

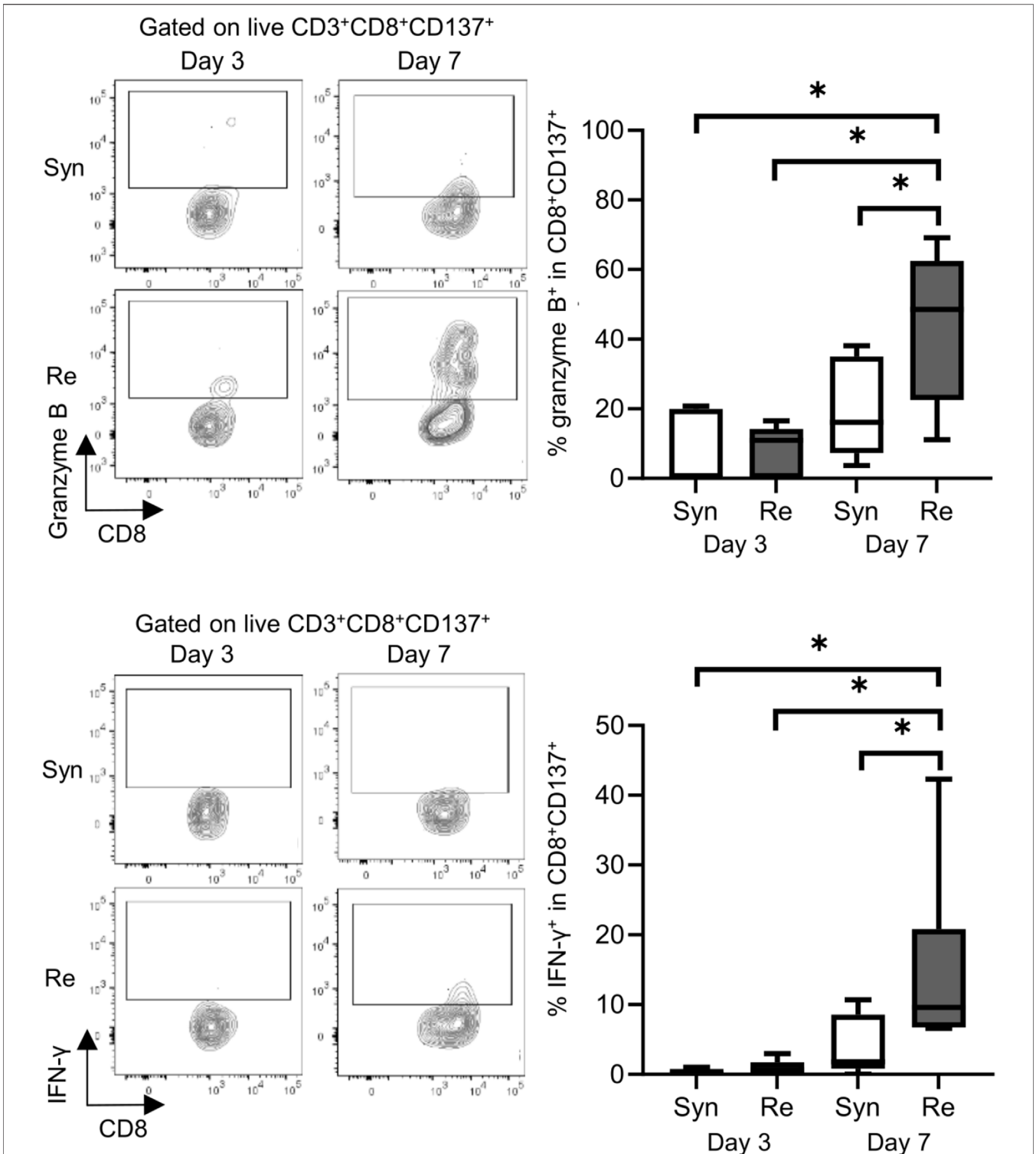


FIGURE 3 | Functional analysis of donor-reactive T cells in the mouse skin transplant model. The representative flow plots show the expression of granzyme B (upper) and interferon gamma (IFN-γ) (lower) in CD137$^+$ donor-reactive CD8$^+$ T cells from recipients of the syngeneic model (Syn, C57BL/6 into C57BL/6) and rejection model (Re, BALB/c into C57BL/6). *$p < 0.05$. The data were generated from four independent experiments (n = 6). One-way ANOVA and Tukey's multiple-comparison test were employed for statistical analysis.

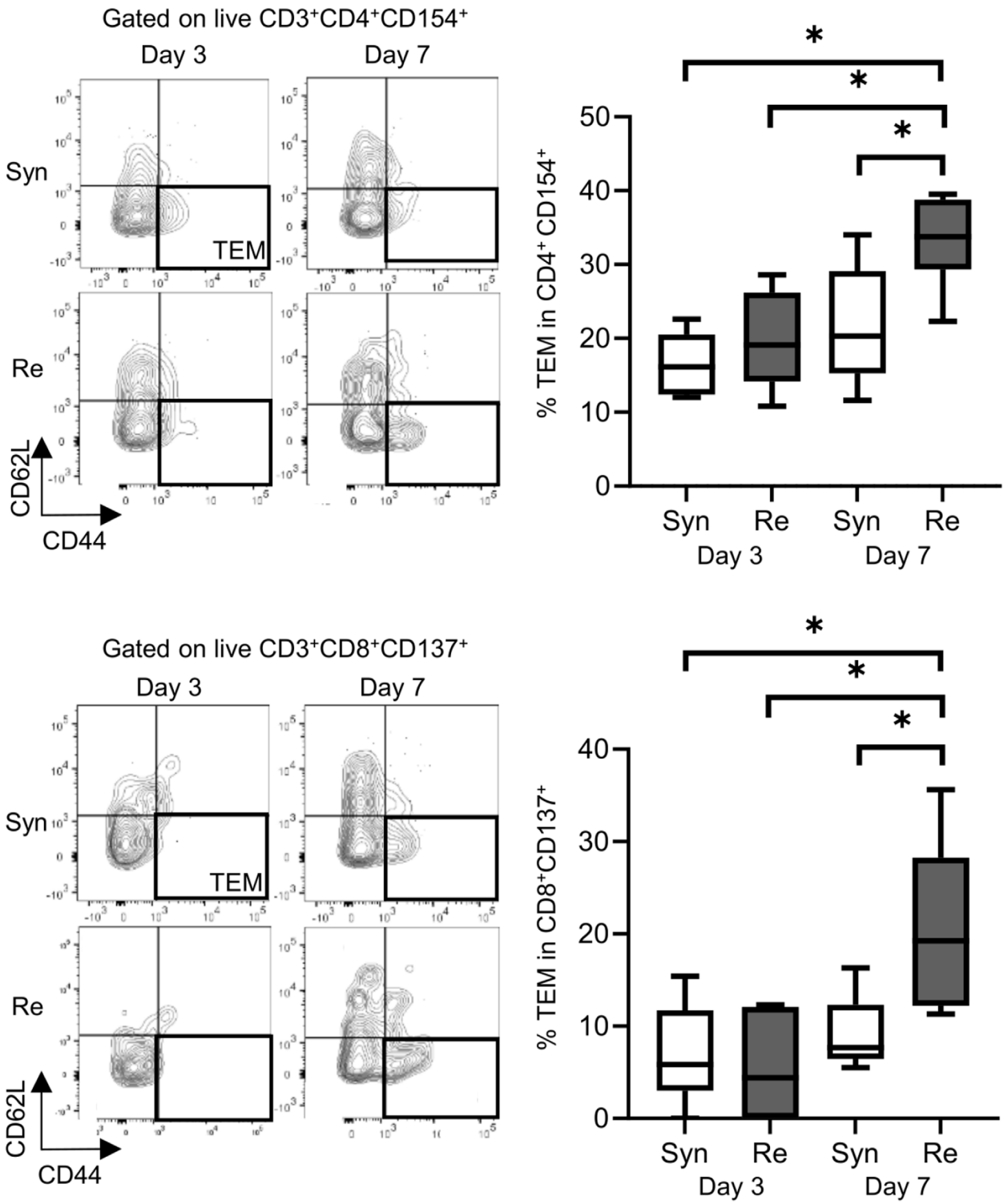


FIGURE 4 | Proportions of effector memory T (TEM) cells (represented by $CD44^+$ and $CD62L^-$) in the mouse skin graft syngeneic model (Syn, C57BL/6 into C57BL/6) and rejection model (Re, BALB/c into C57BL/6). Proportions of TEM cells among $CD154^+$ alloreactive $CD4^+$ T cells (upper). Proportions of TEM cells among $CD137^+$ alloreactive $CD8^+$ T cells (lower). *$p < 0.05$. The data were generated from four independent experiments ($n = 6$). One-way ANOVA and Tukey's multiple-comparison test were employed for statistical analysis.

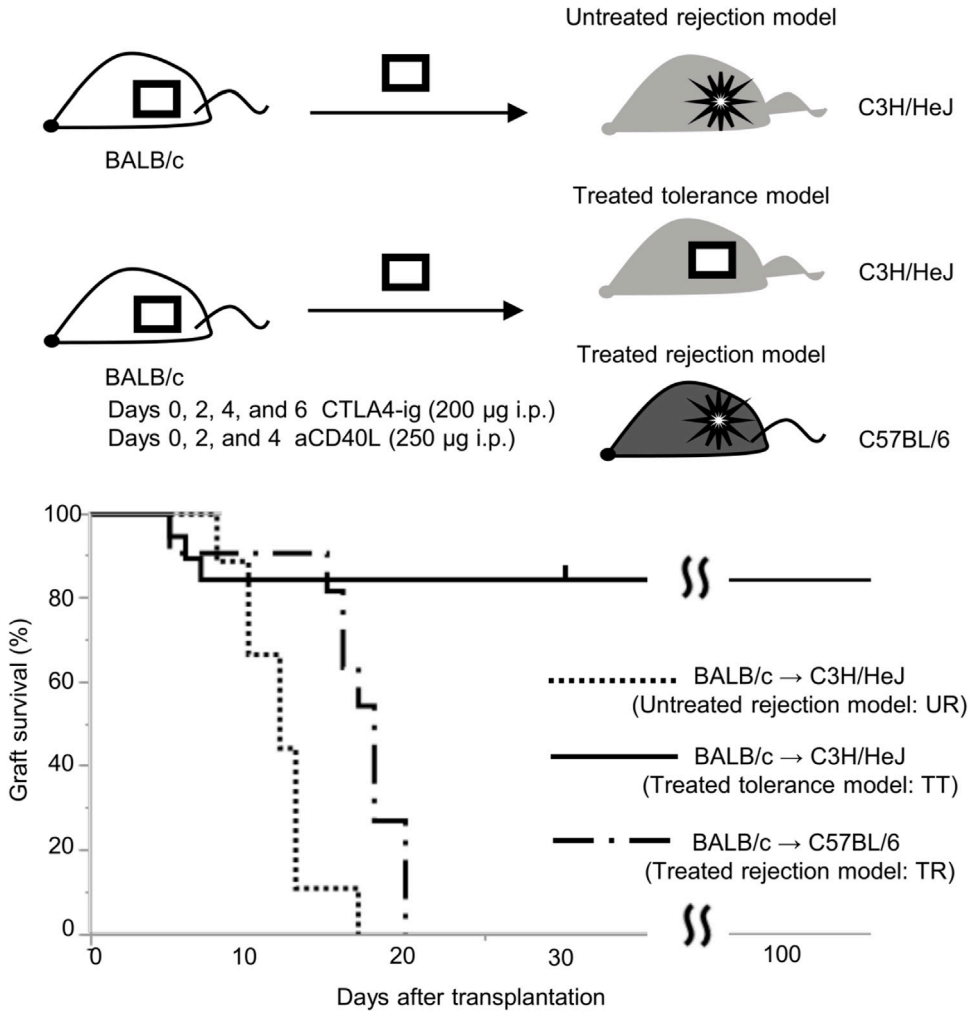


FIGURE 5 | Graft survival curve of the skin transplantation mouse model with immunosuppression. Long-term engraftment was observed in C3H/HeJ recipients with BALB/c graft treated with CTLA-4 IgG and anti-CD154 antibody (treated tolerance model, TT, n = 19), whereas all BALB/c grafts were rejected in untreated C3H/HeJ recipients (untreated rejection model, UR, n = 9, MST: 12 days) and C57BL/6 recipients treated by tolerance induction (treated rejection model, TR, n = 12, MST: 18 days).

$CD4^+CD154^+/CD8^+CD137^+$ T cells than that in the syngeneic model at 7 days after transplantation (syngeneic vs. rejection model, $CD4^+CD154^+$ in total $CD4^+$; 1.0% vs. 1.4%, $p < 0.05$ (upper) and $CD8^+CD137^+$ in total $CD8^+$; 0.27% vs. 0.53%, $p < 0.05$ (lower), **Figure 2**). Donor-reactive T cells identified in this assay showed an increase in proportion and enhancement in function under antigen-specific stimulation in recipients sensitized with BALB/c mouse graft (**Supplementary Figure S2**). The multiparametric flowcytometric analyses demonstrated a unique functionality of donor-reactive $CD8^+$ T cells in the rejection model; for instance, the production ability of the crucial effectors, GZMB and IFN-γ, was specifically enhanced in donor-reactive $CD8^+$ T cells in the rejection model at 7 days after transplantation (syngeneic vs. rejection model, % positive for GZMB 16.1% vs. 54.7%, $p < 0.05$ (upper), and IFN-γ 1.82% vs. 8.18%, $p < 0.05$ (lower), **Figure 3**). As a proof of sensitization, effector memory T (TEM; $CD44^+CD62L^-$) cells were enriched in the donor-reactive population after transplantation (syngeneic vs. rejection model, median % TEM in $CD4^+CD154^+$ 20.2% vs. 32.9%, $p < 0.05$ (upper), and $CD8^+CD137^+$ 9.2% vs. 19.5%, $p < 0.05$ (lower), **Figure 4**).

Quantitative and Qualitative Analyses of Donor-Reactive T Cells for Monitoring Tolerance Induction in the Treated Mouse Skin Transplantation Model

Permanent engraftment was observed in C3H/HeJ recipients of the full MHC mismatched BALB/c graft treated with CTLA-4 IgG and anti-CD154 antibody (treated tolerance (TT) model, ≥30-day survival was recorded in 16/19 animals, 84.2%), whereas all

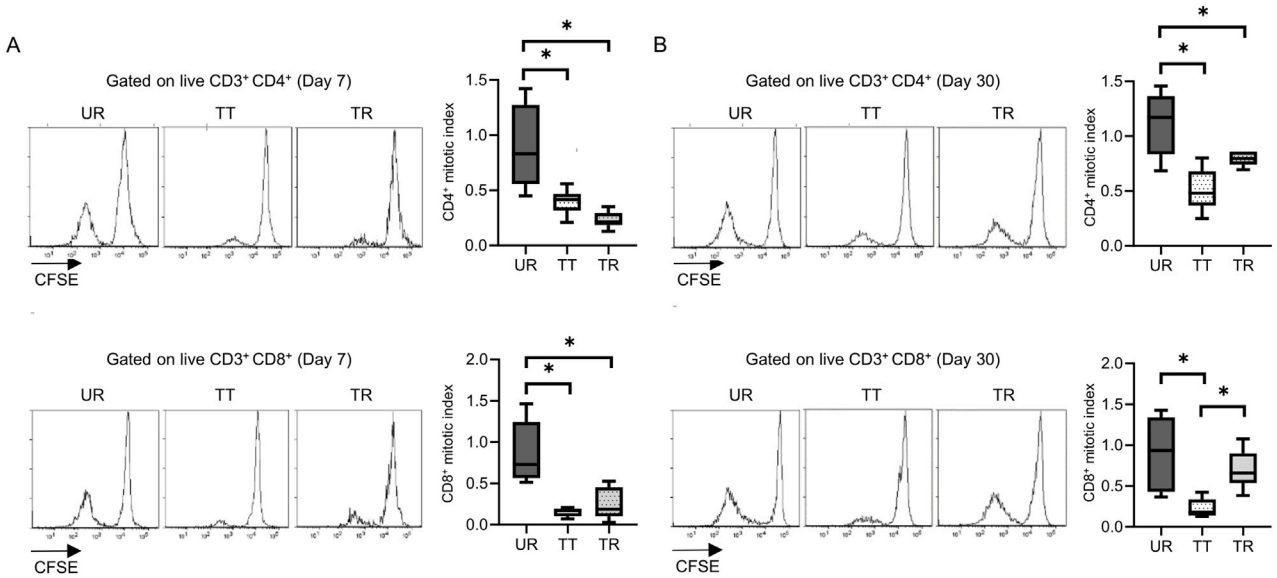


FIGURE 6 | Proliferation assay after mouse skin transplantation with immunosuppression. Representative flow plots and box-and-whisker plots of the mitotic index show the proliferation capacity of CD4 and CD8 T cells from the untreated rejection model (UR, BALB/c into C3H/HeJ) and model treated with CTLA-4 IgG and anti-CD154 antibody (TT, BALB/c into C3H/HeJ or TR, BALB/c into C57BL/6) at **(A)** 7 and **(B)** 30 days after transplantation. *p < 0.05. The data were generated from three independent experiments (n = 6). One-way ANOVA and Tukey's multiple-comparison test were employed for statistical analysis.

C57BL/6 recipients with the same immunosuppression eventually experienced allograft rejection within 20 days (treated rejection (TR) model, **Figure 5**). We investigated the immunological status at 7 and 30 days after transplantation, that is, before and after rejection, respectively. The proportion of FOXP3$^+$ Tregs in CD4$^+$ T cells was comparable, despite tolerance induction, between 7 and 30 days after transplantation (**Supplementary Figure S3**). The proliferation assay conducted at 7 days after transplantation showed a significant reduction in response to immunosuppression in both C3H/HeJ and C57BL/6 recipients compared with that in an untreated rejection (UR) model. However, the conventional proliferation readout results did not show the differential immune response at 7 days after transplantation between C3H/HeJ and C57BL/6 recipients, despite a different outcome (TT model vs. TR model, median MI; CD4$^+$ 0.41 vs. 0.21, p = 0.44 and CD8$^+$ 0.15 vs. 0.19, p = 0.70, respectively, **Figure 6A**). The cATD assay revealed that the proportion of CD8$^+$ donor-reactive T cells in the TT model was lower than that in the TR model at 7 days after transplantation (TT model vs. TR model, median % donor-reactive CD8$^+$; 0.18% vs. 0.35%, p < 0.05, **Figures 7A, B**). The GZMB- and IFN-γ-producing capacity of the CD8$^+$ donor-reactive T cells was comparatively low in the three groups (**Figures 7C, D**). Regardless of the final outcome, models with immunosuppression exhibited impaired memory formation in donor-reactive T cells (**Supplementary Figure S4**). At 30 days after transplantation, the proliferation assay showed a lower MI of CD8$^+$ T cells for the response of the TT model than that for the response of both UR and TR models (TT model vs. UR and TR models, median CD8$^+$ MI: 0.18 vs. 0.93 and 0.66, p < 0.05, respectively, **Figure 6B**). The cATD assay performed at 30 days after transplantation revealed that donor-reactive T cells were detectable in the TT model, similar to those in the UR and TR models (UR vs. TT vs. TR, %CD4$^+$CD154$^+$ in total CD4$^+$ was 2.01%, 1.77%, and 2.35%, %CD8$^+$CD137$^+$ in total CD8$^+$ was 1.00%, 0.7%, and 0.81%, respectively, **Figures 8A, B**). As expected, the functionality of donor-reactive CD8$^+$ T cells in the TT model was lower than that in the UR model (UR vs. TT model, % positive in donor-reactive CD8$^+$ T cells, GZMB; 31.9% vs. 11.2%, p < 0.05, and IFN-γ; 33.9% vs. 3.04%, p < 0.05, respectively, **Figures 8C, D**). However, there were no differences in functionality and memory formation between the TT and TR models (**Supplementary Figures S4, S5**).

DISCUSSION

Allogeneic reactive T cells play a pivotal role in the process of promoting or conversely regulating rejection in allogeneic solid organ transplantation [1]. Understanding the characteristics and behavior of alloreactive T cells is vital for assessing the immune response after allogeneic transplantation [4]. MLR is a classical but practical method to assess allo-response. The precursor frequency of alloreactive T cells has been reported to be 1%–10% under various assay conditions and readouts in both murine and human T-cell repertoires [14–17]. Proliferation, which requires a culture period of 4–5 days, has been widely used as an accessible readout to visualize and quantify the responsiveness of alloreactive T cells using MLR. However, with advancements in flow cytometry technology, it has become feasible to perform multiparametric evaluations of rare populations of less than 1%. This finding suggests the possibility of assessing these infrequent

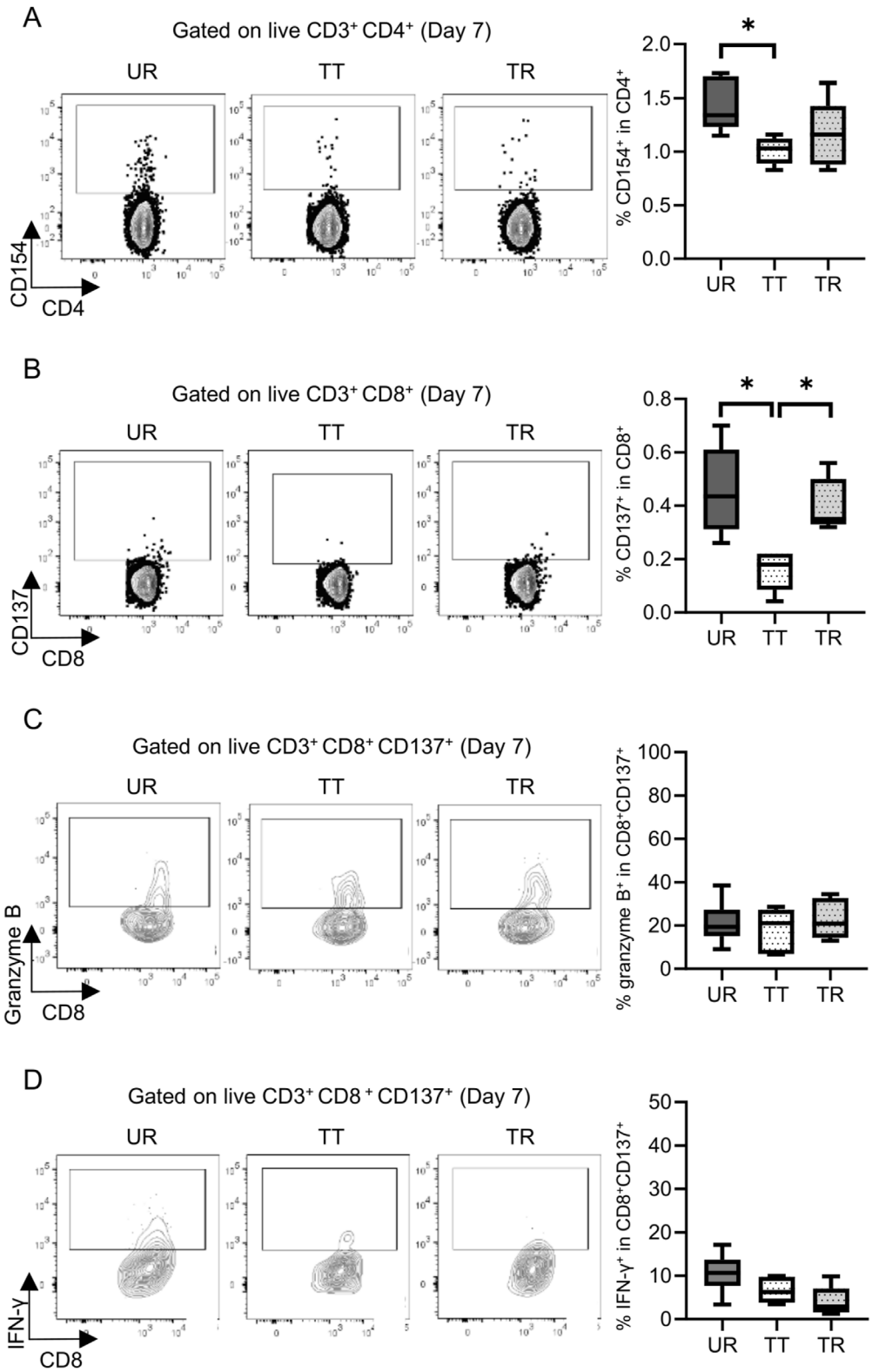


FIGURE 7 | Detection and functional analysis of donor-reactive T cells using an alloreactive T-cell detection assay in the mouse skin transplant model at 7 days after transplantation. Representative flow plots show the alloreactive population defined by **(A)** $CD154^+$ in $CD4^+$ T cells and **(B)** $CD137^+$ in $CD8^+$ T cells and the expression of **(C)** granzyme B and **(D)** interferon gamma (IFN-γ) in $CD137^+$ donor-reactive $CD8^+$ T cells from the untreated rejection model

(*Continued*)

FIGURE 7 | (UR, BALB/c into C3H/HeJ) and model treated with CTLA-4 IgG and anti-CD154 antibody (TT, BALB/c into C3H/HeJ or TR, BALB/c into C57BL/6). *p < 0.05. The data were generated from three independent experiments (n = 6). One-way ANOVA and Tukey's multiple-comparison test were employed for statistical analysis.

alloreactive T cells without the need for proliferation. In line with this prospect, a previous study demonstrated that the cATD assay, using activated allogeneic B-cell stimulators and very early activation markers, enables the detection of alloreactive T cells with high precision in a short-term culture system [8]. In the present study, we validated the utility of the cATD assay for rapid evaluation of donor-reactive T cells in an *in vivo* transplantation model. The usefulness of CD154 and CD137 for detecting antigen-specific $CD4^+$ and $CD8^+$ T cells as rapid-activating molecules has been demonstrated using viral peptides and toxins, respectively [18–20]. CD154 is preferentially expressed on effector $CD4^+$ T cells and memory $CD8^+$ T cells [21]. Although CD137 expression can be induced on $CD4^+$ T cells, the combination of $CD137^+CD154^-$ expression after allo-stimulation has been reported to delineate activated $FOXP3^+$ regulatory T cells that exhibit a specific suppressive capacity against corresponding allo-stimulation [22, 23]. Single-cell TCR analysis has revealed that CD137 expression on $CD8^+$ T cells after allogeneic stimulation is a marker for oligoclonal expanded alloreactive T cells during acute cellular rejection (ACR) after lung transplantation [24]. Moreover, alloreactive CD154 expression on $CD8^+$ memory T cells has been reported to be associated with acute rejection after pediatric liver, intestine, and kidney transplantation [25–27]. Although CD154 could be used as a candidate for predicting rejection by analyzing memory $CD8^+$ T cells, CD137 can be used as a marker to detect a variety of $CD8^+$ T-cell subsets including a substantial portion of naïve populations [20]. Consistent with the results of the previous study, we observed a considerable proportion of a naïve phenotype in donor-reactive $CD8^+$ T cells using CD137 detection. CD137 alloreactive $CD8^+$ T cells showed greater functional molecule expression than those detected by CD154 in our rejection model mice (**Supplementary Figure S6**).

In clinical settings, the cATD assay enables repeated monitoring of circulating alloreactive T cells. The significance of alloreactive T-cell clones in circulation as the pathological effector of rejection after transplantation may be controversial. A recent TCR repertoire analysis using next-generation sequencing revealed that expanded circulating T-cell clones during ACR were observed in the circulation before ACR after lung [24], liver [28], and kidney transplantation [29, 30]. Furthermore, expanded clones in circulation have been reported to overlap with infiltrated T-cell clones in the liver [28] and kidney allografts [29, 30]. An interesting case report of malignant melanoma treated with an immune checkpoint inhibitor after kidney transplantation indicated that the alloreactive T-cell cluster in renal biopsy identified through single-cell RNA sequencing overlapped with circulating clones, which were identified both before and after rejection of the allograft [29]. According to these observations, we believe that circulating alloreactive T cells reflect immune responses after solid organ transplantation.

In the current era where organ transplantation is a standard therapy for patients with organ failure, a standard approach to monitor harmful alloimmune responses is lacking [31]. A previous study reported the usefulness of quantified proliferation in MLR to diagnose immunological rejection [32]. The proliferation and cATD assays assess different time points and readouts, suggesting that they can identify different T-cell populations. During the proliferation assay, *in vitro* culture of T cells is performed over several days to amplify them and obtain T cells of various developmental stages. On the contrary, the cATD assay detects the population that responds rapidly in MLR initiated through overnight culturing, which may indicate a highly primed status and is directly linked to impending rejection. As this assay assesses alloreactivity through a direct pathway, missing the component through indirect pathways could be a limitation when monitoring long-term allo-response after transplantation. However, we believe that its relevance to *in vivo* acute rejection models makes it a useful tool for immune monitoring.

We observed different outcomes and immunological findings in tolerance induction between C3H/HeJ (TT) and C57BL/6 (TR) recipients. C3H/HeJ mice express a dysfunctional toll-like receptor 4, which reduces macrophage and B-cell proliferation and antigen-presenting capabilities, possibly leading to different immune responses and outcomes [33]. Interestingly, the cATD assay showed quantitatively different priming status of donor-reactive $CD8^+$ T cells between the TT and TR models before rejection. After rejection when the rejected graft was lost, the cATD assay did not show differential findings between the TT and TR models; however, the proliferation assay reliably showed sensitization potential in the TR model, based on the results obtained 30 days after transplantation. These findings may be attributed to the feature of alloreactive T cells detected using the cATD assay. This study has some limitations. Notably, the immunological response in skin transplantation is potentially different from that in organ transplantation. Investigation of other organ transplant models and clinical samples could further validate the relevance of the findings of the present study across diverse transplantation settings. However, the cATD assay, which enables real-time and repeatable detection of donor-reactive effectors, might be clinically relevant in diagnosing harmful allo-responses directly linked to the region responsible for rejection. Future research should compare the TCR repertoire of reactive T cells at rejection or upon achieving tolerance between proliferation and cATD assays to obtain differential immunological information. Multifaceted evaluation through the cATD assay facilitates the investigation of superior functional molecules and biomarkers for monitoring clinical conditions such as tolerance status. Additionally, it enables the retrieval of rare live alloreactive T-cell populations for downstream investigation via fluorescence-activated cell sorting and provides valuable information for further studies in the field of translational research.

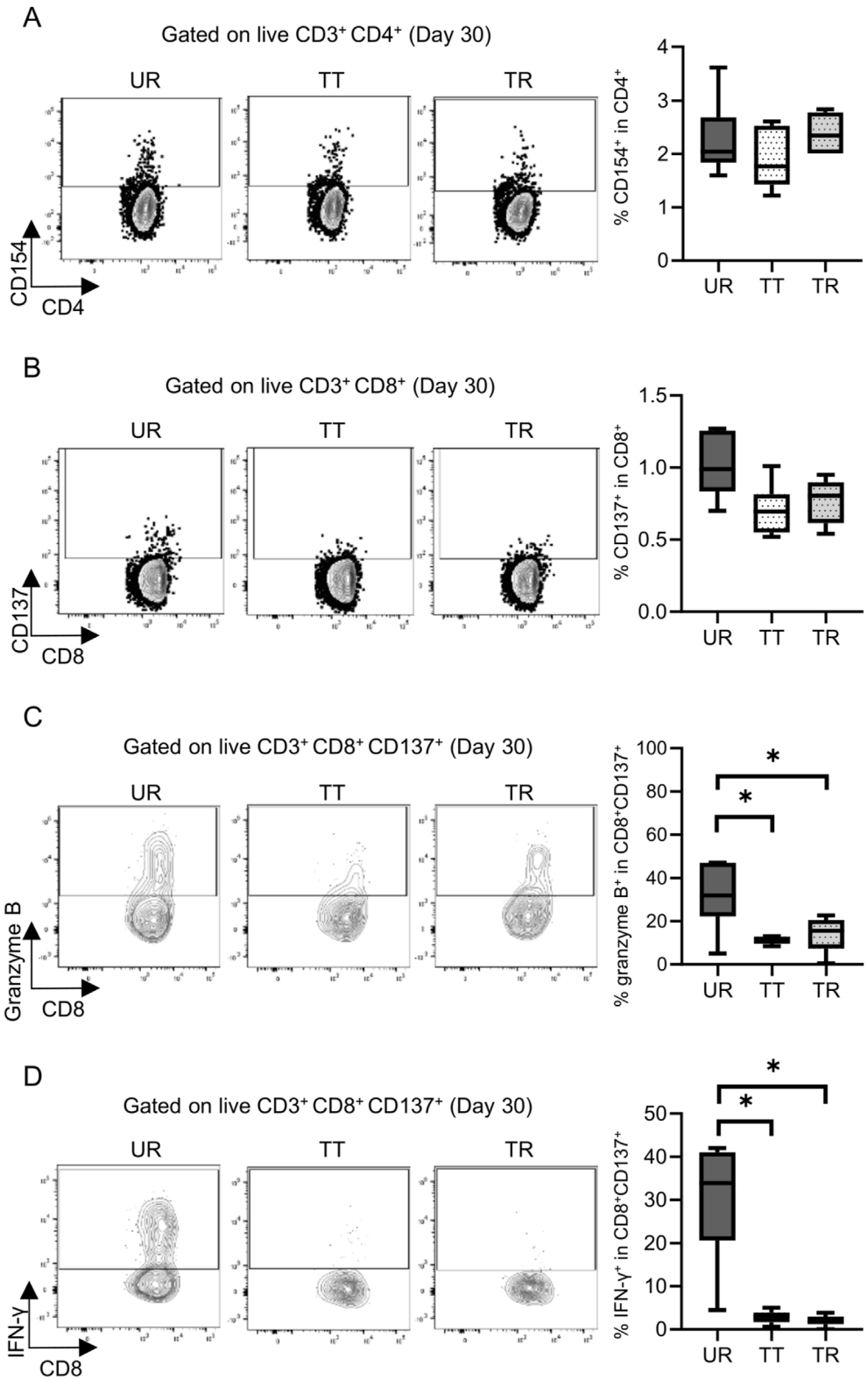


FIGURE 8 | Detection and functional analysis of donor-reactive T cells using an alloreactive T-cell detection assay in the mouse skin transplant model at 30 days after transplantation. Representative flow plots show the alloreactive population defined by **(A)** $CD154^+$ in $CD4^+$ T cells and **(B)** $CD137^+$ in $CD8^+$ T cells and the expression of **(C)** granzyme B and **(D)** interferon gamma (IFN-γ) in $CD137^+$ donor-reactive $CD8^+$ T cells from the untreated rejection model

(*Continued*)

FIGURE 8 | (UR, BALB/c into C3H/HeJ) and model treated with CTLA-4 IgG and anti-CD154 antibody (TT, BALB/c into C3H/HeJ or TR, BALB/c into C57BL/6). *p < 0.05. The data were generated from three independent experiments (n = 6). One-way ANOVA and Tukey's multiple-comparison test were employed for statistical analysis.

In conclusion, the cATD assay using CD154 and CD137 as alloreactive markers effectively distinguished immune responses in *in vivo* mouse transplantation models, highlighting its potential to facilitate prompt quantitative and qualitative estimation of alloreactive T cells after allogeneic transplantation.

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 animal study was approved by Ethics Review Committee for Animal Experimentation of the Graduate School of Biomedical Sciences, Hiroshima University. The study was conducted in accordance with the local legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

Conceptualization, NT; methodology, NT; formal analysis, RA; investigation, RA, AS, and YT; data curation: RA and NT; writing–original draft preparation, RA and NT; writing–review and editing, NT, KI, YT, and HO; visualization, RA and NT; supervision, HO; project administration, HO; funding acquisition, NT and HO. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

GENERATIVE AI STATEMENT

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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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.2025.13879/full#supplementary-material

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Desensitization With Imlifidase for HLA-Incompatible Deceased Donor Kidney Transplantation: A Delphi International Expert Consensus

Lucrezia Furian[1†], Uwe Heemann[2†], Mats Bengtsson[3†], Oriol Bestard[4†], Isabelle Binet[5†], Georg A. Böhmig[6†], John Boletis[7†], David Briggs[8†], Frans H. J. Claas[9†], Lionel Couzi[10†], Emanuele Cozzi[11†], Marta Crespo[12†], Aiko P. J. De Vries[13†], Fritz Diekmann[14†], Magdalena Durlik[15†], Denis Glotz[16†], Ilkka Helantera[17†], Annette Jackson[18†], Stanley C. Jordan[19†], Dirk Kuypers[20,21†], Carmen Lefaucheur[22†], Christophe Legendre[23†], Tomas Lorant[24†], Umberto Maggiore[25†], Nizam Mamode[26†], Smaragdi Marinaki[27†], Annick Massart[28†], Thomas Müller[29†], Rainer Oberbauer[30†], Lutz Renders[31†], Dave Roelen[32†], Jean-Luc Taupin[33†], Ondřej Viklický[34†], Angeliki Vittoraki[35†], Annelies E. de Weerd[36†] and Maarten Naesens[21†]*

[1]Kidney and Pancreas Transplantation Unit, Department of Surgery, Oncology and Gastroenterology DISCOG, University Hospital of Padova, Padova, Italy, [2]Abteilung für Nephrologie, Klinikum Rechts der Isar, Technische Universität München, Munich, Germany, [3]Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden, [4]Nephrology and Kidney Transplant Department, Vall d'Hebron University Hospital, Barcelona, Spain, [5]Clinic of Nephrology and Transplantation Medicine, Cantonal Hospital St Gallen, St. Gallen, Switzerland, [6]Division of Nephrology and Dialysis, Department of Medicine III, Medical University Vienna, Vienna, Austria, [7]Department of Nephrology and Renal Transplantation, National and Kapodistrian University of Athens, Laiko Hospital, Athens, Greece, [8]Histocompatibility and Immunogenetics Laboratory, Birmingham Centre, NHS Blood and Transplant, UK NHS Blood and Transplant, Birmingham, United Kingdom, [9]Eurotransplant Reference Laboratory, Department of Immunohaematology and Blood Transfusion, Leiden University Medical Center, Albinusdreef, Netherlands, [10]Department of Nephrology, Transplantation, Dialysis and Apheresis, Bordeaux University Hospital, Bordeaux, France, [11]Transplant Immunology Unit, Department of Cardiac, National Transplant Centre (CNT), Thoracic and Vascular Sciences Padua University Hospital - Ospedale Giustinianeo, Padova, Italy, [12]Department of Nephrology, Hospital del Mar, Nephropathies Research Group, Hospital del Mar Research Institute, Barcelona, Spain, [13]Division of Nephrology, Department of Medicine, and

***Correspondence**
Lucrezia Furian,
lucrezia.furian@unipd.it

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ORCID: Lucrezia Furian, orcid.org/0000-0002-2264-7986; Uwe Heemann, orcid.org/0000-0002-3886-2517; Mats Bengtsson, orcid.org/0000-0003-2612-0724; Oriol Bestard, orcid.org/0000-0001-9468-7920; Isabelle Binet, orcid.org/0000-0002-2697-9822; Georg A. Böhmig, orcid.org/0000-0002-7600-912X; John Boletis, orcid.org/0000-0003-4664-8921; David Briggs, orcid.org/0000-0002-6796-7086; Frans H. J. Claas, orcid.org/0000-0003-4157-6201; Lionel Couzi, orcid.org/0000-0002-9213-6196; Emanuele Cozzi, orcid.org/0000-0001-7855-5055; Marta Crespo, orcid.org/0000-0001-6992-6379; Aiko P. J. De Vries, orcid.org/0000-0002-9284-3595; Fritz Diekmann, orcid.org/0000-0001-6199-3016; Magdalena Durlik, orcid.org/0000-0002-4798-1497; Denis Glotz, orcid.org/0000-0001-5258-4237; Ilkka Helantera, orcid.org/0000-0002-2954-2538; Annette Jackson, orcid.org/0000-0003-2648-2944; Stanley C. Jordan, orcid.org/0000-0002-0456-8635; Dirk Kuypers, orcid.org/0000-0001-5546-9680; Carmen Lefaucheur, orcid.org/0000-0002-6244-0795; Christophe Legendre, orcid.org/0000-0002-1743-2948; Tomas Lorant, orcid.org/0000-0002-2466-6259; Umberto Maggiore, orcid.org/0000-0002-7468-9600; Nizam Mamode, orcid.org/0000-0001-8549-9098; Smaragdi Marinaki, orcid.org/0000-0002-5920-3086; Annick Massart, orcid.org/0000-0002-6196-8555; Thomas Müller, orcid.org/0000-0003-2236-2312; Rainer Oberbauer, orcid.org/0000-0001-7544-6275; Lutz Renders, orcid.org/0000-0003-1846-0374; Dave Roelen, orcid.org/0000-0002-1846-1193; Jean-Luc Taupin, orcid.org/0000-0002-5766-046X; Ondřej Viklický, orcid.org/0000-0003-1049-2195; Angeliki Vittoraki, orcid.org/0009-0009-0653-3258; Annelies E de Weerd, orcid.org/0000-0001-5134-2025; Maarten Naesens, orcid.org/0000-0002-5625-0792

Abbreviations: ACCORD, Accurate Consensus Reporting Document; ADCC, antibody-dependent cellular cytotoxicity; AMR, antibody-mediated rejection; CDC, complement-dependent cytotoxicity; CDCXM, complement-dependent cytotoxicity crossmatch; CIT, organ cold ischemia time; cPRA, calculated panel reactive antibody; DD, deceased donor; EDTA, ethylenediaminetetraacetic acid; ESOT, European Society for Organ Transplantation; FC, flow cytometric; FCXM, flow cytometric crossmatch; FSGS, focal segmental glomerulosclerosis; HLA, human leukocyte antigen; HLAi KTx, HLA-incompatible kidney transplantation; HS, highly sensitized; IdeS, IgG-degrading enzyme of *S. pyogenes*; IgG, immunoglobulin G; IVIg, intravenous immunoglobulin; IS, immunosuppressive; KTx, kidney transplantation; MDT, multidisciplinary team; rATG, rabbit anti-human thymocyte globulin; SAB, single-antigen bead; SoC, standard of care; TCMR, T-cell-mediated rejection; vPRA, virtual panel reactive antibodies.

Leiden Transplant Center, Leiden University Medical Center, Leiden, Netherlands, [14]Department of Nephrology and Kidney Transplantation, Hospital Clínic Barcelona, Barcelona, Spain, [15]Klinika Transplantologii, Immunologii, Nefrologii i Chorób Wewnętrznych Warszawski Uniwersytet Medyczny ul, Warszawa, Poland, [16]Department of Nephrology and Renal Transplantation, Saint-Louis Hospital in Paris, Paris, France, [17]Transplantation and Liver Surgery, Helsinki University Hospital and University of Helsinki, Helsinki, Finland, [18]Department of Surgery, Duke University, Durham, NC, United States, [19]Nephrology and Transplant Immunology Medical Director Kidney Transplant Program Cedars-Sinai Medical Center, Pediatrics and Medicine David Geffen School of Medicine at UCLA, Los Angeles, CA, United States, [20]Department of Nephrology and Renal Transplantation, University Hospitals Leuven, Leuven, Belgium, [21]Department of Microbiology, Immunology and Transplantation, KU Leuven, Leuven, Belgium, [22]Nephrologist and Head of the Nephrology and Kidney Transplantation Department, Saint-Louis Hospital-APHP, Paris, France, [23]Nephrology at Université Paris Cité and Head of Nephrology and Transplantation Unit at Necker Hospital in Paris, Paris, France, [24]Uppsala University, Department of Surgical Sciences, Section of Transplant Surgery, Uppsala, Sweden, [25]Dipartimento di Medicina e Chirurgia, Università di Parma, UO Nefrologia - Trapianti Rene Pancreas, Programma Regionale Trapianti Emilia-Romagna, Azienda Ospedaliero-Universitaria di Parma, Parma, Italy, [26]Department of Transplantation, Transplant Surgery at Guy's and Great Ormond Street Hospitals, London, United Kingdom, [27]National and Kapodistrian University of Athens, Clinic of Nephrology and Transplantation, "Laiko" General Hospital, Athens, Greece, [28]Department of Nephrology, UZ Antwerpen, Antwerpen, Belgium, [29]Clinic for Nephrology, Renal Transplant Program, Transplant Institute, University Hospital Zurich, Zurich, Switzerland, [30]Medical University of Vienna, Vienna, Austria, [31]Department of Nephrology of Technische Universität München, München (TUM), Munich, Germany, [32]Department of Immunology, Leiden University Medical Center, Leiden, Netherlands, [33]Laboratory of Immunology and Histocompatibility, Hôpital Saint-Louis, APHP Paris, Paris, France, [34]Department of Nephrology, Institute for Clinical and Experimental Medicine, Prague, Czechia, [35]Immunology Department and National Tissue Typing Center, "G.Gennimatas" Hospital, Athens, Greece, [36]Erasmus MC Transplant Institute, Department of Internal Medicine, University Medical Center, Rotterdam, Netherlands

Highly sensitized (HS) patients in need of kidney transplantation (KTx) typically spend a longer time waiting for compatible kidneys, are unlikely to receive an organ offer, and are at increased risk of antibody-mediated rejection (AMR). Desensitization using imlifidase, which is more rapid and removes total body immunoglobulin G (IgG) to a greater extent than other methods, enables transplantation to occur between HLA-incompatible (HLAi) donor–recipient pairs and allows patients to have greater access to KTx. However, when the project was launched there was limited data and clinical experience with desensitization in general and with imlifidase specifically. Hence, this Delphi methodology was used to reach a consensus from a multi-disciplinary team (MDT) of experts from 15 countries on the management of HS patients undergoing imlifidase HLAi from a deceased donor (DD) KTx. This Delphi consensus provides clinical practice guidance on the use of imlifidase in the end-to-end management of HS patients undergoing an HLAi DD KTx and supports centers in the development of guidelines for the utilization and integration of imlifidase into clinical practice.

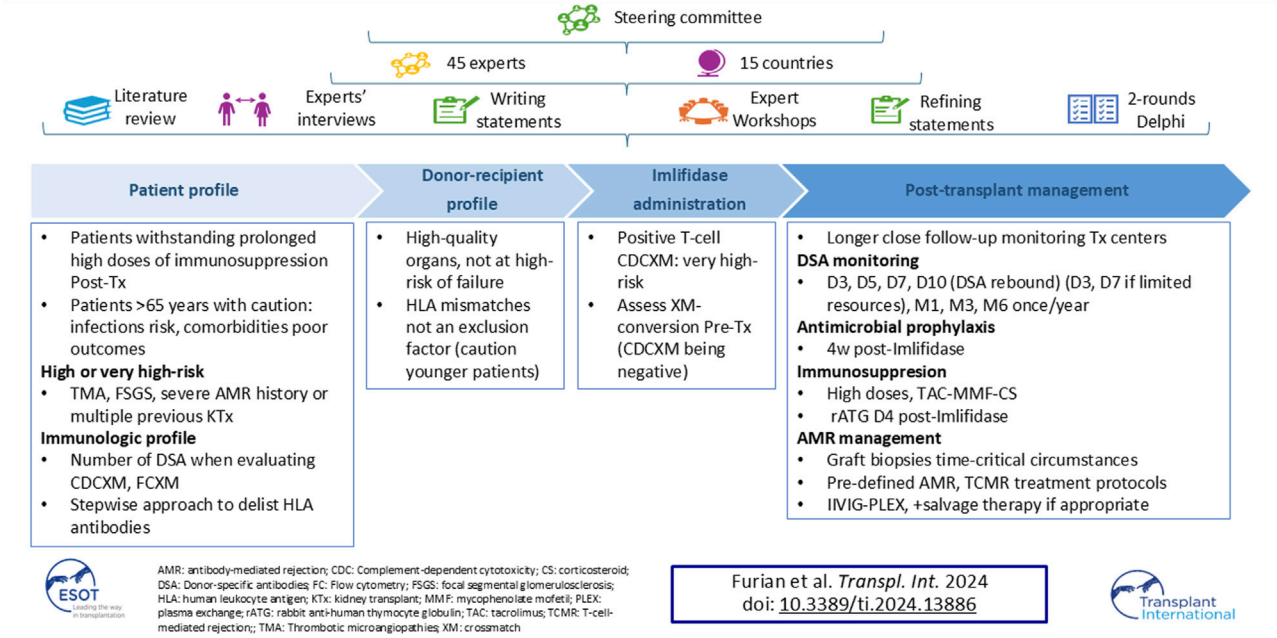
Keywords: desensitization, HLA incompatible, HLAi, kidney transplantation, imlifidase

INTRODUCTION

Sensitized patients with preformed human leukocyte antigen (HLA) antibodies, still face a curious situation, with longer waiting times and higher rejection risks [1–5]. Up to one-third of KTx candidates are sensitized [6], accumulating on waiting lists despite priority allocation programs [6–9]. The definition of HS may vary between countries and allocated regions [10], and patients wait longer for KTx and have higher AMR risks [1–5].

Worldwide, 5%–15% of patients are HS (panel reactive antibodies [cPRAs] ≥85%) [6, 7, 9, 11] and struggle to find compatible donors [8, 12, 13]. There is an increasing number of HS patients waitlisted worldwide with limited access to transplantation [14]. In Europe, Eurotransplant Kidney Allocation System data show that transplantation rates decrease as virtual panel reactive antibodies (vPRA) scores rise: 23% lower for scores ≤50%, 51% for 75%–85%, 65% for >85–95%, and 94% for 99%–100% compared with unsensitized candidates [1]. In the US, 2024 OPTN data showed that 11% of waiting for KTx candidates are HS (cPRA >80%, only 5% cPRA>98%), and 45% show some sensitization (at least cPRA >1%) [14]. Despite prioritization efforts in allocation programs in Europe and the U.S., 35% of HS patients rarely find compatible donors [15].

For HS KTx candidates, advances in desensitization have helped to enable transplantation mainly from living donors [16–18], although there are no drugs formally approved for this indication. Furthermore, protocols are often center-specific and comparisons between them are difficult. The preferred option for HS patients is to receive a compatible



GRAPHICAL ABSTRACT

transplant through available kidney allocation systems, including prioritization programs [9, 14].

However, there is still a population of HS patients who are either not served or not eligible by prioritization programs who remain on waiting lists and for whom novel desensitization therapies are needed [1, 9].

Imlifidase (Idefirix®) is a cysteine proteinase derived from the IgG-degrading enzyme of *Streptococcus pyogenes* (IdeS) that cleaves IgG into F(ab′)2 and Fc fragments, inhibiting complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC) within hours [19], converting positive cross matches to negative, avoiding hyperacute rejection and enabling HLAi transplantation and [20–23], completely removing within hours total body IgG. It is well tolerated.

Imlifidase is conditionally approved by the EMA for desensitization before DD KTx, allowing patients to have greater access to KTx [22]. The reported 3 and 5-year data on Imlifidase HLAi KTx [15], showed positive outcomes with 90% patient and graft survival (death censored) of 84% and 82% at 3 and 5 years respectively [11, 15, 23]. The ESOT ENGAGE initiative reported consensus for imlifidase as a desensitization strategy for DD KTx in highly selected patients with no other options [14]. Although imlifidase is a potent option for overcoming significant immunologic barriers, data and clinical experience with desensitization and imlifidase specifically, remain limited, with countries developing their own consensus guidelines on its use [16, 24].

Aims

To consolidate expert opinion on the evaluation and management of HS patients undergoing HLAi KTx from DD after imlifidase desensitization and to guide transplant physicians in identifying and managing these patients and integrating imlifidase into their center's protocols.

MATERIALS AND METHODS

The international expert panel consisted of 45 European and U.S. transplant nephrologists, surgeons and HLA specialists. Experts were selected based on imlifidase experience or expertise in the field of KTx and/or HLAi transplantation and AMR management.

An iterative approach was developed to reach consensus, following a series of qualitative and quantitative methods based on the Accurate Consensus Reporting Document (ACCORD) guidelines [25], summarized in **Table 1**.

Delphi Methodology

The Delphi methodology [26, 27] was employed to gather global insights on managing HS patients receiving imlifidase HLAi KTx. It was performed in May 2022, when only 46 clinical trial patients were treated with imlifidase, mostly in the U.S. and Sweden. The questionnaire included six sections on imlifidase KTx (see **Figure 1**).

The online survey was completed in two rounds. In the first round, experts voted on the degree of agreement with each

TABLE 1 | Iterative approach to reaching a consensus on a series of statements.

Step	Description
1	To identify a **multidisciplinary Steering Committee** to lead and coordinate the guideline development process
2	To **identify the key topics** involved in the transplant physician's decision-making process when evaluating and managing a highly sensitized patient for an HLAi KTx from a DD with imlifidase
3	**Literature review** to identify the current body of research and the major gaps and inconsistencies in the HLAi KTx clinical practice guidelines
4	**Interviews** with three experts to explore and challenge initial assumptions
5	**The Steering Committee meeting** to discuss experts' views on three predefined risk categories of highly sensitized patients (moderate, high, and very-high risk) was explored
6	**Interviews** with three additional experts to refine and validate the outputs and assumptions from the Steering Committee
7	**First pan-EU Expert Workshop** with 45 expert participants from Europe and the USA to discuss and test these outputs and assumptions. This provided a broader first view of the level of consensus that started to be built on key topics and considerations in the clinical decision-making and risk stratification process of transplant physicians during HLAi KTx
8	Analysis of the insights from the pan-EU Expert Workshop and consolidation into discrete "**expert opinion statements**". A framework for the initial list of statements was defined, enabling structured thinking and the involvement of experts in their areas of expertise
9	**Nine 1-h Expert Review Sessions** in which experts further updated and refined the expert opinion statements in an iterative manner. This culminated in the third iteration of the Imlifidase Clinical Workbook, which consisted of refined expert opinion statements and open-ended questions based on feedback from all experts
10	Finally, these statements were evaluated and responded to in the next phase of the project using a **Delphi methodology with two rounds** of surveys • Following the first round of surveys, the results were analyzed, and the statements and questions were prioritized for discussion during the second Pan-EU Expert Workshop; the prioritization was based on the level of discrepancy and disagreement among panelists, with the aim of challenging and further validating expert consensus and non-consensus. The outputs were used to update and finalize the expert opinion statements which were tested again in the second round of surveys. In this second round, experts had the opportunity to compare their own initial responses and reconsider agreement levels based on the group response from the first round of surveys
11	A thorough qualitative and quantitative analysis of the responses from the second survey was conducted, which ultimately informed the **final content** and respective level of consensus of all the expert opinions

Bold text was simply to facilitate the reading.

statement using a 5-point Likert scale (1 = strongly disagree; 2 = disagree; 3 = neutral; 4 = agree; 5 = strongly agree). Statements reaching ≥75% agreement were considered consensual, while for others, members explained their disagreement.

Statements with lack of agreement were re-written and clarified by the expert panel and re-evaluated in the second round. The results show the percentage of agreement for each final statement after the two rounds.

RESULTS

The consensus statements representing the opinions of the 45 experts from 15 countries who participated in the modified Delphi study are gathered in **Supplementary Tables S1-S5**, with their corresponding levels of agreement.

DISCUSSION

HLAi KTx Infrastructure and Team Resources

There was broad consensus on the need for an optimal infrastructure and MDT to initiate an HLAi KTx program in a transplant center. DD HLAi KTx protocols should be in place for organ retrieval, equitable organ allocation and organ preservation, together with appropriate imlifidase protocols to facilitate transplantation for HS patients who might otherwise be considered unsuitable (87.5% consensus) (**Supplementary Table S1**).

It is advised that an integrated approach among centers be taken with DD HLAi KTx (90.6% consensus) and referring nephrologists and dialysis centers should be informed about the possibility of imlifidase HLAi KTx so that potential patients can be referred to an HLAi KTx expert center to further evaluate their eligibility (90.6% consensus) (**Supplementary Table S1**).

Experts advised that centers should have 24/7 access to HLA laboratory services to address the need for close monitoring of HS patients potentially undergoing HLAi KTx (93.8% consensus) (**Supplementary Table S1**). Indeed, when considering imlifidase HLAi KTx, access to an HLA laboratory is considered essential for the appropriate selection of donor–recipient pairs [6, 28] Assessment of a potential recipient's sensitization history and degree of HLA mismatch with the DD is critical prior to accepting an offer [29]. Post transplantation, appropriate patient monitoring including access to an HLA laboratory allows for monitoring of donor-specific antibodies (DSA), renal function assessment and graft biopsy, to diagnose early AMR and initiate appropriate treatment as soon as possible [30]. According to a clinical study, DSA rebound following imlifidase occurs in 80% of the patients at 3–14 days post-treatment [20]. Hence, immediate access to HLA assessment facilities is critical for effective patient management.

A multidisciplinary approach is advised for evaluating patients' physiological status (87.1% consensus). Similarly, an MDT comprising transplant surgeons, nephrologists, HLA

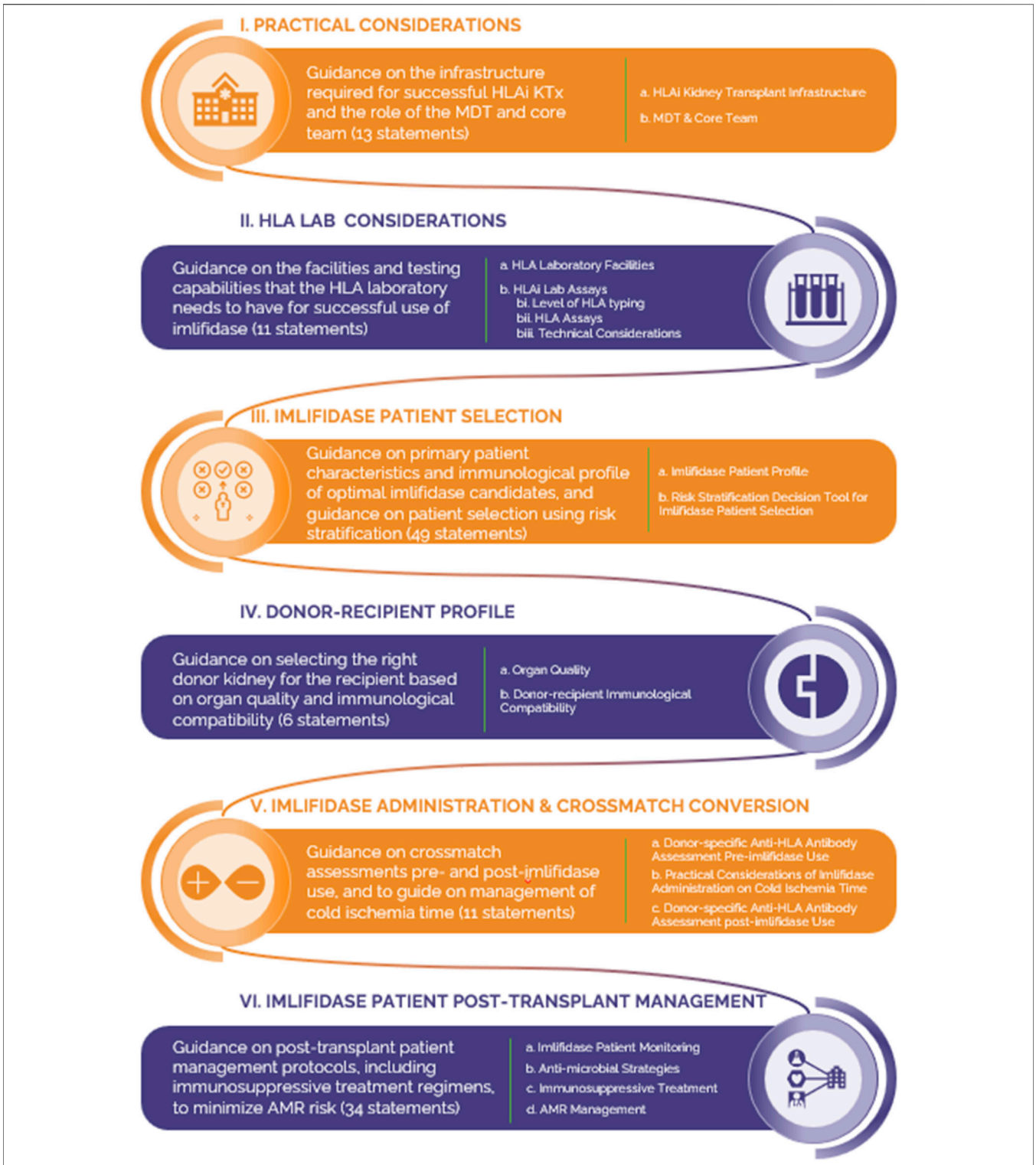


FIGURE 1 | The six sections of the Delphi questionnaire that evaluated the various aspects of kidney transplantation and the utilization of imlifidase.

specialists, transplant coordinators, pathologists, specialized nurses, pharmacists, and ICU specialists should be established to evaluate patient eligibility and progress with HLAi KTx (90.6% consensus), and MDT members should be trained and prepared for imlifidase HLAi KTx, including awareness of center-specific patient management protocols and procedures (87.5%

Key agreements on the clinical practice guidance on the use of imlifidase in the end-to-end management of highly sensitized patients undergoing an HLA-incompatible deceased donor kidney transplantation.

Key practical considerations

HLAi kidney transplant infrastructure

- Deceased donor HLAi KTx protocols in place
- 24/7 access to HLA lab services

MDT and Imlifidase expert core team

- ***MDT*** in place for Imlifidase HLAi KTx incl. Tx surgeons, nephrologists, HLA spec., Tx coordinators, Tx nurses, pharmacist, ICU spec., pathologist and all members adequately trained incl. on center's specific patient management protocol and procedures
- ***MDT*** approach is taken when evaluating the immunological risk that the pre-formed DSA could potentially pose to the patient.
- Dedicated HLAi KTx ***imlifidase expert core team*** in place (comprising Tx surgeon, nephrologist, HLA spec.) available 24/7
- ***Imlifidase expert core team*** is expected to advise on key decisions regarding patient eligibility and management, particularly when evaluating and approving organ suitability at the time of the offer.
- Donor–recipient immunological compatibility and transplantation risk assessed prior to administering imlifidase
- Sufficient time dedicated to educate potential imlifidase patients on the risks, side effects and adherence requirements prior to HLAi transplantation

Key HLA laboratory considerations

HLA laboratory facilities

- Available 24/7 for crossmatch and HLA antibody testing

HLAi laboratory assays

HLA Typing: Type at least 11 HLA loci, including DQ and DP- Perform allelic or high-resolution typing

Crossmatch Assessment:

- Use a serum treatment method to mitigate complement and non-complement-mediated prozone effect and improve accurate HLA antibody detection
- Avoid using an Fc-detecting antibody-based SAB in the first few hours post-imlifidase as this can lead to false positive signals

Key primary characteristics of imlifidase patient

Patient profile

- Select patients capable of withstanding prolonged high doses of immunosuppression following transplantation
- Chronological age not restrictive; considered on physiological age in the context of comorbidities; patients >65 years considered with caution because of the higher risk of infections and poor outcomes
- Characteristics of patients at high or very high risk include TMA, FSGS, history of severe AMR or multiple previous kidney transplantations

Immunologic profile

- Conduct HLA Ab screening for all patients, using a SAB assay, at regular intervals, preferably every 3 months, and 15 days following desensitization and immunization events
- Evaluate historic DSA data and circulating pre-formed anti-HLA specific antibodies as part of the pre-transplant immunologic risk assessment
- Each center should have their own lab reference values to enable estimating how a DSA MFI value will predict reactivity to donor tissue, and thus likelihood of rejection.
- Assess Ab strength based on MFI values and adjust for contributing factors (including number of DSA, Ab class, route of sensitization) to better estimate immunological risk
- Use a step-wise approach to delist as many HLA antibodies as deemed appropriate

Donor–recipient profile

Organ quality

- Select high-quality organs that are not at high risk of failure
- Organ quality and function should be validated by the recipient transplant center administrating imlifidase

Donor–recipient immunological profile

- The number of HLA mismatches should not be an exclusion factor for accepting a donor kidney, provided there is sufficient prior experience in HLAi transplants, although whenever possible it is advised to aim for fewer mismatches in younger recipients

Imlifidase administration and crossmatch conversion

Donor-specific anti-HLA antibody assessment pre-*imlifidase* administration

- Assess donor–recipient immunologic compatibility according to local lab protocols /capabilities, performing at least a FC- or a CDC-XM with a recent (<8 weeks) SAB assay
- Each center should have pre-defined criteria for the FC crossmatch being borderline positive, clearly positive or very positive
- Consider the number and type of DSA loci when evaluating risk of a positive physical crossmatch, CDCXM or FCXM.
- Consider a positive T-cell CDCXM as very high risk and avoid conducting HLAi KTx following a positive T-cell CDCXM at least in the beginning, while building experience

Crossmatch conversion assessment post-imlifidase administration

- Assess XM conversion prior to proceeding with the transplant by doing a physical crossmatch, FCXM or CDCXM, according to local practice. In addition, if possible, it is advised considering performing a Luminex SAB assay post-imlifidase pre-transplantation.

Imlifidase patient post-transplant management

Patient monitoring

- Arrange longer close patient monitoring FU visits post HLAi KTx at Tx center according local protocols, preferably, at least:
  For the first 1-2 months, twice a week-For the following 3-4 months, twice a month-For the following 5 months, once per month for stable patients and twice a month for patients at higher risk of AMR-Once a year, past the first year, provided they regularly visit their local nephrologist
- Establish open communication channels between local hosp/Tx center to ensure best practice protocols are in place around post-Tx management and emergency response.

DSA monitoring

- Closely monitor the DSA to identify the DSA rebound or Ab rebound (using SAB assay according local lab protocol) and ensure that AMR is identified as early as possible; by preferably assess DSA at D3, D5, D7 and D10 (D3 and D7 if limited resources), M1, M3, M6 and once a year
- It is advised to consider the potential interference effect of IVIg on SAB assay results and adapt DSA monitoring frequency appropriately.

Antimicrobial prophylaxis

- Provided to all patients prior to and following HLAi KTx and maintained for at least 4 weeks post-imlifidase administration
- All patients receive vaccination against infections such as influenza, pneumonia, and COVID-19 prior to imlifidase

Immunosuppressive therapy (IST)

- Induction and maintenance protocol tailored to the needs of patients; steroids used for all patients and early withdrawal of steroids avoided
- It is advised that high doses of IST, preferably triple-agent regimen (TAC-MMF-CS), is provided to all patients according to local protocols and their individual risk factor needs.
- When rabbit ATG is used over ATGAM, it is advised to start after day 4 after imlifidase use
- When considering the use of IVIG, anti-CD20 and IL-6 receptor antagonists (if available), no consensus was reached in regard to administration scheme for IVIG and for integrating anti-CD20/IL-6 Rec antag into IS scheme

AMR management

- Pre-determined protocols for treatment of rejection (AMR, T-cell- mediated rejection) should be defined in advance and ready in place to ensure an immediate clinical response
- should be similar to a standard KTx, following local AMR protocols, but implemented earlier with a more rapid stepwise approach, PLEX is considered as part of the AMR management standard of care protocols
- Perform graft biopsies in time-critical circumstances and cases of severely impaired renal function and AMR suspicion, to directly proceed with anti-rejection treatment, but also in case of acute unprecedentedly high antibody production during the first week post-transplantation with normal creatinine levels.

AMR: antibody-mediated rejection; CDC: Complement-dependent cytotoxicity; DSA: Donor-specific antibodies; FC: Flow cytometry; HLA: human leukocyte antigen; HLAi: HLA incompatible; IgG: immunoglobulin G; KTx: kidney transplant; MDT: multidisciplinary team; MFI: mean fluorescence intensity; SAB: single-antigen bead.

FIGURE 2 | Visual abstract.

consensus) (**Supplementary Table S1**). Furthermore, experts advised that a dedicated HLAi KTx imlifidase expert core team (comprising a transplant surgeon, nephrologist, and HLA specialist) be in place and available 24/7 in the case an offer occurs (93.6% consensus) (**Supplementary Table S1**). This core team of experts would advise on key decisions regarding patient eligibility and management, particularly when evaluating and approving organ suitability at the time of the offer (96.8% consensus).

There was 100% consensus that a multidisciplinary approach should be taken in the case of an HLAi donor offer to assess the individual patient (immunological) risk that a pre-formed DSA might pose and to ensure appropriate management when the donor offer comes in (**Supplementary Table S1**).

Experts also recommended that the MDT dedicate sufficient time to educate potential imlifidase patients on the risks and adherence requirements prior to HLAi KTx and throughout the process (96.9% consensus) (**Supplementary Table S1**). This is likely to require several sessions as the majority of these patients are on long-term dialysis and are not expecting transplantation to be an option, therefore they have to adjust to this to evaluate the risk–benefit of treatment and post-transplantation immunosuppressive therapy [28]. Long-term immunosuppression carries risks of adverse events [31] that patients need to be aware of, although many have previous experience with immunotherapy, together with the importance of treatment adherence to improve long-term outcomes and long-term tacrolimus and mycophenolic acid exposure target levels to prevent rejection [32].

At the first use of imlifidase, experts advised treating one patient at a time. This would enable the practical application of HLAi KTx processes into clinical practice (87.5% consensus) (**Supplementary Table S1**), which is likely to increase the chance of successful transplantation, build the experience of the MDT at the center and allow amendment of any protocols should it be necessary.

HLA Laboratory Facilities and Assays

Focusing on technical support/facilities within the transplant centers, the laboratory/testing facilities should have rapid turnaround times particularly for crossmatch evaluation to limit organ cold ischemia time (CIT) (100% consensus) (**Supplementary Table S2**). Furthermore, crossmatch conversion from positive to negative in patients treated with imlifidase should be confirmed before transplantation [23]; therefore, in addition to having HLA assessment facilities, rapid assay turnaround times are also important when performing an imlifidase transplant to keep CIT as short as possible because CIT impacts kidney graft survival rates [33]. To increase this speed, some centers are deciding to transplant based on virtual crossmatch conversion, i.e., single-antigen bead (SAB) data showing a significant decrease in DSA with FCXM as a retrospective test.

Experts advised that HLA typing at the resolution of the recipient or donor profile is sufficient to determine compatibility for each case, preferably typing for all 11 HLA loci (HLA-A, HLA-B, HLA-C, DPA1, DPB1, DQA1, DQB1, DRB1, DRB3, DRB4, and DRB5) (90.6% consensus) (**Supplementary Table S2**). It was also recommended that allelic, high-resolution typing be performed whenever possible (93.6% consensus) and that this should become the future standard for all HS patients (90.6% consensus) (**Supplementary Table S2**).

Experts advised that HLA laboratories follow a method of serum treatment for all HS patient samples to reduce complement interference (93.3% consensus) and non-complement-mediated prozone effects to improve accurate HLA antibody detection (87.1%) (**Supplementary Table S2**). Technical issues impact single antigen assays and may confound assay interpretation. For example, false negative results may occur due to complement interference. Prozone is reportedly very frequent in HS patients (87%), particularly in those with a history of previous transplantation [34].

In the first few (<4) hours post-imlifidase administration, experts advised against the use of an Fc-detecting antibody-based SAB assay as this can lead to false positive signals due to the high amount of single-cleaved IgG (80% consensus) (**Supplementary Table S2**). As other treatments used in conjunction with imlifidase may also interfere with assay results, experts advised that post-imlifidase HLAi KTx, potential effects of intravenous immunoglobulin (IVIg), rabbit anti-human thymocyte globulin (rATG) or anti-CD20 mAb (rituximab) on assay results should be considered (86.7% consensus) (**Supplementary Table S2**).

Primary Characteristics of the Imlifidase Patient Profile

Primary Patient Characteristics

Experts recognized the importance of selecting only those HS patients who are considered capable of tolerating prolonged high doses of immunosuppression following transplantation (88.9% consensus) (**Supplementary Table S3**) since imlifidase administration does not reduce the immunosuppressive burden required in HLAi KTx both in terms of induction and maintenance therapy.

Patient characteristics such as comorbidity, primary renal disease, immunological risk, dialysis/previous transplant history and psychosocial factors may influence the potential outcomes of HLAi KTx [35]. Older patients may be more susceptible to infection following KTx [36] and more likely to have comorbidities. While experts advised that chronological age should not be restrictive and that patients should be considered primarily based on their physiological age in the context of other comorbidities (88.9% consensus), they also advised that patients older than 65 years should be approached with extra caution considering the higher risk of infection and poor outcomes associated with this age group (75% consensus) (**Supplementary Table S3**). The assessment and risk stratification of HS patients has become even more challenging as the number of transplant recipients over 60 years of age increases resulting in an increased incidence of comorbidities contributing to kidney failure, such as diabetes, hypertension, and obesity [37].

Often associated with age is frailty, and while experts advised that patient frailty status be assessed by the MDT and should include physical and cognitive evaluation (88.6% consensus), consensus was

not reached (61.1%) on whether a validated frailty score should be developed specifically for HS patients, given the complexity and higher HLAi KTx risk and lack of standardized frailty evaluation across centers (**Supplementary Table S3**).

Experts advised considering patients with an expected survival rate of ≥5 years unless there are pressing reasons for transplantation or a significantly high unmet need (90.6% consensus) (**Supplementary Table S3**). Other characteristics to be considered when stratifying patients as being at high or very high risk that were confirmed and highlighted by experts here include thrombotic microangiopathy (75%) and primary focal segmental glomerulosclerosis (FSGS) (83.3% consensus) (**Supplementary Table S3**). However, no consensus was reached on original kidney disease with a high recurrence risk as a (relative) contraindication for HLAi KTx (71% consensus). HS patients with severe AMR history (84.4%) or multiple previous KTx should be considered at high risk for AMR after HLAi KTx (90.6% consensus), while patients who have exhausted standard routes of vascular access are at high risk for adverse outcomes on dialysis and should be prioritized for an HLAi KTx (80.6% consensus) (**Supplementary Table S3**).

Patient Immunological Profile

Experts advised conducting HLA antibody screening using SAB for all HS patients at regular intervals according to national and local guidelines, preferably every 3 months, and after 2-3 weeks following desensitization and immunization events (94.4% consensus).

In addition, historical DSA data and screening for circulating preformed anti-HLA specific antibodies should be part of the pre-transplant immunological risk assessment for all HS patients (100% consensus) (**Supplementary Table S3**). Furthermore, considering the different protocols and assays across countries and transplant centers, it was advised that each center has its own reference values to estimate the likelihood of rejection (93.8% consensus) (**Supplementary Table S3**).

Similarly, when assessing a patient's sensitization level, it is important to integrate the strength of the antibody response assessed using mean fluorescence intensity (MFI) in undiluted serum, the breadth of sensitization (assessed using cPRA) and the specificities to create an immunological risk profile.

DSA Characteristics

It was explored whether patient sera should be treated appropriately according to local laboratory protocols when assessing DSA strength to ensure prozone effect inhibition. There was consensus regarding the use of ethylenediaminetetraacetic acid (EDTA) treatment (83.9% consensus) but not on serial dilutions (61.3%) or heat activation (45.2%) (**Supplementary Table S3**).

Despite these results, serial dilutions have been reported to help estimate true cPRA in HS candidates and in evaluating DSA strength. Furthermore, pretransplant serum dilutions can be used to determine unacceptable antigens, and the likelihood of successful HLA antibody reduction with desensitization [24].

Antibody specificities should be confirmed using a physical crossmatch assay to prevent considering non-relevant antibodies directed against denatured HLA as a risk. When discussing DSA strength in terms of MFI value, the following thresholds were used as guidance for the discussion: <3,000 – low; 3,000–5,000 – intermediate; 5,000–10,000 – high; and >10,000 – very high clinical significance and immunological risk.

Delisting unacceptable antigens that are considered lower risk allows transplant physicians to amend a patient's profile within reasonable limits, removing barriers to receiving a transplant despite immunological incompatibilities [38]. When delisting is permitted by the allocating organization, experts have recommended a stepwise approach to delisting as many unacceptable HLA antigens as deemed appropriate according to these parameters: a) start with delisting unacceptable HLA antigens with low-risk DSA (MFI values < 3,000, never crossmatch positive) and then proceed with delisting unacceptable HLA antigens for DSA with intermediate MFI values; b) avoid delisting unacceptable HLA antigens for repeated mismatches and for DSA with a historically positive crossmatch or C1q or C3d assay taking into account memory B cells; and c) take into consideration the additional contributing risk factors when assessing the antibody titers and potential post-transplant rebound risk (83.9% consensus) (**Supplementary Table S3**).

Donor–Recipient Profile

Organ Quality

Focusing on DD kidneys, experts advised selecting high-quality organs that are not at high risk of failure (no signs of severe acute tubular necrosis or acute kidney injury) unless there are pressing reasons to consider otherwise (77.8% consensus), and that organ quality and function be validated by the recipient transplant center administering imlifidase (88.9% consensus) (**Supplementary Table S4**).

For successful long-term transplant outcomes irrespective of the patient's degree of sensitization, it is critical to begin with good organ quality. A donor's kidney needs to have sufficient nephron mass to meet the increased and long-term metabolic demands and stress that a single kidney will incur in the recipient [39]. Kidneys at high delayed graft function risk and with a reduced functional reserve will have a more negative impact in this population of patients [40]. In addition, delayed graft function will also make rebound DSA and AMR assessment more complicated as no clinical parameters of renal function or laboratory values can be followed during this time period. Hence, assessment of kidney quality is critical at the time of transplantation, particularly in donors with suboptimal conditions (older age, uncertain medical history, pre-donation renal failure) [39].

Donor–Recipient Immunological Profile

As advised by experts, HLA polymorphism poses a significant risk in transplantation due to incompatible HLA profiles between recipient and donor (86.1% consensus) (**Supplementary Table S4**), and the greater the disparity in HLA the greater the risk of graft failure regardless of the presence of DSA prior to transplantation [41]. Experts also advised that the number of HLA mismatches should not be an exclusion factor for accepting a donor's kidney, provided there

is sufficient prior experience with HLAi transplants (86.1% consensus), although whenever possible it is advised to aim for fewer mismatches in younger recipients due to their potential need for future transplant(s) (86.1% consensus) (**Supplementary Table S4**).

Imlifidase Administration and Crossmatch Conversion

As mentioned, before Imlifidase administration, experts advised that donor–recipient immunological compatibility be assessed according to the local laboratory protocols and that at least one flow cytometric-crossmatch (FCXM) or a CDC-crossmatch (CDCXM) be performed paired with a fresh or recent (<6 weeks) SAB assay (83.9% consensus) (**Supplementary Table S5**).

Such data will provide more assurance around risk assessment and generate evidence to further support risk stratification and interpretation across patients. Experts advised that each center has pre-defined criteria for assessing FCXM as borderline positive, clearly positive or very positive. It is advised that HLAi KTx with *borderline positive FCXM* undergo transplantation with or without imlifidase, but post-transplant management with higher levels of immunosuppression compared with FCXM negative HLAi KTx; *clearly positive FCXM* be considered to be at high immunological risk and treated using imlifidase; *very positive FCXM* (positive CDCXM) be considered to be at very high immunological risk and either not proceed with the transplant or be treated with imlifidase, provided there are significant pressing reasons and prior experience with HLAi KTx (77.4% consensus) (**Supplementary Table S5**). This is consistent with the agreement reached by the ENGAGE Delphi consensus, where experts agreed that imlifidase could be considered as a desensitization strategy for DD KTx in patients with positive CDCXM or patients with positive FCXM at day 0 who have no other treatment options.[25of]

Provided there is sufficient time and donor/recipient cells, experts advised crossmatch conversion assessment via a physical crossmatch (CDCXM or FCXM), after a second dose of imlifidase according to local practice before proceeding with transplantation (82.7% consensus) (**Supplementary Table S5**).

In patients treated with imlifidase, CDCXM conversion from positive to negative should be confirmed before transplantation [23]. It should be noted that consensus was not reached on a second dose of imlifidase being administered within 24 h of the first dose if the crossmatch had not been converted (71% consensus) (**Supplementary Table S5**), despite this being within the product label [23].

Post-Transplant Management, Monitoring and Follow-Up of Imlifidase Patients

Experts recommended that patients be kept at the transplant center for as long as possible immediately following HLAi KTx to ensure close monitoring is conducted and optimal care is provided during the first 10–15 days (75% consensus), and that open communication channels be established between the hospital and transplant center (should they be separate) to ensure best practice protocols are in place for post-transplant management and emergency response (87.5% consensus) (**Supplementary Table S6**).

It is also advised that monitoring of kidney function, infections and overall clinical status of the patients post-transplantation be conducted in line with local and national guidelines (97.1% consensus) (**Supplementary Table S6**). Longer-term follow-up post-HLAi KTx is also advised, and patients should visit the transplant center at regular intervals following their transplant, preferably at least: twice a week for the first 1–2 months; twice a month for the following 3–4 months; once (stable patients) or twice a month (patients at higher risk of AMR) for the following 6 months; and once a year after this (87.1% consensus), although initially every 3 months may be more appropriate (**Supplementary Table S6**).

DSA Monitoring

Experts recommended close monitoring of DSA using an SAB assay to increase the likelihood of identifying DSA rebound (93.8% consensus) or antibody rebound (93.8% consensus) as close to the time of occurrence as possible (**Supplementary Table S6**). The aim is to ensure early identification of AMR and that treatment to prevent chronic AMR is initiated in a timely manner. It is recommended to assess DSA following the transplant on Days 3, 5, 7, and 10 (not if IVIG is given on Days 9 and 10); Months 1, 3 and 6; and then once a year (87.1% consensus) (**Supplementary Table S6**).

Experts also advised considering the potential interfering effect of IVIg on SAB assay results and adapting the frequency of DSA monitoring accordingly (81.3% consensus) (**Supplementary Table S6**).

Antimicrobial Prophylaxis

Experts advised that antimicrobial prophylaxis be provided to all patients prior to and following HLAi KTx, according to local protocols and individual patient risk factors (96.8% consensus), and that antimicrobial prophylaxis be maintained for at least 4 weeks post-imlifidase transplantation (77.4% consensus) (**Supplementary Table S6**).

It is also advised that all patients receive vaccination against infections such as influenza, pneumonia, and COVID-19 before imlifidase treatment, and at least 2 weeks apart from any cell-depleting therapy (100% consensus) (**Supplementary Table S6**). These strategies align with protection against infections that may occur because of the long-term immunosuppression that is required post-transplantation to prevent graft rejection. Imlifidase temporarily reduces IgG levels (hypogammaglobulinemia), and the most common infections associated with this are respiratory tract infections. Therefore, in addition to the standard antimicrobial prophylaxis in KTx (*Pneumocystis carinii*, cytomegalovirus and oral *candida*), imlifidase patients may require antimicrobials to treat respiratory tract pathogens [23]. Should a patient for any reason not be transplanted after receiving imlifidase treatment, prophylactic oral antimicrobials should still be given for 4 weeks [23].

Immunosuppressive Therapy

It is advised that the induction and maintenance IS protocol be tailored to the needs of HS patients (93.6% consensus), that steroids be used in all patients regardless of risk profile and that early withdrawal of steroids be avoided (94.5% consensus) (**Supplementary Table S6**).

It is advised that high doses of immunosuppression, preferably a triple-agent regimen (tacrolimus, mycophenolate and corticosteroid), be provided to all patients according to local protocols and their individual risk factor needs (94.4% consensus), and that calcineurin inhibitors (100% consensus) and IMDH inhibitors (e.g., MMF) be considered as part of the immunosuppression regimen according to standard of care (SoC) protocols (91.7% consensus) (**Supplementary Table S6**).

AMR Management

Should acute graft rejection occur, it may be T-cell-mediated rejection (TCMR), AMR or both [42]. Confirmation of AMR is provided by kidney biopsy and the presence of microvascular inflammation, an accumulation of inflammatory cells in the graft capillaries (glomerulitis and/or peritubular capillaritis ≥2), with or without the presence of deposits of the complement fraction C4d in the peritubular capillaries, and with circulating DSA against donor HLA antigens [42, 43]. In centers where molecular assessment is available its utilization to detect early stages of AMR, especially early after HLAi KTx, would be beneficial. Experts advised that plasmapheresis should be considered as part of the SoC protocols for AMR management and that the patient's individual risk factor should be assessed (93.8% consensus). Experts also advised that any arising immunological complications should be managed exclusively by the transplant center regardless of the time passed since the HLAi KTx (86.1% consensus) (**Supplementary Table S6**).

Experts advised that predetermined protocols for the treatment of AMR (91.7%) or TCMR (94.5% consensus), acute and chronic, should be well defined in advance and in place for Imlifidase KTx, according to national and local guidelines, to ensure an immediate clinical response can occur (**Supplementary Table S6**). Biopsies should be performed in time-critical circumstances and cases of severely impaired renal function and suspected AMR anti-rejection treatment should be initiated directly, prior to performing or receiving results from a biopsy (96.8% consensus) (**Supplementary Table S6**). Experts also advised that AMR management should follow local AMR protocols but be implemented earlier and with a more rapid stepwise approach, including earlier initiation of a complement inhibitor if needed. If AMR is still not appropriately managed, it is advised to consider alternative options such as splenectomy (87.1% consensus) (**Supplementary Table S6**) or targeting plasma cells in refractory patients.

CONCLUSION

HS patients in need of KTx spend a longer time waiting for compatible kidneys and are often unlikely to receive them. Imlifidase desensitization, which is more rapid and removes total body IgG to a greater extent than other methods, may offer a unique opportunity, especially for DD transplantation, to significantly reduce, albeit only transiently, the risk of hyperacute and accelerated graft rejection and may provide access to transplantation [14, 22, 23]. This Delphi consensus provides clinical practice guidance on Imlifidase use in the management of HS patients undergoing HLAi DD KTx and supports centers in the development of guidelines for imlifidase use and its integration into clinical practice (**Figure 2**). Due to the limited data available at the time of the development of this study and the subsequent uncertainty about the use of imlifidase for desensitization for KTx, increasing clinical experience will further refine the therapeutic guidelines.

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

All authors made substantial contributions to the conception and design of the work as well as the acquisition, analysis and interpretation of the data. All authors contributed to the drafting of the work and undertook critical review for important intellectual content and approved the final version to be published. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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CONFLICT OF INTEREST

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

GENERATIVE AI STATEMENT

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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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.2024.13886/full#supplementary-material

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ORIGINAL RESEARCH
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Only IF/TA in the Histological Evaluation of Post-Reperfusion Baseline Biopsies Correlates With Kidney Transplant Outcome

Quirin Bachmann[1], Carlos Torrez[1], Maike Büttner-Herold[2], Bernhard Haller[3], Flora Haberfellner[1], Renate Hausinger[1], Volker Assfalg[4], Lutz Renders[1], Kerstin Amann[2], Uwe Heemann[1], Christoph Schmaderer[1†] and Stephan Kemmner[1,5†]*

[1]Department of Nephrology, University Hospital Rechts der Isar, Technical University of Munich, TUM School of Medicine and Health, Munich, Germany, [2]Department of Nephropathology, Institute of Pathology, Friedrich-Alexander-University Erlangen-Nuremberg (FAU) and University Hospital, Erlangen, Germany, [3]Institute of AI and Informatics in Medicine, University Hospital Rechts der Isar, Technical University of Munich, TUM School of Medicine and Health, Munich, Germany, [4]Department of Surgery, University Hospital Rechts der Isar, Technical University of Munich, TUM School of Medicine and Health, Munich, Germany, [5]Transplant Center, University Hospital Munich, Ludwig-Maximilians-University (LMU), Munich, Germany

Here, we retrospectively evaluated the informational yield of 338 post-reperfusion kidney transplant biopsies (including 95 living donations) assessed according to BANFF for the histological characteristics interstitial fibrosis and tubular atrophy (IF/TA), glomerulosclerosis, arteriosclerosis, and acute tubular injury (ATI). Associations with delayed graft function (DGF) and death-censored graft survival were explored through Cox-regression analyses. The maximum follow-up time was 11.4 years, with DGF observed in 108 (32%) cases. After deceased donation there was no association between DGF and histologic parameters. Univariable Cox-regression unveiled an association of IF/TA and glomerulosclerosis with long-term death-censored graft survival (HR per 10% increase: IF/TA 1.63; 95% CI 1.17–2.28; p = 0.003; glomerulosclerosis 1.19; 95% CI 1.01–1.39; p = 0.031). In multivariable Cox regression analyses, adjusted for recognized clinical risk variables like expanded criteria donor-status, donor age, history of diabetes, and HLA-mismatches, only IF/TA maintained association over the total observation period in deceased donations and in the total cohort. Arteriosclerosis and ATI were not associated with clinical outcome after deceased donation. Especially ATI did not affect delayed graft function if only deceased donations were considered. Our data underlines the role of organ quality for transplant outcome prior to acute lesions such as ATI during the transplantation process.

Keywords: kidney transplantation, ischemia-reperfusion injury, delayed graft function, donor quality, interstitial fibrosis and tubular atrophy, glomerulosclerosis, arteriosclerosis, acute tubular injury

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***Correspondence**
Stephan Kemmner,
stephan.kemmner@tum.de

[†]These authors have contributed equally to this work

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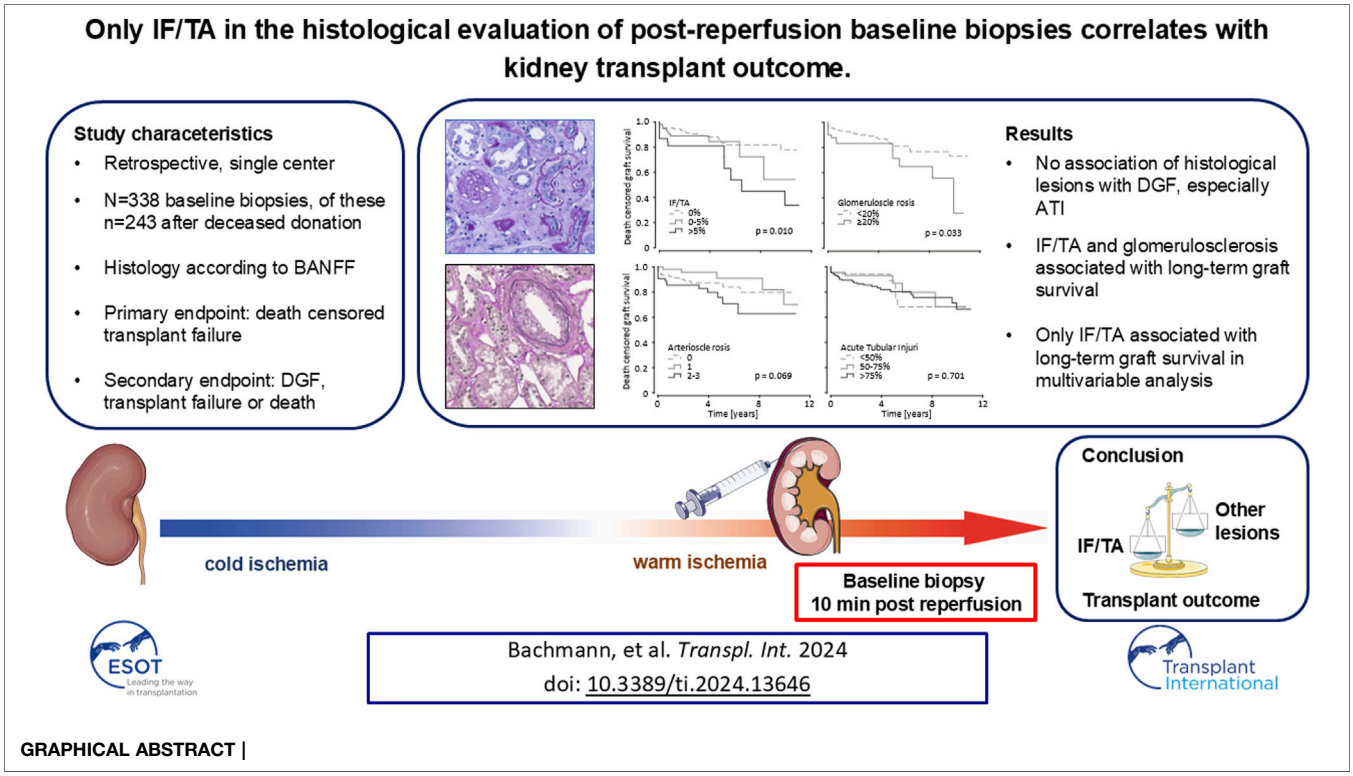
Abbreviations: ATI, acute tubular injury; BPR, biopsy proven rejection; DCD, donation after cardiac death; DBD, donation after brainstem death; DGF, delayed graft function; ECD, expanded criteria donor; eGFR, estimated glomerular filtration rate; HR, hazard ratio; HLA, human leukocyte antigen; IF/TA, interstitial fibrosis and tubular atrophy; KDPI, kidney donor profile index; OR, odds ratio; SCD, standard criteria donor; PNF; primary non-function; PRA, panel-reactive antibodies.



GRAPHICAL ABSTRACT |

INTRODUCTION

Kidney transplantation is the leading therapeutic option for patients with end-stage kidney disease. However, a persistent global challenge is the limited availability of donor kidneys, which fails to meet the increasing demand [1]. This discrepancy has led to an increased acceptance of kidneys from expanded criteria donors and a growing number of those allocated after rescue protocols [2–4]. However, it is noteworthy that over half of the kidneys harvested from donors aged 55 and above were not utilized in the United States as of 2021, underscoring the prevailing challenge [5, 6]. The evaluation of organ quality and the decision-making process regarding donor kidney acceptance or decline remain complex and controversial.

The utility of baseline biopsies in kidney transplantation remains unclear [7]. Baseline biopsies are routinely performed in various transplant centers at different time points, serving as valuable tools to assess graft quality and provide information about the donor kidney [8]. There are different types of baseline biopsies used in transplantation, each serving specific purposes. Procurement biopsies are employed to determine organ quality and inform decisions regarding kidney acceptance or rejection. Interestingly, procurement biopsy findings were the most common reason for discard in a retrospective analysis by Mohan et al., making them a critical factor in donor kidney allocation [9]. In contrast, pre-implantation biopsies are utilized as reference baseline biopsies for potential subsequent biopsies during the clinical follow-up. Reperfusion biopsies, taken intraoperatively after the reperfusion of the donor kidney, are also used as reference biopsies for clinical monitoring [10]. Evidence shows that punch biopsies compared to wedge biopsies are not only as save but yield higher numbers of diagnostically adequate samples according to the Banff criteria [11].

In this study, we focus on the histological finding of acute tubular injury ATI and chronic changes in post-reperfusion baseline biopsies and their potential association with long-term kidney transplant survival. We leverage a robustly characterized cohort, enabling a comparative analysis of the predictive fidelity of histologic indices against a backdrop of established clinical parameters. By elucidating these associations, we seek to compare the histological characterization of kidney grafts during the transplantation process with clinical outcome.

MATERIAL AND METHODS

Data Collection

This retrospective analysis evaluated all kidney transplantations performed between 1st January 2006, and 31st December 2016, at Klinikum rechts der Isar, Munich, Germany, both from deceased and living donors, in which a baseline biopsy was obtained during the transplant surgery via core-needle biopsy.

The analysis was approved by the local ethics committee of the Technical University of Munich, Germany (Approval No. 178/

21s). Exclusion criteria were age <18 years and transplant failure due to surgical complications. Given the context in Germany where non-heart-beating kidney donation is not allowed, all deceased donations in this cohort exclusively resulted from donation after brainstem death (DBD) and will be referred to as such.

Data collection was conducted using the hospital information system, patient records, routine clinical follow-up from external nephrologists, and the Eurotransplant Network Information System - ENIS for donor and recipient data. Patient follow-up extended until 30th June 2017, which served as the data lock point.

For the subsequent statistical analysis, recipients experiencing early graft failure due to perioperative complications, including surgical and non-immunological factors, were excluded from the study.

Endpoints

The primary endpoint of this study was death-censored transplant failure, which encompassed the permanent need for dialysis after transplantation. This includes cases of primary non-function, defined as the absence of initial allograft function with need for dialysis and without perioperative complications, confirmed by ultrasound examination showing adequate organ perfusion. Additionally, the primary endpoint also comprised cases of follow-up end-stage transplant failure, necessitating the reinstitution of dialysis. In the event of recipient death with a functioning graft, the follow-up period was censored at the date of death [12]. Patients were censored at the last day of reported kidney function during the follow-up examination within the follow up period. Primary analysis was performed including transplantations after deceased donation only. Secondary analysis included the total cohort with transplantations after deceased and living donation.

As a secondary endpoint, we considered non-death-censored transplant failure, which included a composite of primary non-function, follow-up end-stage transplant failure necessitating dialysis reinstitution, and recipient death with functioning graft.

Delayed graft function (DGF) was defined as proposed by the Organ Procurement and Transplantation Network - OPTN: need for dialysis during the first week after transplantation [13]. Recipients were subclassified whether they received an organ from standard criteria donors (SCD) or expanded criteria donors (ECD) according to the definition by Port et al. [14]. Thereby, ECDs are defined as donors who are either older than 60 years, or 50–59 years old and meet at least two of the following criteria: cerebrovascular death, history of hypertension, or last serum creatinine >1.5 mg/dL.

Histopathology

The baseline biopsies were routinely taken 10 min after the onset of graft reperfusion using a core needle (18G) biopsy, following the clinic's internal standard of care protocol to assess graft quality through baseline histology [15]. The samples were prepared as paraffin sections with a thickness ranging from 2 to 4 μm. These sections were then stained using hematoxylin and eosin as well as periodic acid–Schiff stains. Biopsy specimens were meticulously evaluated by an experienced renal pathologist (M.B.-H.), who remained blinded to the patients' clinical data. All specimens were presented at the same time to decrease intra observer variability.

The degree of interstitial fibrosis and tubular atrophy (IF/TA) was reported as a percentage, representing the proportion of the affected cortical area in the biopsy sample. Severity of arteriosclerosis was evaluated using a semi-quantitative scoring system (0–3) also based on the Banff classification [16]. Glomerulosclerosis, on the other hand, was expressed as a percentage of the total number of glomeruli observed in the biopsy. The scoring of ATI was carried out following previously described criteria [15]. The assessment involved the identification of specific histologic features, such as apical blebbing, epithelial hydropic swelling with cytoplasmic lucency, loss of brush border, luminal dilatation with flattening of the epithelium, cytoplasmic vacuolization, and sloughing of tubular cells. ATI was diagnosed whenever one or more of these features were observed, and the extent of ATI was categorized as "mild" (<50%), "moderate" (50%–75%), or "severe" (>75%) tubular injury, thus generating 3 groups of comparable size.

Statistics

Continuous data with a normal distribution are presented as mean ± standard deviation, while skewed data are summarized as median and interquartile range (IQR), represented by the first quartile to the third quartile. Categorical data are presented as absolute numbers (n) and percentages (%). Missing data was handled via available case analysis.

To compare baseline characteristics between different groups, Kruskal-Wallis and Mann-Whitney U tests were used for non-normally distributed data, univariable ANOVA and t-tests were used for normally distributed data, and chi-square (χ^2) tests were used for categorical data. For further analysis, patients were stratified according to transplantation type (living/deceased) and according to histological outcome. ATI and arteriosclerosis were divided in groups as described above. IF/TA was analyzed in 3 groups as well: 0%, >0–5% and >5%. Glomerulosclerosis was analyzed as 2 groups: <20% and ≥20%. eGFR was then compared between histological groups at certain time points and statistical significance was calculated using Mann-Whitney U and Kruskal-Wallis test where appropriate. Patients were not included into eGFR-analysis after transplant failure and death.

Spearman rank correlation was used for associations between metric and ordinal data, and the Chi-test was used for associations between ordinal and nominal scaled variables. To assess association between histological parameters they were included into a Spearmen correlation as continuous variables (amount of change as % area), since histological outcome is not normally distributed. Mann-Whitney U and Kruskal-Wallis tests were used to compare the amount of DGF between groups. Univariable and multivariable Cox proportional-hazards models were fitted to the stratified data as described above. The Cox proportional-hazards models included recipient and donor-associated risk factors which are known to be predictive for graft survival after kidney transplantation (**Table 3**). IF/TA

TABLE 1 | Demographic and clinical characteristics of donors and recipients in the total cohort and in kidney transplantations.

Characteristics	All	Living	Deceased	*p*-value
Number, n (%)	338 (100)	95	243	
Living donors, n (%)	95 (28)			
Donor				
Female, n (%)	154 (46)	55 (58)	99 (41)	0.004
Age (years)	53 ± 15	55 ± 11	52 ± 16	n.s.
BMI (kg/m^2)	27 ± 5	27 ± 4	27 ± 5	n.s.
Cause of death (n)				
- Trauma			55 (23)	
- CVA			143 (59)	
- Other			45 (31)	
History of				
- hypertension	136 (40)	36 (38)	100 (41)	n.s.
- diabetes	32 (10)	0 (0)	32 (13)	<0.001
Last SCr (mg/dL)	0.9 [0.7; 1.1]	0.8 [0.7; 0.9]	0.9 [0.7; 1.3]	0.010
ECD	139 (41)	32 (34)	107 (44)	n.s.
Process				
HLA-Mismatch	4 [3; 5]	4 [3; 5]	4 [3; 5]	n.s.
CIT (h)	8 [2; 13]	2 [2; 2]	11 [8; 15]	<0.001
Recipient				
Female, n (%)	122 (36)	35 (37)	87 (36)	n.s.
Age (years)	52 ± 13	47 ± 13	54 ± 12	<0.001
BMI (kg/m^2)	25 ± 5	25 ± 5	25 ± 5	n.s.
Caucasian	331 (98)	94 (99)	237 (98)	n.s.
First transplantation	282 (83)	86 (91)	196 (81)	0.028
Induction therapy	87 (26)	25 (26)	62 (26)	n.s.
Reason for ESKD				
- Glomerulonephritis	98 (29)	32 (34)	66 (27)	n.s.
- Diabetes	40 (12)	7 (7)	33 (14)	n.s.
- Hypertension	50 (15)	13 (14)	37 (15)	n.s.
- Other	150 (44)	43 (45)	107 (44)	n.s.
Dialysis vintage (months)	48 [18; 86]	3 [0; 17]	69 [38; 94]	<0.001
Immunosuppression				
- Glucocorticoids	337 (100)	95 (100)	243 (100)	n.s.
- Cni	337 (100)	95 (100)	243 (100)	n.s.
- Tacrolimus	265 (78)	89 (94)	176 (72)	<0.001
CCI	2 [2; 4]	2 [2; 3]	3 [2; 4]	0.004
kidney-pancreas transplantation	7 (2)	0 (0)	7 (3)	<0.001
Results				
Transplant failure				
- After 1 year	21 (6)	1 (1)	20 (8)	0.015 n.s.
- After 5 years	34 (10)	4 (4)	31 (13)	
- Maximum follow-up	48 (14)	7 (7)	41 (17)	0.024
Death with functioning transplant				
- After 1 year	12 (4)	1 (1)	11 (4)	0.012
- After 5 years	29 (9)	2 (2)	27 (11)	<0.001
- Maximum follow-up	38 (11)	4 (4)	34 (14)	0.001
Delayed graft function	108 (32)	12 (13)	96 (40)	<0.001
Primary non-function	12 (4)	1 (1)	11 (4)	n.s.
Patients with rejections after 1 year	93 (27)	32 (34)	61 (25)	n.s.
eGFR (ml/min/1,73 m^2)				
- After 1 year	44 (33; 60)	50 (39; 61)	42 (32; 59)	0.032
- After 3 years	46 (36; 61)	57 (42; 68)	42 (35; 60)	0.002
Histology				
Interstitial fibrosis and tubular atrophy				n.s.
0%		64 (67)	148 (61)	
0%–5%		23 (24)	55 (23)	
>5%		8 (8)	38 (16)	
Glomerulosclerosis				0.022
<20%		84 (89)	183 (79)	
≥20%		10 (11)	50 (24)	
Arteriosclerosis grade				0.045
0		39 (44)	95 (44)	
1		33 (38)	55 (26)	
2–3		16 (18)	64 (30)	

(Continued on following page)

TABLE 1 | (*Continued*) Demographic and clinical characteristics of donors and recipients in the total cohort and in kidney transplantations.

Characteristics	All	Living	Deceased	*p*-value
Acute tubular injury				<0.001
<50%		48 (51)	36 (15)	
50%–75%		27 (29)	47 (20)	
>75%		19 (20)	158 (66)	

n (%) for categorical data, mean ± standard deviation for normally distributed data, median [interquartile range] for skewed data. BMI, Body Mass Index; CCI, Charlson Comorbidity; CIT, cold ischemia time; eGFR, estimated glomerular filtration rate; ECD, expanded criteria donor; ESKD, end stage kidney disease; HLA, Human leukocyte antigen; SCr, Serum creatinine. Chi-squared test was used to compare frequencies, t-test was used to compare normally distributed metric data, Mann-Whitney-U-test was used to compare nominal and not normally distributed metric data.

and glomerulosclerosis were included as a continuous variable in the Cox proportional-hazards analysis. For time-to-event analysis, Kaplan-Meier analysis and log-rank tests were employed to compare 1-year and long-term death censored graft survival between the histologically stratified groups for deceased donation only and the total cohort. Additionally, a multivariable Cox proportional-hazard analysis including HLA-mismatches and panel reactive antibodies (PRA) was applied to assess immunological factors in comparison to the histological outcome. Since exact timepoints of biopsy proven rejections (BPR) were not available, Spearman correlation and not Cox-analysis was used to assess association between BPRs and immunological factors. All statistical tests were performed two-sided with a significance level (α) of 0.05.

Statistical analyses were carried out using "IBM SPSS Statistics" version 29 (IBM Corp., NY, United States) and "R" version 3.4.4 (R development team, Vienna, Austria). For data visualization Adobe Illustrator, version 26.5 was utilized.

RESULTS

Patients

A total of 338 kidney transplantations from living and deceased donors with baseline biopsies fulfilled the inclusion criteria for our analysis. Detailed baseline demographics are presented in **Table 1**.

The median follow-up time for recipients at the time of data extraction from the clinical follow-up database was 3.4 (0.0–11.4) years. During observation, three patients were lost to follow-up and censored: one patient after deceased donation after 54 days and two patients after living donation (after 342 and 428 days). Patients without event were censored after follow-up.

Transplant Outcomes

In the study, primary non-function (PNF) was observed in 12 (4%) of the transplantations, while DGF was experienced in 108 (32%) of the transplantations.

The median estimated glomerular filtration rate (eGFR) at various post-transplantation intervals was assessed, registering 43 [32; 54] mL/min/1.73 m^2 at 3 months, 44 [34; 60] mL/min/1.73 m^2 at 1 year, and escalating to 46 [36; 61] mL/min/1.73 m^2 at the 3-year mark. Living donations presented an eGFR of 48 [36; 57] mL/min/1.73 m^2 after 3 months, significantly higher than the 40 [30; 53] mL/min/1.73 m^2 recorded for deceased donations (p = 0.017). This trend persisted, with living donations registering 50 [39; 61] mL/min/1.73 m^2 after 1 year and 57 [42; 68] mL/min/1.73 m^2 after 3 years, compared to 42 [32; 59] mL/min/1.73 m^2 at 1 year and 42 [34; 60] mL/min/1.73 m^2 and 3 years for deceased donations (**Table 1**).

To discern the interrelationships between histological parameters, a nonparametric correlation analysis was employed. A noteworthy observation was the minimal yet significant association between ATI and IF/TA (r = 0.11; p = 0.042). In alignment with anticipatory postulations, pronounced correlations were evident between IF/TA and glomerulosclerosis (r = 0.44; $p < 0.001$) and between IF/TA and arteriosclerosis (r = 0.25; $p < 0.001$). Moreover, a small but significant association was delineated between glomerulosclerosis and arteriosclerosis (r = 0.15; p = 0.012).

Intriguingly, a comparative evaluation between living and deceased donations revealed no significant disparities in the prevalence of IF/TA although there was a trend towards better outcomes after living donation. Glomerulosclerosis and arteriosclerosis proved to be of lower level in living donation as well. Greatest differences were observed in the incidence of ATI, which was conspicuously elevated in deceased donations ($p < 0.001$), suggesting a potential implication of the donation and preservation process on acute renal histological manifestations.

Predictive Value of Baseline Biopsies

When analyzing transplantations after deceased donations only, IF/TA, glomerulosclerosis and arteriosclerosis did not significantly impact the amount of DGF, although a trend towards higher rates of DGF with increasing histological damage was visible (**Figure 1A**). Surprisingly, there also was no association between ATI and DGF. Only after inclusion of living donations into the analysis, higher grades of ATI caused more DGF (**Supplementary Figure S1A**). Though this surely only corresponds to the procedural differences between living and deceased donations.

In this cohort, there was no difference in death censored graft survival between transplants with and without DGF after 1 year. After the full observation period transplants without DGF had a significantly better survival (p = 0.045; **Supplementary Figure S2A**), suggesting an influence only on long-term graft survival.

ATI as well as IF/TA, glomerulosclerosis and arteriosclerosis did not influence 1-year eGFR after deceased donation. When

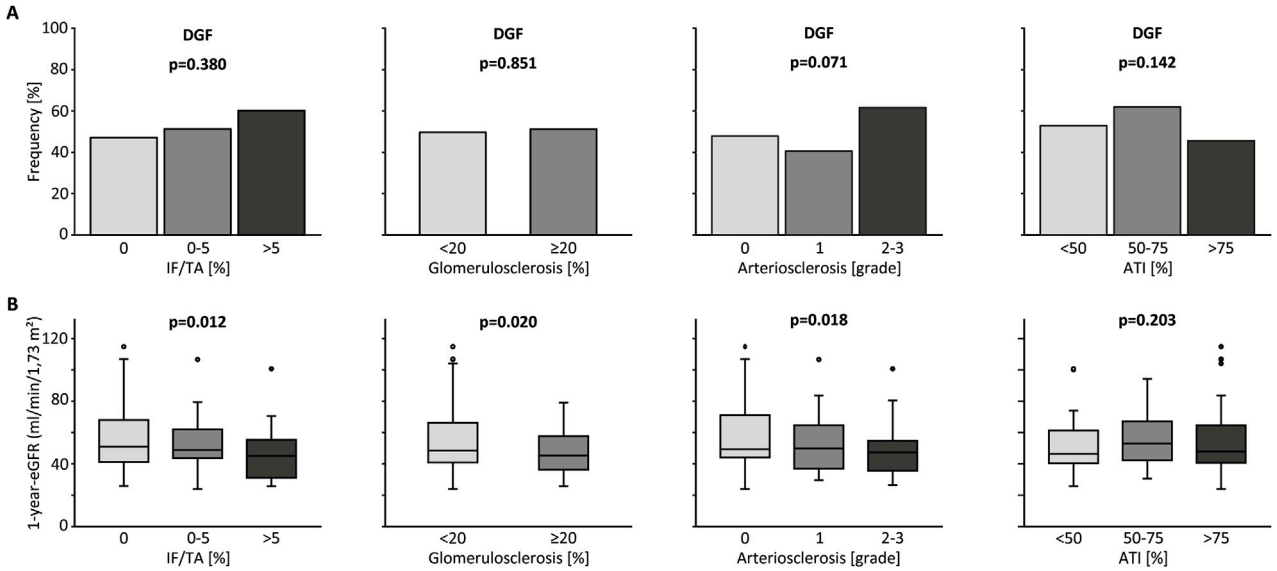


FIGURE 1 | Kidney graft tissue was taken 10 min after reperfusion by 18G core needle biopsy. Histological evaluation was performed by one experienced renal pathologist. A semi-quantitative score according to the Banff Classification was used to assess arteriosclerosis (AS). Interstitial fibrosis and tubular atrophy (IF/TA), glomerulosclerosis (GS), and acute tubular injury (ATI) are shown as percentage of the entire area used for histological investigation. Only data from transplantation after deceased donation is included in this analysis. **(A)** Percent stacked column chart of the amount of delayed graft function (DGF) for different amounts of IF/TA, GS, AS, and ATI. **(B)** Boxplots of the eGFR or kidney transplant recipients after living and deceased donation 1 year after transplantation. Groups are divided by histological categories as in **(A)**. Chi-squared test was used to compare categorial data. Kruskal-Wallis test was used for comparison of >2 groups with metric variables. Mann-Whitney U test was used for comparison of 2 groups with metric variables.

including living donations, these results were no different, except ATI proving to show an association with eGFR, again likely caused by the procedural factors (**Figure 1B**; **Supplementary Figure S1B**). No associations between any histological parameters and proteinuria (mg/g creatinine), which was recorded up to 5 years after transplantation were found for deceased donations.

For Kaplan-Meier analysis, ATI and arteriosclerosis were divided into groups as described above. IF/TA was analyzed in 3 groups as well: 0%, >0–5% and >5%. Glomerulosclerosis was analyzed as 2 groups: <20% and ≥20%. With lower degree of IF/TA short-term (1 year) and long-term (full follow-up period) death censored graft survival improved as shown in Kaplan-Meier analysis in deceased donations as well as living and deceased donations together. The same was observed for glomerulosclerosis. Arteriosclerosis only influenced short-term graft survival after deceased donation. ATI did not have any relevant influence on death censored graft survival for short- and long-term observation for deceased donations and the total cohort (**Figure 2**; **Supplementary Figure S3**). This was also the case, if only kidneys from ECD-donors were taken into consideration (**Supplementary Figure S2B**).

In univariable Cox proportional hazard analysis of transplantations after deceased donation, IF/TA showed a higher association than glomerulosclerosis with long-term graft survival (IF/TA: HR per 10% increase 1.63; 95% CI 1.17–2.28; p = 0.003; glomerulosclerosis: HR per 10% increase 1.19; 95% CI 1.01–1.39; p = 0.031). For short-time graft survival only IF/TA proved an association (IF/TA: HR per 10% increase 1.70; 95% CI 1.10–2.62; p = 0.016). ATI in baseline biopsies does not appear to be associated in univariable Cox proportional hazard models (**Table 2**). Arteriosclerosis grades 2 and 3 combined showed a significant HR compared with lower grades of arteriosclerosis only for long-term death censored graft survival after including living donations as well (HR 2.10; 95% CI 1.03–4.25; p = 0.040). IF/TA and glomerulosclerosis were also significantly associated with death censored graft survival when including all transplantations (**Supplementary Table S1**). **Table 3** shows the hazard ratio for previously identified factors influencing kidney transplantation outcomes for long- and short-term death-censored graft survival.

In multivariable Cox regression models that included ECD-status, history of diabetes, number of human leukocyte antigen (HLA)-mismatches, or recipient age, none of the tested histological parameters showed a significant association with 1-year death-censored graft survival when including only deceased donations or all transplantations (**Table 4**; **Supplementary Table S2**, data for ATI and arteriosclerosis not shown). However, in a model focused on immunological co-variates with the number of HLA-mismatches, percentage of panel reactive antibodies, and ECD-status, IF/TA was significantly associated with long-term death-censored graft survival in deceased donations and the total cohort (deceased donation: HR 1.05; 95% CI 1.01–1.09; p = 0.007). IF/TA was also associated with long-term death-censored graft survival in models that included ECD-status, donor history of diabetes, and recipient age or number of HLA-mismatches in deceased donations (model including recipient age: HR 1.04; 95% CI

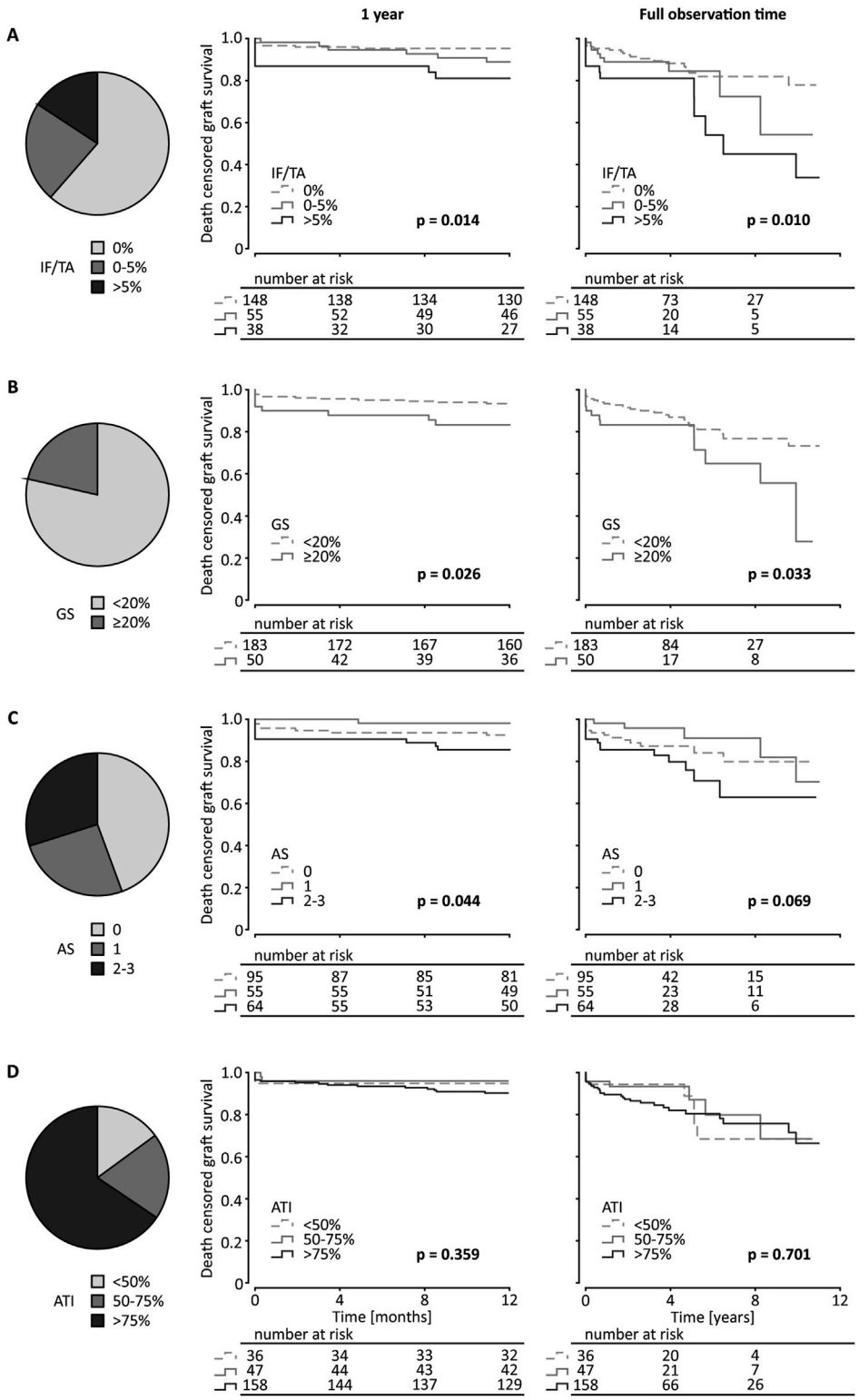


FIGURE 2 | Kidney graft tissue was taken 10 min after reperfusion by 18G core needle biopsy. Histological evaluation was performed by one experienced renal pathologist. **(A)** semi-quantitative score according to the Banff Classification was used to assess arteriosclerosis (AS). Interstitial fibrosis and tubular atrophy (IF/TA), glomerulosclerosis (GS), and acute tubular injury (ATI) are shown as percentage of the entire area used for histological investigation. Only data from transplantation after deceased donation is included in this analysis. **(A)** Pie chart of the distribution of IF/TA in 3 categories (0%, 0%–5%, >5%). Kaplan-Meier estimates for short (1 year) and long term (11.4 years) death censored graft survival for the 3 IF/TA categories. **(B)** Pie chart of the distribution of GS in 2 categories (<20%, ≥20%) and Kaplan-Meier

(*Continued*)

FIGURE 2 | estimates for short- and long-term death censored graft survival for the 2 categories of GS. **(C)** Pie chart of the distribution of AS in 3 categories (grades 0, 1, 2–3) and Kaplan-Meier estimates for short- and long-term death censored graft survival for the 3 categories of arteriosclerosis. **(D)** Pie chart of the distribution of ATI in 3 categories (<50%, 50%–75%, >75%) and Kaplan-Meier estimates for short- and long-term death censored graft survival for the 3 ATI categories. Log-rank testing was used for calculation of each *p*-value.

TABLE 2 | Univariable Cox proportional hazards models for 1-year and total-observation time for death censored graft survival of deceased donations with hazard ratios (HR) and 95% confidence intervals (CI) for post-reperfusion biopsy outcomes.

	1 year	*p*-value	Total observation time	*p*-value
IF/TA				
per 10% increase	1.70 (1.10–2.62)	**0.016**	1.63 (1.17–2.28)	**0.003**
Glomerulosclerosis				
per 10% increase	1.23 (0.98–1.56)	0.076	1.19 (1.01–1.39)	**0.031**
Arteriosclerosis				
grade 0	Reference		Reference	
grade 1	0.24 (0.03–1.96)	0.183	0.64 (0.23–1.79)	0.391
grades 2 + 3	1.98 (0.74–5.32)	0.175	1.85 (0.87–3.96)	0.112
ATN				
0%–50%	Reference		Reference	
51%–75%	0.76 (0.11–5.38)	0.781	0.76 (0.27–2.60)	0.757
76%–100%	1.86 (0.43–8.08)	0.409	1.19 (0.50–2.88)	0.692

p-*values <0.05 are highlighted in bold.*

TABLE 3 | Univariable Cox proportional hazards models for 1-year and total-observation time for death censored graft survival with hazard ratios (HR) and 95% confidence intervals (CI) for donor, recipient and transplant associated factors.

	1 year	p-value	Total observation time	*p*-value
Donor associated				
Age	1.07 (1.03–1.11)	<**0.001**	1.05 (1.02–1.07)	<**0.001**
Gender (f)	0.88 (0.37–2.08)	0.766	0.90 (0.51–1.60)	0.729
BMI	1.03 (0.95–1.12)	0.440	1.01 (0.95–1.06)	0.853
ECD	4.74 (1.74–12.93)	**0.002**	3.24 (1.79–5.87)	<**0.001**
History of				
- hypertension	3.48 (1.34–9.05)	**0.011**	2.62 (1.45–4.75)	**0.012**
- diabetes	3.50 (1.26–9.69)	**0.016**	3.86 (1.95–7.65)	<**0.001**
- smoking	0.40 (0.13–1.19)	0.099	0.46 (0.22–0.97)	**0.042**
Cause of death: CVA	2.85 (0.96–3.47)	**0.041**	1.91 (0.98–3.76)	0.059
last SCr	0.82 (0.49–8.60)	0.059	0.76 (0.42–1.20)	0.353
Recipient associated				
Age	1.06 (1.02–1.11)	**0.006**	1.03 (1.01–1.06)	**0.013**
BMI	1.06 (0.98–1.15)	0.158	1.07 (1.01–1.13)	**0.030**
Gender (f)	0.40 (0.14–1.20)	0.102	0.95 (0.53–1.71)	0.864
CCI	1.05 (0.72–1.52)	0.801	1.00 (0.78–1.29)	0.978
Reason for ESKD				
- glomerulonephritis	0.95 (0.37–2.45)	0.913	0.99 (0.53–1.85)	0.976
- diabetes	0.79 (0.18–3.37)	0.745	0.87 (0.34–2.19)	0.759
- hypertension	0.29 (0.04–2.13)	0.222	0.64 (0.25–1.61)	0.340
Duration of dialysis	1.01 (1.00–1.02)	0.320	1.01 (1.00–1.01)	0.086
Transplant associated				
Donation type deceased	8.07 (1.08–60.14)	**0.042**	2.05 (0.92–4.58)	0.081
CIT	1.04 (0.97–1.11)	0.270	1.02 (0.98–1.07)	0.260
Number of HLA-mismatches	1.71 (1.19–2.45)	**0.004**	1.35 (1.08–1.69)	**0.008**
PRA	1.01 (1.00–1.02)	0.230	1.01 (1.00–1.02)	**0.004**
DGF	1.65 (0.42–6.13)	0.458	1.91 (1.41–2.61)	<**0.001**
Number of BPR in first year	2.19 (1.55–3.10)	<**0.001**	1.80 (1.48–2.19)	<**0.001**
Number of all BPR			0.74 (0.49–1.11)	0.149

BMI, Body Mass Index; BPR, biopsy-proven rejection; CCI, Charlson Comorbidity Index; CIT, cold ischemia time; CVA, cerebro-vascular accident; DGF, delayed graft function; eGFR, estimated glomerular filtration rate; ECD, expanded criteria donor; ESKD, end stage kidney disease; HLA, Human leukocyte antigen; PRA, panel-reactive antibody; SCr, Serum creatinine; TX, transplantation.

p-*values <0.05 are highlighted in bold.*

TABLE 4 | Multivariable Cox-regression model for 1-year and total-observation time for death censored graft survival of deceased donations with hazard ratios (HR) and 95% confidence intervals (CI) including prognostic factors for reduced graft survival.

Variables	1 year		1 year		max. follow-up		max. follow-up		max. follow-up	
	Model 1		Model 2		Model 1		Model 2		Model 3	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
IF/TA	1.05 (0.99–1.11)	0.081	1.03 (0.99–1.09)	0.171	1.04 (1.01–1.08)	**0.022**	1.04 (1.01–1.08)	**0.023**	1.05 (1.01–1.09)	**0.007**
ECD	2.31 (0.72–7.34)	0.158	2.82 (0.82–9.72)	0.101	2.97 (1.41–6.29)	**0.004**	3.76 (1.72–8.23)	**<0.001**	3.03 (1.51–6.01)	**0.002**
Number HLA-miss matches	2.15 (1.35–3.43)	**0.001**			1.30 (0.99–1.72)	0.061			1.28 (0.98–1.67)	0.072
PRA									1.01 (1.00–1.02)	**0.005**
Recipient age			1.03 (0.98–1.09)	0.246			1.00 (0.97–1.03)	0.873		
h.o. diabetes	1.42 (0.48–4.12)	0.526	1.71 (0.69–4.87)	0.319	2.42 (1.18–5.00)	**0.016**	2.53 (1.23–5.20)	**0.012**		
Glomerulo-sclerosis					1.01 (0.99–1.03)	0.432	1.01 (0.99–1.03)	0.349	1.01 (1.00–1.03)	0.141
ECD					2.80 (1.32–5.95)	**0.007**	3.26 (1.50–7.01)	**0.003**	2.84 (1.41–5.72)	**0.001**
Number HLA-miss matches					1.28 (0.97–1.68)	0.086			1.24 (0.96–1.62)	0.101
PRA									1.01 (1.00–1.02)	**0.005**
Recipient age							1.00 (0.97–1.03)	0.851		
h.o. diabetes					2.56 (1.24–5.30)	**0.011**	2.66 (1.28–5.50)	**0.009**		

Models of 1 year graft survival including Glomerulosclerosis were neglected since univariable analysis showed no association. IF/TA, Interstitial fibrosis and tubular atrophy; HLA, Human leukocyte antigen; PRA, panel-reactive antibody.
p-*values <0.05 are highlighted in bold.*

1.01–1.08; $p = 0.023$; model including HLA-mismatches: HR 1.04; 95%-CI 1.01–1.08; $p = 0.022$) as well as the total cohort. Glomerulosclerosis did not prove to be prognostic for long-term graft survival in any of the above-described models (**Table 4**; **Supplementary Table S2**).

No influence of IF/TA, glomerulosclerosis, arteriosclerosis, or ATI on the appearance of the first BPR was revealed by univariable Cox proportional hazard analysis (**Supplementary Table S3**).

Influence of Immunological Parameters

As expected, the number of biopsy proven rejections during the first year after transplantation was highly associated with 1-year and long-term death censored graft survival (1 year: HR 2.19; 95% CI 1.55–3.08; $p < 0.001$; long-term: HR 1.80; 95% CI 1.48–2.19; $p < 0.001$). Interestingly, there was only a weak association between the number of HLA-mismatches and the number of biopsy proven rejections during the first year (r = 0.11; $p = 0.042$) which also persisted when including deceased donations only (r = 0–15; $p = 0.024$), and no association between percentage of PRA and BPR during the first year (r = 0.04; $p = 0.51$).

In a multivariable Cox-regression analysis with these 3 parameters, the number of BPR during the first year after transplantation and the number of HLA-mismatches were independently associated with 1-year death censored graft survival. In the same model, all 3 parameters were independently associated with long-term graft survival (**Supplementary Table S4**).

DISCUSSION

In this single-center retrospective study, we assessed the predictive value of post-transplant protocol biopsies conducted 10 min after onset of reperfusion, a standard practice in our transplant center. We leveraged a well-characterized cohort of kidney transplant recipients from both living and deceased donors. This allowed us to evaluate the relevance of histological findings against established clinical parameters encompassing donor and transplant characteristics, as well as immunological factors.

Studies investigating the influence of histological lesions in baseline biopsies on transplant success, DGF, and renal function have yielded heterogeneous results. A retrospective analysis found no association between ATI and DGF, acute rejection and graft survival in reperfusion biopsies [17]. Contrarily, increased risk for DGF was reported in donation after cardiac death (DCD) in grafts with reported ATI compared to no ATI [18]. Other data suggests reduced graft and recipient survival in severe chronic allograft injury in pre-transplant biopsies [19]. In another retrospective study glomerulosclerosis was the only histologic parameter associated with 5-year kidney allograft outcomes but did not outperform clinical parameters [20].

Complicating this narrative is the contentious backdrop of procurement biopsies. The intrinsic procedural demands accentuate cold ischemia times, with attendant augmentation in hemorrhage risks, as evidenced in a Portuguese study [21]. The interpretive acumen of histological assessments is also dependent

on the expertise of the evaluating pathologist, with specialized pathologists delivering enhanced diagnostic insights compared to general pathologists [22].

In our study, all reviewed chronic parameters (IF/TA, glomerulosclerosis, arteriosclerosis) proved to have some association with death-censored graft survival in Kaplan-Meier analysis for short- and long-term observation in deceased donations only as well as the whole cohort. ATI solely did not offer any information about transplant survival. Opposing previous opinions, although arteriosclerosis showed a slight yet statistically significant association on renal transplant survival, it failed to achieve statistical significance when evaluated using Cox-regression, casting doubt on its actual influence [23, 24]. Given the intrinsic association between histological changes, we prioritized analysis of the correlation of each parameter individually rather than using a composite score.

The absence of an association between ATI and graft survival, corroborated by prior literature, challenges the notion that targeting ATI might enhance graft quality [2, 18, 25]. While ATI undeniably plays a pivotal role in DGF, and DGF is a recognized independent predictor of graft survival, our findings suggest that DGF's impact operates independently of ATI, a finding recently confirmed by Wang et al. [26]. Instead, the repercussions of DGF may be more strongly influenced by organ quality metrics such as IF/TA and glomerulosclerosis. These metrics may heighten the graft's vulnerability to ischemia-reperfusion injury. Ischemia-reperfusion injury, a principal driver for ATI, has been extensively researched in mouse models over recent years, primarily to identify therapeutic targets that bolster graft survival. However, none of these proposed targets have achieved clinical relevance so far [15, 27, 28]. Some authors argue in favor of interventions, especially for kidneys from marginal donors, to ameliorate ATI. Yet, our data does not support this perspective, particularly as ATI also did not correlate with graft survival even in ECD-grafts alone [29, 30].

Regardless of accumulating evidence challenging the utility of preimplantation biopsy findings, particularly due to the questionable predictive value from on-call pathologists lacking specialized renal pathology training, biopsy results still stand as the predominant reason for organ discard [31–33]. Our observation that only IF/TA demonstrates an association with long-term graft survival after adjusting for clinical parameters, in deceased donations only as well as the whole cohort, necessitates a strict reevaluation of the routinely employed procurement biopsies. Advocates for procurement or post-transplantation protocol biopsies often emphasize their potential in enabling personalized patient care, such as tailoring immunosuppression [34]. Indeed, the standalone association of IF/TA with long-term graft survival, coupled with the association with DGF based on conventional biopsy parameters, could bolster this argument. However, our data did not indicate a correlation between biopsy results and the occurrence of rejections. Consequently, the tangible additional insight offered by the biopsy appears limited. It's conceivable that the inclusion of further immunologic histological parameters could enhance its value.

Our data confirmed the significance of established predictors for transplant survival after living and deceased donation. Next to donor history of diabetes, especially immunological parameters, meaning HLA-mismatches, PRA, and the number of biopsy proven rejections in the first year after transplantation proved to be strong and reliably associated with death censored graft survival in our cohort. Nonetheless, existing composite scores of these parameters fail to attain a concordance statistic above 0.7 [35, 36]. The mounting evidence favoring superior survival post-transplantation, compared to dialysis—even with organs deemed unsuitable for transplant, such as those labeled by the SCD/ECD classification—calls for strategies to avoid discarding potentially viable organs, particularly those of better quality [37–39]. In line with this, we found comparable 5-year graft and patient survival between standard and rescue allocation within our cohort which was previously published [40]. While histology provides valuable insights into organ quality without necessarily outperforming other parameters, we suggest that procurement biopsies could be particularly beneficial for organs typically overlooked. This notion warrants further exploration, as current guidelines for decision-making in this context are lacking.

This study warrants several critical discussions. We analyzed a single-center cohort comprising a moderate sample size, which included kidney transplants from both deceased and living donors. Without access to comparable data from other centers it what not possible to validate our findings against a different background. The differential selection processes and the potential variability in data availability between living and deceased donations may result in more detailed information for living donations. To reduce histological bias, the pathologist was entirely blinded to all patient-specific details. However, potential personal biases and biases by intra-observer variability might arise given that a single pathologist graded all biopsy samples. To decrease intra-observer variability the biopsies were not graded at the time of transplantation but at a single time point after collection of all samples. Previous data revealed insufficient diagnostic validity in histology performed by general pathologists, thus a highly specialized and experienced renal pathologist participated in this analysis [31]. The study's follow-up lacked data on a substantial number of patients at the endpoints, possibly introducing a selection bias towards patients who were more adherent to their treatment regimens. Moreover, the inherent limitations of a retrospective design mean our study cannot achieve the rigor of a prospective observational study.

In conclusion, our findings support the persistent utility of established clinical and donor characteristics as primary predictors of kidney graft survival, with histological parameters playing a supplemental role [41]. Our findings indicate that while histological markers, specifically IF/TA, are associated with transplant outcomes, they do not surpass the predictive ability of established clinical indicators.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because they were created for this retrospective analysis, thus we regard it as our intellectual property. It will be made available by the authors upon reasonable request. Requests to access the datasets should be directed to the corresponding author.

ETHICS STATEMENT

The studies involving humans were approved by Ethics Committee of the Technical University of Munich, Germany. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SK initiated the realization of the retrospective study and wrote the ethics proposal. QB, CT, FH, and SK collected clinical data. QB and CT performed statistical analysis. BH and SK reviewed and discussed statistical analysis. MB-H analyzed the biopsies. VA is responsible for the routinely performance of surgical biopsies during the transplant process. QB wrote the first draft of the manuscript. SK edited the first draft of the manuscript. MB-H, BH, VA, RH, KA, LR, UH, and CS oversaw the study and critically discussed the manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

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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.2024.13646/full#supplementary-material

DATA SHEET 1 | Supplemental tables and supplemental figure legends.

IMAGE 1 | Supplemental figure 1.

IMAGE 2 | Supplemental figure 2.

IMAGE 3 | Supplemental figure 3.

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ORIGINAL RESEARCH
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Endovascular Preparation With Innovative Custom-Made Stent-Graft Before Kidney Transplantation: The Solution for Patients With Hostile Iliac Calcification

Matthieu Arsicot[1,2], Raffaele Pio Ammollo[1], Marine Bordet[1,3], Leila Dehina-Khenniche[1], Olivier Thaunat[4], Nellie Della Schiava[1,3], Antoine Millon[1], Emilien Seizilles De Mazancourt[5], Lionel Badet[6] and Xavier Matillon[6]*

[1]Service de Chirurgie Vasculaire et Endovasculaire, Hospices Civils de Lyon, Lyon, France, [2]Centre de Référence des Infections Vasculaire Complexes (CRIVasc Network), Hospices Civils de Lyon, Lyon, France, [3]Laboratoire de Genie Electrique et Ferroélectrique (LGEF), Institut National des Sciences Applicquées (INSA)-Lyon, University Lyon, Villeurbanne, France, [4]Service de Transplantation, Néphrologie et Immunologie Clinique, Hôpital Edouard Herriot, Hospices Civils de Lyon, Lyon, France, [5]Service d'Urologie, Hôpital Saint Louis, Paris, France, [6]Service d'Urologie et de Transplantation, Hospices Civils de Lyon, Lyon, France

The increasing age of patients with end-stage renal disease raises the issue of hostile arterial access for transplantation, with technical difficulties associated with clamping and suturing the iliac artery. Some of these patients - who theoretically represent those who would benefit the most from transplantation in terms of mortality - are contraindicated because of anatomical and medical issues. In this context, a specific endovascular device called EndoPreKiT (Endovascular Preparation for Kidney Transplantation) has been designed, enabling arterial access for transplantation via a mini-invasive procedure. It consists of a woven Dacron supported by self-expanding nitinol rings, ensuring anchorage and allowing arterial clamping. The middle part of the anterior face of the device is stentless, enabling the anastomosis directly onto the Dacron once the calcified artery wall has been removed. After a cadaveric study validating its technical feasibility, such device was successfully implanted in 10 patients considered unfit for transplantation due to severe wall calcification. Two of them have been successfully transplanted with excellent outcomes after 13 and 3 months of follow-up. EndoPreKiT device may be a significant breakthrough in transplant surgery, that could expand the horizon of eligibility to include even the most fragile patients with challenging arterial access.

Keywords: atherosclerosis, graft survival, kidney transplantation, vascular calcification, vascular surgical procedures, iliac endoprothesis, endovascular procedures

***Correspondence**
Matthieu Arsicot,
matthieu.arsicot@chu-lyon.fr

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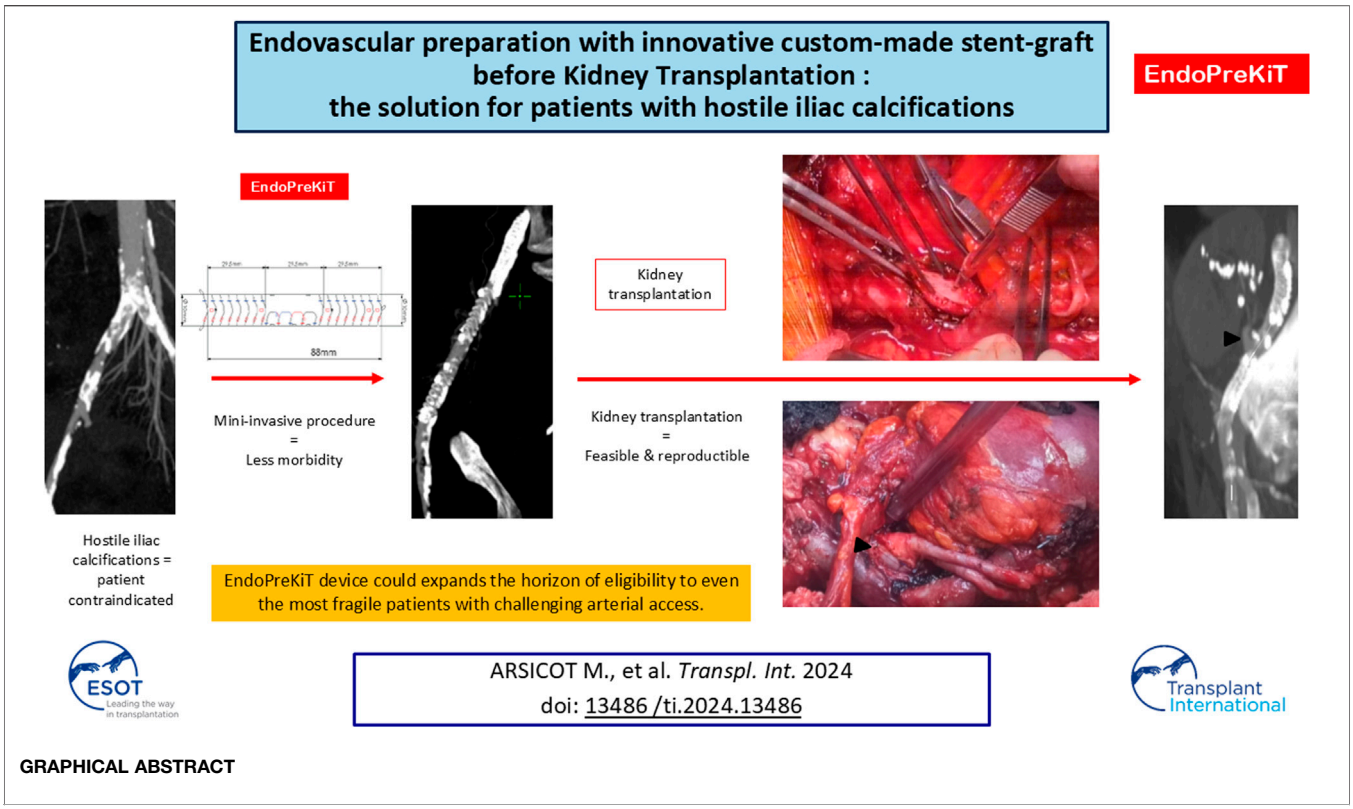
INTRODUCTION

The increasing age of patients with end-stage renal disease (ESRD) raises the issue of hostile arterial access for transplantation, since the perturbations of phosphocalcic metabolism associated with chronic renal disease are a key factor favouring mediacalcosis [1]. Because of the technical difficulties involved in clamping and suturing the iliac artery, many patients are contraindicated for Kidney Transplantation (KT) [2, 3].



GRAPHICAL ABSTRACT

In such cases, an aorto-iliac bypass may be offered, providing a space for clamping and anastomosis at the time of subsequent KT. However, the morbi-mortality associated with this procedure is substantial (estimated 10%–30% [4]) in these high-risk cardiovascular patients [5, 6]. Moreover, KT is usually made more difficult in case of previous abdominal surgery. However, these high-risk cardiovascular patients who are considered unfit for transplantation, are precisely those who would benefit most from such intervention in terms of survival [7]. In this context, a specific endovascular device has been designed to prepare the arterial access for kidney transplantation using a minimally invasive endovascular procedure.

This paper aims to delineate the development of this innovative device, from its conceptualization to rigorous validation via cadaveric studies. Furthermore, we offer insights garnered from our initial clinical endeavours, encompassing the implantation of the device in ten patients previously deemed unsuitable for transplantation, and the inaugural kidney transplantation in utilising the device in two of them.

MATERIALS AND METHODS

EndoPreKiT Device

The Anaconda endoprosthesis, produced by Terumo Aortic is a custom-made device (**Figure 1**). Its use is currently approved for use in aortic and iliac artery aneurysmal disease. Under the regulations of tailor-made Medical Devices, EndoPreKiT is a custom made device produced by Terumo Aortic at the surgeon's request on the basis of the patient's specific anatomical criteria and for a single and precise indication, that is the vessel preparation for kidney transplantation in case of severe iliac atherosclerotic occlusive disease. It consists of a woven Dacron supported by self-expanding nitinol rings, which seal the device proximally and distally (**Figures 1.1, 1.3**) to have sealing and clamping. In the EndoPreKiT module, the middle segment (**Figure 1.2**) is lined with half stents in the posterior wall only to avoid the collapse and thrombosis of the graft; the stent-less anterior wall allows graft anastomosis directly on the Dacron, after removal of the calcified artery wall.

The manufacturer allows the combination of different proximal and distal diameters, with a minimum total length of 88 mm. The endoprosthesis is designed for deployment in the external iliac artery without internal iliac artery coverage, but it can be placed more proximally depending on the patient's anatomy. Several radiopaque markers were placed on the device to ensure deployment in the correct orientation, in which the platform receiving the graft anastomosis is anterior (**Figure 1**). These markers will also allow fluoroscopic detection of the anastomosis site during KT.

Proof of Concept: Cadaveric Study

A preliminary study on human cadaver was carried out to verify the technical feasibility of endovascular implantation as well as the suturability of the device, in an anatomical dissection laboratory equipped with a C-arm on a human female cadaver

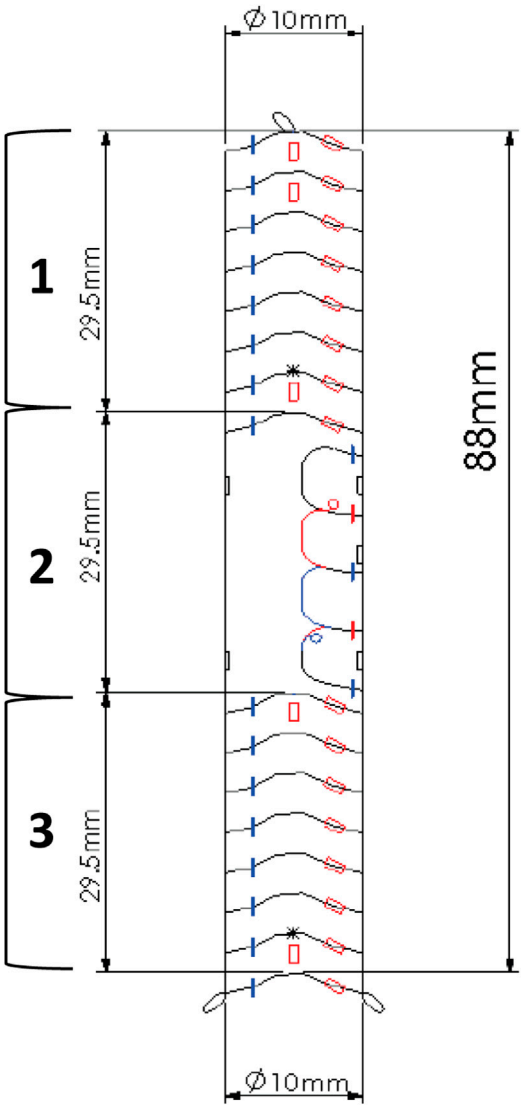


FIGURE 1 | Example of an EndoPreKiT planning design, left side view. The proximal and distal parts (**1** and **3**) are reinforced by the stent rings for sealing and clamping. The anterior face of the middle part (**2**) is free of any stent to receive the future renal artery anastomosis.

of 83 years of age. The body was not perfused, as we did not aim to test device patency. This study was financed by internal funding.

EndoPreKiT Implantations in Patients on the Waiting List

After multidisciplinary team discussions, patients can be judged unfit for KT and are contraindicated due to massive aorto-iliac calcifications (**Figure 2**). A total of ten patients (median age: 64 years old, range 43–74; 50% male) were contraindicated and deemed suitable for EndoPreKit implantation, from January 2021 to January 2024, due to arterial access problems: calcifications (impossibility to clamp and suture the future kidney graft), with or without stenosis. If significant stenoses

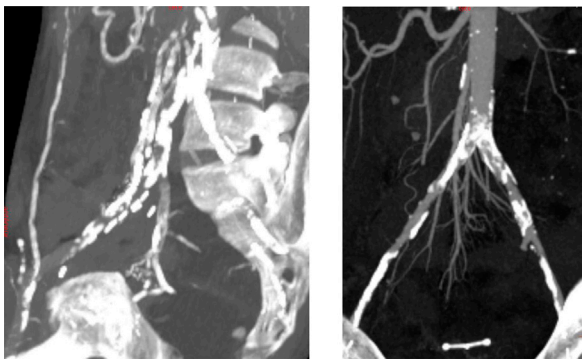


FIGURE 2 | Two examples of massive aorto-iliac calcification with technical contraindications to kidney transplantation.

were associated, additional stenting during the procedure was planned to ensure flow through the endoprosthesis. In this case, the custom-made endoprosthesis could be adapted to position the graft platform before or after the stenosis. The institutional ethics committee allowed EndoPreKiT implantation as the procedure in this extremely selected population outside of a phase 1 study because in the context of a therapeutic impasse. Informed consent was obtained from all ten patients undergoing the procedure over a period of 34 months (April 2021 – February 2024). A multicenter study is ongoing (NCT06677437). As authorized by French law, this is a retro and prospective data study. This study was financed by internal funding. Clinical characteristics are described in **Table 1**. This study was validated by the methodological and regulatory support teams of the HCL sponsor, as well as by the Scientific and Ethics Committee of the Hospices Civils de Lyon (autorisation no. AGORA 23-5431 and avis CFE 23-431).

First Two Cases Patients

The first transplantation was performed on a 70-year-old woman, with a Nephroangiosclerosis (NAS) and diabetic nephropathy, on haemodialysis since 2010. She was considered unsuitable for KT because of circumferential aorto-iliac calcifications and a hostile abdomen due to previous iterative surgeries, with haemorrhagic and infectious complications after a gastric bypass for class III obesity.

The second transplantation was performed on a 37-year-old woman with an autosomal dominant polycystic kidney disease. She was treated with peritoneal dialysis since April 2021. She was also considered unsuitable for KT because of bilateral circumferential aorto-iliac calcifications.

RESULTS

Cadaveric Study

We used two custom-made endovascular legs dedicated to kidney transplantation (Anaconda® Vascutek, Terumo Aortic), of 10 mm of both proximal and distal diameter, and 100 mm of

TABLE 1 | Clinical characteristics of patients treated with EndoPreKiT module.

Patient number	Sex	Age at EndoPreKiT implantation	HTA	Diabetes	Dyslipidemia	Obesity	Respiratory failure/COPD	CAD	ASA score	Preoperative anticoagulant/antiplatelet therapy	Dialysis type	Type of renal insufficiency	Previous renal transplantations	Number of previous renal transplantations	Previous iliac interventions	Previous abdominal interventions	Preoperative GFR
1	F	43	-	-	-	-	-	✓	3	DAPT	HD	Nephrotic syndrome	No	-	✓	✓	19
2	F	54	✓	-	✓	✓	-	-	3	SAPT	HD/PD	Interstitional nephropathy from uretral reflux	✓	1	-	-	7
3	M	64	✓	✓	✓	-	-	-	3	SAPT	HD	Diabetic nephropathy	No	-	✓	-	6
4	M	72	-	-	✓	-	-	✓	2	AVK	HD	Primary glomerulonephritis	✓	2	-	✓	14
5	M	66	✓	✓	-	-	-	-	3	SAPT	HD	Nephroangiosclerosis/iatrogenic nephropathy	No	-	-	✓	13
6	F	70	✓	✓	✓	✓	-	-	3	SAPT	HD	Diabetic nephropathy	No	-	-	✓	11
7	M	74	✓	-	✓	-	-	✓	3	SAPT + AVK	HD/PD	Interstitial nephropathy	No	-	✓	✓	4
8	M	64	✓	-	-	-	✓	✓	3	SAPT	HD	Iatrogenic nephropathy	No	-	-	-	13
9	F	46	✓	-	-	-	-	-	2	SAPT	HD	ADPKD	No	-	-	-	9
10	F	62	✓		-	-	-	✓	3	SAPT	HD	Primary glomerulonephritis	✓	1	✓	✓	5
Total/Mean		61,5	8	3	5	2	1	5	-	-	-	-	3	-	4	7	-

Abbreviations: HTA, arterial hypertension; COPD, chronic occlusive pulmonary disease; CAD, coronary artery disease; eGFR, estimated glomerular filtration rate; DAPT, double antiplatelet therapy; SAPT, single antiplatelet therapy; AVK, anti-vitamin K; HD, hemodialysis; PD, peritoneal dialysis.

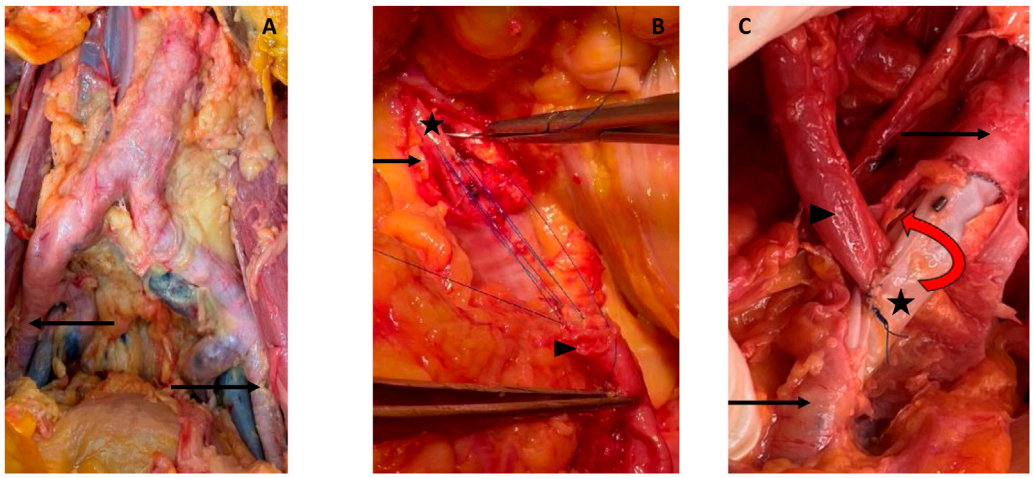


FIGURE 3 | Cadaveric study. **(A)** Calcified cadaveric iliac arteries (black arrows). **(B)** Anastomosis of the renal artery (black arrowhead) to the anterior middle segment of the endoprothesis (black star) after removal of the anterior wall of the iliac artery. Parachute tension suture to test the resistance of the graft. **(C)** Final control of the end-to-side anastomosis with a twist to test the resistance of the graft.

length (30, 30, and 40 mm proximal, medium, and distal parts, respectively) **Figure 3**.

The First Step Vessel Exposure

Through a conventional laparotomy, iliac vessels were dissected on both sides, showing a high degree of calcification. After an open bilateral exposure of the femoral arteries, the two superficial femoral arteries (SFA) were retrogradely punctured with a 16G needle. Common femoral artery puncture was avoided due to major circumferential calcifications. Under fluoroscopy, the aorta was catheterised with a Terumo®O35 260 cm stiff guide wire. Through a 65 cm multi-graded straight catheter, a straight O35 180 cm ultra-stiff guidewire (Lunderquist, Cook Medical) was positioned in the descending thoracic aorta. We used a 16Fr sheath (Dryseal, Gore Vascular) to verify the navigability in the distal external iliac artery (EIA). As no friction was encountered, no preventive dilatation was necessary. The stent graft was thus placed into the proximal EIA. The internal iliac artery was identified on the angiogram and the correct position of the stent graft was verified according to the alignment of the markers. Once the device was completely released, a post-dilation with a 10 × 40 mm balloon was performed along the entire length of the device for impaction and sealing. The same procedure was repeated on the contralateral iliac side. The final arteriographic control was satisfactory on both sides.

Second Step: Kidney Autotransplantation on the Endograft

Through the laparotomy, a conventional bilateral nephrectomy was performed. The renal arteries were only slightly calcified.

Using fluoroscopy guidance, the markers of the middle segment were identified, and the vessel was marked with a sterile pencil. Through a longitudinal arteriotomy, the stentless anterior part of the endograft was exposed. After an external cross-clamping of both artery and graft on the proximal and distal portions of the device, a longitudinal incision of the anterior surface of the middle segment of the endograft was carried out to allow the suturing of the renal artery, with an end-to-side suture (**Figure 3**). During suturing time, we did not experience any difficulty in needle penetration through the graft; there was no tissue weakness, no tearing, and no need to reinforce the suture line. Anastomosis was then checked with a high-pressure serum injection through the femoral introducer sheath.

EndoPreKiT Implantations in Patients on the Waiting List: Endovascular Procedure

All the procedures were performed by the same experienced operator in a hybrid room and fusion imaging (**Figure 4**). Open femoral access was necessary for one patient to perform a prosthetic femoral bypass, under general anesthesia. The remaining 9 cases were performed under local anesthesia, through a percutaneous femoral access (3/9 common; 6/9 superficial) obtained with ultrasound guidance and preclosed with two percutaneous closure devices (Proglide; Abbott Vascular Inc., Santa Clara, CA, United States).

The procedure systematically began with predilatation of the iliac artery using an 8 mm balloon. The stent graft was then advanced into the iliac artery, and the precise alignment of the stent graft was verified thanks to the radio-opaque markers, as follows:

- From the front, five markers positioned on the left side of the stent mark the beginning and end of the proximal and distal stents. A PAIR of proximal marker ensures that the stent graft was loaded correctly into its launcher. At the opposite distal part of the endoprothesis is present only one lateral marker on the patient's left (**Figure 4A**).

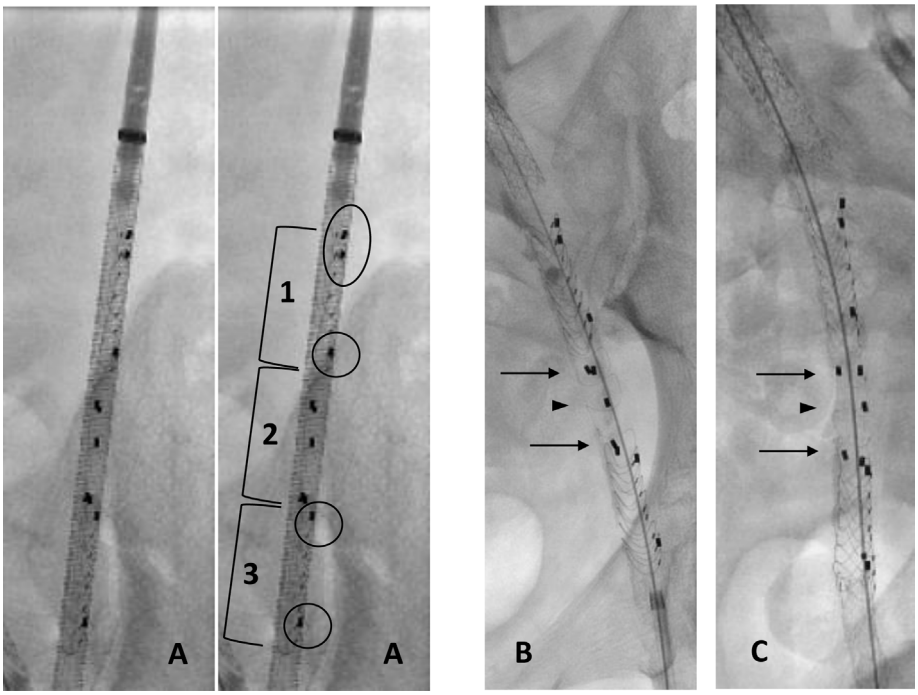


FIGURE 4 | EndoPreKiT during implantation with angiography. **(A)** Front view before deployment: **1**: proximal part of endoprosthesis with two first left markers to avoid loading errors (*black oval*); only one distal lateral marker on the patient's left (*black circle*). **2**: middle part of the endoprosthesis, waiting for future anastomosis, marked by two pairs of anteroposterior markers. **3**: distal part of endoprosthesis with only one proximal and one distal lateral marker on the patient's left (*black circle*). **(B)** Front view after deployment: superposition of the two pairs of anteroposterior markers to place the part free of any stent anteriorly (*black arrows*). **(C)** Lateral view after deployment: good opposition of 2 pairs of anteroposterior markers; the single marker in the middle is well posterior (*black arrowhead*).

- Middle part of the endoprosthesis, waiting for future anastomosis, marked by two pairs of anteroposterior markers. From the front the three posterior and two anterior markers of the middle segment must be aligned to ensure correct orientation of the free stent platform that will receive the graft anastomosis (**Figure 4B**). **Figures 4B, C** shows front view after deployment (superposition of the two pairs of anteroposterior markers to place the part free of any stent anteriorly) and lateral view after deployment (good opposition of 2 pairs of anteroposterior markers). Note that the single marker in the middle is well posterior.

In the case of the patent internal iliac artery, this was identified by angiography and the stent graft deployed in the external iliac (in 5/10 patients, right). Finally, balloon angioplasty was systematically performed to obtain expansion and correct apposition of the stent graft to the vessel.

Technical success was 100%, with a mean operative time of 98.7 min (range: 52–260), mean radiation dose of 2907.0 μGy/m^2 (range: 465.5–9722.6), mean radiation time of 15.62 min (range: 9.7–25.2) and a mean 45.8 mL of contrast volume injection (range: 20–71). No device misalignments nor vessel ruptures were seen intraoperatively. No deterioration in renal function was observed in the non-dialyzed patients.

In 7/10 patients, a planned adjunctive procedure was necessary: 1 common femoral artery prosthetic bypass for access creation due to severe atherosclerosis enabling common femoral artery percutaneous access; 1 ilio-femoral stenting to allow device sheath progression (paving/cracking); 4 common iliac stent deployments to improve inflow due to significant stenosis; 1 hypogastric artery embolization and common iliac stenting to create a safe proximal landing zone ensuring correct sealing.

No postoperative transfusion was needed. All patients were discharged without complications after a median hospital stay of 2 days (range: 2–4), under double oral antiplatelet therapy of 75 mg acetylic acid plus 75 mg clopidogrel for 3 months, and single antiplatelet therapy thereafter. All patients underwent CT angiography and US Doppler at 1-month post-procedure to remove the contraindication to KT.

Kidney Transplantation on EndoPreKiT: The First Two Cases

The first KT was performed 121 days after the implantation of *EndoPreKiT* using a graft with Extended Criteria (ECD) and Donation after Brain Death Donor (DBD) (**Figure 5**). The right external iliac artery was dissected through a retroperitoneal access. A longitudinal arteriotomy of about 3 cm and the partial ablation of the superior vessel wall allowed the

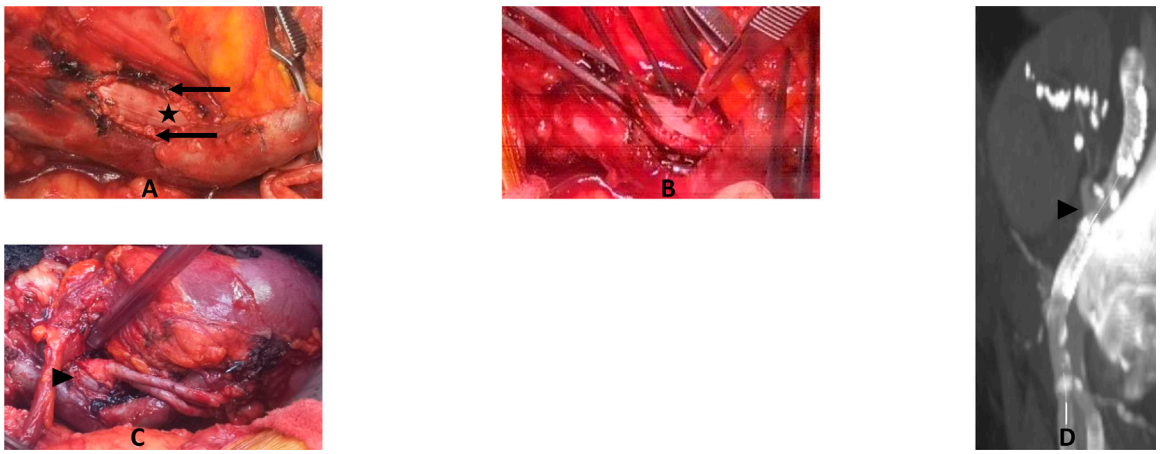


FIGURE 5 | *In vivo* kidney transplantation. **(A)** Arteriotomy and removal of the anterior wall of the iliac artery (*black arrow*) for EndoPreKiT exposition (*black star*) after venous anastomosis. **(B)** Prothetotomy on the anterior part of the middle segment without stent, during a cross-clamping of the iliac artery, ignoring calcifications. **(C)** *In vivo* kidney transplantation. Renal artery anastomosis (*black arrowhead*), venous anastomosis and ureteroneocystostomy done. **(D)** Angiographic CTscan: arterial anastomosis control (*black arrowhead*) with endoprosthesis's markers.

exposure of the middle segment of the device, localised under fluoroscopy. After 100 mg acetylsalicylic acid and 25 UI/kg IV heparin infusion, the iliac artery was cross-clamped at the level of the proximal ring segment. At the distal part, we had to use an endoclamping technique with ballon due to insufficient cross-clamping. Then, arterial anastomosis was performed on the graft with a 6-0 Prolene overlock, with a cold and warm ischemia time of 11.5 h and 37 min, respectively. There were no endoleaks at the end of the procedure, the nitinol rings had no clamping lesions, and no balloon dilation was necessary. Total blood loss was 400 mL and operative time 157 min. After an ICU unit stay of 3 days, spontaneous diuresis was obtained on the 5th day and the double J stent was removed on the 9th day postoperatively. There was no Delayed Graft Function (no need for dialysis during the first week after transplantation). The patient was dismissed under 75 mg PO acetylsalicylic acid after a total hospital stay of 13 days, with a serum creatinine of 175 µmol/L; eGFR 25 mL/min/1,73 m^2). A non-infected peri-vesical hematoma drainage was necessary 29 days after KT, with a total hospital stay of 15 days.

Follow-up Doppler ultrasounds performed at 1, 2, 3, 4, 7, and 8 months postoperatively, all showed a good intra-parenchymatous vascularisation pattern, as well as a patent graft, without in-stent or anastomotic stenosis. The CT scan performed at the time of the peri-vesical hematoma showed no complications of the EndoPreKiT module or vessel anastomoses. At the last follow-up visit (13 months), graft function was quite stable (creatinine 200 µmol/L; eGFR 20 mL/min/1.73 m^2).

Our second KT was performed 133 days after the implantation of EndoPreKit. It was an ABO-incompatible living-donor KT. The same surgical technique was performed. EndoPreKiT was cross-clamped: no endoclamping was required. Cold and warm ischemia times were 260 and 51 min, respectively. Total blood loss was 300 mL, and total operative time was 146 min. After a total hospital stay of 7 days, she was dismissed under PO acetylsalicylic acid 75 mg PO, with an excellent graft function (creatinine 70 µmol/L; eGFR >90 mL/min/1.73 m^2).

Day 0, 1 and 2 months post-operative Doppler ultrasounds showed adequate intra-parenchymatous vascularisation pattern. During the first 2-month follow-up, graft function remained stable without major or minor surgical complications.

DISCUSSION

This original work confirms the proof of concept of a device, usually used in another situation, which could make some patients eligible for KT in case of severe aorto-iliac calcification.

The number of patients unfit for KT due to severe peripheral artery disease (PAD) is increasing as a consequence of the demographic evolution of patients on the waiting list of KT and of the organ shortage [8]. According to the 2020 Kidney Disease Improving Global Outcomes (KDIGO) Guidelines on the Evaluation and Management of Kidney Transplantation, peripheral artery disease is one of the main areas of examination before such intervention [3]. A recent survey among 939 kidney transplant surgeons has shown a large variability in both diagnosis and treatment of peripheral arterial disease in this setting: 67.7% of respondents rated technical problems as the most important concern, followed by increased mortality risk because of cardiovascular comorbidity and ethical issues [9].

However, KT in older patients and patients with severe aorto-iliac calcification, seems to improve patient and graft survival compared to the renal replacement therapy population [10]. Although it is a recognised source of surgical complexity, severe aorto-iliac artery disease is not an absolute contraindication for kidney transplantation. It is generally considered that at least 3 cm of a disease-free vessel is needed to perform a vascular anastomosis and that in the absence of this criteria, a procedure for vessel

preparation (aorto-femoral bypass or iliac endarterectomy) is needed in about 3% of kidney transplant recipients [11, 12].

Particularly in younger patients judged unfit for KT, it is important to offer a solution to overcome severe aorto-iliac calcifications. In preparation for subsequent KT, the aortobifemoral bypass has always been considered the only technique available [10]. In case of the need for vessel preparation, a vascular open surgery can be performed simultaneously or preventively to a kidney transplant [13]. A simultaneous procedure may be beneficial in patients with a high cardiovascular risk, and may as well limit the risk of pretransplantation HLA sensitisation due to blood transfusions [14]. On the other hand, a preventive surgery is associated with a lower risk of kidney thrombosis and vascular prosthesis infection, and benefits from a better stabilisation of the prosthesis within surrounding tissue before transplantation. In the absence of clear guidelines about the optimal delay between a preventive vascular surgery procedure and the transplantation, an interval of less than 1 year is generally considered advisable [13].

In the field of aortoiliac artery disease, endovascular surgery has broadly shown an advantage of reduced morbidity and mortality compared to open surgery, especially in frail patients [15]. In kidney transplant recipients, calcification progression is speeded up by phosphocalcic metabolism changes, and a previous bare metal stent placement makes impossible future arterial sutures.

We designed the EndoPreKit from an original custom-made stent graft whose indication has been changed to make KT possible in this clinically complex population, characterised by a very high cardiovascular risk, through a solution which would appear to be simple, of low-morbidity, reproducible and effective. EndoPreKiT also makes it possible to perform KT under simple and reproducible surgical conditions. To our knowledge, this is the world's first experience with a custom-made endoprosthesis in the field of transplantation, as it is already known for abdominal aortic aneurysm complex surgery.

The EndoPreKiT module has been conceived to limit most of the technical pitfalls of both open and endovascular arterial preparation of kidney transplantation. The percutaneous device placement leaves the abdomen "free" for any further surgical procedure and can be performed on an outpatient basis, which significantly improves clinical tolerance. Of note, since ESRD patients are already under haemodialysis for the majority, a contrast-enhanced CT scan can be safely performed to proceed to stent-graft sizing. Due to its nitinol stent structure, the EndoPreKiT module is supposed to show the same good long-term patency rates observed for the newer generations of Anaconda iliac limbs [16, 17]. Moreover, as found during our cadaver study, the middle anterior segment of EndoPreKiT shows excellent properties in terms of suture. In our first two cases in living patients, we also confirmed a good sealing of the stent graft to the arterial wall without endoleaks. Cross-clamping of the arterial axis at the proximal and distal part was safely performed without device collapse, ring fracture or residual stenosis requiring balloon angioplasty or re-stenting.

Our study has some limitations. The device in itself can be an obstacle: the 16 Fr sheath progression can be difficult in case of severely calcified arteries, requiring an additional peroperative angioplasty. This device has a cost, its production usually requires 6 weeks, and should be implanted at least 3 months before KT, the duration of the dual antiplatelet therapy to obtain the full endothelialisation of the stent-graft. Transplant surgeons must be aware of the theoretical risk of proximal or distal endoleak, which can be managed with complementary procedures (balloon angioplasty, relining with covered stent. . .). Other limitations are the study design, its low power and its non-comparative nature. Due to the small sample size, it was not possible to measure the incidence of endoleaks, stent fractures or migration. Further studies are required to assess the long-term patency of the device and other complications.

CONCLUSION

The EndoPreKiT device could mark a substantial leap forward in the landscape of kidney transplantation, offering a minimally invasive alternative that extends treatment options to previously marginalised patients with compromised iliac access. Further studies are warranted to comprehensively assess its applicability, long-term efficacy, potential enhancements to quality of life, and overall impact on healthcare expenditure. These endeavours are required to fully comprehend the transformative potential of this innovation and optimise its integration into clinical practice.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The institutional ethics committee allowed EndoPreKiT implantation as the procedure in this extremely selected population outside of a phase 1 study because in the context of a therapeutic impasse. Informed consent was obtained from all ten patients undergoing the procedure over a period of 34 months (April 2021 – February 2024). A multicenter study is ongoing (NCT06677437). As authorized by French law, this is a retro and prospective data study. This study was validated by the methodological and regulatory support teams of the HCL sponsor, as well as by the Scientific and Ethics Committee of the Hospices Civils de Lyon (autorisation no. AGORA 23-5431 and avis CFE 23-431).

AUTHOR CONTRIBUTIONS

MA and XM conducted the surgeries, designed the prototype, designed the study, acquired the data, interpreted the data and wrote the manuscript. OT, MB, LD-K, ND, AM, LB, RA, and ES: designed the study, interpreted the data, critically reviewed

the data and drafted a final version of the manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

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A Global Survey of Self-Reported Cancer Screening Practices by Health Professionals for Kidney Transplant Candidates and Recipients

Nida Saleem[1,2,3], Wai H. Lim[4,5], Jacqueline H. Stephens[1,2], Annabelle Wilson[1], Billie Bonevski[1], Allison Jaure[2,6], Armando Teixeira-Pinto[2,6], Eleonora Dal Grande[1,2], Martin Howell[2,6], Farzaneh Boroumand[2,6], Anita van Zwieten[2,6], Chandana Guha[2,6], Nicole Scholes-Robertson[1,2], Steven J. Chadban[6,7], Carmel M. Hawley[8,9], Jonathan C. Craig[1], Jeremy R. Chapman[3], Danyal Hassan[10], Greg Knoll[11,12], Naoka Murakami[13] and Germaine Wong[2,3,6]*

[1]Flinders Health and Medical Research Institute, College of Medicine and Public Health, Flinders University, Bedford Park, SA, Australia, [2]Centre for Kidney Research, Kids Research Institute, The Children's Hospital at Westmead, Westmead, NSW, Australia, [3]Department of Renal and Transplantation Medicine, Westmead Hospital, Westmead, NSW, Australia, [4]Department of Renal Medicine, Sir Charles Gairdner Hospital, Perth, WA, Australia, [5]Faculty of Health and Medical Science, University of Western Australia, Perth, WA, Australia, [6]Sydney School of Public Health, University of Sydney, Sydney, NSW, Australia, [7]Department of Renal Medicine, Kidney Centre, Royal Prince Alfred Hospital, Sydney, NSW, Australia, [8]Department of Nephrology, University of Queensland, Brisbane, QLD, Australia, [9]Department of Nephrology, Princess Alexandra Hospital, Brisbane, QLD, Australia, [10]Department of Renal and Transplantation Medicine, Shifa International Hospital, Islamabad, Pakistan, [11]Division of Nephrology, Department of Medicine, The University of Ottawa, Ottawa, ON, Canada, [12]Division of Nephrology, Kidney Research Centre, The Ottawa Hospital Research Institute, Ottawa, ON, Canada, [13]Division of Nephrology, Washington University in St. Louis, St. Louis, MO, United States

Cancer is a major cause of morbidity and mortality in kidney transplant recipients. Health professionals have a critical role in promoting cancer screening participation. From March 2023 to February 2024, an online survey was distributed to kidney transplant health professionals globally to assess their screening practices. We compared their reported screening practices to recommended guidelines and analyzed factors associated with these practices. We received 97 responses, and most were nephrologists (70%), and around 80% recommended breast, colorectal, and cervical cancer screening for kidney transplant candidates and recipients. About 85% recommended lung cancer screening for higher-risk individuals. Skin cancer screening recommendations varied from 69% for transplant candidates and 84% for recipients. Self-reported cervical cancer screening practices were most concordant with recommended guidelines, followed by breast and skin cancers. Barriers reported included a lack of cancer screening awareness (28%),

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***Correspondence**
Nida Saleem,
nida.saleem@health.nsw.gov.au

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Abbreviations: ANZSN, Australian and New Zealand Society of Nephrology; AST, American Society of Transplant; BEAT-CKD, Better Evidence and Translation-Chronic Kidney Disease; CI, Confidence Interval; CRE-PACT, Centres of Research Excellence: Partnering with Patients with Chronic Kidney Disease; CST-CSN, Canadian Society of Transplantation and the Canadian Society of Nephrology; CT, Computed tomography; DNA, Deoxyribonucleic acid; EBPG, European Best Practice Guidelines; FIT, Faecal Immunohistochemical Test; FOBT, Faecal Occult Blood test; GP, General practitioner; HPV, Human Papillomavirus; KDIGO, Kidney Disease: Improving Global Outcomes; KHA-CARI, Kidney Health Australia-Caring for Australasians with Renal Impairment; MRI, Magnetic resonance imaging; OR, Odds Ratio; PTLD, Post-Transplant Lymphoproliferative Disorder; RA, Renal Association; SIR, Standardised Incidence Ratio; SONG, Standardised Outcomes in Nephrology; TSANZ, Transplantation Society of Australia and New Zealand; TTS, The Transplantation Society; UK, United Kingdom; USA, United States of America.

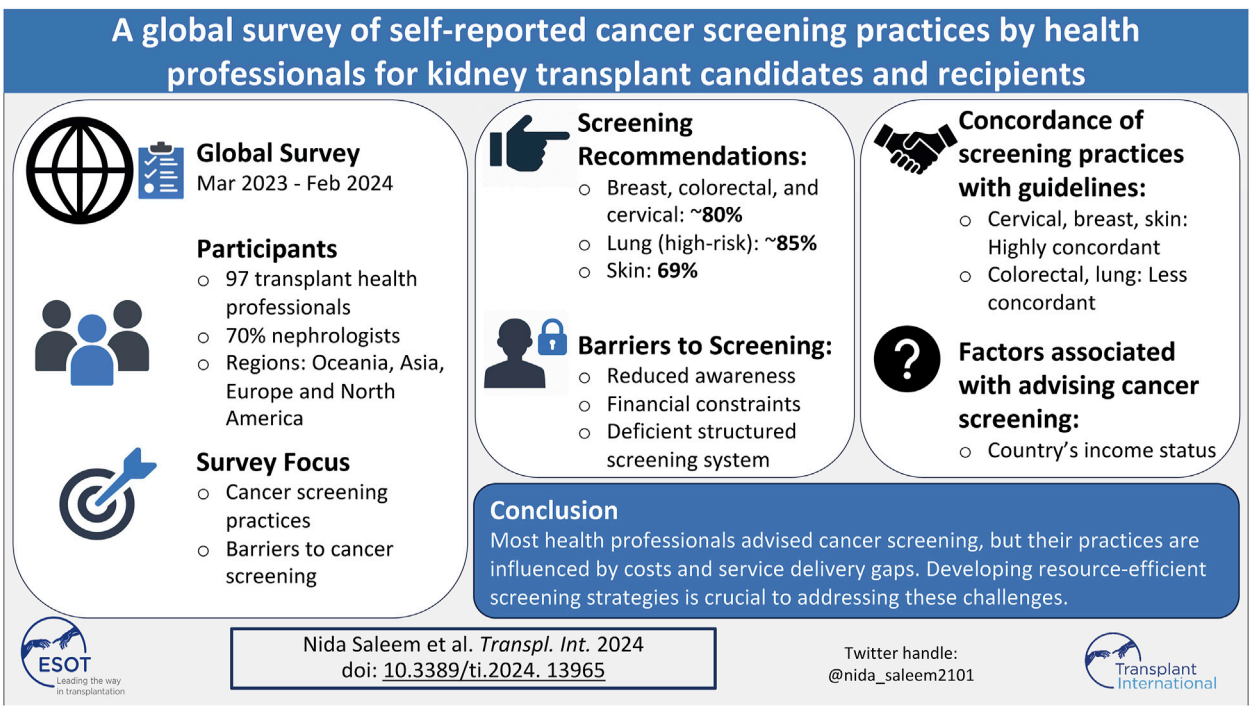
perceived financial constraints (35%), and deficient structured cancer screening systems (51%). Professionals from high-income countries were more likely to advise screening than those from lower-middle-income countries, with odds ratios ranging from 2.9 to 12.3. Most health professionals reported recommending cancer screening for kidney transplant candidates and recipients. However, recommendations were influenced by costs and service delivery gaps within health systems.

Keywords: kidney transplantation, cancer screening, transplant candidates, transplant recipients, cancer

INTRODUCTION

In kidney transplant recipients, cancer is a critically important outcome as it is one of the leading causes of death and the most feared complication of long-term immunosuppression [1, 2]. Compared to the age and sex-matched general population, the overall cancer incidence rates are at least double in kidney transplant recipients, with the increased risk varying depending on the cancer type. Kidney transplant recipients are particularly susceptible to virus-related and non-virus-related cancers, such as melanoma, non-melanoma skin cancers, cervical cancer, and post-transplant lymphoproliferative disease (PTLD) [3, 4]. The standardized incidence ratios (SIR) for these cancers range from 2.5 to 9.8 [5]. For non-immune-related cancers like colorectal and lung cancers, the risk is also elevated by approximately 2-3 times compared to the general population. Once cancer develops, the risk of death for kidney transplant recipients is about twice as high as for the general population [6]. This heightened mortality is due to the aggressiveness of cancers resulting from long-term immunosuppression and impaired immune surveillance. Additionally, the fear of acute allograft rejection from cancer-directed systemic therapies may jeopardize ongoing treatments for these high-risk patients.

To improve cancer-related outcomes, cancer screening plays a crucial role by facilitating early cancer detection and effective treatment before advanced-stage and aggressive diseases. Trial-based evidence in the general population has shown proven long-term mortality benefits with cancer screening, particularly concerning breast, colorectal, and cervical cancers [7–9]. Following recommendations from general population guidelines and evidence from observational studies, several transplant guidelines, like the Kidney Disease Improving Global Outcomes (KDIGO), the American Society of Transplantation (AST), and the European Best Practice Guidelines (EBPG), have recommended



GRAPHICAL ABSTRACT

age-appropriate cancer screening for kidney transplant candidates and recipients [10]. However, prior research has indicated that guidelines are not often applied.

Despite recommendations by clinical practice guidelines, uptake of cancer screening remains low among transplant recipients [11, 12]. In Canada, less than 50% of women with kidney transplants participated in routine cervical and breast cancer screening, whereas over 70% of women without chronic kidney disease received regular Pap smears or human papillomavirus (HPV) tests and mammography [12]. Patients with kidney transplants may face many challenges, including concurrent comorbid conditions such as infections and cardiovascular diseases, limited access to preventive care, and prioritization of ongoing health issues, such as maintaining optimal allograft function, over other less imminent problems, such as cancer [13]. Similarly, a lack of health providers' cancer screening recommendations and follow-up, limited knowledge, and health literacy may impact screening participation [14]. Delayed diagnosis and treatment may result in poorer outcomes.

One of the key elements for successful implementation involves identifying and understanding the potential barriers at the patient, provider, and organizational levels and devising strategies to address these barriers [15]. Many studies have emphasized the pivotal role that health professionals play in improving cancer screening participation for their patients, as they are a direct and trusted source of health information [16, 17]. In transplantation, health professionals' knowledge, practices, and the challenges they encounter in clinical settings are unknown.

This study aimed to gain insights into the disparities and gaps in cancer screening implementation among transplant health providers by describing their global cancer screening practices for kidney transplant candidates and recipients, identifying barriers, and evaluating factors influencing their cancer screening behaviours.

MATERIALS AND METHODS

Study Design

We formulated a questionnaire that assessed the cancer screening practices of health professionals working in nephrology, including nephrologists, nephrology trainees, transplant surgeons, nurses, and transplant coordinators, for kidney transplant candidates and recipients. After reviewing literature and cancer screening guidelines, the survey was developed with our patient partners (Consumer representatives at the Center for Kidney Research and members of key consumer groups; Better Evidence and Translation-Chronic Kidney Disease (BEAT-CKD) [18] and Centers of Research Excellence: Partnering with Patients with Chronic Kidney Disease (CRE-PACT) [18]) and piloted among fifteen experts from a local health district and a kidney research center in Sydney, Australia, to ensure its appropriateness, ease, and understandability. The survey was modified according to the suggestions of these research experts.

The survey contained three sections. The first included nine questions regarding the respondents' demographic and professional details. The second section assessed their referral patterns and barriers that may influence the participants' choices. Lastly, the third component included questions regarding their site-specific screening practices, including their advice regarding types of screening, modality, and frequency for both transplant candidates and recipients. The detailed survey is attached to the **Supplementary Material** (**Supplementary Material S1**).

Informed consent was obtained electronically from the survey participants. We then used adaptive questioning, a survey technique where survey questions are tailored based on the participants' previous responses, to minimize the number of questions and enhance the relevance of the survey experience for each respondent [19]. Responders had the opportunity to check the completeness of their responses and review them using the back buttons. To prevent duplicative responses, the survey was distributed exclusively through unique invitation links.

Participants

A closed web-based questionnaire was sent via email to all members of the Australia and New Zealand Society of Nephrology (ANZSN), Transplantation Society of Australia and New Zealand (TSANZ), BEAT-CKD, and The Transplantation Society (TTS) working network contact directory and through personal contacts from March 2023 to February 2024. Global health professionals currently working in nephrology, including nephrologists, nephrology professionals in training (trainee, resident, fellow), kidney transplant surgeons, nurses, and transplant coordinators, were invited to participate. After the initial post, one reminder email was sent to those who had yet to respond. All information on the questionnaire was de-identified to ensure confidentiality. No financial incentives were provided to the respondents. Ethics approval for this study was obtained from the University of Western Australia Human Ethics Committee (Approval Number: 2022/ET000790) following the guidelines set forth by the National Health and Medical Research Council (NHMRC). Reciprocal approval was granted by Flinders University's Research Ethics and Compliance Office (Approval Number: 5966). We followed the Checklist for Reporting Results of Internet E-Surveys (CHERRIES) checklist to report this study [19].

Statistical Analysis

Cancer screening practices of health professionals were analyzed using descriptive statistics. The proportions of participants who advised breast, colorectal, cervical, skin, lung, prostate and kidney cancer screening were estimated and graphically represented using clustered bar charts. Missing data was excluded while calculating these proportions.

Similarly, the proportion of site-specific cancer screening practices of transplant health professionals was described and compared with widely accepted transplant guidelines such as Kidney Disease: Improving Global Outcomes (KDIGO), American Society of Transplant (AST), Canadian Society of Transplantation and the Canadian Society of Nephrology (CST-CSN), European Best Practice Guidelines (EBPG), Kidney Health Australia-Caring for Australasians with Renal Impairment (KHA-CARI) and Renal Association (RA) Clinical Practice Guidelines. Specifically, we reviewed practices against the recommendations for breast, colorectal, cervical, skin, and lung

TABLE 1 | Clinical practice guidelines on cancer screening in kidney transplant recipients.

Guidelines	AST [20–22] (2000, 2009)[a]	CST-CSN [23, 24] (2010)[a]	KDIGO [10, 25, 26] (2009)[a]	RA [27, 28] (2017)[a]	KHA-CARI [29, 30] (2012)[a]	EBPG [31, 32] (2002)[a]
Breast cancer	Every 1–2 yearly mammography between 50 and 69 years	Every **2–3** yearly mammography between **50** to **74** years[b]	Annual mammography above **50** years[b]	Every **3** yearly mammography between **50** to **70** years[b]	Every **2** yearly mammography between **50** to **74** years[b]	Mammography between **45** to **74** years[b]
Cervical cancer	Annual PAP cytology between 18 to **65** years OR Every 3 yearly HPV testing[c] [33, 34]	Annual PAP cytology between **25** to **69** years[b]	Every 3 yearly PAP cytology between **21** to **65** years OR Every **5** yearly **HPV** testing till **65** years[b] [26]	Every **3** yearly PAP cytology between **25** to **49** years than **5** yearly till **65** years[b]	PAP cytology between **25** to **74** years OR Every 3 yearly HPV testing[c] [35]	Annual PAP cytology between **25** to **65** years OR Every **5** yearly **HPV** testing between **30** to **65** years[b] [32]
Colorectal cancer	Annual FIT[d] OR Every 5 yearly sigmoidoscopy between 50 to **75** years	Every **2** yearly FIT[d] OR Every **10** yearly sigmoidoscopy between **50** to **74** years[b]	Annual FIT[d] OR Every **5–10** yearly sigmoidoscopy between **50** to **75** years Colonoscopy if FIT[d] positive[b]	Every **2** yearly FIT[d] between **50** to **74** years Colonoscopy if FIT[d] positive[b]	Every **2** yearly FIT[d] between **45** to **74** years[b] Colonoscopy if FIT[d] positive[b]	FIT[d] between **50** to **74** years[b] Colonoscopy if FIT[d] positive[b]
Skin cancer	Monthly skin self-exam Annual physician exam	Skin self-exam Annual Specialist exam	Skin self-exam Annual Specialist exam	Biennial physician exam for 5 years post-transplant than annually	Skin self-exam Annual Specialist exam	—
Lung cancer	**Annual** CT-chest between **50** to **80** years	—	—	—	—	—

[a]*Bold indicates screening modalities, frequencies, starting and stopping ages following the KDIGO, transplant candidate guidelines [26] and current American [22], Canadian [24], United Kingdom [28], Australian [30], and European general population guidelines [32].*
[b]*Cancer screening as per general population guidelines.*
[c]*HPV, testing frequency based on American Society of Transplantation Infectious Disease guidelines and Australian Cancer Council guidelines.*
[d]*Faecal Immunochemical test.*

cancer screening modalities, frequencies, and starting and stopping ages (**Table 1**). We noted the proportions of health professionals' cancer screening responses and defined concordance between their screening practices and the guidelines as strong (>75%), moderate (50- ≤ 75%), weak (25- ≤ 50%), and very weak (≤25%).

We used logistic regression modeling to determine the association between demographic and clinical factors and the willingness to recommend screening for breast, colorectal, and cervical cancers. We also included factors such as clinicians' work experiences, their cancer screening system, and countries of practice, categorizing based on income status according to the World Bank classification [36]. An odds ratio (OR) of >1, with a 95% confidence interval, indicated a greater likelihood of cancer screening advice compared to the reference group. All statistical analyses were performed using SPSS [37].

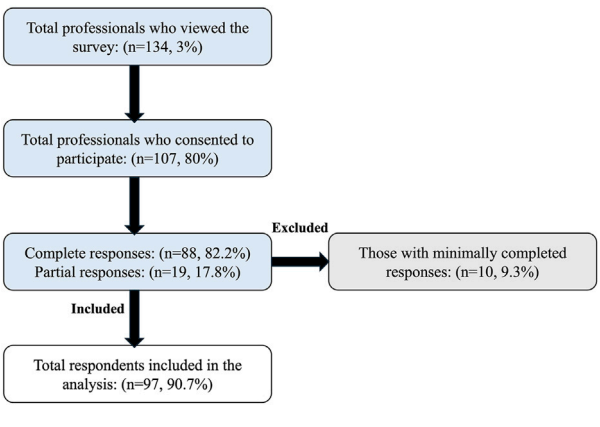


FIGURE 1 | A flow diagram showing health professionals' inclusion process.

RESULTS

Approximately 4500 health professionals working in the kidney transplant setting were invited to participate, with 134 viewing the survey (view rate 3%). Of the 134 survey viewers, 107 consented to participate (participation rate 80%). Among 107 participants, 88 completed the survey (completion rate 82%), while 19 (18%) provided partial responses. Of the 19 respondents who provided partial responses, 10 were excluded for completing less than 30% of the survey. As a result, data from 97 respondents were included in the final analysis, as illustrated in the flow diagram (**Figure 1**).

Table 2 shows the demographic characteristics of all respondents. About half of the responders were males (53%), in the age 31–40 years category (50%), and worked in Australia (56%). Most were nephrologists (70%), with clinical experience of

TABLE 2 | Demographic characteristics of the survey respondents (n = 97).

Characteristics	n	%
Gender		
Male	51	53
Female	45	46
Prefer not to say	1	1
Age groups (years)		
18–30	7	7
31–40	48	50
41–50	18	19
51–60	17	18
61–70	7	7
Country of residence based on income status		
High-income countries		
Australia	54	56
New Zealand	5	5
USA	5	5
Others[a]	8	8
Lower-middle income countries		
Pakistan	24	25
Vietnam	1	1
Primary role		
Nephrologist	68	70
Transplant nurse	15	16
Nephrology trainee/resident	8	8
Others	6	6
Work experience (years)		
<10	62	64
11–20	18	19
21–30	12	12
>30	5	5
Location of work		
Urban	80	83
Rural and remote	10	10
Both	7	7
Practice setting[b]		
Transplant centre	53	59
Private dialysis centre	22	24
Public dialysis centre	30	33

[a]*Includes health professionals from the United Kingdom, Saudi Arabia, Canada, Germany.*
[b]*Missing data for seven respondents (Percentage calculation excludes missing data).*

less than 10 years (64%) and working in urban (83%) and transplant settings (59%). In addition to their clinical roles, many reported contributing to the formulation of clinical practice guidelines (42%), and some reported working as a policymaker (13%) and holding governmental/institutional funding in kidney research (9%).

Self-Reported Frequency of Providing Cancer Screening Advice To Kidney Transplant Candidates and Recipients

Among 97 respondents, 92 (95%) reported recommending cancer screening for kidney transplant candidates. Eighty-two (85%) reported recommending breast cancer screening, 78 (81%) cervical cancer screening, 76 (79%) colorectal cancer screening, 66 (69%) skin cancer screening, and 51 (53%) lung cancer screening. Only 11 (12%) respondents would recommend prostate cancer screening, and four (4%) would recommend kidney cancer screening.

When asked about their practices for kidney transplant recipients, 90 out of 95 respondents reported recommending cancer screening (95%, with two missing responses). Similarly, out of 91 respondents (six missing), 80 (88%) reported recommending breast cancer screening, 78 (86%) cervical cancer screening, 77 (85%) colorectal cancer screening, 76 (84%) skin cancer screening, 42 (46%) lung cancer screening, 11 (12%) prostate cancer screening and 4 (4%) kidney cancer screening. The overall proportion of reported screening recommendations for all cancers was higher for kidney transplant recipients than candidates, except for lung cancer. A graphical representation of these cancer screening recommendations is shown in **Figure 2**.

Site-Specific Cancer Screening Practices of Transplant Health Professionals

Table 3 shows site-specific cancer screening practices of transplant health professionals. Most respondents reported recommending biennial breast cancer screening (60%) by mammography (96%). Many respondents reported recommending a broad age range for breast cancer screening. Around 50% would initiate breast screening at the age of 40 and extend screening beyond 80. Some (34%) would continue breast cancer screening irrespective of age.

The majority of respondents reported recommending cervical cancer screening by conventional cytology (56%), commencing at 18–25 years or when sexually active (92%), and stopping at over 70 years (48%). In addition to cytological evaluation, some (29%) professionals also advised HPV-DNA testing. However, their reported cervical cancer screening frequency was less than guideline recommendations (46%).

For colorectal cancer screening, many respondents advised fecal immunochemical testing (FIT) (66%) commencing at the age of 50 years (54%), and around 50% would suggest less frequent screening (less than biennial screening), and the majority (52%) would advocate for ongoing screening regardless of age.

Approximately 46% of health professionals reported recommending lung cancer screening among average-risk kidney transplant candidates and recipients. The most common screening modality was low-dose computer tomography (CT) chest in high-risk transplant candidates and recipients, defined as current smokers or have quit smoking in the past 15 years, with a 20-pack year smoking history. Many health professionals expressed uncertainty regarding the commencement, frequency, and cessation of lung cancer screening.

Many professionals advised skin cancer screening using whole-body skin examinations conducted by a dermatologist (52%) or a non-skin specialist (36%). Reported screening intervals were typically annual (76%) for average risk and six-monthly (49%) for high-risk transplant candidates and recipients.

Comparison of Transplant Health Professionals' Responses With the Recommendations by Clinical Practice Guidelines

Figure 3 shows the concordance of reported screening practices for kidney transplant candidates and recipients with clinical

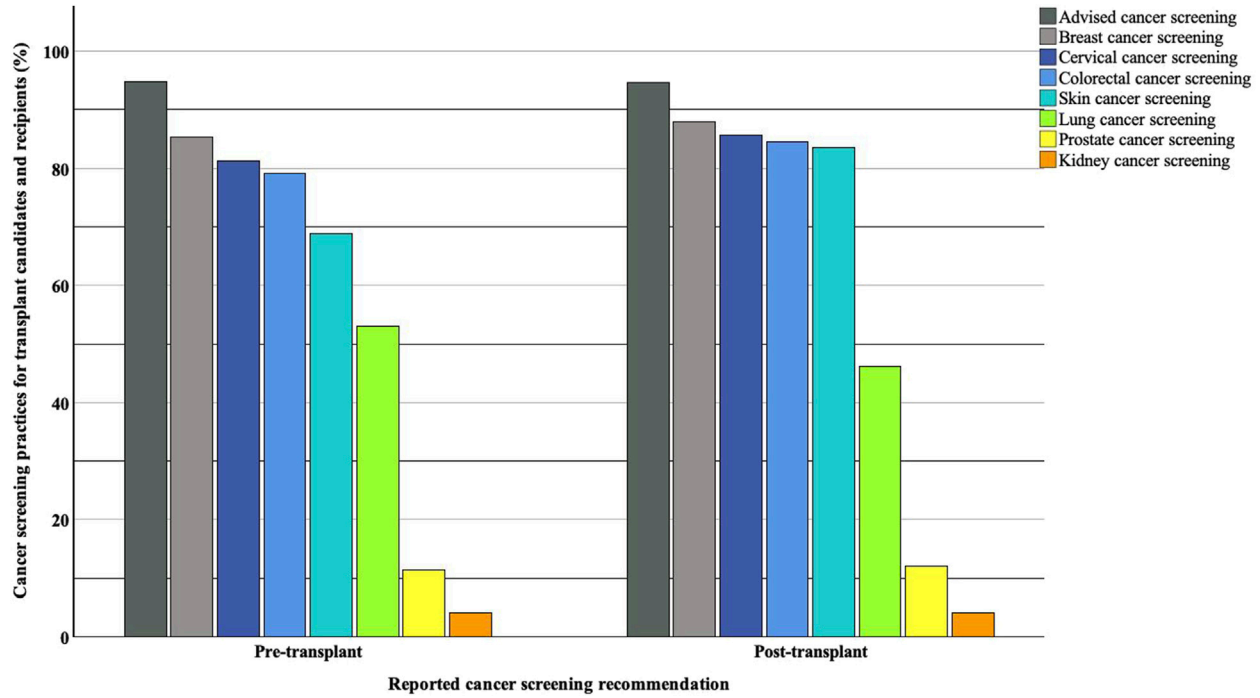


FIGURE 2 | Reported cancer screening recommendations for kidney transplant candidates and recipients.

practice guidelines. Cervical cancer screening reported practices were most concordant with international clinical practice guidelines, followed by breast and skin cancer screening practices.

Conversely, health professionals reported a lower level of conformity regarding their practices for colorectal and lung cancer screening compared to established clinical guidelines. Specifically, more than 50% of respondents favored continuing colorectal screening regardless of age. Also, only 40% advised CT-chest for lung cancer screening. Less than 20% of the participants reported recommending annual colorectal and lung cancer screenings, showing very weak concordance with many guidelines' recommendations.

Barriers Influencing Transplant Health Professionals' Cancer Screening Recommendations

Financial constraints (35%), lack of patient awareness (28%), and the lack of specialized cancer screening units (28%) were frequently reported barriers to screening. Another prevailing barrier impacting their cancer screening advice was the lack of a structured screening system, especially in the post-transplant setting. While 76% of respondents indicated having a structured screening system for transplant candidates, the majority (51%) reported a lack of a structured screening system for transplant recipients in their clinical setting.

In contrast, most health professionals denied having inadequate skills, training, and time as barriers to recommending screening. The majority acknowledged their duties to discuss cancer screening with transplant candidates and recipients (**Figure 4**).

Factors Aligned With Reported Breast, Colorectal, and Cervical Cancer Screening Recommendations

As seen from **Table 4**, professionals from high-income countries (HIC), such as Australia, New Zealand, Canada, Germany, the United States, the United Kingdom, and Saudi Arabia, were more likely to recommend cancer screening in pre- and post-transplant settings than health professionals working in low-to middle-income countries (LMIC) such as Pakistan and Vietnam (odds ratios ranging between 2.9 and 12.3). Those with a working experience of greater than 10 years and those with a structured pre-transplant cancer screening system were more likely to advocate cancer screening for kidney transplant candidates, especially for cervical cancer (odd ratios of 5.9, CI: 1.3–27.3 and 9.2, CI: 2.2–38.3). However, these factors did not influence reported cancer screening recommendations for transplant recipients.

DISCUSSION

Consistent with clinical practice guidelines for cancer screening in kidney transplant candidates and recipients, our study found that most transplant health professionals reported recommending breast, colorectal, and cervical cancer screening

TABLE 3 | Reported site-specific cancer screening practices among transplant health professionals.

Cancer screening practices		n	%
Breast cancer screening (n = 82)[a]			
Starting age, years	<40	13	16
	40	34	42
	50	30	37
	Unsure	5	6
Modality	Mammography	79	96
	Ultrasound	28	34
	MRI[b] breast	9	11
	Breast self-examination	32	39
	Clinical breast examination	35	43
Frequency	Annually	21	26
	Biennially	49	60
	Others	6	7
	Unsure	6	7
Stopping age, years	>70	32	39
	>80	12	15
	Continue regardless of age	28	34
	Unsure	10	12
Male breast cancer screening	Yes	12	15
	No	52	63
	Unsure	18	22
Cervical cancer screening (n = 82)[a]			
Starting age, years	<18	2	2
	18–25	26	32
	When sexually active	49	60
	Unsure	5	6
Modality	Conventional cytology	46	56
	HPV[c] testing	24	29
	Liquid-based cytology	8	10
	Unsure	4	5
Frequency	Annually	16	20
	Every 2–3 years	38	46
	Every 5 years	18	22
	Other	6	7
	Unsure	4	5
Stopping age, years	>70	39	48
	>80	3	4
	Continue regardless of age	23	28
	Unsure	17	21
Colorectal cancer screening (n = 82)[a]			
Starting age, years	<40	10	12
	40	12	15
	50	44	54
	60	7	9
	>60	1	1
	Unsure	8	10
Modality	Blood plasma test	3	4
	Colonoscopy	6	7
	CT[d] colonoscopy (Virtual colonoscopy)	5	6
	FOBT[e] (guaiac or immunohistochemical)	54	66
	Sigmoidoscopy (rigid or flexible)	7	9
	Stool DNA test (FIT[f]-DNA test)	3	4
	Unsure	4	5
Frequency	Annually	14	17
	Every 2–3 years	42	51
	Every 5 years	18	22
	Other	3	4
	Unsure	5	6
Stopping age, years	>70	26	32
	Continue regardless of age	43	52
	Unsure	13	16

(Continued on following page)

TABLE 3 | (*Continued*) Reported site-specific cancer screening practices among transplant health professionals.

Cancer screening practices		n	%
Lung cancer screening (n = 82)[a]			
Average risk	Yes	38	46
	No	36	44
	Unsure	8	10
High risk[g]	Yes	70	85
	No	5	6
	Unsure	7	9
Starting age, years	<40	15	18
	40	13	16
	50	21	26
	60	3	4
	>60	1	1
	Unsure	29	35
Modality	Chest radiography	32	39
	Low-dose CT chest	33	40
	Other	1	1
	Unsure	16	20
Frequency	Annually	15	18
	Every 2–3 years	24	29
	Every 5 years	15	18
	Other	6	7
	Unsure	22	27
Stopping age, years	>70	19	23
	>80	7	9
	Continue regardless of age	30	37
	Unsure	26	32
Skin cancer screening (n = 75)[a]			
Modality	Full body skin check by dermatologist	39	52
	Full body skin check by GP[h]/non-skin specialist	27	36
	Skin self-check	8	11
	Unsure	1	1
Frequency-average risk	Annually	57	76
	Every 2–3 years	10	13
	Every 5 years	3	4
	Other	1	1
	Unsure	4	5
Frequency-high risk[i]	Every 3 months	11	15
	Every 6 months	37	49
	Annually	20	27
	Every 2-3 years	1	1
	Other	3	4
	Unsure	3	4

[a]*Sample size varies due to missing data (Percentage calculation excludes missing data).*
[b]*Magnetic resonance imaging.*
[c]*Human papillomavirus.*
[d]*Computerised tomography.*
[e]*Faecal occult blood test.*
[f]*Faecal immunochemical test.*
[g]*Defined as a current smoker or someone who has quit smoking in the past 15 years and has a smoking history of at least a 20-pack year.*
[h]*General practitioner.*
[i]*Defined as a personal or family history of skin cancer, a skin type more sensitive to UV damage, a history of severe sunburns, spending a lot of time outdoors, or using a solarium.*

to their patients. Transplant health professionals were more likely to recommend skin cancer screening for kidney transplant recipients than for candidates, while lung cancer screening was less frequently recommended, accompanied by a lot of reported uncertainties. Transplant health professionals proposed a broader age range for starting and stopping cancer screening compared to clinical practice transplant guidelines. Screening practices were influenced by factors such as cancer screening awareness among patients, the availability of health system resources, and the financial constraints faced by both patients and health facilities.

Studies to date have reported that transplant recipients can benefit from age-appropriate population-based screening practices, including breast, colorectal, and cervical cancers [38]. Similarly, our study findings showed cervical, breast, and skin cancer screening practices among health professionals were consistent with published guidelines. A higher conformity with cervical screening practices may be influenced by improved knowledge regarding test performance, the cost-benefit ratios of screening using HPV-DNA testing [39], especially self-testing, and access to updated cervical cancer screening transplant

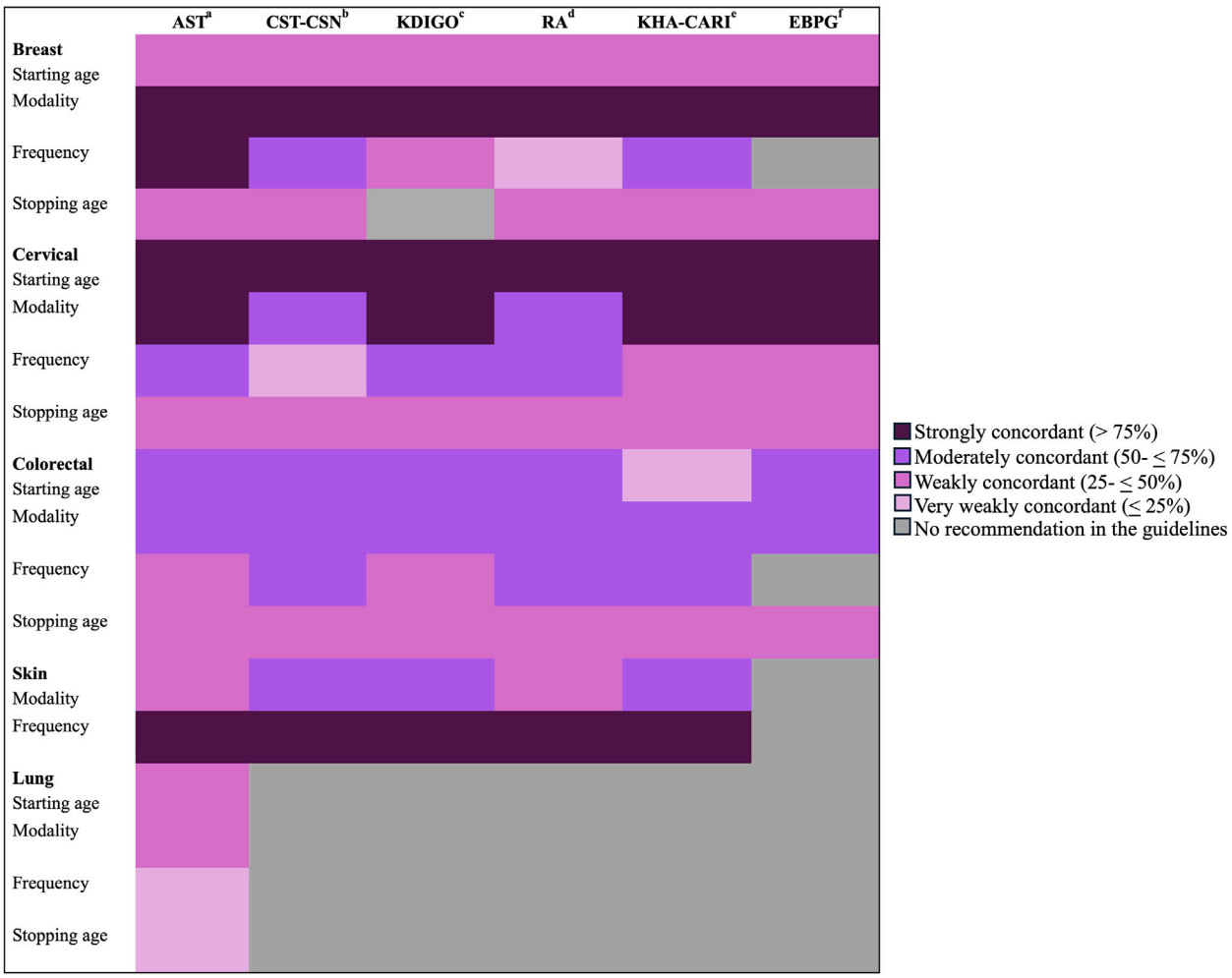


FIGURE 3 | Concordance between reporting screening recommendations and clinical practice guidelines. a: American Society of Transplant, b: Canadian Society of Transplantation and the Canadian Society of Nephrology, c: Kidney Disease: Improving Global Outcomes, d: Renal Association Clinical Practice Guidelines, e: Kidney Health Australia-Caring for Australasians with Renal Impairment, f: European Best Practice Guidelines.

guidelines [33, 34], in contrast to most transplant guidelines published before 2012. Similarly, a high concordance with breast cancer screening guidelines was observed, likely attributed to greater awareness and robust trial-based evidence showing mortality benefits in the general population [40]. Likewise, a greater alignment of skin cancer screening practices and guidelines may be due to the uniform advocacy of skin cancer screening by transplant health professionals. This advocacy is driven by heightened awareness of the disease burden and higher cumulative incidence of skin cancers compared to the age and sex-matched general population [3, 41].

Several inconsistencies were evident between the self-reported screening practices of transplant health professionals and the recommended guidelines. For instance, most transplant health professionals suggested broader age ranges for screening, particularly for colorectal and breast cancers. There were also discrepancies across the recommended frequency for colorectal screening and uncertainties regarding the frequency and timing of lung cancer screening. Clinicians would also recommend less frequent screening for colorectal and lung cancers. Only 29% would recommend HPV-DNA testing for cervical cancer screening in addition to PAP cytology, and 40% would use low-dose CT chest for lung cancer screening for high-risk individuals.

There are likely to be many reasons for the observed discordance. Health professionals may prioritize other competing health issues experienced by transplant recipients, such as maintaining optimal graft function, over future events, such as cancer, that are not immediately imminent [42, 43]. Similarly, the screening practices among health professionals are also highly variable. These practices are largely driven by their patients' cancer risk, expected survival, preferences, comorbidities and ongoing treatment burden, leading to inconsistent screening advice [16, 43]. Furthermore, the lack of uniformity between cancer screening guideline recommendations likely contributes to their inconsistent

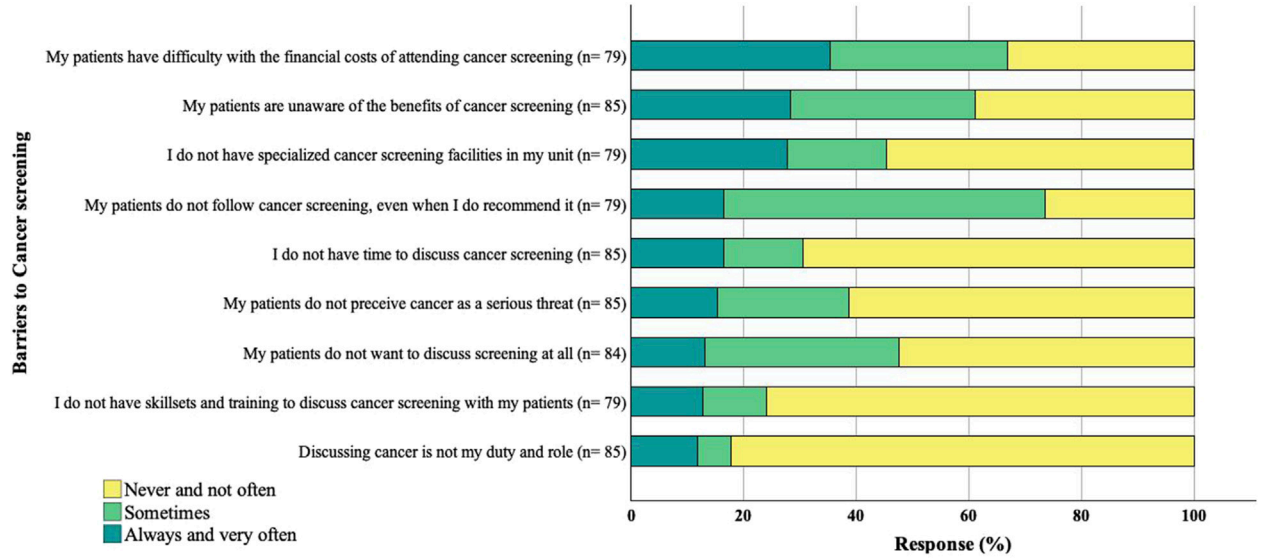


FIGURE 4 | Reported barriers to cancer screening. The sample size for each response varies due to missing data.

screening advice. The recommendations to target broader age ranges for cancer screening may be influenced by the growing evidence suggesting heightened cancer risk and a more aggressive cancer course in younger transplant recipients [44]. Current clinical guidelines suggest primary HPV testing every 3–5 years for transplant recipients compared to conventional cytology screening biennially [45]. However, the transition to HPV testing from conventional cytology has not been universally adopted by LMIC due to various barriers, including a lack of infrastructure for high-complex molecular testing and equipment, limited screening system, laboratory capacity, skilled expertise, and human resources, and centralization of laboratories [45, 46]. While there is now robust trial-based evidence to suggest lung cancer screening using low-dose CT among high-risk individuals reduces the risk of lung cancer-related death by 20%–24% compared to no screening [47, 48], population-based screening programs have not commenced in many countries. For instance, in Australia, the proposed launch date for the National lung cancer screening program is in July 2025 [49]. Similarly, in the United Kingdom (UK), the National Health Service will roll out the program in 2025, with full coverage anticipated in 2030 [50].

Other factors, such as economic deprivation, inadequate healthcare funding, infrastructure, and resources, may also impact cancer screening decisions [12, 51–53]. A robust, well-organized, well-governed, publicly funded population-based screening program is needed to maximize uptake and participation in cancer screening. However, these systems are lacking in many LMICs [52], as reported by health professionals residing in countries such as Pakistan and Vietnam in our study. In addition to reliable health investments, education about the potential benefits and harms of routine screening and recommendations is crucial. Misinformation and the lack of awareness among patients and clinicians may lead to under-utilization and inappropriate screening [52, 54]. Prior research has indicated many transplant recipients underestimated the importance of cancer screening [55, 56].

Other strategies that may facilitate the successful implementation of cancer screening for transplant recipients within both income settings include involving primary care physicians in screening advice [17], ensuring a continuum of care at transplant centers [17], adopting an individualized risk-based approach to screening, and promoting shared decision-making by considering various factors including patients' life expectancy, graft health, comorbidities, and the recipients' age in cancer screening decisions [17, 43, 57]. Also, there is a need for regularly updating society-based guidelines, ensuring the recommendations remain aligned with the most current evidence. Furthermore, incorporating and educating about self-testing for HPV-DNA and FIT may improve screening compliance and limit the burden on providers and health resources [52, 58]. Other interventions like mobile screening to mitigate travel costs and employing patient navigators may improve screening adherence [52, 59].

This study has several limitations. Despite developing a well-designed survey, conducting thorough pilot testing, and sending reminder emails [60], limited survey view rates remain a key limitation, likely due to professionals' lack of interest or time for cancer screening [61], impacting the generalizability of our study findings. However, we have not explicitly investigated the factors that may contribute to these limited survey view rates. Also, most of the respondents were nephrologists (70%) with less than 10 years of work experience (63%) and primarily practicing in urban settings (80%), which may not fully reflect the cancer screening practices of more experienced transplant health professionals from rural and remote settings. While we attempted to sample participants from the relevant global transplant societies, we do not have representation from

TABLE 4 | Factors impacting transplant health professionals' site-specific cancer screening practices in pre- and post-transplant settings[a].

Factors[b]	p-value	OR[c] (95% CI[d])
For kidney transplant candidates		
Pre-transplant reported breast cancer screening recommendation		
Gender[e]		
Male (n = 51)		1.0
Female (n = 44)	0.38	0.6 (0.2–1.9)
Country of practice		
Lower-middle-income[f] (n = 25)		1.0
High income[g] (n = 71)	**<0.001**	5.1 (1.6–16.7)
Work experience		
Less than 10 years (n = 61)		1.0
Greater than 10 years (n = 35)	0.08	4.0 (0.9–19.2)
Have a structured cancer screening system[h]		
No (n = 16)		1.0
Yes (n = 65)	0.13	3.5 (0.7–17.7)
Pre-transplant reported cervical cancer screening recommendation		
Gender[e]		
Male (n = 51)		1.0
Female (n = 44)	0.17	0.5 (0.2–1.4)
Country of practice		
Lower-middle-income[f] (n = 25)		1.0
High income[g] (n = 71)	**<0.001**	5.3 (1.8–15.6)
Work experience		
Less than 10 years (n = 61)		1.0
Greater than 10 years (n = 35)	**0.02**	5.9 (1.3–27.3)
Have a structured cancer screening system[h]		
No (n = 16)		1.0
Yes (n = 65)	**0.00**	9.2 (2.2–38.3)
Pre-transplant reported colorectal cancer screening recommendation		
Gender[e]		
Male (n = 51)		1.0
Female (n = 44)	0.17	0.5 (0.2–1.4)
Country of practice		
Lower-middle-income[f] (n = 25)		1.0
High income[g] (n = 71)	**<0.001**	7.3 (2.5–21.3)
Work experience		
Less than 10 years (n = 61)		1.0
Greater than 10 years (n = 35)	0.09	2.8 (0.8–9.0)
Have a structured cancer screening system[h]		
No (n = 16)		1.0
Yes (n = 65)	0.21	2.4 (0.6–9.2)
For kidney transplant recipients		
Post-transplant reported breast cancer screening recommendation		
Gender[e]		
Male (n = 49)		1.0
Female (n = 41)	0.21	2.5 (0.6–10.0)
Country of practice		
Lower-middle-income[f] (n = 23)		1.0
High income[g] (n = 68)	0.11	2.9 (0.8–10.5)
Work experience		
Less than 10 years (n = 58)		1.0
Greater than 10 years (n = 33)	0.50	0.7 (0.2–2.3)
Have a structured cancer screening system[i]		
No (n = 44)		1.0
Yes (n = 28)	0.17	0.3 (0.1–1.7)
Post-transplant reported cervical cancer screening recommendation		
Gender[e]		
Male (n = 49)		1.0
Female (n = 41)	0.58	1.4 (0.4–4.7)
Country of practice		
Lower-middle-income[f] (n = 23)		1.0
High income[g] (n = 68)	0.07	3.1 (0.9–10.4)

(Continued in next column)

TABLE 4 | (*Continued*) Factors impacting transplant health professionals' site-specific cancer screening practices in pre- and post-transplant settings[a].

Factors[b]	p-value	OR[c] (95% CI[d])
Work experience		
Less than 10 years (n = 58)		1.0
Greater than 10 years (n = 33)	0.86	0.9 (0.3–3.0)
Have a structured cancer screening system[i]		
No (n = 44)		1.0
Yes (n = 28)	0.16	0.3 (0.1–1.5)
Post-transplant reported colorectal cancer screening recommendation		
Gender[e]		
Male (n = 49)		1.0
Female (n = 41)	0.83	1.1 (0.4–3.6)
Country of practice		
Lower-middle-income[f] (n = 23)		1.0
High income[g] (n = 68)	**<0.001**	12.3 (3.3–45.3)
Work experience		
Less than 10 years (n = 58)		1.0
Greater than 10 years (n = 33)	0.52	1.5 (0.4–5.3)
Have a structured cancer screening system[i]		
No (n = 44)		1.0
Yes (n = 28)	0.28	0.5 (0.1–1.9)

Bold values indicate significant p-values for factors.
[a]Calculated through univariate logistic regression modelling.
[b]Sample size varies due to missing data.
[c]Odds ratio.
[d]Confidence interval.
[e]One health professional hasn't mentioned gender.
[f]Transplant health professionals from Pakistan and Vietnam.
[g]Transplant health professionals working in Australia, New Zealand, the United States of America, the United Kingdom, Canada, Germany and Saudi Arabia.
[h]Five professionals were unsure of the pre-transplant structured cancer screening system.
[i]14 professionals were unsure of the post-transplant structured cancer screening system.

countries in Africa and other parts of Asia (including India and China). This study relied on self-reported data for assessing the cancer screening practices of health professionals, potentially introducing a reporting bias by overestimating their inclination towards cancer screening and underestimating their actual screening behaviours. This study, however, has several strengths. The survey was distributed globally to health professionals working in the field of post and pre-transplant care. The self-reported survey approach allowed us to gain insights into their perceived barriers to screening, which would not be possible by merely observing screening practices. Prior to dissemination, the survey was extensively reviewed by consumer representatives and pilot-tested among clinicians, patients, and caregivers. The survey was conducted via a secured online portal, ensuring the confidentiality and anonymity of the respondents.

In conclusion, our study has provided an overview of the key factors influencing cancer screening practices among transplant health professionals. Most respondents acknowledged the importance of screening among at-risk individuals and recognized their pivotal role in providing screening advice. However, the lack of resources and inadequate cancer screening systems significantly impacted their screening decisions, highlighting the need for attention in these areas.

Implementing the widely accepted screening guidelines' recommendations developed in high-income countries may not be feasible in low-resource settings, and there is an urgent need to implement cancer screening programs desired for low-income transplant settings. Future studies are imperative to develop and evaluate cost-effective screening strategies in LMIC, ensuring equitable and accessible post-transplant care for all.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the corresponding author upon request.

ETHICS STATEMENT

This study involved human participants and was approved by the University of Western Australia Human Ethics Committee (Approval Number: 2022/ET000790). Reciprocal approval was obtained from Flinders University's Research Ethics and Compliance Office (Approval Number: 5966). The study was conducted in accordance with local legislation and institutional requirements. All participants provided written informed consent prior to their participation.

AUTHOR CONTRIBUTIONS

NS: Participated in research performance, data analysis, and article writing. WL: Participated in research design, performance, and manuscript editing. JS: Participated in research design, data analysis, and article writing. AW: Participated in research design and article writing. BB: Participated in research design and article writing. AJ: Participated in research design and manuscript editing. AT-P: Participated in data analysis and manuscript editing. ED: Participated in data analysis and manuscript editing. MH: Participated in research design, performance, and manuscript editing. FB: Participated in data analysis and manuscript editing. AvZ: Participated in research design, performance, and manuscript editing. CG: Participated in research design and research performance. NS-R: Participated in research design and research performance. SC: Participated in article writing. CH: Participated in research design. JoC: Participated in article writing. JeC: Participated in research performance and manuscript editing. DH: Participated in research performance. GK: Participated in research performance. NM: Participated in research performance and manuscript editing. GW: Participated in research design, performance and article writing.

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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.

GENERATIVE AI STATEMENT

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2024.13965/full#supplementary-material

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Associations of Pretransplant Patient-Reported Outcomes Measurement Information System Physical Function Score With Kidney Transplant Outcomes

*Junji Yamauchi[1], Amy M. Cizik[2], Katalin Fornadi[3], Dominik Thomas[1], Divya Raghavan[1], Duha Jweehan[1], Suayp Oygen[1], Silviana Marineci[1], Michelle Buff[3], Motaz Selim[3], Michael Zimmerman[3], Istvan Mucsi[4,5] and Miklos Z. Molnar[1]**

[1]Department of Internal Medicine, Division of Nephrology and Hypertension, Spencer Fox Eccles School of Medicine at the University of Utah, Salt Lake City, UT, United States, [2]Department of Orthopedics, Spencer Fox Eccles School of Medicine at the University of Utah, Salt Lake City, UT, United States, [3]Department of Surgery, Division of Transplantation and Advanced Hepatobiliary Surgery, Spencer Fox Eccles School of Medicine at the University of Utah, Salt Lake City, UT, United States, [4]Temerty Faculty of Medicine, University of Toronto, Toronto, ON, Canada, [5]Ajmera Transplant Centre, University Health Network, Toronto, ON, Canada

Simple and validated physical function measures are needed for kidney transplant candidates because pretransplant low physical function is a common and potentially modifiable risk factor. This single-center retrospective study investigated the associations between pretransplant physical function assessed by the Patient-Reported Outcomes Measurement Information System® Physical Function (PROMIS-PF) computer adaptive testing and early posttransplant outcomes. We analyzed 154 adult kidney-alone transplant recipients. The median pretransplant PROMIS-PF score was 43 (interquartile range, 39–47). Patient characteristics were not significantly different across the score category (normal, score ≥45; mild, score of 40–45; and moderate/severe, score <40). The PROMIS-PF score was not associated with length of transplant hospital stay, delayed graft function, 6-month and 12-month graft function, or 12-month patient and graft survival. However, a lower PROMIS-PF score was significantly associated with a higher risk of emergency room visits [adjusted odds ratios compared to normal: mild, 1.68 (95% confidence interval, 0.76–3.83); moderate/severe, 3.23 (1.34–7.79)] and rehospitalization [adjusted odds ratios: mild, 2.61 (1.16–5.90); moderate/severe, 2.53 (1.07–6.00)] within 1 month posttransplant. Results suggest that PROMIS-PF is a practical tool for assessing physical function in kidney transplant candidates. Larger studies are needed to confirm the utility of PROMIS-PF to identify transplant candidates who would benefit from pretransplant prehabilitation.

Keywords: kidney transplantation, transplant outcomes, PROMIS®, Patient-Reported Outcomes Measurement Information System®, physical function

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***Correspondence**
Miklos Z. Molnar,
miklos.molnar@hsc.utah.edu

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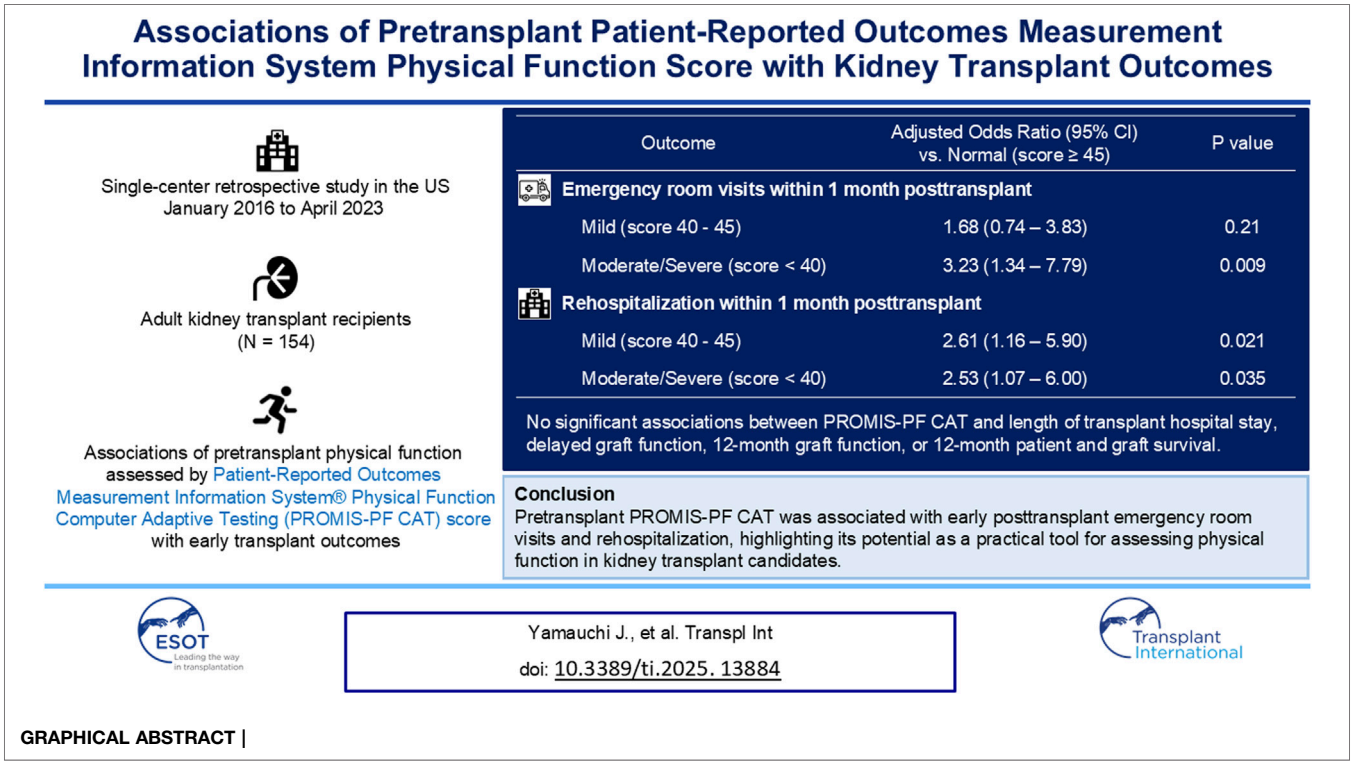
Abbreviations: CAT, computer adaptive testing; eGFR, estimated glomerular filtration rate; IQR, interquartile range; IRT, item response theory; LOS, length of transplant hospital stay; PROMs, Patient-reported outcome measures; PROMIS-PF, Patient-Reported Outcomes Measurement Information System Physical Function.



GRAPHICAL ABSTRACT |

INTRODUCTION

Low physical function is common among individuals with kidney failure and is associated with poor prognosis after kidney transplantation [1–5]. Therefore, physical function assessment may help identify kidney transplant recipients who might be candidates for pretransplant rehabilitation (prehabilitation). However, performance-based physical function assessments, such as the short physical performance battery or the 6-Minute Walk Test, require training to administer and can be time-consuming, more efficient, validated, assessment tools are needed for daily clinical use [6]. Patient-reported outcome measures (PROMs), such as the 36-item Short Form Health Survey (SF-36), are also utilized to evaluate physical function [7]. Although they are easier to administer than physical performance tests, the burden of completing extensive questionnaires remains a significant barrier to widespread use. Furthermore, the reliability of these tools is limited in patients with markedly below-average physical functioning [8].

The Patient-Reported Outcomes Measurement Information System (PROMIS®) was developed with the support of the National Institutes of Health to establish standardized, generic patient-reported outcome measures [9]. PROMIS offers fixed-length testing and computer adaptive testing (CAT). In fixed-length short form testing, using 4-item or 8-item short forms, a predetermined set of questions is administered irrespective of the respondent's functional status. Conversely, PROMIS CAT utilizes item banks and administers questions that are optimized by item response theory (IRT) and selected based on previous answers using score estimation algorithms [10]. With CATs, all participants begin with the first item, targeting the midpoint of the T-score (functional) range. Subsequent items are selected by an algorithm based on responses to previous items until a stopping rule (reliability >90% or completing 12 items) is satisfied. PROMIS CAT requires fewer questions compared to other PROMs not developed using IRT, thereby substantially reducing the question burden. The CAT system can yield highly precise results with an average of only 4–6 questions. PROMIS CAT and short forms produce comparable scores [11].

PROMIS Physical Function (PROMIS-PF) measures the domain of physical function and has been validated in several disease conditions including chronic kidney disease [12–15]. A recent study found that a lower pretransplant PROMIS-PF 4-item short form score was significantly associated with a higher risk of rehospitalization within 1 month after kidney transplantation [16]. However, the utility of pretransplant PROMIS-PF CAT assessments or their associations with posttransplant outcomes have not been evaluated in kidney transplant recipients. We therefore investigated the associations of the pretransplant PROMIS-PF CAT scores with transplant outcomes within 12 months posttransplant. Our hypothesis was that a lower pretransplant PROMIS-PF CAT score is associated with worse early posttransplant outcomes, such as higher hospitalization rates and longer transplant hospital stays.

MATERIALS AND METHODS

Data Source and Study Population

This retrospective study included adult kidney-alone transplant recipients who underwent transplantation at the University of Utah hospital from January 2016 to April 2023 and received a PROMIS-PF CAT within 12 months pretransplant. Recipients less than 18 years of age or those who underwent multi-organ transplantation were excluded. Patient data were extracted from our enterprise data warehouse. This study was approved by the University of Utah Institutional Review Board (IRB_00162331), which also granted an exemption from informed consent.

Measurement and Interpretation of the PROMIS-PF Score

PROMIS-PF item banks version 1.2 or version 2.0 were administered as CAT at outpatient clinics for non-research, clinical purposes using our proprietary university-developed system, My Evaluation (mEVAL), which was introduced at University of Utah Health in 2015 to facilitate standardized PROM assessments across various care settings [17]. The PROMIS-PF item banks consist of 165 items across four subdomains: instrumental activities of daily living, mobility or lower extremity function, back and neck (central) function, and upper extremity function [18]. Responses to the items range from 1 ("cannot do") to 5 ("not at all" or "without any difficulty"). PROMIS-PF was scored using the T-score metric. The PROMIS-PF score ranges from 20 points to 80 points, with the US general population mean ± standard deviation of 50 ± 10. A higher score indicates better physical function. The PROMIS scoring guidelines classify PROMIS-PF scores into no significant physical function impairment (normal, score ≥45), mild (40 to <45), moderate (30 to <40), and severe (<30) [19]. In the current study, PROMIS-PF scores were categorized into normal, mild, and moderate/severe because only six patients fell into the severe category. For patients with multiple measurements within 12 months preceding the index kidney transplantation, the PROMIS-PF score closest to the transplant date was used for analysis. We did not perform the psychometric property testing because it has been already established in the chronic kidney disease population [12].

Outcomes

The outcomes of interest were associations between the pretransplant PROMIS-PF score and early post-transplant outcomes, including length of transplant hospital stay (LOS), delayed graft function defined as any dialysis in the first week post-transplant, emergency room visits and rehospitalization for any reason within 1 month posttransplant, 6-month and 12-month estimated glomerular filtration rate (eGFR) calculated via the Chronic Kidney Disease Epidemiology Collaboration equation 2021 [20], and 12-month patient and graft survival. We collected data on emergency room visits and rehospitalizations to our hospital because our clinical protocol required all recipients to remain near our hospital and contact us directly during the first month of post-transplant. All rehospitalizations were included regardless of the length of hospital stay.

Covariates for Multivariable Regression Analysis

For multivariable linear and logistic regression analyses, we selected covariates based on published literature and theoretical considerations [16, 21, 22]. We adjusted for donor factors (age, donor type, donation after brain death/circulatory death, and cold ischemia time) and recipient variables (age, sex, race, Charleson Comorbidity Index [23, 24], prior organ transplant, preemptive transplant, calculated panel reactive antibody, and lymphocyte-depleting antibody induction). In the logistic regression for emergency room visits and rehospitalization, due to the limited number of events, we first calculated propensity scores for each outcome using all the covariates and then calculated odds ratios, adjusting only for the propensity score.

Statistical Analysis

We used mean ± standard deviation or median and interquartile range (IQR) for summarizing continuous variables and number (%) for categorical variables. Patient characteristics at transplantation and observed outcomes were delineated in accordance with the PROMIS-PF score category (normal, mild, and moderate/severe). We used the Jonckheere–Terpstra trend test to analyze the trends of baseline characteristics and outcomes across the PROMIS-PF score category. We used linear regression to analyze the associations of the PROMIS-PF score with LOS and 6-month and 12-month eGFR. Logistic regression was used to analyze the associations of the PROMIS-PF score with the presence/absence of emergency room visits and rehospitalization within 1 month posttransplant. We used the two-sided p-value of <0.05 to adjudicate statistical significance. STATA Version 18 was used for all statistical analyses (STATA Corporation, College Station, TX).

RESULTS

Patient Characteristics

Among 1,012 kidney transplant recipients, a total of 154 kidney recipients had the PROMIS-PF score evaluated within 12 months pretransplant (**Figure 1**). The median number of PROMIS-PF tests was 1 (IQR, 1–2), with 113 recipients (73%) undergoing one assessment within 12 months pretransplant. PROMIS-PF was assessed at a median of 5 (IQR, 3–8) months prior to transplantation. The median number of questions answered was 4 (IQR, 4–4), with 139 recipients (90%) required to answer four questions. The maximum number of questions answered was 11. **Table 1** shows patient characteristics at kidney transplantation according to the pretransplant PROMIS-PF score category [normal (n = 61, 40%), mild (n = 52, 34%), and moderate/severe (n = 41, 27%)]. Median pretransplant PROMIS-PF score was 43 (IQR, 39–47).

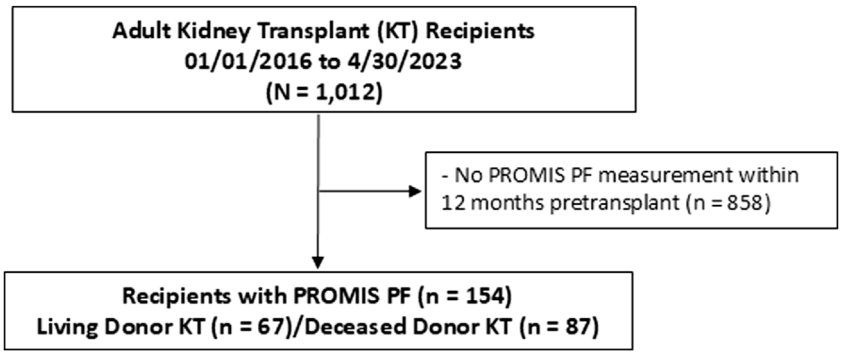


FIGURE 1 | Study flowchart. PROMIS PF, Patient-Reported Outcomes Measurement Information System Physical Function.

Recipients had a mean age of 52 ± 14 years; 41% were female; and the majority were white (68%). History of diabetes was reported in 38% and 32% had kidney failure from diabetes. Median Charleson Comorbidity Index was 5 (IQR, 3–6). Additionally, 25% underwent preemptive transplant. Lymphocyte-depleting antibody induction (anti-thymocyte globulin or alemtuzumab) was administered to 62% of recipients. The majority received tacrolimus (91%), mycophenolate (100%), and steroids (100%) as maintenance immunosuppression at transplant hospitalization discharge. Donors were living in 44%, with a mean age of 39 ± 15 years, and 50% were female. Overall, no significant trends were found in recipient and donor characteristics across the PROMIS-PF category.

Early Posttransplant Outcomes

Table 2 summarizes the observed outcomes. The median LOS was 3 (IQR, 3–4) days. Delayed graft function was reported in 6 recipients (4%). The mean 6-month and 12-month eGFR were 63 ± 21 and 63 ± 20 mL/min/1.73 m^2. Patient death and death-censored graft failure were reported in 10 (6%) and 2 (1%) recipients at 12 months. Biopsy-proven rejection and *de novo* donor-specific antibody class I and class II were observed in 15 (10%), 11 (7%), and 18 (12%) recipients at 12 months, respectively. There were no significant trends in these outcomes across the PROMIS-PF category. Emergency room visits and rehospitalization within 1 month posttransplant were observed in 58 (38%) and 59 (38%) recipients, respectively; and proportions of these outcomes were significantly higher in the mild and moderate/severe groups than the normal group (Jonckheere–Terpstra test for trend, p = 0.018 and 0.024, respectively). Reasons for emergency room visits and rehospitalizations are summarized in **Supplementary Table S1**. Infection was the most common reason for emergency room visits (n = 14, 9%), including urinary tract infections (n = 9, 6%) and other infections (n = 5, 3%). Surgical complications were the second most frequent cause (n = 13, 8%), primarily related to surgical wounds (n = 11, 7%) and other surgical complications (n = 2, 1%). Similarly, rehospitalization was most often due to infection (n = 16, 10%), with urinary tract infections (n = 8, 5%) being the primary contributor. Surgical complications were also the second leading cause of rehospitalization (n = 10, 6%), due to surgical wound problems (n = 6, 4%) and other complications (n = 4, 3%).

Associations of Pretransplant PROMIS-PF Score and Transplant Outcomes

In the multivariate regression analysis, the pretransplant PROMIS-PF score was not significantly associated with LOS or 6-month/12-month eGFR (**Table 3**; coefficients for covariates are available in **Supplementary Tables S2–S4**). However, significant associations were found between the pretransplant PROMIS-PF score and emergency room visits and rehospitalization within 1 month (**Table 4**). Adjusted odds ratios for emergency room visits increased with the decrease in the pretransplant PROMIS-PF score [1.68 (95% confidence interval, 0.74–3.83) and 3.23 (1.34–7.79) in the mild and moderate/severe groups, respectively, with the normal category as the reference]. The risk of rehospitalization was significantly higher both in the mild and moderate/severe groups [adjusted odds ratios, 2.61 (1.16–5.90) and 2.53 (1.07–6.00)]. Multivariate regression analysis was not performed for other outcomes due to the small event numbers.

Comparison Between Recipients With and Without the PROMIS-PF Assessment

Given that only a subset of all transplant recipients completed the PROMIS-PF prior to their kidney transplantation, we compared the characteristics and transplant outcomes of recipients with and without the PROMIS-PF assessment (**Supplementary Tables S5, S6**). Compared to the recipients without the assessment, those with the assessment were more likely to have diabetes (31% vs. 38%) and exhibited higher Charlson Comorbidity Index values [median (IQR), 4 (2–5) vs. 5 (3–6)]. Recipients with the assessment more frequently received living-donor kidneys (34% vs. 44%) and less likely received the lymphocyte-depleting antibody induction (76% vs. 62%). Additionally, those with the score experienced higher rates of 1-month rehospitalization (22% vs. 38%) and 12-month mortality (3%

TABLE 1 | Patient characteristics.

Characteristic	Total	PROMIS PF category			p for trend
		Normal (≥45)	Mild (40–<45)	Moderate/Severe (<40)	
	N = 154	N = 61	N = 52	N = 41	
Recipient					
Pretransplant Physical Function score	43 (39–47)	48 (46–52)	41 (40–43)	36 (34–38)	<0.001
Number of PROMIS-PF questions answered	4 (4–4)	4 (4–4)	4 (4–4)	4 (4–4)	0.13
Months from pretransplant assessment to transplant	5 (8–3)	6 (8–4)	5 (8–2)	4 (7–3)	0.25
Age (years)	52 (14)	53 (15)	52 (15)	51 (14)	0.44
Sex					0.40
Female	63 (41%)	23 (38%)	21 (40%)	19 (46%)	
Male	91 (59%)	38 (62%)	31 (60%)	22 (54%)	
Body mass index (kg/m^2)	28.2 (5.2)	27.3 (4.8)	28.8 (5.1)	28.7 (5.6)	0.15
Race					0.26[a]
White	105 (68%)	42 (69%)	35 (67%)	28 (68%)	
Black	5 (3%)	1 (2%)	0 (0%)	4 (10%)	
Hispanic	21 (14%)	7 (11%)	9 (17%)	5 (12%)	
Asian	5 (3%)	1 (2%)	3 (6%)	1 (2%)	
American Indian/Alaska Native	4 (3%)	1 (2%)	2 (4%)	1 (2%)	
Native Hawaiian/other Pacific Islander	13 (8%)	8 (13%)	3 (6%)	2 (5%)	
Multiracial	1 (1%)	1 (2%)	0 (0%)	0 (0%)	
History of diabetes	59 (38%)	23 (38%)	21 (40%)	15 (37%)	0.96
Prior organ transplantation	15 (10%)	9 (15%)	4 (8%)	2 (5%)	0.085
Dialysis duration					0.73
Preemptive	38 (25%)	15 (25%)	14 (27%)	9 (22%)	
≤1 year	25 (16%)	9 (15%)	10 (19%)	6 (15%)	
1–3 years	32 (21%)	13 (21%)	10 (19%)	9 (22%)	
3–5 years	27 (18%)	13 (21%)	7 (13%)	7 (17%)	
>5 years	32 (21%)	11 (18%)	11 (21%)	10 (24%)	
Cause of kidney failure					0.62[a]
Diabetes	50 (32%)	19 (31%)	19 (37%)	12 (29%)	
Hypertension	19 (12%)	6 (10%)	5 (10%)	8 (20%)	
Glomerulonephritis	22 (14%)	9 (15%)	5 (10%)	8 (20%)	
Cystic disease	27 (18%)	10 (16%)	11 (21%)	6 (15%)	
Others	36 (23%)	17 (28%)	12 (23%)	7 (17%)	
Hepatitis B virus core antibody	12 (9%)	9 (17%)	0 (0%)	3 (8%)	0.060
Hepatitis C virus antibody	5 (3%)	2 (3%)	1 (2%)	2 (5%)	0.74
Human immunodeficiency virus antibody	2 (1%)	0 (0%)	2 (4%)	0 (0%)	0.14
Charlson Comorbidity Index at transplant	5 (3–6)	5 (3–6)	5 (4–7)	5 (3–7)	0.55
Charlson Comorbidity Index category (tertile)					0.44
2–4	69 (45%)	30 (49%)	21 (40%)	18 (44%)	
5–6	47 (31%)	18 (30%)	17 (33%)	12 (29%)	
7–16	38 (25%)	13 (21%)	14 (27%)	11 (27%)	
Calculated panel reactive antibody (%)	0 (0–0)	0 (0–16)	0 (0–0)	0 (0–0)	0.35
Human leucocyte antigen mismatch					0.74
0	8 (5%)	5 (8%)	1 (2%)	2 (5%)	
1	7 (5%)	4 (7%)	1 (2%)	2 (5%)	
2	16 (10%)	8 (13%)	4 (8%)	4 (10%)	
3	23 (15%)	8 (13%)	7 (13%)	8 (20%)	
4	31 (20%)	10 (16%)	15 (29%)	6 (15%)	
5	49 (32%)	15 (25%)	19 (37%)	15 (37%)	
6	20 (13%)	11 (18%)	5 (10%)	4 (10%)	
Induction immunosuppression					
Lymphocyte depleting induction	95 (62%)	40 (66%)	29 (56%)	26 (63%)	0.69
Anti-thymocyte globulin	56 (36%)	20 (33%)	21 (40%)	15 (37%)	0.61
Alemtuzumab	41 (27%)	21 (34%)	8 (15%)	12 (29%)	0.34
Basiliximab	10 (6%)	3 (5%)	4 (8%)	3 (7%)	0.58
Maintenaice immunosuppression at discharge					
Tacrolimus	140 (91%)	57 (93%)	46 (88%)	37 (90%)	0.50
Cyclosporine	2 (1%)	1 (2%)	0 (0%)	1 (2%)	0.86
Everolimus	1 (1%)	0 (0%)	1 (2%)	0 (0%)	0.81
Belatacept	19 (12%)	6 (10%)	8 (15%)	5 (12%)	0.62
Mycophenolate	154 (100%)	61 (100%)	52 (100%)	41 (100%)	—
Steroids	154 (100%)	61 (100%)	52 (100%)	41 (100%)	—

(Continued on following page)

TABLE 1 | (*Continued*) Patient characteristics.

Characteristic	Total	PROMIS PF category			p for trend
		Normal (≥45)	Mild (40–<45)	Moderate/Severe (<40)	
	N = 154	N = 61	N = 52	N = 41	
Donor					
Donor type					0.80
Living donor	67 (44%)	27 (44%)	23 (44%)	17 (41%)	
Deceased donor	87 (56%)	34 (56%)	29 (56%)	24 (59%)	
Age (years)	39 (15)	39 (15)	38 (15)	41 (15)	0.62
Sex					0.90
Female	77 (50%)	29 (48%)	30 (58%)	18 (44%)	
Male	77 (50%)	32 (52%)	22 (42%)	23 (56%)	
Terminal serum creatinine (mg/dL)	0.96 (0.48)	0.90 (0.29)	0.99 (0.66)	1.00 (0.46)	0.44
Kidney Donor Profile Index[b]	39 (23)	39 (24)	35 (22)	46 (22)	0.34
Donation after circulatory death[b]	30 (34%)	11 (32%)	9 (31%)	10 (42%)	0.51
Donor kidney on-pump	86 (56%)	35 (57%)	28 (54%)	23 (56%)	0.85
Cold ischemia time (hours)	9 (8)	9 (8)	10 (9)	8 (8)	0.83

Recipients were stratified into three groups: no significant physical function impairment (PROMIS-PF score ≥45), mild (40–<45), and moderate/severe (<40). Values are expressed as mean (standard deviation), median (interquartile range), or number (%). The Jonckheere–Terpstra trend test was used to calculate p-values for trend.

[a]*P-values for race and cause of kidney failure were calculated via Chi-square tests.*

[b]*Only for deceased donors.*

PROMIS PF, Patient-Reported Outcomes Measurement Information System Physical Function.

TABLE 2 | Transplant outcomes according to the PROMIS-PF score category.

Outcome	Total	PROMIS PF category			p for trend
		Normal (≥45)	Mild (40–<45)	Moderate/Severe (<40)	
	N = 154	N = 61	N = 52	N = 41	
Length of hospital stay (days)	3 (3–4)	3 (3–4)	3 (3–4)	3 (3–4)	0.68
Length of hospital stay ≥7 days	13 (8%)	3 (5%)	7 (13%)	3 (7%)	0.49
Delayed graft function	6 (4%)	2 (3%)	3 (6%)	1 (2%)	0.95
Any emergency room visit within 1 month	58 (38%)	17 (28%)	20 (38%)	21 (51%)	**0.018**
Any rehospitalization within 1 month	59 (38%)	16 (26%)	24 (46%)	19 (46%)	**0.024**
6-month eGFR (mL/min/1.73 m^2)	63 (21)	64 (18)	61 (22)	64 (24)	0.79
12-month eGFR (mL/min/1.73 m^2)	63 (20)	62 (20)	62 (21)	66 (21)	0.63
6-month death-censored graft failure	1 (1%)	0 (0%)	1 (2%)	0 (0%)	0.81
12-month death-censored graft failure	2 (1%)	0 (0%)	2 (4%)	0 (0%)	0.73
6-month mortality	2 (1%)	1 (2%)	0 (0%)	1 (2%)	0.87
12-month mortality	10 (6%)	3 (5%)	5 (10%)	2 (5%)	0.85
Rejection within 12 months	15 (10%)	5 (8%)	7 (13%)	3 (7%)	0.96
de novo DSA class I within 12 months	11 (7%)	4 (7%)	4 (8%)	3 (7%)	0.86
de novo DSA class II within 12 months	18 (12%)	4 (7%)	9 (17%)	5 (12%)	0.26

Recipients were stratified into three groups: no significant physical function impairment (PROMIS-PF score ≥45), mild (40-<45), and moderate/severe (<40). Values are expressed as mean (standard deviation), median (interquartile range), or number (%). The Jonckheere–Terpstra trend test was used to calculate p-values for trend. Bold values denote statistically significant differences with p-values <0.05.

DSA, donor-specific antibody; PROMIS PF, Patient-Reported Outcomes Measurement Information System Physical Function.

vs. 6%). Other characteristics and outcomes did not show substantial differences between the groups.

DISCUSSION

Physical function is a significant and potentially modifiable prognostic factor among kidney transplant recipients [25, 26]. Low physical function is a major component of frailty, a condition common in kidney failure that is characterized by declines in physiological and cognitive states, associated with reduced physiologic reserve [1, 27]. Frailty is also associated with poor posttransplant outcomes and prehabilitation is being explored to improve outcomes [26, 28]. Therefore, it is imperative to establish simple and feasible physical function assessment tools to efficiently identify transplant candidates who may benefit from pretransplant prehabilitation. In this retrospective exploratory study, we investigated the associations between pretransplant PROMIS-PF scores and early transplant outcomes among kidney transplant recipients. While the pretransplant PROMIS-PF score was not associated with LOS or graft function, it was significantly associated with emergency room visits and rehospitalization within 1 month posttransplant. To our knowledge, this is the first study

TABLE 3 | Multivariate liner regression analysis of length of hospital stay and graft function.

PROMIS-PF score	Coefficient	95% CI	p-value
Length of stay (days, natural log-transformed)			
PROMIS-PF score (per 1-point increase)	–0.01	(–0.02, 0.00)	0.20
PROMIS-PF score category			
Normal (≥45): reference	ref		
Mild (40–<45)	0.14	(–0.05, 0.32)	0.15
Moderate/Severe (<40)	0.05	(–0.15, 0.25)	0.65
6-month eGFR (mL/min/1.73 m^2)			
PROMIS-PF score (per 1-point increase)	0.0	(–0.5, 0.5)	0.90
PROMIS-PF score category			
Normal (≥45): reference	ref		
Mild (40–<45)	–5.1	(–12.7, 2.4)	0.18
Moderate/Severe (<40)	–0.2	(–8.3, 7.9)	0.96
12-month eGFR (mL/min/1.73 m^2)			
PROMIS-PF score (per 1-point increase)	–0.1	(–0.6, 0.3)	0.61
PROMIS-PF score category			
Normal (≥45): reference	ref		
Mild (40–<45)	–2.3	(–9.7, 5.0)	0.53
Moderate/Severe (<40)	2.5	(–5.4, 10.4)	0.53

Linear regression was conducted for continuous PROMIS-PF scores and separately for the PROMIS-PF score category, adjusting for donor factors (age, donor type, donation after brain death/circulatory death, and cold ischemia time) and recipient variables (age, sex, race, Charleson Comorbidity Index, prior organ transplant, preemptive transplant, calculated panel reactive antibody, and lymphocyte-depleting antibody induction). Length of stay (days) was natural log-transformed to achieve a normal distribution.
CI, confidence interval; PROMIS PF, Patient-Reported Outcomes Measurement Information System Physical Function.

TABLE 4 | Multivariate logistic regression analysis for emergency room visits and rehospitalization within 1-month posttransplant.

PROMIS-PF score	Odds ratio	95% CI	p-value
Emergency room visits within 1 month			
PROMIS-PF score (per 1-point increase)	0.94	(0.89, 0.99)	**0.023**
PROMIS-PF score category			
Normal (≥45): reference	ref		
Mild (40–<45)	1.68	(0.74, 3.83)	0.21
Moderate/Severe (<40)	3.23	(1.34, 7.79)	**0.009**
Rehospitalization within 1 month			
PROMIS-PF score (per 1-point increase)	0.94	(0.90, 1.00)	**0.033**
PROMIS-PF score category			
Normal (≥45): reference	ref		
Mild (40–<45)	2.61	(1.16, 5.90)	**0.021**
Moderate/Severe (<40)	2.53	(1.07, 6.00)	**0.035**

Associations between PROMIS-PF scores and events were analyzed using logistic regression, adjusting for the propensity scores that were calculated using donor factors (age, donor type, donation after brain death/circulatory death, and cold ischemia time) and recipient variables (age, sex, race, Charleson Comorbidity Index, prior organ transplant, preemptive transplant, calculated panel reactive antibody, and lymphocyte-depleting antibody induction). Logistic regression was performed for continuous PROMIS-PF scores and separately for the PROMIS-PF score category. Bold values denote statistically significant differences with p-values <0.05.
CI, confidence interval; PROMIS PF, Patient-Reported Outcomes Measurement Information System Physical Function.

evaluating the PROMIS-PF CAT score in this patient population.

Previous studies have indicated that pretransplant low physical function and frailty are linked to longer LOS after kidney transplantation [29]. Lorenz et al. and Nastasi et al. conducted single-center studies that demonstrated a significant association between longer LOS and lower extremity functional impairment assessed using the Short Physical Performance Battery [30, 31]. In contrast, we found no association between the pretransplant PROMIS-PF score and LOS. This might be partly attributable to differences in the study periods because LOS has decreased over time [29]. Our study, covering kidney transplants between 2016 and 2023, reported a median LOS of 3 days. In comparison, the studies by Lorenz et al. and Nastasi et al., including kidney transplants before 2016, had median LOS of 4 and 8 days, respectively. Shorter LOS and improvements in patient care might have minimized LOS differences in our study. Additionally, variations in clinical practices and eligibility criteria for kidney transplantation between transplant centers could also explain the lack of association in the present study.

We did not find associations between the pretransplant PROMIS-PF score and graft function in this study. As serum creatinine concentration is influenced by muscle mass, the eGFR may be overestimated in recipients with lower physical function due to potentially reduced muscle mass. However, our findings are consistent with those of Lorenz et al., who also found no

association between the Short Physical Performance Battery score and 12-month graft function measured via iothalamate clearance [30].

In line with previous studies that utilized different physical function measures such as the Kidney Disease Quality of Life Short Form and the Short Physical Performance Battery [30, 32, 33], the pretransplant PROMIS-PF score was significantly associated with posttransplant emergency room visits and rehospitalization. The odds ratios of emergency room visits increased with decreasing PROMIS-PF scores, indicating that the pretransplant PROMIS-PF score effectively captures these risks. The risk of rehospitalization was higher even in the mild group compared with the normal group. Our findings also align with those of Lorenz et al., who similarly reported a significant association between lower pretransplant PROMIS-PF 4-item short form scores with a higher risk of early rehospitalization after kidney transplantation [16]. Notably, they also found that the predictive value of the PROMIS-PF 4-item short form was comparable to frailty measures, including the physical frailty phenotype and the Short Physical Performance Battery. According to the study by Brodke et al., which documented the real-life physical ability indicated by the PROMIS-PF score, the physical function of score 45, distinguishing the normal from mild categories, corresponds to "Some difficulty with 2 h of physical labor and yard work; little difficulty with household chores and walking greater than 1 mile." [34] Similarly, a score of 40, making the threshold of the mild and moderate/severe categories, corresponds to "Some difficulty with 2 h of physical labor, household chores, yard work, and walking greater than 1 mile." These levels of pretransplant physical function may serve as a risk indicator for early posttransplant emergency room visits and rehospitalization.

Previous research on physical function and frailty demonstrated significant associations with delayed graft function, mortality, and graft survival [25, 28, 35]. Our study, however, could not evaluate these associations due to the small number of events observed. Larger-scale studies powered to detect clinician-driven outcomes are needed. However, these outcomes, such as graft function and survival, may not be as important to the patient as the quality-of-life health outcomes that are measured using PROMIS-PF. Measures such as PROMIS-PF allow patients to self-report their health status and subsequently one can assume they measure values and preferences that matter most to patients. A patient may care more about improving their ability to do physical labor, household chores, and yard work from much difficulty to little difficulty than whether they had delayed graft function. A preference elicitation study by Genie, et al. revealed that patient preferences among individuals with kidney failure are heterogeneous based on the patient's age and duration of dialysis [36]. They found that graft survival did matter to patients and that patients were willing to wait an additional 29 months for transplantation for a graft that survived 5 more years (15 years vs. 10 years graft survival). Future preference elicitation studies should include quality of life outcomes and tradeoffs between clinical and graft survival outcomes among kidney transplant patients.

Given that PROMIS CAT demonstrates superior accuracy in measuring physical functioning across a broader range compared to other PROMs and achieves more precise results with fewer questions compared to most short forms, PROMIS CAT is considered particularly advantageous in the following situations: (1) assessing individuals with extremely poor health, (2) accurately measuring individuals with very good health, and (3) administering a small number of items [8, 11]. In situations with a broad range of anticipated physical functioning, CAT provides an accurate assessment with fewer items by tailoring questions to the individual's functional level, avoiding asking irrelevant questions. This is relevant when assessing kidney transplant candidates. Furthermore, advantage (3) is a key feature for implementing universal and prospective physical function assessments in patients with kidney failure throughout the disease continuum, minimizing the burden on both patients and providers, particularly in high-volume centers. Our findings support the rationale for introducing PROMIS-PF CAT in such settings.

This study has several limitations. As this is a single-center retrospective study with a relatively small sample size and predominantly white patients, the generalizability of our findings may be limited. Selection bias is a potential concern given that PROMIS-PF tests were administered for clinical purposes and that only a portion of our patients were included in this study. Indeed, the comparison between recipients with and without the PROMIS-PF assessment suggested higher risk profiles among those with the assessment. Thus, the PROMIS-PF scores presented in this study may be worse than those of the general kidney transplant population. However, we believe that these relatively small differences do not have a substantial impact on our results. We were unable to adjust for all potential confounding factors due to the small sample size and limited event numbers. We also could not analyze important outcomes, such as mortality and graft failure. Additionally, because we had no standardized criteria for emergency room visits or admissions, these outcomes are subject to subjective decisions and may not be considered as strict research endpoints. While PROMIS CAT is suggested to provide more accurate results than fixed-length testing [11], we were unable to compare PROMIS-PF CAT with other physical function and frailty measures because we did not have these data.

In conclusion, a lower pretransplant PROMIS-PF CAT score was associated with a higher risk of emergency room visits and rehospitalization within 1 month posttransplant. Our findings indicate that PROMIS-PF could be a valuable physical function assessment tool in kidney transplant candidates. Further research with extended follow-up and larger sample sizes is needed to confirm the utility of the PROMIS-PF assessment in this population.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by University of Utah Institutional Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because this was a retrospective study using de-identified datasets.

AUTHOR CONTRIBUTIONS

JY, AC, IM, and MM conceived this study. JY, AC, IM, and MM conducted the statistical analysis. JY, AC, IM, and MM analyzed the data. JY, AC, KF, DT, DR, DJ, SO, SM, MB, MS, MZ, IM, and MM interpreted the results. JY, AC, and MM drafted the manuscript. KF, DT, DR, DJ, SO, SM, MB, MS, MZ, and IM critically revised the manuscript. All authors read and approved the final 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.

GENERATIVE AI STATEMENT

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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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.2025.13884/full#supplementary-material

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ORIGINAL RESEARCH
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Pre-Transplant Hypoalbuminemia Is Not Associated With Early Key Outcomes Among Simultaneous Pancreas and Kidney Transplant Recipients

Ekaterina Fedorova[1], *Sofia Nehring Firmino*[2], *Dixon B. Kaufman*[1], *Jon S. Odorico*[1], *David Aufhauser*[1], *Carrie Thiessen*[1], *David P. Al-Adra*[1], *Didier Mandelbrot*[3], *Brad C. Astor*[3,4] *and Sandesh Parajuli*[3]*

[1]*Division of Transplant Surgery, Department of Surgery, University of Wisconsin School of Medicine and Public Health, Madison, WI, United States,* [2]*University of Wisconsin-Madison School of Medicine and Public Health, Madison, WI, United States,* [3]*Division of Nephrology, Department of Medicine, University of Wisconsin School of Medicine and Public Health, Madison, WI, United States,* [4]*Department of Population Health Sciences, University of Wisconsin School of Medicine and Public Health, Madison, WI, United States*

The role of pre-transplant hypoalbuminemia and its impact on post-transplant outcomes in patients undergoing simultaneous pancreas-kidney (SPK) transplantation remains unclear. We analyzed all SPK recipients at our center, who had at least 2 weeks of pancreas and kidney graft survival and had serum albumin measured within 45 days pre-transplant. Recipients were categorized based on pretransplant albumin level as normal (≥4.0 g/dL, N = 222, 42%), mild hypoalbuminemia (≥3.5–<4.0 g/dL, N = 190, 36%), and moderate hypoalbuminemia (<3.5 g/dL, N = 120, 23%). Kidney delayed graft function (DGF), length of stay (LOS) after transplant, re-hospitalization within 30 days after discharge, and need for a return to the operating room (OR) related to transplant surgical complications, acute rejection, and uncensored and death-censored graft failure, within the first years post-transplant were outcomes of interest. A total of 532 SPK recipients were included. Mild or moderate hypoalbuminemia was not associated with DGF, LOS, re-hospitalization, or return to the OR in unadjusted or adjusted analyses. Similarly, mild or moderate hypoalbuminemia was not associated with a risk of graft rejection or graft failure. Among SPK recipients, pre-transplant hypoalbuminemia was not associated with worse outcomes and should not be the determining factor in selecting patients for SPK transplant.

Keywords: hypoalbuminemia, SPK, DGF, patient survival, graft survival

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***Correspondence**
Sandesh Parajuli,
sparajuli@medicine.wisc.edu

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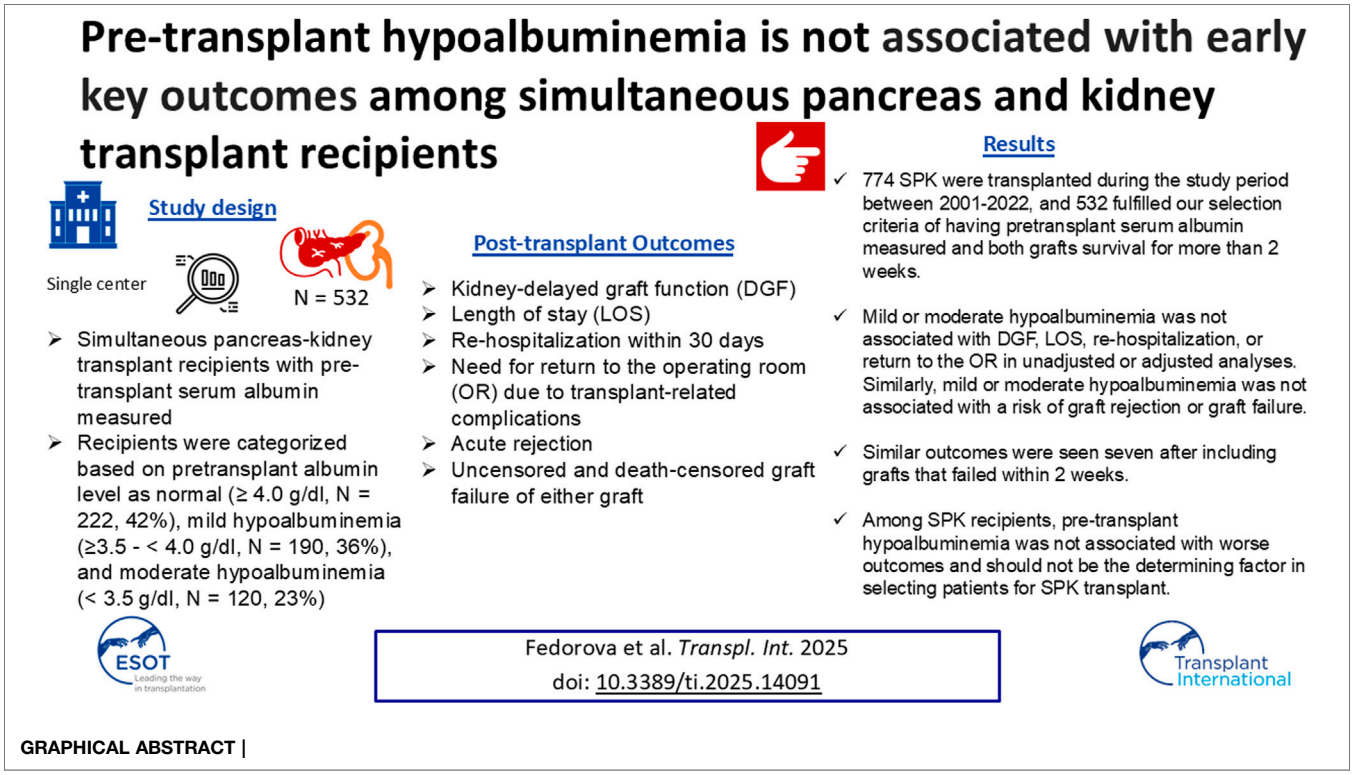
Abbreviations: ABMR, Antibody-Mediated Rejection; aHR, Adjusted Hazard Ratio; BMI, Body Mass Index; CIT, Cold Ischemia Time; CMV, Cytomegalovirus; cPRA, Calculated Panel Reactive Antibody; DCGF, Death Censored Graft Failure; DCD, Donation After Circulatory Death; ESRD, End Stage Renal Disease; HLA, Human Leukocyte Antigen; KDPI, Kidney Donor Profile Index; KDGF, Kidney Delayed Graft Function; LOS, Length of Stay; SPK, Simultaneous Pancreas and Kidney; TCMR, T-cell Mediated Rejection.



GRAPHICAL ABSTRACT |

INTRODUCTION

Albumin is the most abundant plasma protein in the human body and has many functions, including maintaining plasma colloid osmotic pressure, acting as a transport protein, buffering antioxidants, and having anticoagulant effects [1]. Hepatic synthesis of albumin depends on nutritional status and insulin stimulation; it can be decreased due to inflammatory cytokines, such as IL-6 and tumor necrosis factor-alpha [1, 2].

Hypoalbuminemia is multifactorial and results in increased capillary permeability and leakage. It may be related to the release of inflammatory cytokines, decreased nutritional intake, increased excretion, and altered rate of synthesis and catabolism of albumin [1–3]. Hypoalbuminemia is commonly seen in critically ill patients and is often associated with worse outcomes for a variety of medical and surgical conditions [4–9]. Hypoalbuminemia is associated with increased cardiovascular mortality as well as all-cause mortality in patients with end-stage renal disease (ESRD) undergoing hemodialysis [4]. Additionally, pre-operative hypoalbuminemia is associated with increased surgical morbidity, mortality, and increased risk of surgical complications [5–7]. In kidney-only transplantation, patients with pre-transplant hypoalbuminemia have an increased risk of BK and cytomegalovirus (CMV) infection [10]. However, pre-transplant hypoalbuminemia in kidney-only transplant recipients is not associated with an increased rate of re-admission, reoperation, or delayed graft function [11].

Simultaneous pancreas-kidney transplant (SPK) is a form of treatment for those with diabetes mellitus and ESRD as it restores euglycemia and slows the progression of diabetes complications [12, 13]. In these patients, hypoalbuminemia has been proposed to be caused by chronic systemic inflammation and reduced synthesis of albumin [14]. Additionally, post-operative hypoalbuminemia in SPK recipients is associated with an increased rate of CMV virus infection, graft loss, and a trend toward decreased survival [15]. However, the relationship between the severity of pre-transplant hypoalbuminemia and post-transplant outcomes among SPK recipients has not been thoroughly studied. We hypothesize that pre-transplant hypoalbuminemia is associated with an increased rate of postoperative complications in SPK recipients.

MATERIALS AND METHODS

Study Population and Design

This single-center study from the University of Wisconsin-Madison included all adult SPK transplant recipients performed between 01/01/2001 and 12/31/2022. We excluded all recipients whose pancreas or kidney graft failed within 2 weeks of transplant, multiorgan transplant recipients (i.e., simultaneous liver - pancreas), or pancreas after kidney transplant recipients. In subgroup analysis, we analyzed SPK recipients even with graft failure within 2 weeks of transplant. This study was approved by the University of Wisconsin Institutional Review Board (IRB protocol number: 2014-1072). This study followed the Declaration of Helsinki. The clinical and research activities being reported were consistent with the Principles of the

Declaration of Istanbul as outlined in "The Declaration of Istanbul on Organ Trafficking and Transplant Tourism."

Only recipients with serum albumin levels measured within 45 days before transplant were included. In patients with multiple albumin levels measured within 45 days before transplant, we used the measurement closest to the transplant date. SPK recipients without albumin levels within the 45-day timeframe before transplant were excluded. Recipients were categorized based on pretransplant albumin levels as normal (≥4.0 g/dL), mild hypoalbuminemia (≥3.5–< 4.0 g/dL), and moderate hypoalbuminemia (<3.5 g/dL). Although, we initially planned to categorize the severe hypoalbuminemia group defined as <3.0 g/dL, this was not possible due to the extremely small sample size in this group with limited outcomes of interest, so for this reason they were included in the moderate hypoalbuminemia group. Pre or peri-transplant albumin transfusion to correct hypoalbuminemia has never been the current or past practice at our center, although few recipients may have received albumin infusion intraoperative or post-operative for various other indications. At no time during this study period or currently in our program was there a protocolized criterion for pretransplant serum albumin level to approve or disapprove SPK transplant eligibility.

Length of stay (LOS) after index transplant, kidney delayed graft function (DGF), and re-hospitalization within 30 days after discharge were perioperative outcomes of interest followed by the need for a return to the operating room (OR) related to transplant surgical complications, acute rejection of either grafts and uncensored and death-censored graft failure, within the 12 months post-transplant were short-term outcomes of interest. We limited outcomes to the first 12 months post-transplant to better correlate pre-transplant serum albumin levels and early post-transplant outcomes.

Variables and Definitions

Donor characteristics such as age, sex, race, body mass index (BMI), death due to cardiovascular causes, terminal serum creatinine, kidney donor profile index (KPDI), donation after circulatory death (DCD), pancreas cold ischemia time (CIT), and kidney CIT were reported. Information on recipients included age, sex, race, BMI, diabetes type, induction therapy, pre-emptive transplant, and early steroid withdrawal. Immunologic factors reported were panel reactive antibody (cPRA) > 20%, average Human Leukocyte Antigen (HLA) mismatch (of 6), and whether primary or prior transplant.

DGF was defined as a need for dialysis within one-week post-transplantation. Re-hospitalizations within 30 days post initial discharge and any unanticipated return to the operating room within 12 months post-SPK were included. Return to the operating/procedure room for an anticipated ureteric stent removal was not considered a return to the OR. All episodes of rejections were biopsy-proven within 12 months of transplant. Kidney uncensored graft failure was defined as all causes of graft failure including death, while death-censored graft failure (DCGF) was defined as a return to dialysis or re-transplantation, all within 2 weeks–12 months post-transplant. Similarly, pancreas uncensored graft failure was defined as all causes of pancreas graft failure including death. And pancreas DCGF was defined based on the current United Network for Organ Sharing criteria for pancreas graft failure, which include removal of the pancreas graft, re-registration for a pancreas transplant, registration for an islet transplant after receiving pancreas, or an insulin requirement that is ≥0.5 units/kg/day for 90 consecutive days [16, 17].

Serum Albumin Measurement

At our institution, serum albumin concentration measurements are conducted utilizing bromocresol assays. Before 2008, albumin was measured using bromocresol green assays. Since 2008, our institution has used bromocresol purple to assess serum albumin concentrations.

Reference ranges were adjusted to reflect the methodology of each test; prior institutional comparison of both assays on patient samples showed an average negative 0.56-g/dL bias in the newer bromocresol purple assay. This difference was considered to be within allowable limits for general clinical use.

Immunosuppressive Protocols

Our center-specific induction immunosuppression therapy was consistent throughout the study period, either a depleting agent (alemtuzumab or anti-thymocyte globulin) or a non-depleting agent (basiliximab) was utilized. Triple immunosuppression with tacrolimus, mycophenolic acid and prednisone taper was standard for all recipients. Few had early steroid withdrawal based on the immunological risk and patient request as previously described [18].

Biopsy and Rejection Protocols

The two most common indications for kidney biopsy were an unexplained rise in creatinine and proteinuria or the development of *denovo* DSA against HLA as described before [19]. Similarly, the common indications for pancreas biopsy were an unexplained rise in pancreatic enzymes, development of *denovo* DSA, and unexplained hyperglycemia [20]. If possible, we attempt to perform both graft biopsies in the setting of dual graft dysfunction [20].

Similarly, the management of rejections was based on the severity and proximity from the transplant to the diagnosis of rejection as described before [19, 20]. Briefly, kidney T cell-mediated rejection (TCMR) was treated with steroid pulse plus/minus anti-thymocyte globulin. And antibody-mediated rejection (AMR) was treated with steroid pulse, intravenous immunoglobulin (IVIG), plus/minus rituximab, plus/minus plasmapheresis. Treatment of pancreas rejection was based on the type and severity of rejection and was graded by the Banff criteria. TCMR was treated with IV steroid pulse with or without anti-thymoglobulin 6–12 mg/kg in 4–10 divided doses, while mixed rejection was treated with steroids, anti-thymoglobulin, IVIG, and plasmapheresis. Early AMR was treated with steroids, IVIG, and plasmaphereis [20].

Statistical Analysis

Continuous data were compared using Student's *t*-test or the Wilcoxon rank-sum test, where appropriate. Categorical data

TABLE 1 | Baseline characteristics of participants.

	Variables	Overall (N = 532)	<3.5 (n = 120)	≥3.5–<4.0 (n = 190)	≥4.0 (n = 222)	P
Donor Factors	Mean age (yrs)	28.0 (12.2)	27.7 (12.6)	26.3 (11.0)	29.7 (12.8)	0.06
	Female (%)	38.0	40.8	33.7	40.1	0.88
	Non-white (%)	13.2	12.5	13.7	13.1	0.92
	Mean body mass index (kg/m^2)	23.7 (4.3)	23.8 (4.0)	23.8 (4.2)	24.1 (4.5)	0.35
	Cause of death: Cardiovascular (%)	19.0	17.5	13.2	24.8	**0.01**
	Terminal serum Creatinine (mg/dL)	1.03 (0.89)	0.90 (0.50)	1.08 (1.05)	1.06 (0.91)	0.15
	Mean kidney donor profile index %	20.2 (16.5)	21.3 (17.8)	17.3 (15.4)	21.8 (16.2)	0.79
	Donation after circulatory death (%)	14.3	19.2	15.3	10.8	**0.03**
	Pancreas Cold ischemia time (hrs)	14.5 (4.5)	13.2 (4.2)	14.5 (4.0)	15.2 (4.8)	**<0.001**
	Kidney Cold ischemia time (hrs)	15.3 (4.4)	14.8 (4.3)	15.2 (3.9)	15.6 (4.9)	0.14
Immunologic Factors	cPRA >20% (%)	14.2	11.5	17.9	12.2	0.95
	Mean HLA mismatch (of 6)	4.3 (1.2)	4.5 (1.2)	4.3 (1.3)	4.3 (1.2)	0.23
	Previous transplant (%)	16.9	6.7	18.4	21.1	0.001
Recipients Factors	Means pre-transplant serum albumin level	3.8 (0.6)	3.0 (0.4)	3.7 (0.1)	4.3 (0.3)	**<0.001**
	Mean age (yrs)	43.2 (9.3)	43.3 (9.8)	44.3 (9.7)	42.2 (8.7)	0.17
	Female (%)	39.7	47.5	41.6	33.8	**0.01**
	Non-white (%)	9.0	14.2	8.4	6.8	**0.03**
	Mean body mass index (kg/m^2)	25.9 (3.9)	25.2 (3.9)	26.4 (4.0)	25.7 (3.9)	0.48
	Diabetes type					
	Type I	89.8	85.0	57.9	94.1	**0.02**
	Type II/Other	10.2	15.0	12.1	5.9	
	Induction Immunosuppression (%)					
	Alemtuzumab	35.5	24.2	32.1	44.6	**0.003**
	Anti-thymocyte globulin	24.3	30.0	26.3	19.4	
	Basiliximab	40.2	45.8	41.6	36.0	
	Early steroid withdrawal (%)	3.6	4.2	3.7	3.2	0.62
	Pre-emptive transplant	44.5	28.2	45.7	52.4	<0.001

Bold signifies statistical sigificant with p < 0.05.

were analyzed using Fisher's exact test or chi-square test. *p*-Values ≤ 0.05 were considered statistically significant. Multivariable logistic regression and Cox proportional hazard models were used to analyze associations of the pre-transplant serum albumin levels with various outcomes of interest. Variables considered to be associated with outcomes of interest from baseline characteristics were included in adjusted models. Outcomes of interest were also analyzed by Kaplan-Meier survival analysis. Analyses were performed in Stata SE 18.0[1].

RESULTS

774 SPK recipients were transplanted during the study period, 23 were excluded due to early graft failure or early patient death all within 2 weeks of transplant, and 219 did not have serum albumin levels measured during the timeframe and were excluded from the study. With this, a total of 532 SPK recipients fulfilled our selection criteria. The details of recipient and donor baseline characteristics are summarized in **Table 1**. Recipient's and donor's characters differed across albumin categories in multiple variables, including the donor's cause of death, the proportion of DCD donors, pancreas cold ischemia time, the proportion of previous transplant recipients, recipient's sex, types of diabetes, induction immunosuppression and proportion of pre-emptive transplant recipients.

[1]http://www.stata.com

Kidney Delayed Graft Function

A total of 7.3% of recipients had DGF (**Table 2**). 4.5% of recipients with normal albumin levels, 9% with mild, and 10% with moderate hypoalbuminemia group experienced DGF. Mild hypoalbuminemia (OR: 2.08; 95% CI: 0.93–4.67; p 0.07) and moderate hypoalbuminemia were not associated with risk of DGF (OR: 2.36; 95% CI: 0.99–5.63; p = 0.05) in an unadjusted model. This was further confirmed after adjustment of multiple variables in mild (OR: 1.69; 95% CI: 0.65–4.37; p = 0.28); moderate (OR: 1.52; 95% CI: 0.55–4.20; p = 0.42) hypoalbuminemia groups (**Table 3**).

Length of Stay

The mean LOS among the entire cohort after index transplant was 11.1 ± 7.9 days (**Table 2**). The mean LOS in normal, mild, and moderate hypoalbuminemia recipients was 11.2 ± 6.5 days, 10.4 ± 6.0 days, and 12.1 ± 12.0 days, respectively. Mild hypoalbuminemia was not associated with increased or decreased LOS in unadjusted (OR: −0.78; 95% CI: −2.31, 0.76; p = 0. 32) or adjusted models (OR: −0.94; 95% CI: −2.49, 0.61; p = 0. 24). Moderate hypoalbuminemia was also not associated with LOS in unadjusted (OR: 0.92; 95% CI: −0.84, 2.61; p = 0. 31) or adjusted models (OR: 0.22; 95% CI: −1.61, 2.06; p = 0. 81) (**Table 4**).

Rehospitalization

A total of 34.2% were re-hospitalizations within 30 days after the initial discharge from the index transplant (**Table 2**). The rate of rehospitalization in normal, mild, and moderate

TABLE 2 | Complications, DGF, LOS, rehospitalization, Re-operation.

Complications		DGF, %	LOS, mean (sd)	Rehospitalization, %	Re-operation, %
	Pre-Tx albumin (g/dL)				
	≥4.0	4.5	11.2 (6.5)	32.4	25.7
	≥3.5–<4.0	9.0	10.4 (6.0)	35.3	20.0
	<3.5	10.0	12.1 (12.0)	35.8	20.8
	Overall	7.3	11.1 (7.9)	34.2	22.6

TABLE 3 | Delayed graft function (n = 39).

Complications	Pre-Tx albumin	Unadjusted			Adjusted		
		OR	95% CI	p-value	OR	95% CI	p-value
DGF	≥4.0	Ref	Ref	Ref	Ref	Ref	Ref
	≥3.5–<4.0	2.08	0.93, 4.67	0.07	1.69	0.65, 4.37	0.28
	≥3.0–<3.5	2.36	0.99, 5.63	0.051	1.52	0.55, 4.20	0.42

Adjusted for age, sex, race, diabetes type, pre-emptive transplant, induction immunosuppression, pancreas cold time, donor age.

TABLE 4 | Length of stay, mean LOS.

Complications	Pre-Tx albumin	Unadjusted			Adjusted		
		Coef	95% CI	p-value	Coef	95% CI	p-value
Length of stay	≥4.0	Ref	Ref	Ref	Ref	Ref	Ref
	≥3.5–<4.0	–0.78	–2.31, 0.76	0.32	–0.94	–2.49, 0.61	0.24
	<3.5	0.92	–0.84, 2.61	0.31	0.22	–1.61, 2.06	0.81

Adjusted for age, sex, race, diabetes type, pre-emptive transplant, induction immunosuppression, pancreas cold time, donor age.

TABLE 5 | Re-hospitalization within 30 days after initial discharge (n = 182).

Complications	Pre-Tx albumin	Unadjusted			Adjusted		
		OR	95% CI	p-value	OR	95% CI	p-value
Re-hospitalization	≥4.0	Ref	Ref	Ref	Ref	Ref	Ref
	≥3.5–<4.0	1.13	0.75, 1.71	0.55	1.31	0.86, 2.82	0.21
	<3.5	1.16	0.73, 1.86	0.53	1.34	0.81, 2.23	0.25

Adjusted for age, sex, race, diabetes type, pre-emptive transplant, induction immunosuppression, pancreas cold time, donor age.

hypoalbuminemia recipients were 32.4%, 35.3%, and 35.8% respectively. Mild hypoalbuminemia was not associated with re-hospitalization rates in unadjusted (OR: 1.13; 95% CI: 0.75–1.71; p = 0.55) or adjusted models (OR: 1.31; 95% CI: 0.86–2.82; p = 0.21). Similarly, moderate hypoalbuminemia was not associated with re-hospitalization rates in unadjusted (OR: 1. 16; 95% CI: 0.73–1.86; p = 0.53) or adjusted models (OR: 1. 34; 95% CI: 0.81–2.23; p = 0. 25) (**Table 5**). The most common indications for re-hospitalization were gastrointestinal symptoms including, nausea, vomiting, diarrhea, and abdominal pain followed by hypo or hypertension.

Reoperations Within 2 Weeks–12 Months

A total of 22.6% of recipients returned to the operating room within 2 weeks–12 months post-transplant (**Table 2**). 25.7% of patients with normal albumin levels, 20% with mild, and 20.8% with moderate hypoalbuminemia were reoperated within the stated timeframe. Mild hypoalbuminemia was not associated with increased reoperation rates in unadjusted (OR: 0.59; 95% CI: 0.29–1.18; p = 0.14) or adjusted models (OR: 0.54; 95% CI: 0.26, 1.12; p = 0.10). Similarly, moderate hypoalbuminemia was not associated with increased reoperation rates in unadjusted (OR: 1.04; 95% CI: 0.52–2.04; p = 0.92) or adjusted models (OR: 0.80; 95% CI: 0.38–1.66; p = 0. 54) (**Table 6**). This was further confirmed by the Kaplan-Meier survival analysis curve (**Figure 1**). The most common indications for return to the operating room were intraabdominal fluid collection followed by intrabdominal bleeding.

Acute Rejection Within 2 Weeks–12 Months

A total of 19.4% of SPK recipients developed pancreas rejection and 12.2% developed kidney rejection. Pre-transplant hypoalbuminemia, either mild or moderate was not associated with acute rejection in either adjusted or unadjusted models

TABLE 6 | Need to go to OR related to transplant within 12 months (n = 48).

Complications	Pre-Tx albumin	Unadjusted			Adjusted		
		HR	95% CI	p-value	HR	95% CI	p-value
Re-operation	≥4.0	Ref	Ref	Ref	Ref	Ref	Ref
	≥3.5–<4.0	0.59	0.29, 1.18	0.14	0.54	0.26, 1.12	0.10
	<3.5	1.04	0.52, 2.04	0.92	0.80	0.38, 1.66	0.54

Adjusted for age, sex, race, diabetes type, pre-emptive transplant, induction immunosuppression, pancreas cold time, donor age.

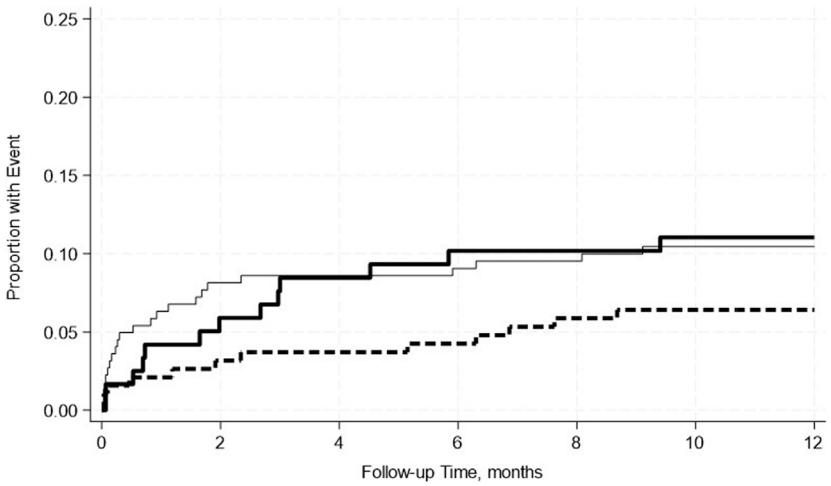


FIGURE 1 | No significant differences in the incidence rate ratio of re-operation within 2 weeks–12 months post-transplant by serum albumin levels. The thin solid line represents the normal pre-transplant serum albumin levels group, the thick dashed line represents the mild hypoalbuminemia group and the thick solid line represents the moderate hypoalbuminemia group.

TABLE 7 | Acute rejection within 12 months.

Complications	Pre-Tx albumin	Unadjusted			Adjusted		
		HR	95% CI	p-value	HR	95% CI	p-value
Pancreas acute rejection (n = 103)	≥4.0	Ref	Ref	Ref	Ref	Ref	Ref
	≥3.5–<4.0	1.13	0.74, 1.74	0.57	1.16	0.75, 1.71	0.51
	<3.5	0.86	0.51, 1.47	0.58	0.81	0.46, 1.41	0.45
Kidney acute rejection (n = 65)	≥4.0	Ref	Ref	Ref	Ref	Ref	Ref
	≥3.5–<4.0	0.97	0.56, 1.70	0.92	0.98	0.55, 1.73	0.94
	<3.5	1.02	0.55, 1.93	0.94	0.81	0.41, 1.59	0.54

(**Table 7**). This was further confirmed by the Kaplan-Meier Survival analysis curve (**Figures 2**, **3**).

Graft Failures Within 2 Weeks–12 Months

A total of 7.3% of SPK recipients experienced uncensored graft failure and 4.7% experienced pancreas DCGF. Similarly, 3.3% experienced kidney uncensored graft failure, and 1.1% experienced kidney DCGF (**Table 8**). Pre-transplant hypoalbuminemia either mild or moderate was not associated with either uncensored or death-censored graft failure in either adjusted or unadjusted models. This was further confirmed by the Kaplan-Meier Survival analysis curve (**Figures 4**–**7**).

Also, 3.0% of SPK recipients experienced death with both functional grafts, and similar to the previous findings, neither mild nor moderate pre-transplant hypoalbuminemia was associated with death with both functional grafts (**Table 8**).

A total of 17 SPK recipients had early pancreas DCGF, 3 had early kidney DCGF, and 3 died with both functional grafts, all within 2 weeks post-transplant, and were not included in the main analysis of the study. When analyzing the data in this subgroup of SPK recipients, pretransplant hypoalbuminemia was not associated with those outcomes of interest either in the mild or moderate hypoalbuminemia group in the unadjusted model (**Supplementary Table S1**).

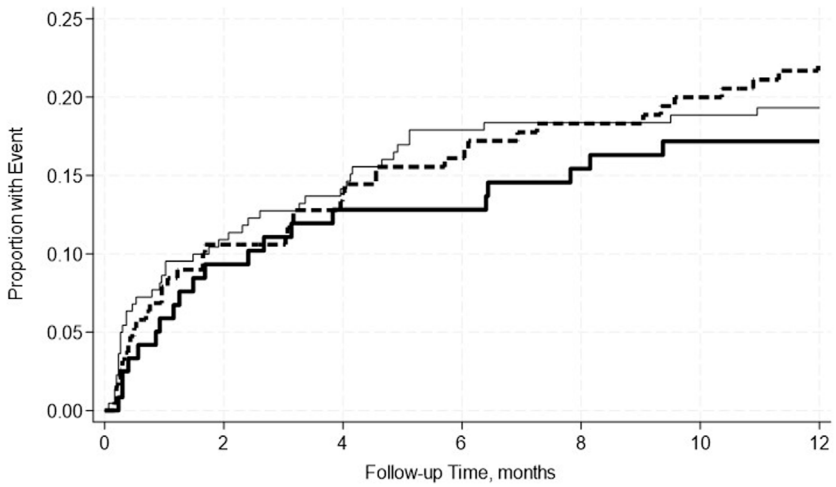


FIGURE 2 | No significant differences in the incidence rate ratio of pancreas rejection within 2 weeks–12 months post-transplant by serum albumin levels. The thin solid line represents the normal pre-transplant serum albumin levels group, the thick dashed line represents the mild hypoalbuminemia group and the thick solid line represents the moderate hypoalbuminemia group.

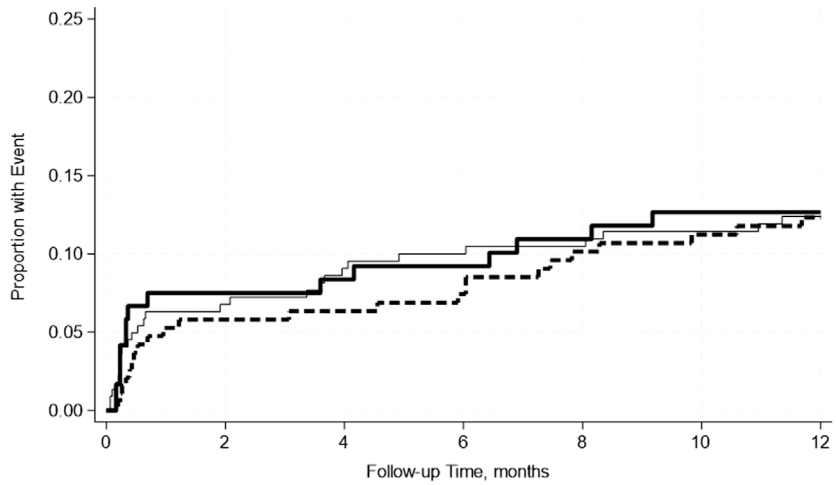


FIGURE 3 | No significant differences in the incidence rate ratio of kidney rejection within 2 weeks–12 months post-transplant by serum albumin levels. The thin solid line represents the normal pre-transplant serum albumin levels group, the thick dashed line represents the mild hypoalbuminemia group and the thick solid line represents the moderate hypoalbuminemia group.

Also when including these recipients with early pancreas graft failure, pretransplant hypoalbuminemia was not associated with either grafts uncensored or DCGF or death either in the mild or moderate hypoalbuminemia group in the unadjusted model or adjusted model (**Supplementary Table S2**).

DISCUSSION

In this large cohort of 532 SPK recipients, we found that pre-transplant hypoalbuminemia was not significantly associated with any detrimental early post-transplant outcomes. We looked for various common early post-transplant outcomes and events including kidney DGF, length of stay, readmission, re-operation, and graft failure, and none of these feared outcomes were associated with pre-transplant hypoalbuminemia.

Hypoalbuminemia is a known risk factor for various detrimental outcomes in patients who suffer from diabetes mellitus and ESRD. Inflammation has been identified to play a key role in the pathogenesis of hypoalbuminemia in this group of patients. Chronic systemic inflammation that suppresses both innate and acquired immunity ultimately places patients with concomitate diabetes and ESRD at higher risk of life-threatening infections, morbidity, and death [4, 21, 22]. Therefore, we

TABLE 8 | Graft failure within 12 months.

Complications	Pre-Tx albumin	Unadjusted			Adjusted		
		HR	95% CI	p-value	HR	95% CI	p-value
Uncensored pancreas graft failure (n = 39)	≥4.0	Ref	Ref	Ref	Ref	Ref	Ref
	≥3.5–<4.0	0.96	0.47, 1.94	0.90	1.10	0.53, 2.31	0.80
	<3.5	0.87	0.38, 2.02	0.75	0.86	0.35, 2.11	0.75
Death censored pancreas graft failure (n = 25)	≥4.0	Ref	Ref	Ref	Ref	Ref	Ref
	≥3.5–<4.0	0.77	0.32, 1.90	0.58	0.97	0.38, 2.49	0.96
	<3.5	0.77	0.27, 2.19	0.63	0.79	0.26, 2.40	0.68
Uncensored kidney graft failure (n = 18)	≥4.0	Ref	Ref	Ref	Ref	Ref	Ref
	≥3.5–<4.0	1.56	0.54, 4.50	0.41	1.52	0.51, 4.57	0.45
	<3.5	1.24	0.35, 4.40	0.74	1.12	0.29, 4.27	0.87
Death censored kidney graft failure (n = 6)	≥4.0	Ref	Ref	Ref	Ref	Ref	Ref
	≥3.5–<4.0	1.17	0.24, 5.79	0.85	X	x	X
	<3.5	x	X	x	x	x	X
Death with functioning graft (n = 16)	≥4.0	Ref	Ref	Ref	Ref	Ref	Ref
	≥3.5–<4.0	1.16	0.37, 3.59	0.80	1.20	0.37, 3.91	0.77
	<3.5	1.24	0.34, 4.39	0.74	1.14	0.30, 4.35	0.84

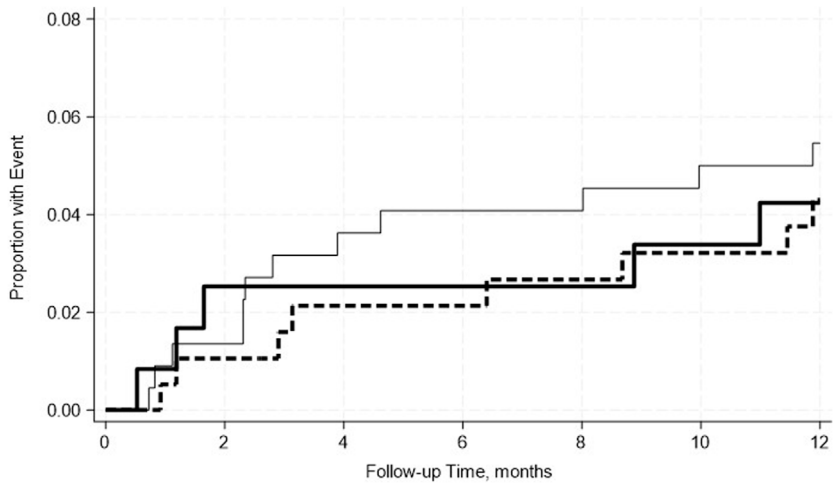


FIGURE 4 | No significant differences in the incidence rate ratio of the pancreas death censored graft failure within 2 weeks–12 months post-transplant by serum albumin levels. The thin solid line represents the normal pre-transplant serum albumin levels group, the thick dashed line represents the mild hypoalbuminemia group, and the thick solid line represents the moderate hypoalbuminemia group.

expected to observe a negative effect of hypoalbuminemia on both patient and graft survival. Breyer et al., also did not observe worse outcomes among kidney transplant recipients, although they report a lower risk of acute rejection based on the degree of hypoalbuminemia [11].

Patients with diabetes and ESRD are at higher risk for malnutrition [23]. Among solid organ transplant recipients, malnutrition has been associated with various poor clinical outcomes among liver, kidney, heart, and lung transplant recipients [23, 24].

In the past, serum albumin was thought to be an acceptable sole marker of nutritional status but has since been widely researched and refuted [23]. A retrospective cohort study from 2006 found no association between serum albumin and mortality risk among lung transplant recipients but found a higher risk of death in recipients with a low prealbumin level (<18 g/dL) [25]. Still, pre-albumin and albumin levels should not be considered valid tools for malnutrition diagnosis, as they are influenced by multiple factors including inflammation and fluid status. According to Evans et al, albumin and prealbumin as acute phase proteins do not consistently or predictably change with weight loss, calorie restriction, or nitrogen balance and more accurately indicate the severity of the inflammatory response rather than poor nutrition status [26]. In October 2020, the American Society for Parenteral and Enteral Nutrition (ASPEN) published a position paper stating that albumin and prealbumin are not components of any accepted definitions of malnutrition [26]. Extrapolating from previous data among various solid organ transplants, even among SPK recipients, our results support that serum albumin levels should not be

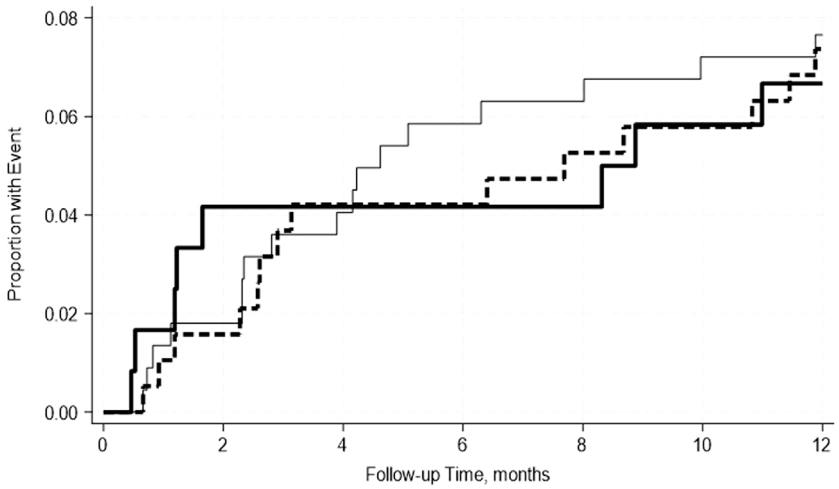


FIGURE 5 | No significant differences in the incidence rate ratio of the pancreas uncensored graft failure within 2 weeks–12 months post-transplant by serum albumin levels. The thin solid line represents the normal pre-transplant serum albumin levels group, the thick dashed line represents the mild hypoalbuminemia group, and the thick solid line represents the moderate hypoalbuminemia group.

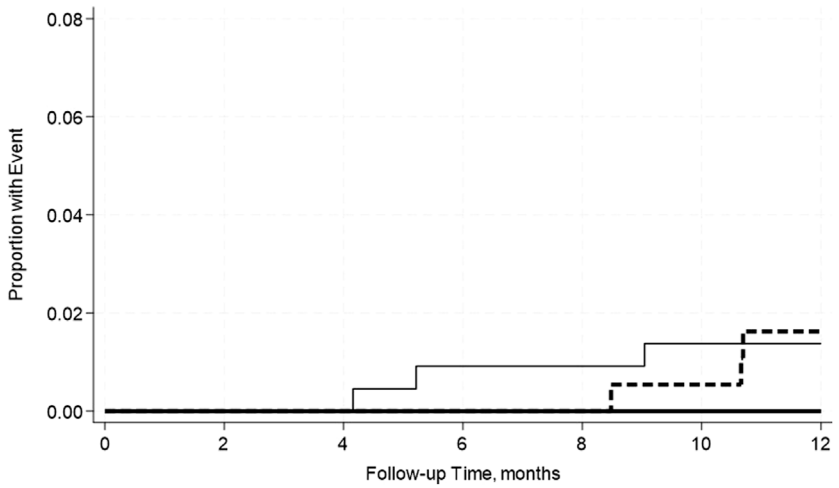


FIGURE 6 | No significant differences in the incidence rate ratio of kidney death censored graft failure within 2 weeks–12 months post-transplant by serum albumin levels. The thin solid line represents the normal pre-transplant serum albumin levels group, the thick dashed line represents the mild hypoalbuminemia group and the thick solid line represents the moderate hypoalbuminemia group.

used to assess nutritional status in this unique population. Although, out of the scope of this study, in the future, if an association between malnutrition and outcomes among SPK recipients is to be sought, should include various other nutritional tools and should not solely rely on the serum albumin levels.

To the best of our knowledge, there is no other study assessing the risk of pre-transplant hypoalbuminemia in SPK recipients with various post-transplant outcomes. In one study, Becker et al reported that persistent post-SPK hypoalbuminemia was associated with an increased risk of CMV infection, both kidney and pancreas graft failure, and a trend toward increased risk of overall patient mortality [15]. In another study, among kidney-only transplant recipients, Anderson et al. reported that hypoalbuminemia was an independent risk factor for overall graft failure after kidney transplantation [27]. Furthermore, several other studies showed that kidney transplant recipients with hypoalbuminemia were at increased risk for all-cause mortality, cardiovascular mortality, graft failure, DGF, acute rejection, BK, and CMV infections [10, 28–31].

To the best of our knowledge, this is the first study that investigated mild and moderate hypoalbuminemia and its associations with numerous post-transplant outcomes among patients undergoing SPK. Data that originated from a single-

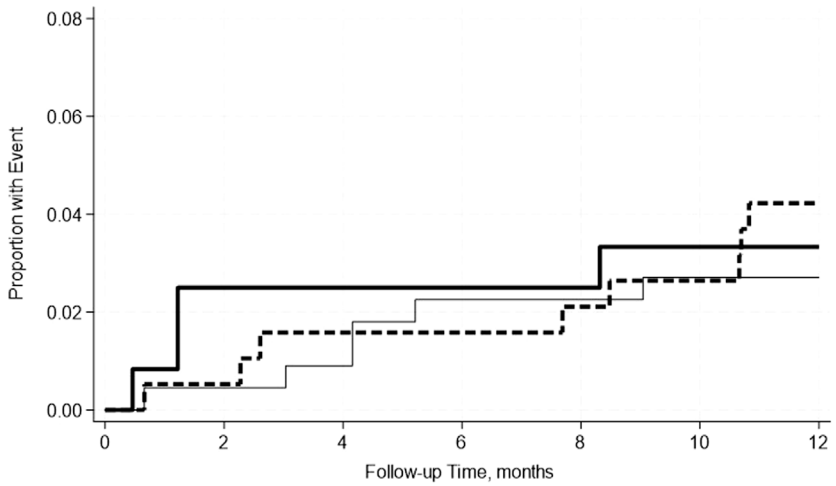


FIGURE 7 | No significant differences in the incidence rate ratio of kidney uncensored graft failure within 2 weeks–12 months post-transplant by serum albumin levels. The thin solid line represents the normal pre-transplant serum albumin levels group, the thick dashed line represents the mild hypoalbuminemia group and the thick solid line represents the moderate hypoalbuminemia group.

center hospital study allowed us to provide nuanced, granular data points, that reflected a homogeneous approach to transplant practices involving both medical and surgical patient management. These unique characteristics are unavailable with large multicenter registry datasets. Nonetheless, our study had several limitations. We were unable to identify the exact causes of hypoalbuminemia. Additionally, it was out of the scope of this study to assess the risk of infections and malignancies based on the pre-transplant serum albumin levels. Also, we did not assess the outcomes based on the changes in serum albumin levels post transplant. Lastly, due to having stringent selection criteria for SPK, most of the recipients were likely to be in relatively good health, and despite having mild/moderate hypoalbuminemia they were able to recover from SPK transplantation no differently than those without hypoalbuminemia. Also, we did not assess the various pre-transplant risk factors that usually coexist with hypoalbuminemia including pre-transplant peritoneal dialysis modality, frailty, nutritional status, muscle mass, fluid overload etc.

To summarize, the outcomes of this study have significant clinical importance showing that various degrees of hypoalbuminemia, were not associated with inferior outcomes particularly when it comes to death-censored and death uncensored graft failure. We conclude that patients with mild or moderate hypoalbuminemia, as defined in this study, who are otherwise acceptable candidates for SPK, should be considered for transplantation. Undoubtedly, future research on this topic is necessary to address the limitations reported above. Also, research to identify some of the easily available biomarkers predicting various post-transplant outcomes in these populations will be beneficial.

Summary

Simultaneous pancreas-kidney transplant (SPK) has become a growing form of treatment for those with diabetes mellitus and ESRD as it restores euglycemia and slows the progression of diabetes complications. In these patients, hypoalbuminemia has been proposed to be caused by chronic systemic inflammation and reduced synthesis of albumin. However, the role of pre-transplant hypoalbuminemia and its impact on post-transplant outcomes in patients undergoing SPK transplantation remains unclear. In this study, we studied 532 SPK recipients at our center with mild and moderate hypoalbuminemia. Outcomes of interest included kidney delayed graft function (DGF), length of stay (LOS) after transplant, re-hospitalization within 30 days after discharge, and need for a return to the operating room related to transplant surgical complications, acute rejection, and uncensored and death-censored graft failure, within the first years post-transplant. Mild or moderate hypoalbuminemia was not associated with DGF, LOS, re-hospitalization, return to the operating room, graft rejection, or graft failure. The outcomes of this study have significant clinical importance showing that mild or moderate hypoalbuminemia, was not associated with inferior outcomes, concluding that these patients are acceptable candidates for SPK.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by University of Wisconsin- Institutional Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the

participants' legal guardians/next of kin because Retrospective study. Written consent particularly to this study was not obtained.

AUTHOR CONTRIBUTIONS

EF: concept, design, and manuscript preparation, editing. SN: manuscript preparation. DK: editing. JO: editing. DA: editing. CT: editing. DA-A: editing. DM: editing. BA: analysis, editing. SP: original concept, design, manuscript preparation, editing.

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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.2025.14091/full#supplementary-material

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ORIGINAL RESEARCH
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Liver Transplantation in Alcohol-Associated Hepatitis. Benefits and Limitations of Psychosocial Selection and Support in Alcohol Relapse. The Experience of a Tertiary Center in Italy

Luca S. Belli[1]†, Adelaide Panariello[2†], Giovanni Perricone[1], Paola Prandoni[2], Raffaella Viganò[1], Chiara Mazzarelli[1], Chiara Becchetti[1], Sara Giacalone[3], Giovanna Donvito[4], Sara Conti[4], Paolo A. Cortesi[4], Elena Roselli[5], Gianpaola Monti[5], Marco Carbone[1], Luciano G. De Carlis[6,7‡] and Mauro Percudani[2‡]*

[1]Hepatology and Gastroenterology Unit, Azienda Socio Sanitaria Territoriale Grande Ospedale Metropolitano Niguarda, Milan, Italy, [2]Mental Diseases Unit, Azienda Socio Sanitaria Territoriale Grande Ospedale Metropolitano Niguarda, Milan, Italy, [3]Unit for Continuity Hospital-Territory Care Azienda Socio Sanitaria Territoriale Grande Ospedale Metropolitano Niguarda, Milan, Italy, [4]Research Centre on Public Health (CESP), University of Milano-Bicocca, Monza, Italy, [5]Intensive Care Unit, Azienda Socio Sanitaria Territoriale Grande Ospedale Metropolitano Niguarda, Milan, Italy, [6]General Surgery and Transplantation Unit, Azienda Socio Sanitaria Territoriale Grande Ospedale Metropolitano Niguarda, Milan, Italy, [7]Department of Medicine and Surgery, University of Milano-Bicocca, Milan, Italy

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***Correspondence**
Luca S. Belli,
luca.belli@ospedaleniguarda.it

†These authors share first authorship

‡These authors share senior authorship

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Patients with severe alcoholic hepatitis SAH may suffer of undiagnosed psychiatric illnesses, typically depression. Assessment of prevalence and potential impact of psychiatric disturbances on alcohol relapse after LT, were the main objectives of this study. One hundred consecutive patients with SAH from April 2016 to May 2023 were analyzed. All patients were evaluated by an integrated team including psychiatrists, addiction specialists and social workers. Thirty (30%) were listed, of whom 25 underwent early liver transplantation (eLT) after a median time of 36 days from the index episode of SAH with a median model for end stage liver disease (MELD) score of 36, whereas 33 (33%) were excluded, with psycho-social issues being the main cause of exclusion in 18 patients (54.5%). Twenty-four patients (96%) are currently alive after a median follow-up of 32 months from LT. Sixteen transplanted patients had major depression with or without anxiety, with 10 patients (33%) being treated with antidepressants post-LT. Overall, 4 patients (16%) relapsed into alcohol consumption after liver transplantation and 1 died of alcohol related liver disease (4%). From this

Abbreviations: ACLF, acute on chronic liver failure; AD, acute decompensation; AH, alcoholic hepatitis; ALD, alcohol-associated liver disease; ALT, alanine transaminase; AST, aspartate transaminase; AUD, alcohol use disorder; CS, corticosteroids; DSM, diagnostic and statistical manual of mental disorders; ETG, ethyl-glucuronide; let, early liver transplantation; GAF, global assessment of functioning; GGT, γ-glutamyl transpeptidase; HAM-A, Hamilton anxiety rating scale; HAM-D, Hamilton depression rating scale; LT, liver transplantation; MDF, Maddrey's discriminant function; MELD, model for end-stage liver disease; MELD-Na, model for end-stage disease - sodium; MT, medical treatment; NIAAA, National institute on alcohol abuse and alcoholism; sLT, standard liver transplantation; WL, waiting list.

experience emerged that psychiatric comorbidities are highly prevalent among patients with SAH and that their diagnosis/treatment contributed to mitigate the risk of alcohol relapse.

Keywords: alcohol-associated hepatitis, liver transplantation, alcohol use disorder, mortality, alcohol relapse, psychiatric conditions

INTRODUCTION

Early liver transplantation (eLT) is considered the treatment option with the best outcomes in terms of survival for a select group of patients with severe alcohol-associated hepatitis (SAH) [1–7]. However, there are barriers to implementing programs due to scarcity of resources, ethical issues, and not least stigma [8]. A key issue is that many patients who develop SAH have unidentified or untreated psychiatric conditions that can range from depression to anxiety and personality disorders [9–12], conditions that hepatologists are not well-trained to treat and that may favor alcohol use disorder (AUD) [13–16]. As a matter of fact, a frequent interplay exists between psychiatric conditions (typically depression), genetic factors (familiarity for AUD), and stressful life events (i.e., deaths in the family, job loss, frustrating life events), with mental disorders (psychiatric illness and AUD) being potentially modifiable with medical treatments (MT) (**Figure 1**). Notably, undiagnosed depression is one of the mental health conditions in which environmental triggers can lead to alcohol abuse with alcohol being the self-medication that helps patients control their profound suffering and sadness. Identifying and implementing a parallel psycho-social pathway for patients before and after liver transplantation (LT) is imperative, to ensure not only the best candidate selection but also to minimize the risk of alcohol relapse after LT.

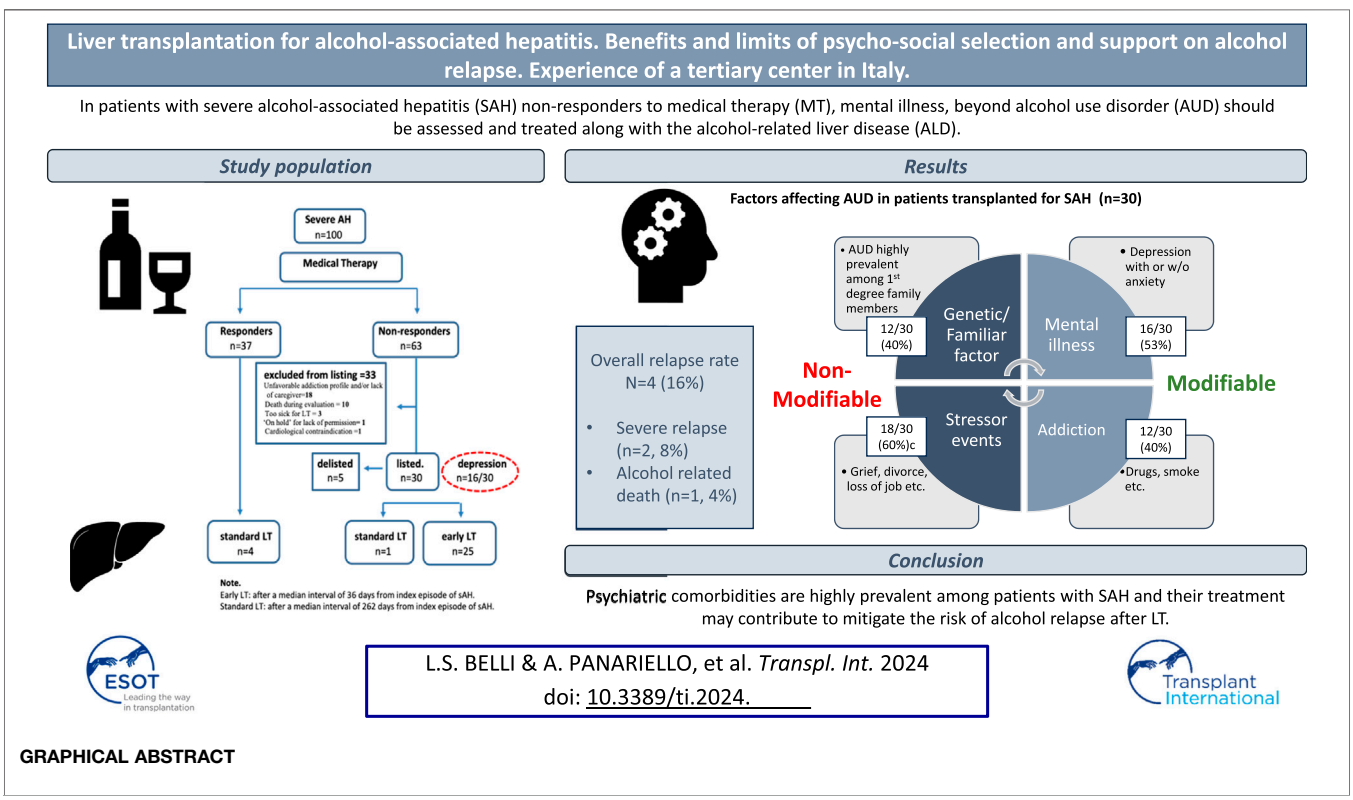
In this study we aimed to describe:

the psychosocial selection process used in a tertiary Center in Italy with a focus on the prevalence of psychiatric conditions and their response to treatment;
the reasons for not listing patients who do not respond to MT;
the benefits and limitations of psychosocial interventions on alcohol relapse after LT in our region.

PATIENTS AND METHODS

Study Population

In total, 100 consecutive patients with clinically diagnosed SAH [17] admitted to our unit between April 2016 and May 2023 were included in this study and clinical and biochemical data were prospectively registered in a bespoke database. The severity of alcoholic hepatitis (AH) was assessed using Maddrey's



GRAPHICAL ABSTRACT

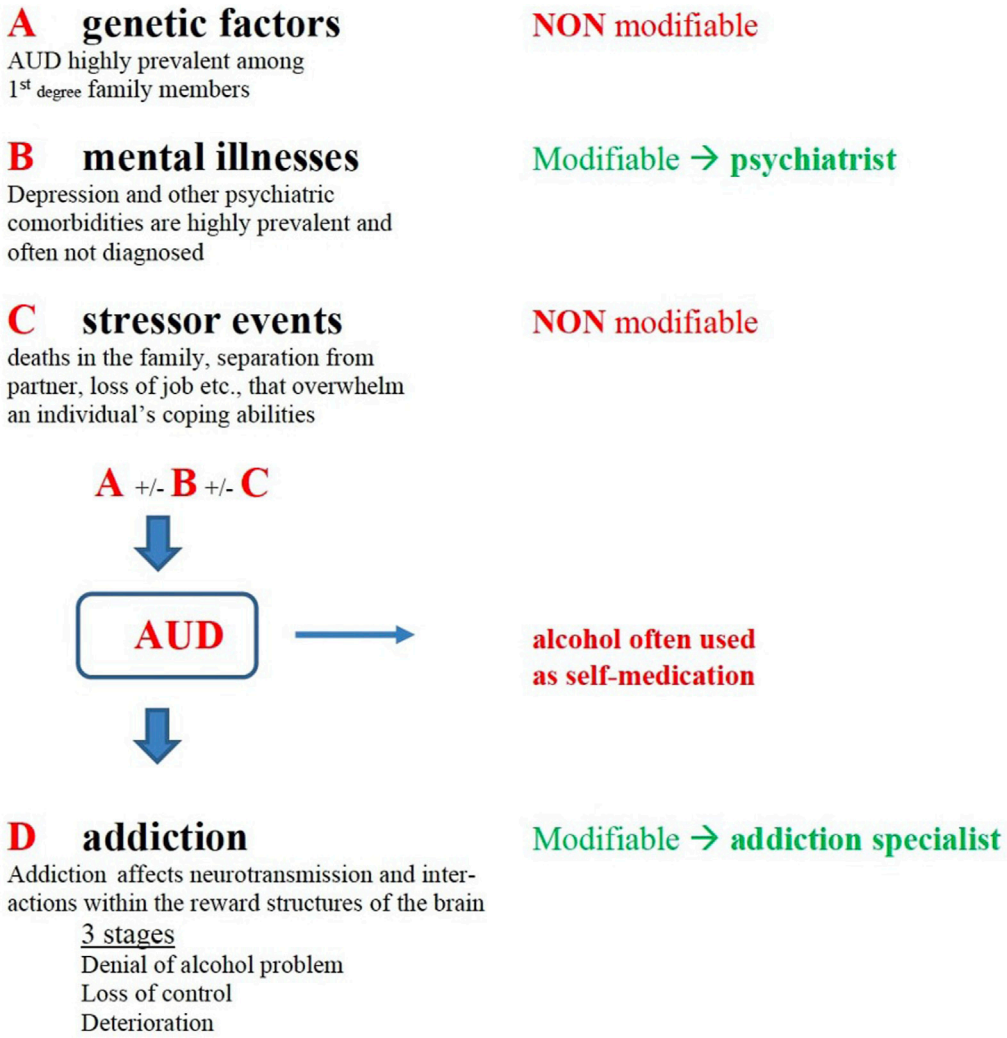


FIGURE 1 | Modifiable and non-modifiable factors in patients with AUD Modifiable and non-modifiable factors in patients with AUD (adapted from the American Society of Addiction Medicine, Board of Directors, April 2, 2011).

discriminant function (MDF) and the model for end-stage disease - sodium (MELD-Na) score.

Data Collection

For each included patient the following parameters were collected: demographic characteristics (age at admission, sex, ethnicity), severity of liver disease (MDF, MELD-Na, Lille score), use of corticosteroids (CS), reasons for not using CS. Information regarding alcohol consumption and behavioral habits before LT, the amount of alcohol consumption in units/day, the duration of alcohol abuse, drug use, tobacco use, previous attempts at alcohol rehabilitation, legal issues, and family history of alcohol misuse were also recorded. In patients who underwent LT the following parameters were recorded: MELD-Na score at LT, explant histology, patient and graft survival, interval from admission to waiting list (WL) registration, interval from WL registration to LT, post-LT major complications, post-LT hospital stay, post-LT alcohol relapse, and interval from discharge to alcohol relapse.

Informed written consent was obtained from patients or their relatives for all participants. The study was approved by the Institutional Review Board and was conducted in accordance with both the Declaration of Helsinki and local law. Six of the 30 patient LT recipients were also included in a previous publication [6].

Definitions

Response to Medical Treatment

Response to MT was based on the Lille model or a continuous reduction in MELD score reflecting a favorable trajectory of liver function.

Early vs. Standard LT

We adhered to the definition of the ACCELERATE study [3] which defines the window for eLT related to AH within 6 months of the index episode of SAH.

Post-LT Alcohol Relapse

Post-LT alcohol relapse was defined as any type of alcohol intake based on patient and family member interviews. Mild relapse: occasional slip, less than 1 per month. Moderate relapse: continuous drinking at daily doses within the recommended standards of up to 4 drinks/day for men and 3 drinks/day for women. Severe relapse: regular use above the recommended standards or with associated morbidity or mortality [18–20].

Risk Factors for Relapse to Alcohol Use

The following risk factors were collected: psychiatric co-morbidities, history of polysubstance abuse, drinking from a young age, family history of AUD, sub-optimal social support, failed attempts at alcohol rehabilitation, and smoking in the 6 months before transplant. A careful assessment of risk factors was used to optimize care before and after LT. All patients were recommended to join the local service for addiction surveillance and behavioral therapy. Since January 2023, patients with more than 2 risk factors were followed up by mental health specialists at the LT center.

Exclusion Criteria for LT

Patients were considered not eligible for liver tranplant if one or more of the following conditions were present 1) poor awareness of AUD and lack of willingness to abstain; 2) unsatisfactory Global Assessment of Functioning or GAF, (lower than 70; see details in the Psychiatric and psychologic evaluation sub-chapter); 3) psychiatric disturbances that are not deemed treatable; 4) presence of significant cognitive impairment as assessed by neuro-psychologic tests prescribed by a dedicated psychiatrist on clinical suspicion; 5) inadequate social support and housing conditions 6) ongoing substance use disorder other than cannabis and methadone. The presence of at least 1 of the aforementioned conditions identified a patient with an unfavorable addiction/social profile.

In contrast with what was established in the study by Mathurin [1], patients with undiagnosed psychiatric disorders deemed treatable and patients with prior liver decompensation in case they had never been evaluated and supported by a mental health professional in the past, were not upfront excluded.

Psychiatric and Psychological Evaluation of the Whole Cohort

A dedicated psychiatrist and a psychologist evaluated all LT candidates in conjunction with the patient's caregiver, usually a family member, and a social assistant when needed. The evaluation focused on four major aspects:

- Severity of AUD according to the criteria of the diagnostic and statistical manual of mental disorders (DSM-5) [21], which identifies 3 classes (mild, moderate, severe) depending on the number of symptoms. Insight into AUD, coping skills, awareness, and agreement to adhere to lifelong alcohol abstinence were also assessed.
- Presence of potentially treatable psychiatric disorders (depression, anxiety, personality disorders). To this aim, a symptom checklist (SCL 90) [22] was administered as a first screening tool to all potential LT candidates who were able to complete it. The Hamilton depression rating scale (HAM-D) and the Hamilton anxiety rating scale (HAM-A) were used to stratify the severity of depression and anxiety and for monitoring.
- Since suffering from AUD does not only negatively affect the health of the patients, but also their social, educational, and occupational functioning, all these domains were evaluated using the "global assessment of functioning" or GAF scale [23], which is commonly utilized by psychiatrists to rate the impact of mental disease on daily life. It is divided into 10 sections and measures the extent to which a person's symptoms affect their day-to-day life on a scale of 0–100. The higher the score, the better the patient can handle daily activities.
- Presence of socioeconomic deprivation, particularly unemployment, poor housing conditions and lack of caregivers [24, 25]. In selected cases, a neuropsychologist was involved to exclude cognitive impairment related to alcohol or other etiologies.

Post-Transplant Follow-Up and Relapse Prevention Interventions

Patients were followed up every week for the first month after LT, then every month until 3 months, and then at least 6, 9, 12, 18, 24, 30, 36, 42 and 48 months thereafter. All patients were recommended to abstain from alcohol after LT and were routinely interviewed to elicit any alcohol use. Specific toxicological tests such as ethyl-glucuronide (ETG) in urine and hair were performed in patients with suspected alcohol relapse. Indirect markers of possible alcohol abuse (mean corpuscular volume [MCV], aspartate transaminase [AST], alanine transaminase [ALT], γ-glutamyl transpeptidase [GGT]) were also evaluated. All patients were evaluated by a dedicated mental health specialist team at the transplant center (psychologists or psychiatrists) at least once a year while also being referred to the local service for addiction. Patients with major depressive disorder were maintained on a predetermined strict surveillance with the dedicated psychiatrist at the transplant center with additional visits that were adjusted as clinically required. If a patient lapsed or relapsed after LT, an intensive individualized program was also initiated at the transplant center.

Statistical Analysis

A descriptive analysis of the cohort was carried out on the total population. Continuous and categorical variables were summarized by absolute and relative frequencies, and median and interquartile range (IQR), respectively. A further analysis focused on non-responders and among them it compared the characteristics of patients listed for early-LT and those not listed. Group characteristics were compared using the Wilcoxon signed-rank test, while categorical ones were compared using Fisher's exact test. Time was measured from the first day of hospitalization to the last known date of follow-up or date of death from any cause. Survival analyses, using the Kaplan-Meier method, were carried out overall and stratified for the following groups: responders, non-responders listed, and non-listed non-

TABLE 1 | Baseline characteristics of 100 patients with severe alcohol-associated hepatitis.

Gender	
Male patients, n (%)	63 (63.0)
Age, median (IQR)	51.5 (44.0–56.5)
Ethnicity, n (%)	
Caucasian	95 (95.0)
Other	5 (5.0)
Referred from another hospital, n (%)	70 (70.0)
Previous episode of liver decompensation, n (%)	29 (29.0)
Maddrey's Discriminant Function, median (IQR)	72.1 (51.9–100.9)
MELD score, median (IQR)	25.9 (22.8–30.1)
MELD-Na score, median (IQR)	28.5 (25.0–32.3)
AH diagnosed as "probable"	71 (71.0)
AH diagnosed as "definite" (histologic confirmation)	29 (29.0)
Underlying cirrhosis, n (%)	98 (98.0)
Confirmed histologically at index episode of AH	30 (30.0)
Psychosocial characteristics	
Alcohol consumption, units/day, median (IQR)	10 (9.0–15.0)
Duration of alcohol consumption, years, median (IQR)	20 (15.0–30.0)
Active smokers, n (%)	45 (45.0)
Illicit substance users, n (%)	10 (10.0)
Living with partner, n (%)	60 (60.0)
AUD in first grade family members, n (%)	40 (40.0)
Previous detoxification attempts, n (%)	30 (30.0)
Psychiatric comorbidities, n (%)	55 (55.0)
Depression	22 (22.0)
Depression and anxiety	13 (13.0)
Depression and personality disorder	6 (6.0)
Anxiety	7 (7.0)
Personality disorder	7 (7.0)

Abbreviations: ACLF, acute on chronic liver failure; GAF, global assessment of functioning; MELD, model for end-stage liver disease; MELD-Na, model for end-stage liver disease-sodium; sAH, severe alcohol-associated hepatitis.

responders. All statistical analyses were conducted using R V.4.3.1 (R Core Team, Vienna, Austria).

RESULTS

During the study period, 100 consecutive patients with SAH were admitted to our Unit; in total, 63 (63%) were men, the median [IQR] age was 51 [44–56] years, 95 (95%) were Caucasian, and 70 (70%) had been referred from other hospitals. A history of prior liver decompensation was present in 29 patients (29%). At admission, the median [IQR] MDF was 72.1 [52–101] and the median [IQR] MELD score was 25.9 [23–30] (**Table 1**). According to the National Institute on alcohol abuse and alcoholism (NIAAA) criteria, the diagnosis of AH was "probable" in 71 patients and "definite" in the 29 patients who underwent a liver biopsy. An underlying liver cirrhosis was present in 98 patients (98%).

The median [IQR] alcohol intake was 10 [9–15] units/day, with a median [IQR] duration of alcohol intake of 20 [15–30] years. A total of 45 patients (45%) were active smokers and 10 (10%) reported the use of illicit drugs. A total of 40 patients (40%) had a first-degree family member suffering from AUD. In total, 30 (30%) patients failed previous attempts of detoxification. All patients met the criteria for severe AUD according to DSM-5 [21] and 55 patients (55%) met the criteria for a mood disorder and/or anxiety disorder (**Table 1**). Depression was the most common mental disorder, observed in 41 (41%) patients in the cohort and categorized as moderate-severe in 34 (34%) cases.

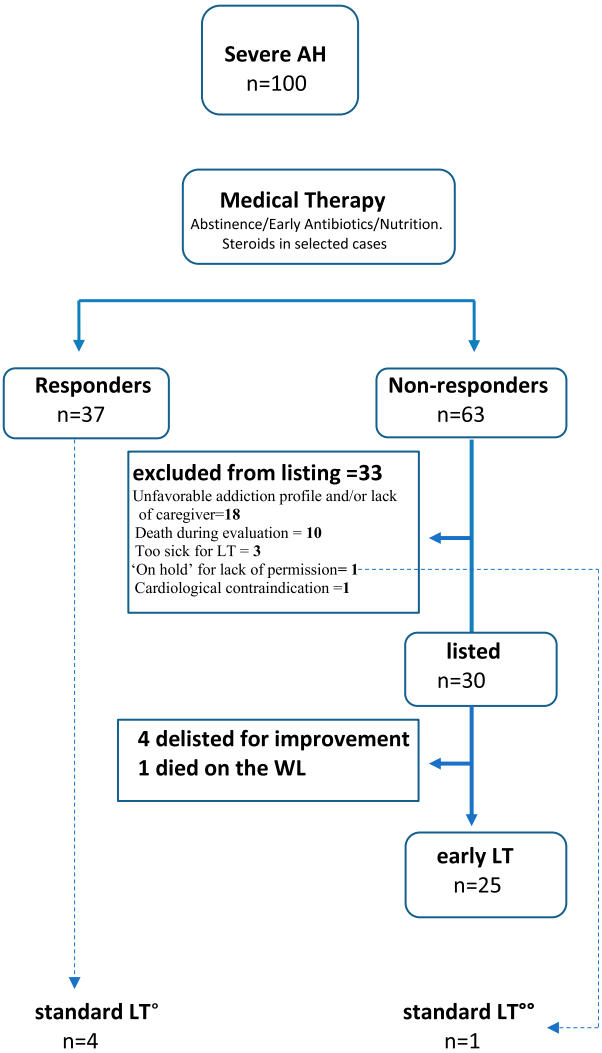


FIGURE 2 | Disposition of patients. Note: Early LT, after a median interval of 36 days from index episode of sAH. Standard LT, after a median interval of 262 days from index episode of sAH. °4 patients who were discharged from hospital re-decompensated again for reasons not related to alcohol relapse and were listed for standard LT. °°One "irregular immigrant" could be listed only when regularized 7 months after index episode of sAH.

Outcome After Response or No Response to Medical Treatment

The main components of MT were alcohol abstinence, early identification and appropriate treatment of infections, nutrition, and the use of CS in selected patients (**Figure 2**). The main reasons for not using CS were spontaneous decrease of bilirubin (25 patients, 25%), confirmed or presumed infection (16 patients, 16%), acute on chronic liver failure (ACLF) grade 3 (17 patients, 17%), rapid worsening (9 patients, 9%), MELD score >30 (7 patients, 7%).

TABLE 2 | Psychosocial characteristics of patients listed for early LT compared to those excluded from placed on the waiting list for transplant.

	Listed for LT n = 30	Excluded from early LT n = 33	Total n = 63	*P*-value
Gender				
Male patients, n (%)	21 (70.0)	19 (57.6)	40 (63.5)	0.447
Age, median (IQR)	51.5 (46.3, 56.8)	53.0 (45.0, 58.0)	52.0 (45.5, 57.5)	0.757
Ethnicity, n (%)				0.675
Caucasian	29 (96.7)	30 (90.9)	59 (93.7)	
Other	1 (3.3)	3 (9.1)	4 (6.3)	
Referred from other hospitals, n (%)	27 (90.0)	25 (75.8)	52 (82.5)	0.248
Previous episode of liver decompensation, n (%)	8 (26.7)	11 (33.3)	19 (30.2)	0.763
Psychosocial characteristics				
Alcohol consumption, units/day, median (IQR)	10.0 (7.25, 15.0)	10.0 (8.75, 15.5)	10.0 (8.0, 15.0)	0.599
Duration of alcohol consumption, years, median (IQR)	22.50 (20.0, 30.0)	20.00 (6.5, 30.0)	20.00 (19.3, 30.0)	0.442
Active smokers, n (%)	10 (33.3)	17 (51.5)	27 (42.9)	0.228
Illicit substance users, n (%)	2 (6.7)	3 (9.1)	5 (7.9)	1.000
Living conditions, n (%)				0.381
Living with partner	23 (76.7)	18 (54.5)	41 (65.1)	
Living alone	4 (13.3)	7 (21.2)	11 (17.5)	
Living with parents	3 (10.0)	5 (15.2)	8 (12.7)	
Working conditions, n (%)				0.680
Actively working	14 (46.7)	12 (36.4)	26 (41.3)	
Retired	3 (10.0)	4 (12.1)	7 (11.1)	
Unemployed	13 (43.3)	15 (45.5)	28 (44.4)	
AUD in first grade family members, n (%)	12 (40.0)	11 (33.3)	23 (36.5)	1.000
Previous detoxification attempts, n (%)	9 (30.0)	9 (27.3)	18 (28.6)	1.000
Psychosocial characteristics leading to exclusion from listing[a]	0			
Poor awareness of AUD	0	18 (54.5)		
Lack of caregiver	0	9 (27.2)		
GAF<70	0	6 (18.1)		
Cognitive impairment	0	2 (6)		
Untreated psychiatric condition or active substance use		0		
Psychiatric comorbidities, n (%)				
Depression	8 (26.7)	6 (18.2)	14 (22.2)	0.659
Depression and anxiety	4 (13.3)	3 (9.1)	7 (11.1)	0.928
Depression and personality disorder	3 (10.0)	2 (6.1)	5 (7.9)	0.940
Anxiety	3 (10.0)	0 (0.0)	3 (4.8)	0.214
Personality disorder	0 (0.0)	4 (12.1)	4 (6.3)	0.138
MELD score at LT, median (IQR)*	36.0 (30–39)	—	—	—

*Abbreviations. IQR: interquartile range; AUD: alcohol use disorder; GAF: global assessment of functioning; MELD, model for end-stage liver disease; sAH, severe alcohol-associated hepatitis. *calculated for the 25 patients who underwent e-LT, after a median interval of 36 days (23–69) after the index episode of SAH.*

[a]*All 18 patients excluded for psychosocial issues, had poor awareness of their AUD; all other psychosocial characteristics are to be considered in addition to poor awareness.*

In total, 63 (63%) patients were non-responders to MT (**Figure 2**). Of these, 30 (30/63, 47%) were listed and 25 (25/63, 39.7%) underwent eLT after a median interval of 36 days after the index episode of SAH with a median MELD score of 36, and 33 patients were excluded from were considered not suitable for a LT for the following reasons: unfavorable addiction profile (lack of awareness of AUD) with or without an adequate caregiver (18 patients); death during the evaluation process [10 patients: 8 due to sepsis, 1 due to acute distress respiratory syndrome (ARDS) and 1 due to liver failure]; too sick for LT (3 patients) severe aortic valve regurgitation (1 patient); and one patient was 'on hold' while waiting for his residency permit (**Figure 2**). This last patient received a standard LT 7 months after the index episode of SAH.

A total of 37 patients responded to medical treatment but four of them decompensated again in the following months for reasons not related to alcohol abuse and received standard LT more than 6 months after the index episode of SAH, after a median of 236 days (**Figure 2**).

When comparing the psycho-social characteristics of patients listed for eLT with those excluded, notably an unfavorable addiction profile and socio-economic deprivation including lack of an adequate caregiver were the main reasons for exclusion in 58% of the cases (**Table 2**).

Psychiatric and Psychological Characteristics of Transplanted Patients

All transplant patients had a GAF higher than 70 points with a median of 80, reflecting some mild symptoms, or some difficulty in social, occupational, or school functioning, but generally functioning well. Depression (with or without concomitant anxiety or personality disorder) was diagnosed in 16 patients and was moderate or severe (according to the HAM-D scale) in 12 patients. Antidepressant medication was administered to 10 patients and 9 showed significant clinical improvement when reassessed 6, 12 and 24 months later (**Table 3**, Panel A).

TABLE 3 | Number of cases with depression and anxiety disorders in the 30 patients who underwent LT stratified by severity (according to HAM-D and HAM_A classification) and their outcome after 6, 12 and 24 months of treatment.

	At LT	After 6 months	After 12 months	After 24 months
Severe Depression, n	2	0	0	0
Moderate Depression, n	10	1	0	0
Mild depression, n	4	0	0	1
Total	16	1	0	0
Severe anxiety, n	0	0	0	0
Moderate anxiety, n	2	0	0	0
Mild anxiety, n	3	0	0	0
Total	5	0	0	0

Anxiety alone was diagnosed in 5 additional patients and was treated with psychological therapy (**Table 3**, Panel B). Patients with depression and anxiety were regularly followed up in the psychiatric clinic at the transplant site.

Psychiatric Disturbances: Treatment and Results

Of the 16 LT recipients suffering from depression, two had severe depression according to the Hamilton Rating Scale (>25) and were treated with escalating doses of escitalopram up to a maximum dosage of 10 mg/day with complete remission of symptoms after 1 month. In total, 10 patients had moderate depression (Hamilton Rating Scale between 18 and 25) and were treated with lower escalating doses of escitalopram up to a maximum dosage of 8 mg/day with remission of symptoms after 1 month. One patient autonomously discontinued antidepressant therapy and had a relapse 6 months after LT. All patients received psychotherapy interventions in case of addiction to psychopharmacotherapy. Patients with mild depression and anxiety disorder received only psychotherapy interventions with a good recovery. No cases of severe anxiety requiring selective serotonin reuptake inhibitors (SSRIs) were present in our cohort. The follow-up period was 24 months with regular checks of improvement on the Hamilton depression rating scale and the Hamilton anxiety rating scale (**Table 3**).

Issues Regarding Adherence to Local Services for Addiction

Once transplanted, all patients were recommended to join the local service for addiction surveillance and behavioral therapies, but long-term adherence was sub-optimal as 10 patients (40%) refused to maintain contact with the Addiction Unit mainly because they did not like it or because they perceived no benefit. Notably, 2 patients in stable condition were discharged by the addiction specialists after a variable period of care, mainly due to the need to devote limited resources to participants with an active issue. These 10 patients were otherwise regularly seen by the mental health specialists at our center at least once a year on the occasion of the visit with the transplant hepatologist. None of them has had an alcohol relapse to date.

Outcomes With and Without LT: Survival and Alcohol Relapse

A total of 30 patients (30%) were transplanted, 25 after a median of 36 days (IQR) after the index episode of sAH (early-LT) and 5 after a median of 236 days (IQR 60–117) (sLT). After a median (IQR) follow-up of 32.2 months (IQR 9.5–61.4), 4 patients (13%) resumed alcohol intake after 3, 6, 30 and 36 months after LT, respectively. Alcohol relapse was harmful in 2 cases and one patient died of end-stage ALD. The 4 patients with alcohol relapse underwent stricter surveillance at our center and two of the three living patients are now abstinent.

Of the eight patients who were transplanted despite previous episodes of liver de-compensation before LT, 5 had undiagnosed moderate or severe depression that was considered curable and all of them had very strong family support. Only 1 relapsed despite regularly attending the local addiction unit and despite being closely followed up by a psychiatrist for his depression. Unfortunately, a very negative stressor event, (abandonment by his wife), occurred 15 months after LT which the patient was not able to cope with. (see AUD story of patient 1).

The 24 month-survival of the patients excluded from transplant was 10% (**Figure 3**)

Brief AUD Histories of the 4 Patients Who Relapsed

Patient n. 1. Before LT he had 2 admissions for AH, but was able to remain abstinent for 15 months between the 2 episodes and suffered from severe depression which was controlled with selective serotonin reuptake inhibitors (SSRIs), 8 mg escitalopram. His GAF was favorable, >70, he had a permanent job and his wife was a very motivated caregiver. After a thorough discussion within the team, he was accepted for LT on the condition that he follow an intensive individualized program of support from mental health specialists. Three months after the transplant while regularly attending the local addiction unit, he had a severe alcohol relapse which was triggered by the separation from his wife. Despite all of our efforts, he died 28 months later of alcohol-related end-stage liver disease. Patient n. 2. He started drinking 20 years before LT and 4 years before LT increased alcohol consumption to 2-3 bottles of wine per day after the death of his mother. His GAF profile was favorable (married, small child, actively working as a

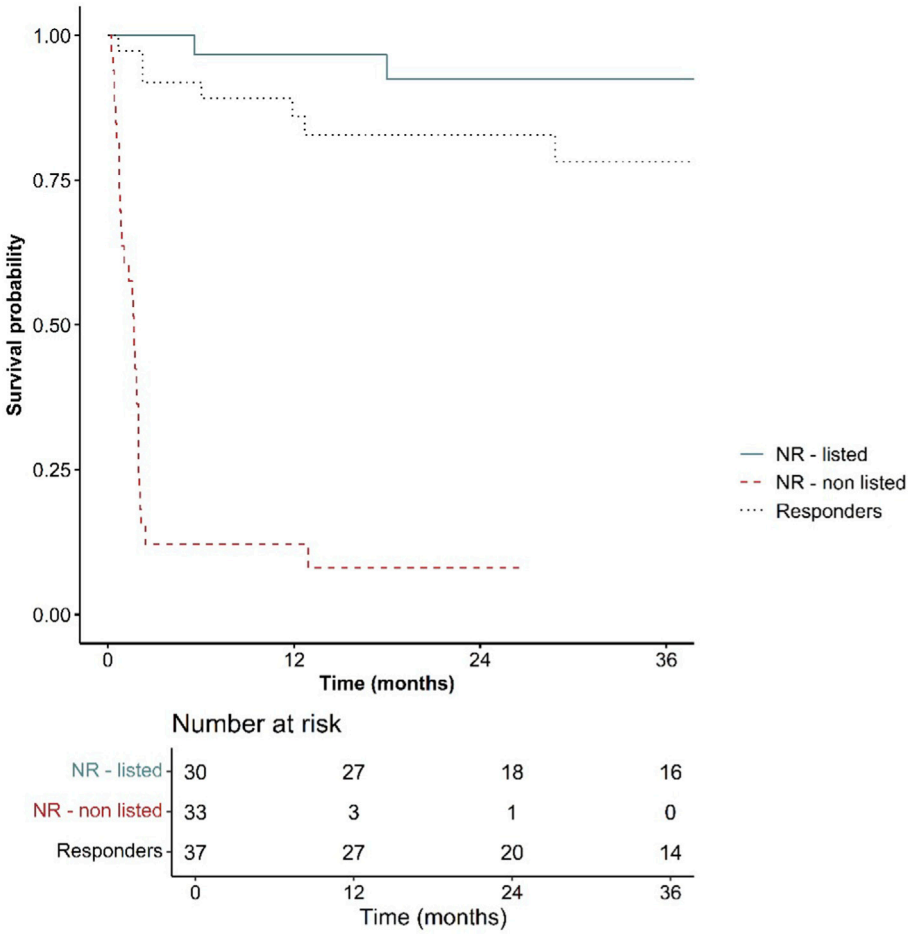


FIGURE 3 | Survival probability.

greengrocer, no psychiatric disorders). Unfortunately, he resumed occasional drinking 30 months after LT which became severe in the following months. His adherence to control visits has remained sub-optimal since then, but he is alive 62 months after LT with normal AST/ALT and slightly elevated GGT, and he keeps working as a "street" greengrocer. He is currently on 5 mg escitalopram, and 600 mg gabapentin. Patient n. 3. He resumed moderate alcohol consumption 6 months after LT, concurrent with a stressful event (separation from his wife and young child who had moved to Morocco). He was then started on an intensive support program with our mental health specialists and has remained abstinent since then. The family was reunited a few months later. At the last visit, he had normal LFTs and a stable job as a metalworker. No need for medication for AUD. Patient 4. Admitted for an index episode of alcohol-associated hepatitis in July 2018 while suffering from moderate depression for which he is currently being treated. Following the liver transplant, he returned to full active work and regularly attended the specialized addiction unit while being strongly supported by his very attentive wife. After 40 months he resumed occasional moderate alcohol consumption with some craving symptoms. He was started in an intensive program but still admits to occasional lapses. He is currently on 8 mg escitalopram and 800 mg gabapentin.

DISCUSSION

This study highlights the importance of integrated collaboration with a psycho-social team that includes dedicated psychiatrists, addiction specialists and social workers in order to identify patients suitable for LT and to implement therapies to help patients manage the risk of alcohol relapse after LT [26–31]. Thanks to this collaboration 96% of our cohort of patients with sAH being offered an LT are currently alive (**Figure 3**) and the alcohol relapse rate was limited (16%) after a median follow-up of 32 months.

Our selection process was different from that used in other Centers [1, 6, 30–33], particularly with respect to the following 2 clinical issues. First, central to the inclusion criteria in many centers was the requirement that the episode of sAH be the first

decompensating event, on the assumption that a history of prior liver decompensation identifies patients who are less likely to remain abstinent after LT. We decided to adopt less stringent criteria since the majority of patients in the present cohort had never been referred to a mental health specialist for the presence of potentially treatable psychiatric disorders predisposing to AUD, nor had they been evaluated and supported by an addiction specialist. In the end, 8 patients with prior liver decompensating events were transplanted. Our experience follows and partly confirms that reported by Weinberg et al. [32], in whom 31 patients with prior decompensation were transplanted in the U.S. and were at significantly higher risk for any alcohol use after LT when compared with those without prior decompensation. Second, patients with moderate-severe depression, which is highly prevalent in patients with AUD, were not excluded.

Depression with or without concomitant anxiety or personality disorders was newly diagnosed in 41 patients (41/100, 41%) including 16 (16/30, 53%) LT candidates, who were effectively treated with antidepressants after LT and maintained on regular follow-up with the dedicated psychiatrist at the LT Center. Of the 4 classes of drugs currently available, namely, SSRIs (such as escitalopram, fluoxetine, paroxetine, sertraline, etc.), Serotonin-norepinephrine reuptake inhibitors (SNRIs, such as duloxetine, venlafaxine, etc.), Monoamine oxidase inhibitors (MAOIs such as isocarboxazid) and Tricyclic antidepressants (TCAs, such as amitriptyline, imipramine, etc.), only SSRIs were used in patients with advanced liver disease or LT recipients, due to safety issues. Even when using SSRIs, clinicians should be aware of possible drug interactions. Fluoxetine and paroxetine may cause a rise in tacrolimus and cyclosporine blood levels through inhibition of cytochrome P450 3A4 enzymes, while citalopram, escitalopram and sertraline have a limited effect on cytochrome P450 enzymes and were the first-line drugs whenever indicated. To our knowledge detailed data on psychiatric comorbidities have not been reported in this specific setting. This less stringent selection led to the applicability of eLT in 39% of non-responder patients, which is higher than what has been observed in other multicenter cohort studies where LT applicability was below 30% [1, 4, 6].

Regarding the main reasons for not listing patients who were non-responders to MT, an unfavorable addiction profile and socio-economic deprivation including lack of an adequate caregiver, accounted for the majority of the exclusions. We believe that an early referral to mental health specialists would be key to preventing a large percentage of patients with AUD from progressing to the more advanced stage of addiction. Unfortunately, early referral is not very common in our area. In the same vein, socio-economic deprivation including unemployment, poor housing conditions and lack of a caregiver are frequently exacerbated by AUD but are only marginally mitigated by current interventions of social assistance.

Alcohol relapse was documented in 4 patients, 13%, with 2 patients experiencing severe relapse and 1 patient dying from end-stage alcohol-related liver disease despite being closely followed up by our addiction specialist and psychiatrist. Notably a severe trigger event, typically a loss in the family, was present in 3 of the 4 patients who relapsed. Overall, only 60% of the patients were regularly followed up by a specialized addiction unit in the territory, as 40% either refused to maintain contact with local services or were discharged by local services after a variable period due to the limited resources allocated to addiction care in our area. This finding points to the limits of the use of addiction specialists outside the LT units, at least in the region of Lombardy. Based on this experience, we have decided that patients who are considered by experience with a higher risk of relapse, typically those with 2 or more risk factors, should be strictly linked to the integrated addiction specialists within our LT unit and that closer collaboration with addiction services in the territory needs to be implemented. Despite these drawbacks overall alcohol relapse and severe alcohol relapse were limited, at 16% and 8%, respectively, after a median follow-up of 32 months and we hypothesize that accurate diagnosis and control of depression were valuable tools in helping patients to maintain abstinence after LT.

We acknowledge some limitations of this study. First, the majority of patients were identified as having probable sAH with the diagnosis confirmed by histological findings in less than one-third of patients with an available pre-LT liver biopsy. We cannot exclude that some patients without a liver biopsy were misclassified although their clinical presentation was typical of AH. Second, biomarkers for the detection of alcohol use were not systematically used after LT which may underestimate alcohol relapse. This limitation was offset by lifelong post-transplant hepatology follow-up care at our transplant center, which consistently included inquiries about alcohol use, laboratory tests and ultrasound examinations for evidence of recurrent disease. Although rare or low-dose drinking was likely to be underreported, relapse with a negative impact on liver function would have been detected by the hepatology team. Third, the denominator in our study included only patients who were transferred to our Liver Unit as it was not possible to capture those patients who were referred to our Center but not transferred, as they were excluded by our mental health specialists after discussion with the mental health specialists of the local hospital or with the addiction specialist in the territory. Fourth, the median follow-up of 32 months after LT with a wide IQR does not allow a reliable assessment of alcohol relapse. In addition, as the vast majority of our patients were Caucasian, 95%, the conclusions of this study cannot be generalized to diverse populations. Finally, a control group of patients with untreated depression/anxiety was not available which limits the understanding of the extent to which diagnosis and treatment of depression/anxiety reduce relapse rates.

In conclusion, integrated collaboration with mental health specialists, psychiatrists, and addiction specialists may have been key to the initial success of the program although referral to addiction specialists outside the LT unit was suboptimal. We highlight the high prevalence of undiagnosed psychiatric comorbidities which are often curable, possibly contributing to mitigating the risk of alcohol relapse after liver transplantation.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by EC Milano 3, ASST GOM Niguarda. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LB and AP conceptualization, supervision, and writing of the article; GP conceptualization, data curation, editing, and critical review of the article; PP, RV, CM, CB, SG, ER, and MC data curation; SC, PC, and GD statistical analysis; GM, MP, and LD review of the manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

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ORIGINAL RESEARCH
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Systematic Sex-Based Inequity in the MELD Score-Based Allocation System for Liver Transplantation in Germany

Leke Wiering[1,2], Annette Aigner[3], Marieke van Rosmalen[4], Brigitta Globke[2,5], Tomasz Dziodzio[5], Nathanael Raschzok[2,5], Münevver Demir[1], Wenzel Schöning[5], Frank Tacke[1], Petra Reinke[6], Johann Pratschke[5], Robert Öllinger[5†] and Paul V. Ritschl[2,5†]*

[1]Charité—Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Department of Hepatology and Gastroenterology, Campus Virchow-Klinikum and Charité Campus Mitte, Berlin, Germany, [2]Berlin Institute of Health at Charité—Universitätsmedizin Berlin, BIH Biomedical Innovation Academy, BIH Charité (Junior) Clinician Scientist Program, Berlin, Germany, [3]Charité—Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Institute of Biometry and Clinical Epidemiology, Berlin, Germany, [4]Eurotransplant International Foundation, Leiden, Netherlands, [5]Charité—Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Department of Surgery, Campus Charité Mitte, Campus Virchow-Klinikum, Berlin, Germany, [6]Charité—Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin Center for Advanced Therapies (BeCAT), Berlin, Germany

In liver allocation systems based on the Model for End-stage Liver Disease (MELD) score, sex inequities have been identified in countries with high organ donation rates. Whether similar inequities exist in regions with average to low donation rates remained unclear. We assessed the impact of sex on transplantation rates, waiting list mortality and post-transplant survival in 25,943 patients waitlisted for liver transplantation in Germany between 2003 and 2017 using competing risk analysis. Women are currently underrepresented on the waiting list (33.3%) and among transplant recipients (31.1%) compared to their proportion of severe liver disease cases (35.1%). The introduction of MELD-based allocation has worsened this disadvantage [HR before: 0.89 (0.81–0.98), after: 0.77 (0.74–0.81)]. Three key factors contribute to this disparity: Women have lower creatinine levels despite worse renal function, reducing their MELD score (median 1, 0–3). Second, exceptional MELD points are more frequently granted to men [HR 1.61 (1.54–1.69) compared to regular allocation]. Third, the small height of women has the highest impact on the probability of not being transplanted [adjusted HR 0.85 (0.81–0.9)]. Even in countries with lower organ donation rates, MELD-based allocation leads to sex inequity. Measures are needed to ensure sex-neutral liver allocation in MELD-based systems worldwide.

Keywords: organ procurement and allocation, model for end-stage liver disease, end stage liver disease, disparities, gender, sex inequity, waiting list, access to transplantation

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***Correspondence**
Paul V. Ritschl,
paul.ritschl@charite.de

†These authors have contributed equally to this work

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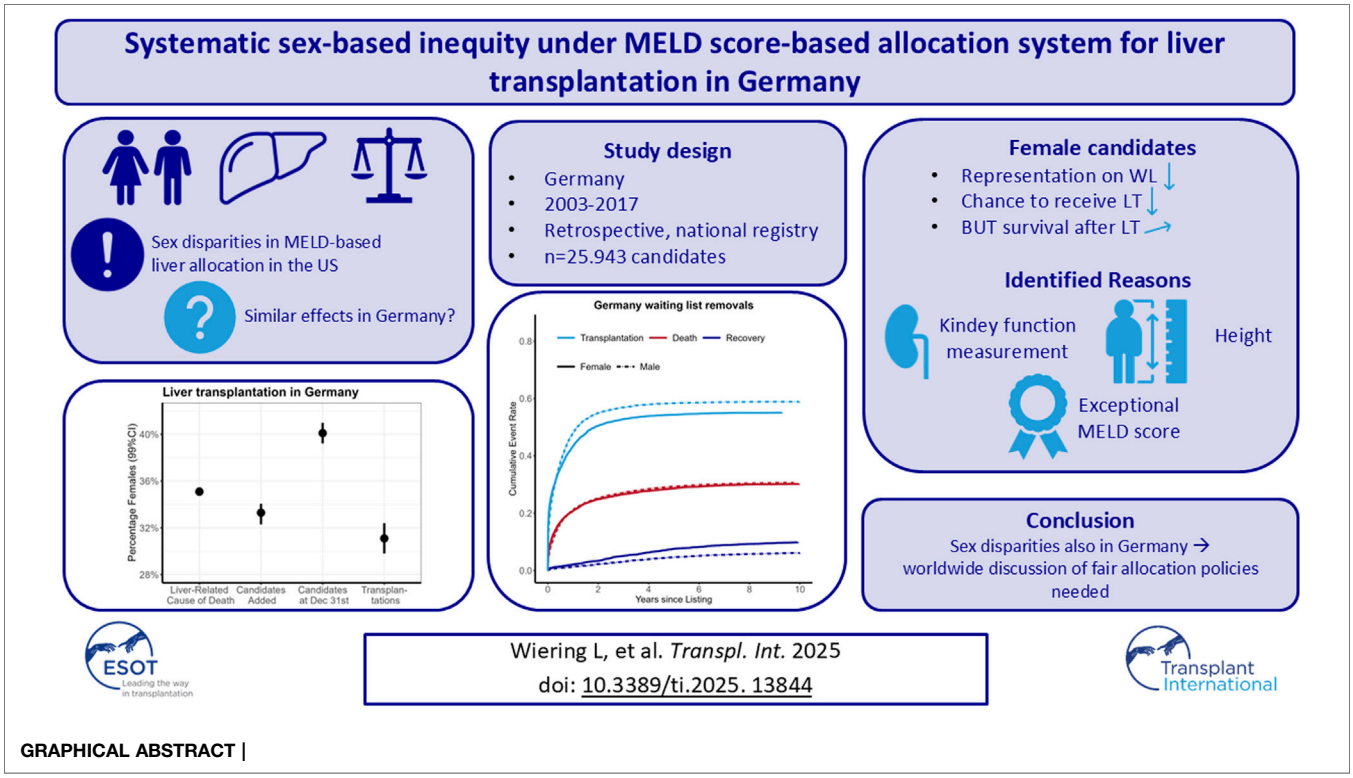
Abbreviations: LT, liver transplantation; MELD, Model for End-stage Liver Disease; tBil, total bilirubin; sCr, serum creatinine; INR, international normalized ratio; HCC, hepatocellular carcinoma; eGFR, estimated glomerular filtration rate; IQR, interquartile range; HR, hazard ratio; CI, confidence interval.



GRAPHICAL ABSTRACT |

INTRODUCTION

In recent years, sex disparities in liver transplantation have been increasingly recognized [1]. Among them, the chances of women to receive life-saving liver transplantation (LT) are reduced compared with those of men [2, 3]. Every year, more than 30.000 patients worldwide undergo LT [4] and the limited availability of deceased donor organs is still a problem of great ethical relevance that has not yet been solved.

In 2002, the United Network for Organ Sharing introduced the Model for End-stage Liver Disease (MELD) score as a new liver allocation policy in the United States [5, 6]. The MELD score is a disease-severity scale and aims to reduce waitlist mortality by using transparent criteria and guaranteeing fair allocation. The MELD formula counts total bilirubin (tBil), serum creatinine (sCr), and international normalized ratio (INR) [5]. Exceptions have been added for individuals whose disease severity is not adequately reflected by their actual calculated MELD score, assigning these patients exceptional MELD points [e.g., hepatocellular carcinoma (HCC), **Supplementary Material S1, S2**]. Today, the majority of countries offering LT have implemented comparable allocation rules [4]. In the United States, the liver allocation policy was recently changed to include sex (MELD 3.0) [7]. Although this represents an important advancement, the available data on sex disparities in liver transplantation are very much limited to the United States [8]. Although these data are crucial, they do not seem sufficient to adapt allocation policies worldwide as countries differ in their allocation procedures, access to healthcare, organ donation availability, and other factors. As a result, algorithms in other countries have not been adjusted for sex equity.

This study aimed to evaluate transplant probability in women in the context of the MELD-based liver allocation system in Germany, a country substantially different from the United States with respect to donation rates and access to healthcare, and to encourage possible amendments to overcome sex-based inequalities in liver transplantation worldwide.

MATERIALS AND METHODS

Study Design and Setting

This study analyzed the German LT program. The primary endpoint was the hazard ratio for women compared with that for men to receive LT before and after the introduction of the MELD-based allocation system. The MELD-based allocation system was implemented on 16 December 2006. The study included patients registered (waitlisted) from 1 January 2003, to 31 December 2017.

Data Sources and Quality

Data on the German LT program were provided by Eurotransplant with the approval of the working group for LT of the German Medical Association on 16 September 2019. Cause of death statistics were obtained from the Federal Statistical Office for Germany (**Supplementary Material S4**). Data were obtained in anonymized form.

Patient Selection and Allocation Rules

To account for the applied allocation rules, pre-MELD and MELD eras were defined as the patients who were removed from the waiting list for any reason before or after the implementation of the MELD-based policy. Patients younger than 18, patients receiving a living donor transplant, patients receiving, or awaiting a multi-organ transplant and those listed with high urgency (equivalent to Status 1 in the United States) were excluded (see **Supplementary Figures S1, S2**).

Variables and Definitions

Epidemiological and procedure-related data are listed in **Supplementary Tables S1–S4**. To address the bias resulting from the unisex use of sCr in the MELD formula despite physiologically lower sCr levels in women [9] their estimated glomerular filtration rates [eGFR using the chronic kidney disease epidemiology collaboration (CDK-EPI) formula [10]] were used. By inserting the female eGFR into the male formula for eGFR and back-calculating to sCr we determined a corrected sCr for women. Finally, a corrected MELD score was computed using this corrected sCr (**Supplementary Material S3**).

Statistical Analysis

Descriptive statistics were carried out according to their level [absolute and relative frequencies for categorical variables, median and interquartile range (IQR) for continuous variables]. Cumulative incidence curves displaying time to transplantation, death or ineligibility (waiting list mortality), and recovery were plotted. To assess the effect of sex on transplantation, waiting list mortality and survival after transplantation we used competing risk analysis and derived cause-specific hazard ratios (HRs) based on multivariable Cox-proportional hazards regression models. Survival after transplantation was additionally depicted using Kaplan-Meier curves. Follow-up for survival analysis began at the time of transplantation and ended at death or was censored at the time of the last documented follow-up. The effect of height on transplantation was modeled using a spline with four degrees of freedom. We additionally ran sensitivity analyses with robust standard errors. Due to a very limited number of missing values in the key parameters of interest all models are based on complete cases (max. 0.04% for German data for all data presented in **Supplementary Tables S1–S4**). Statistical analyses were performed using R [11] (see **Supplementary Material S5** for used packages). According to the local Institutional Review Board (Charité – Universitätsmedizin Berlin), no specific approval was required for this study, which analyzed data already anonymized.

RESULTS

Sex Imbalance in the German Liver Transplantation System

In total, 25,943 patients were registered on the respective waiting lists during the observation period, of which 20,018 met the inclusion criteria. In fact, 10,482, (52.4%) of these patients underwent LT within the observation period. Patient demographics are summarized in **Supplementary Figure S1**; **Supplementary Tables S1–S4** (candidates and recipients).

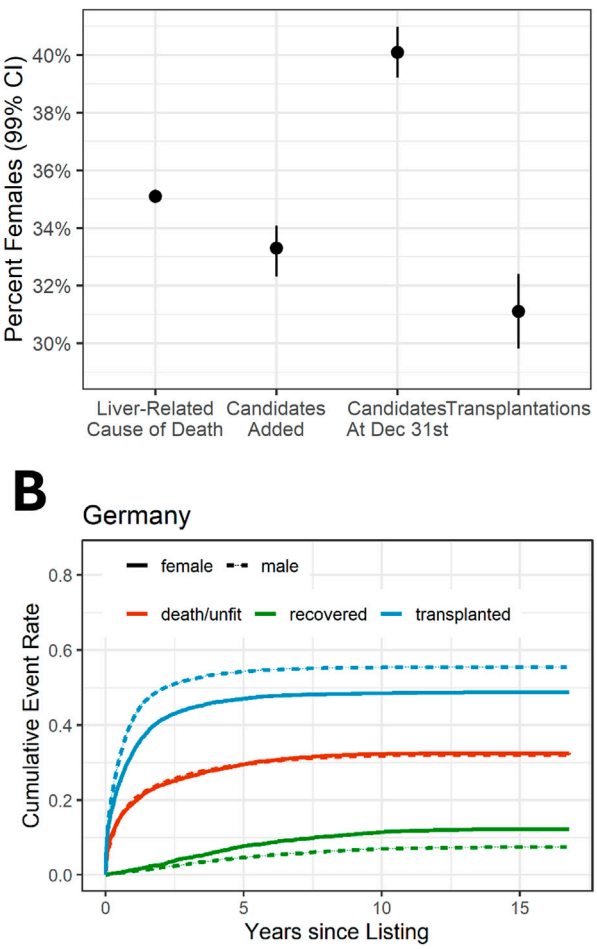


FIGURE 1 | Sex ratio in liver transplantation and probability of transplantation by sex. **(A)** Female proportion in the German liver transplantation program in relation to liver-related causes of death. **(B)** Probability of outcome of candidates on the liver waiting list by time-to-event analysis (cumulative incidence function). The data displayed depict the MELD era for **(A, B)**.

Because the incidence of liver disease is not equally distributed between women and men, the proportion of liver-related causes of death was computed as a benchmark with women steadily representing 35.1%. The ratio of women newly registered on the waiting list and the percentage of waitlisted women who received LT considerably changed after the implementation of the MELD-based allocation system (**Supplementary Figure S2**). Annual waitlist registrations for women decreased, i.e., from 36.2% (95% confidence interval (95%CI): 34.5–37.9) to 33.3% (95%CI: 32.3–34.1), and the annual percentage of actual female transplant recipients decreased, i.e., from 34.4% (95%CI: 32.0–36.8) to 31.1% (95%CI: 29.8–32.4), respectively (**Figure 1A**). In

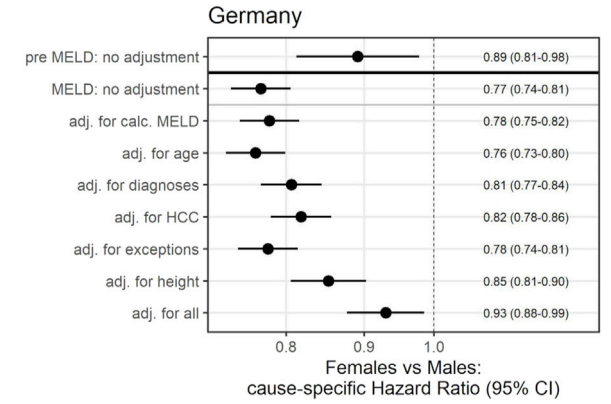


FIGURE 2 | Effects of female sex on transplantation rates before and after the introduction of the MELD-based liver allocation system using competing-risk Cox regression (outcome: time to transplantation). HCC, hepatocellular carcinoma; MELD, Model for End-Stage Liver Disease.

contrast, a snapshot of the actual waiting lists on 31 December of each year revealed an average of 40.1% (95%CI: 39.2–41.0) of female patients.

Reduced Transplantation Rates in Female Candidates

Cumulative incidence analysis revealed that the chances of transplantation are significantly lower for women than for men (**Figure 1B**). Prior to the MELD era (pre-MELD), in waitlisted women had a slightly lower hazard of LT than men (HR = 0.89, 95%CI: 0.81–0.98). However, in the time-to-event model for the period after the MELD-based allocation system was introduced, the hazard of transplantation for women was estimated to be even lower, i.e., only 0.77-fold when compared with that of men (HR = 0.77, 95%CI: 0.74–0.81; **Figure 2**). Depending on the reference baseline, the absolute number of the gap since the introduction of MELD-based allocation until 2017, would be up to 731 transplants not allocated to women corresponding to approximately every 12th transplantation during this period (**Supplementary Figure S3**).

For better understanding, the analysis was adjusted for different covariates (**Figure 2**). Prior to the implementation of the MELD-based allocation system, sex-based discrimination could be explained by differences in height (**Supplementary Figure S4**). In the present allocation policy, differences between women and men in access to LT could be partially explained by adjustments for height (HR = 0.85, 95%CI: 0.81–0.90) as well as HCC, the most prevalent diagnosis for exceptional MELD (HR = 0.82, 95%CI: 0.78–0.86) (**Figure 2**).

Waiting List Mortality and Survival After Transplantation

The MELD-based allocation system was implemented to reduce prolonged waiting times and mortality rates among patients on the waiting list. When examining waiting list mortality independently using competing risk regression with a cause-specific hazard, no significant sex differences were observed before the system's introduction. Following the adoption of the MELD-based allocation system, women exhibited a slightly lower cause-specific hazard ratio for waiting list mortality (before HR = 0.96, 95%CI: 0.85–1.08; after HR = 0.86, 95%CI: 0.81–0.91) (**Supplementary Figure S5**). However, this statistic only captures the instantaneous effect on waiting list mortality. Overall, the reduced ratio does not result in a substantially lower overall waiting list mortality due to the adverse impact on the likelihood of transplantation. As a result, comparable rates of women and men die while waiting for liver transplantation or are removed from the waiting list for becoming unfit for transplantation (2 years after listing: 23.9% of female recipients; 24.3% of male recipients) (**Figure 1B**). Height was found to negatively influence waiting list mortality in women (**Supplementary Figure S5**). Survival after liver transplantation was comparable in the short term for men and women (1-year patient survival HR = 1.02, 95% CI: 0.92–1.12). In the longer term, female transplant recipients showed slightly better survival compared to men. This effect was already found in the pre-MELD era and did not significantly change thereafter (overall survival in women compared to men, pre-MELD HR = 0.85, 95%CI: 0.75–0.96; MELD HR = 0.89, 95% CI: 0.83–0.96) (**Supplementary Figure S6**).

Calculated MELD and Serum Creatinine Withhold MELD Points in Women

Reflecting the differences in transplantation rates by sex, no difference was observed in the calculated MELD score of all candidates at the time point of listing between men (median 17, IQR 10–29) and women (median 17, IQR 10–30). The MELD score of patients who actually received a transplant was higher in women (median 19, IQR 1–32) compared to men (median 17, IQR 11–30). To better understand this difference, the specific laboratory values that define the calculated MELD score were subjected to detailed analysis (**Figure 3**). Of particular interest is sCr as it is used without adaptation to well-known sex-based differences [10]. The cohorts were separated into recipients with and without renal replacement therapy; as for MELD score calculation sCr was set to 4 mg/dL in patients receiving dialysis. Overall, among patients who received a transplant, women were more likely to be on dialysis (20.6% vs. 14.3%, **Figure 3A**). Women who were transplanted without receiving renal replacement therapy had significantly lower sCr values than men (0.94 vs. 1.04 mg/dL; 99%CI = 0.90–0.99 vs. 1.01–1.07, **Figure 3B**), although their actual kidney function, represented by the eGFR, was significantly worse (52.1 vs. 69.0 mL/min; 99%CI = 49.7–54.4 vs. 65.8–72.0). Corrected sCr levels in women were higher than uncorrected levels (0.94 vs. 1.19 mg/dL; 99%CI = 0.90–0.99 vs. 1.14–1.25) and importantly those corrected sCR levels in women were higher compared to uncorrected levels in men (1.19 mg/dL vs. 1.04 mg/dL; 99%CI = 1.14–1.25 vs. 1.01–1.07). Subsequently, also women's corrected MELD scores were higher compared to men's. As an indicator of the need for women to compensate for this sex-unspecific use of sCr, female patients not on dialysis had

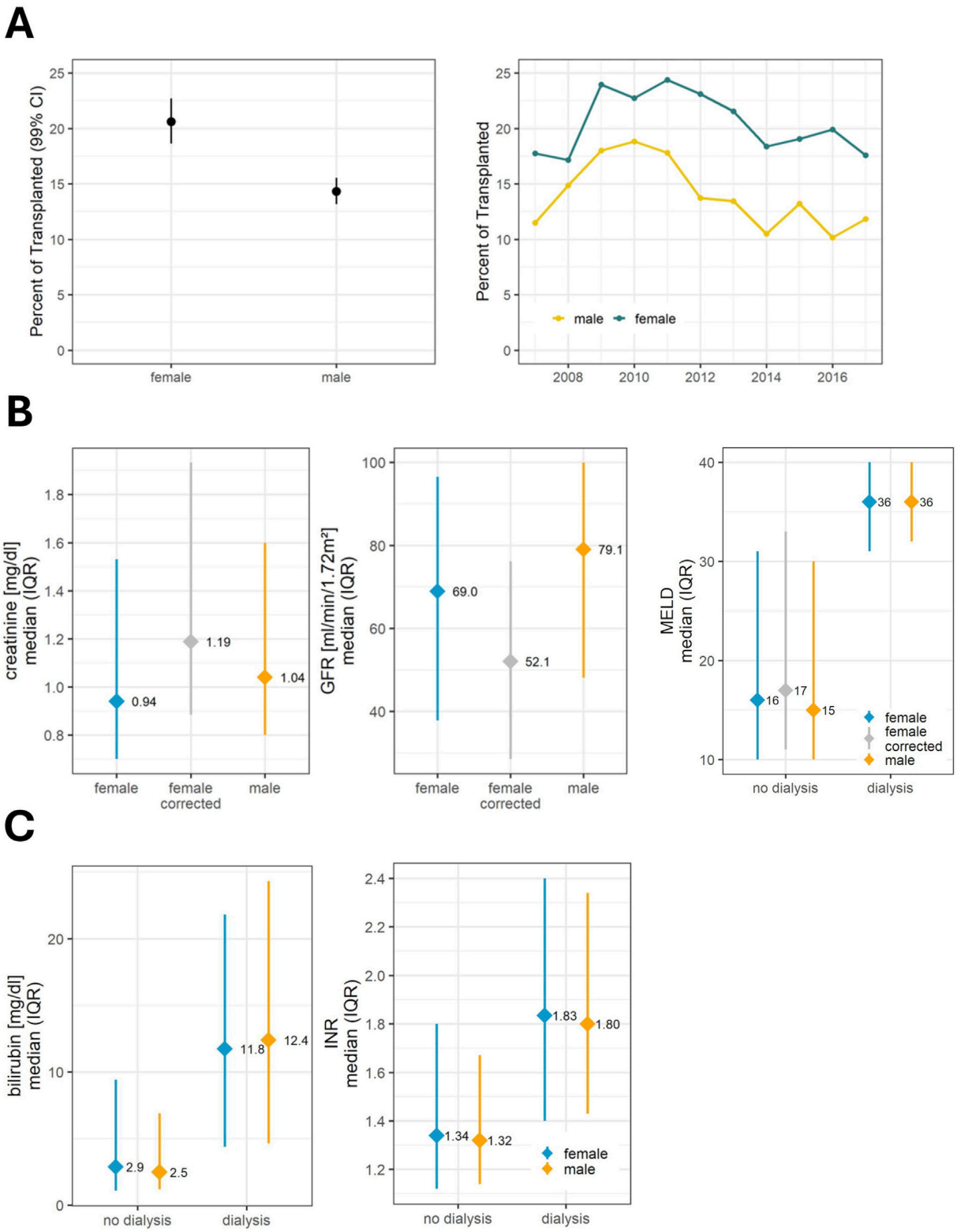


FIGURE 3 | Components of the MELD score and influence of renal function. **(A)** Dialysis: Proportion of patients requiring dialysis at the time of organ allocation and its development over time. **(B)** Renal function and MELD: For patients without the need for renal replacement therapy, female recipients had lower serum creatinine values, but their actual renal function was worse than that of men. A corrected creatinine was used for the MELD calculation. For patients with a need for renal

(*Continued*)

FIGURE 3 | replacement therapy, the creatinine value in the MELD formula was set at 4 mg/dL. In this group, MELD scores do not differ between the sexes. **(C)** Further components of the MELD score: In the non-dialysis group bilirubin and INR were higher in the female cohort to compensate for lower creatinine. In the dialysis group, this was not the case. For the analysis of the MELD score, only the calculated MELD score was used without considering exceptional MELD. GFR, glomerular filtration rate (calculated using the CKD-EPI formula); INR, International normalized ratio; MELD, Model for End-Stage Liver Disease.

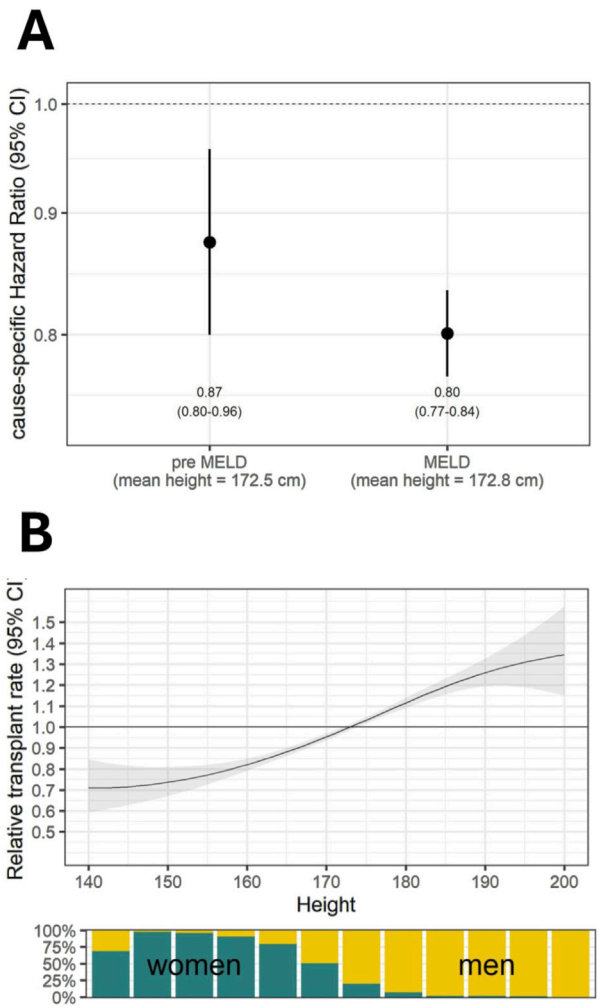


FIGURE 4 | (A) Effects of height on transplantation rates using competing-risk Cox regression models. **(B)** Under the MELD-based allocation policy, the chances of transplantation increase directly with body height. The bar graph indicates the percentage of women in defined height groups.

increased levels of tBil and INR compared to men (tBil: women 2.9 mg/dL, 99%CI = 2.60–3.20, **Figure 3C**; men 2.5 mg/dL, 99% CI = 2.38–2.65; INR: women 1.34, 99%CI = 1.3–1.38; men 1.32, 99%CI = 1.30–1.34; **Figure 3C**). In a model, using a corrected MELD score, based on the eGFR corrected sCR levels as described above, women would gain up to three critical MELD points (**Figure 3B**; **Supplementary Figure S7**). Notably, additional MELD points would be assigned to all women with an eGFR below 85 mL/min. This would include 63.7% of female candidates who do not require renal replacement therapy. In a MELD-based allocation system missing MELD points could tip the balance and lead to lower chances of transplantation and longer waiting times (**Supplementary Figure S8**).

Height-Related Hazards Disadvantage Shorter Candidates

By analyzing the hazard of height, we found that pre-existing height discrimination regardless of sex (HR = 0.87, 95%CI: 0.80–0.96) was exacerbated after implementation of the MELD-based liver allocation system (HR = 0.80, 95%CI: 0.77–0.84) (**Figure 4A**). This effect was found to be directly proportional to the height of the candidates with women constituting the vast majority of short individuals (**Figure 4B**).

Exceptional MELD and Its Impact on Sex Inequity

Certain indications are eligible for exceptional MELD points according to country-specific allocation guidelines. Consequently, the proportion of transplants based on these indications has increased over time (**Figure 5A**). Overall, 39.3% of effectively transplanted patients had a MELD exception. Men were more likely to receive an exceptional MELD (40.2% vs. 37.5% in women, **Supplementary Table S2**) and candidates with an exceptional MELD have a higher chance of undergoing transplantation compared to those without (HR = 1.61, 95% CI: 1.54–1.69; **Figure 5B**). The most frequent diagnosis for the standardized exceptional MELD is HCC and in the group of patients receiving an exceptional MELD for this reason the number of women is disproportionately low (**Figure 5C**).

DISCUSSION

This is the first study to demonstrate that the introduction of a MELD-based liver allocation system has exacerbated an existing disadvantage in the chances of women undergoing deceased donor LT in Germany. Although for the United States, this has been indicated previously [2, 3, 12, 13], very few comparable data are available for other countries [14]. Based on the large cohort data, the similarity of the allocation systems and the identified systematic flaws, we believe that this inequity is of relevance in all countries using similar MELD-based allocation systems [15].

Globally, of the more than 1,3 million deaths per year due to cirrhosis, the proportion of women dying due to liver failure is one-third [16] and the risk of liver-related death is similar to that of men [17]. Despite stable sex proportions of liver-related causes of death, we observed an increase in the effective male-to-female LT ratio over time, to the detriment of women. Certainly, liver-related mortality does not necessarily match exactly with the incidence, prevalence, or burden of end-stage liver disease nor

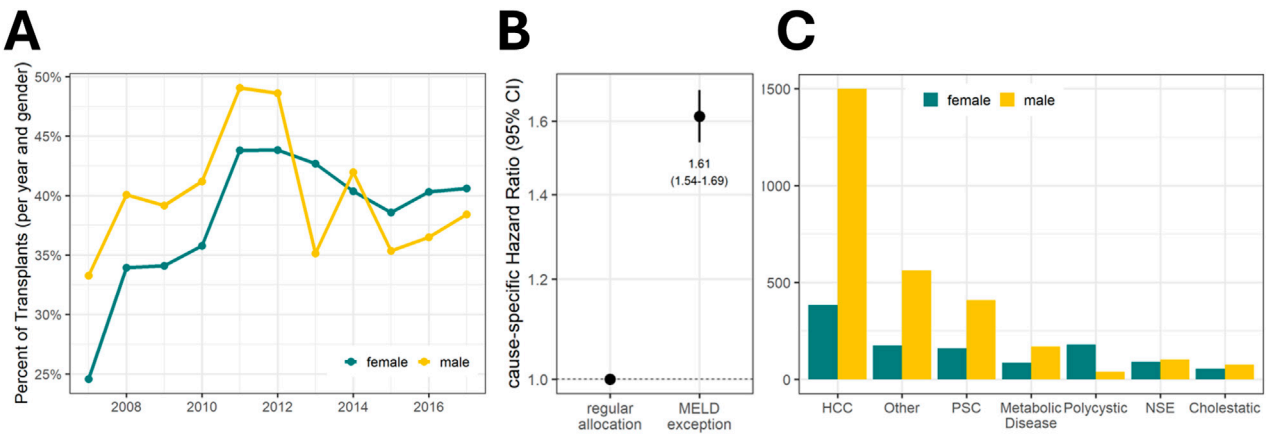


FIGURE 5 | (A) Development of exceptional MELD over time. The percentage of exceptional MELD among transplant recipients increased after its implementation to nearly 50% in 2011 (43.8% for female recipients and 49.1% for male recipients). Overall, the share was higher in men than in women (40.2% vs. 37.5%). **(B)** Transplant probability of candidates by exceptional MELD status. **(C)** Indications for receiving exceptional MELD and their sex ratio. HCC, Hepatocellular Carcinoma; MELD, Model for End-Stage Liver Disease; NSE, Nonstandard Exception; PSC, Primary Sclerosing Cholangitis.

does it implicitly correspond to the indication for LT. However, the HEPAHEALTH project by the European Association for the Study of the Liver [18] and the Global Burden of Disease Study [16] have revealed that the aforementioned sex ratio of liver-related deaths matches the epidemiology of liver disease and that the ratio has remained constant over time. Based on our data, the disadvantage of women undergoing liver transplantation is based on four aspects, namely, reduced waitlisting, calculated MELD, height, and exceptional MELD.

First, women are disproportionately less likely to be listed for liver transplantation. This imbalance may be based on the disadvantages caused by serum sCr and exceptional MELD, as the majority of transplant centers implemented absolute MELD thresholds for waitlisting and/or transplantation [19].

Second, female recipients had lower values of sCr even though their actual renal function was worse than in male patients (**Figure 5**; **Supplementary Figure S9**). This difference can account for up to three or even more MELD points [9, 20]. Because of the lower muscle mass of women, they have lower sCr levels [21] which heavily affect the MELD score [22]. The implementation of MELD-Na in the United States in 2016 has exacerbated this disparity [20]. As we have shown, a woman has to be "sicker" to have the same MELD score as a man, which explains the higher waitlist frailty, mortality, and dropout rate due to ineligibility for LT previously described in women [22, 23].

Third, height is lower in women, which has a negative impact on the probability of receiving LT. Some studies have already highlighted that lower body height in women is associated with a higher waiting list mortality in the United States [20, 24–26]. Although there is no solid evidence of how height affects the chances of organ allocation in this objective system, the most obvious reason is a decrease in organ offers due to fear of large-for-size syndrome [27, 28]. Consequently, the complete spectrum of offered donor livers is accepted in terms of organ volume for male recipients, whereas only a portion is accepted for female recipients [29]. In a recent study this difference in acceptance of organ offers was found to persist even in patients with high disease severity, resulting in a lower chance of receiving a transplant and a higher waiting list mortality rate [30]. Therefore, size compensation may be needed for retributive justice.

Fourth, women are less likely to receive an exceptional MELD. It is known that patients with exceptional MELD are generally more likely to receive LT and have lower waiting list mortality [31, 32]. The most common standardized exception, HCC, is more frequent in men [33]. In the German transplant registry, only 20.4% of all HCC patients were female. In the United States, the rules for HCC exceptions have already been adapted and revised in 2009 and 2015 to address the imbalance between HCC and non-HCC patients.

Consequently, it is essential to optimize current allocation systems worldwide to address these sex inequities. To compensate for the loss of MELD score points due to the use of sCr, either additional MELD score points for women [9, 20], a corrected sCr [34], or the implementation of GFR into the MELD formula have been suggested, partially demonstrating a harmonization of waiting list mortality [35–37]. Our study suggests that the recipient's height should also be considered to counteract the problem of large-for-size [24, 29]. Organs from shorter donors could be allocated preferentially to shorter recipients (regardless of sex) or small people could otherwise receive extra MELD score points as in pediatric transplantation [6, 38]. Finally, exceptional MELD status can be adjusted by policy changes, e.g., reduction of exceptional points. In the United States, following a growing debate [39], the first specific policy modification was adopted in 2023 to minimize sex-based differences by using the so-called MELD 3.0 which assigns an additional 1.33 MELD points to women and adjusts the limits of included laboratory values [7]. This represents a crucial step in addressing the disparities also identified in our study. However, the specific effect of this adjustment remains to be investigated, as the described factors such as height and exceptional MELD are not explicitly addressed. In other countries, such adjustments are still lacking, and data from outside the United States are largely insufficient to justify such modifications. Although MELD-based

allocation is utilized globally, significant differences persist in transplant and healthcare systems. There are notable disparities in organ donation availability, the exact design of MELD-based allocation (e.g., criteria for exceptional MELD points), and financial and socio-economic access to transplantation. Therefore, it is essential to consider and analyze local contexts and potentially tailor guidelines to meet specific regional circumstances. Recently, Tejedor et al. published their findings from an analysis of the Spanish Liver Transplantation Registry [40]. Their study represents the first national investigation outside the United States on this topic and also found lower transplantation rates for women compared to men. Spain and the United States have the highest rates of organ donation internationally and utilize a significant number of donations after circulatory death. Although the Spanish study is an important step, the applicability of the existing findings to many other countries remains uncertain. Our study helps to fill this knowledge gap: Germany, with an average organ donation rate and no current practice of transplanting organs from donors after circulatory death, is more representative of many other countries than Spain and the United States. The fact that we found similar results suggests that sex-based inequity is inherent in the system, highlighting the need for a global discussion and adaptation of allocation rules.

Regarding waiting list mortality, we described comparable absolute waiting list mortality rates for women and men, but we also reported a reduction in the cause-specific hazard ratio for waiting list mortality for women. This may seem contradictory and inconsistent with previous reports from the United States [2, 3, 12]. However, in the present study an effective reduction in waiting list mortality was probably not achieved due to the adverse effect on the likelihood of transplantation. Differences with previous reports may have been influenced by the statistic selected to analyze the competing risks of transplantation and waiting list mortality. In this context, it is also reasonable to assume that the analysis of waiting list mortality is always complicated by changes in allocation policies as waiting list registrations are highly dependent on the chances of transplantation and the majority of transplant centers will only evaluate patients who meet certain criteria (e.g., threshold of MELD score, exceptional MELD). Therefore, a change in allocation rules will alter the listing behavior of transplant centers. The resulting shift in the composition of the waiting list makes direct comparisons challenging. This confirms our approach of additionally relating the sex ratio in the transplant system to the entire patient population.

The quality of our results depends on the quality of data entry. All data shown are analyzed retrospectively and therefore do not provide proof of any causal relationships, although the evidence seems clear. However, these limitations are comparable to similar studies.

In conclusion, women in need of LT face two problems: they are less likely to be waitlisted, and their chances of receiving a transplant are lower than those of men. Although the implementation of a MELD-based liver allocation system aimed to guarantee a fair and objective organ allocation, this was not accomplished in terms of sex-based equity. As the results of our study are in line with other international studies, this sex-based inequity must be resolved worldwide. Possible approaches to improve the allocation system would be to consider the inclusion of the height of the recipients, a reevaluation of the renal function, and a discussion of the priority of patients with HCC in all MELD-based transplantation programs.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: Data is not directly available from the researchers but may be requested from the working group for liver transplantation at the German Medical Association (Bundesärztekammer). Alternatively data may be requested from the German transplant registry. Requests to access these datasets should be directed to https://transplantations-register.de/.

ETHICS STATEMENT

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

Conceptualization: LW, MD, FT, PR, RÖ, and PR formal analysis: AA validation: AA investigation: LW and AA resources: PR and RÖ writing–original draft: LW and PR writing–review and editing: BG, TD, NR, MD, WS, FT, and RÖ supervision: FT and JP. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

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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.2025.13844/full#supplementary-material

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The Clinical Significance of HLA Compatibility Scores in Lung Transplantation

Liesbeth Daniëls[1,2], Hanne Beeckmans[3], Andrea Zajacova[3], Pieterjan Kerckhof[3†], Saskia Bos[4], Maarten Naesens[5], Bart Vanaudenaerde[3], Frans Claas[6,7†] and Robin Vos[3,4]*

[1]Histocompatibility Laboratory (HLA), Clinical Biology, CHU UCL Namur Site Godinne, Namur, Belgium, [2]Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp, Belgium, [3]Department of Chronic Diseases, Metabolism and Ageing, Laboratory for Respiratory Diseases and Thoracic Surgery, Faculty of Medicine, KU Leuven, Leuven, Belgium, [4]Department of Respiratory Diseases, University Hospitals Leuven, Leuven, Brussels, Belgium, [5]Department of Microbiology, Immunology and Transplantation, Faculty of Medicine, KU Leuven, Leuven, Belgium, [6]Eurotransplant and Eurotransplant Reference Laboratory, Department of Immunology, Leiden University Medical Center (LUMC), Leiden, Netherlands, [7]Department of Translational Research in Immunology and Inflammation, University of Antwerp, Antwerp, Belgium

Lung transplantation is a life-saving therapeutic option for many chronic end-stage pulmonary diseases, but long-term survival may be limited by rejection of the transplanted organ. Since HLA disparity between donor and recipient plays a major role in rejection, we performed a single center, retrospective observational cohort analysis in our lung transplant cohort (n = 128) in which we calculated HLA compatibility scores for B-cell epitopes (HLAMatchmaker, HLA-EMMA), T-cell epitopes (PIRCHE-II) and missing self-induced NK cell activation (KIR Ligand Calculator). Adjusted Cox proportional hazards model was used to investigate the association between mismatched scores and time to development of donor-specific antibodies (DSA) post-transplant, time to first biopsy-proven acute rejection episode, freedom from CLAD, graft survival and overall survival. For time to first DSA, HLA-EMMA DQB1 scores and PIRCHE-II DQB1 scores were significantly associated with more rapidly developing anti-HLA-DQ antibodies. HLA-EMMA DQB1 score was significantly associated with worse survival. KIR ligand Host-versus-Graft (HvG) mismatches was significantly associated with worse graft survival (CLAD or death) and shorter time to first biopsy-proven rejection when 2 mismatches were present. We demonstrated that HLA-DQB1 compatibility scores and KIR ligand HvG 2 mismatches may allow for identification of recipients at risk of poor long-term outcomes after lung transplantation.

Keywords: lung transplantation, HLAMatchmaker, HLA-EMMA, PIRCHE-II, KIR ligand calculator

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***Correspondence**
Liesbeth Daniëls,
liesbeth.daniels@chuuclnamur.uclouvain.be

†ORCID:
Pieterjan Kerckhof
orcid.org/0000-0002-3806-4478
Frans Claas
orcid.org/0000-0003-4157-6201

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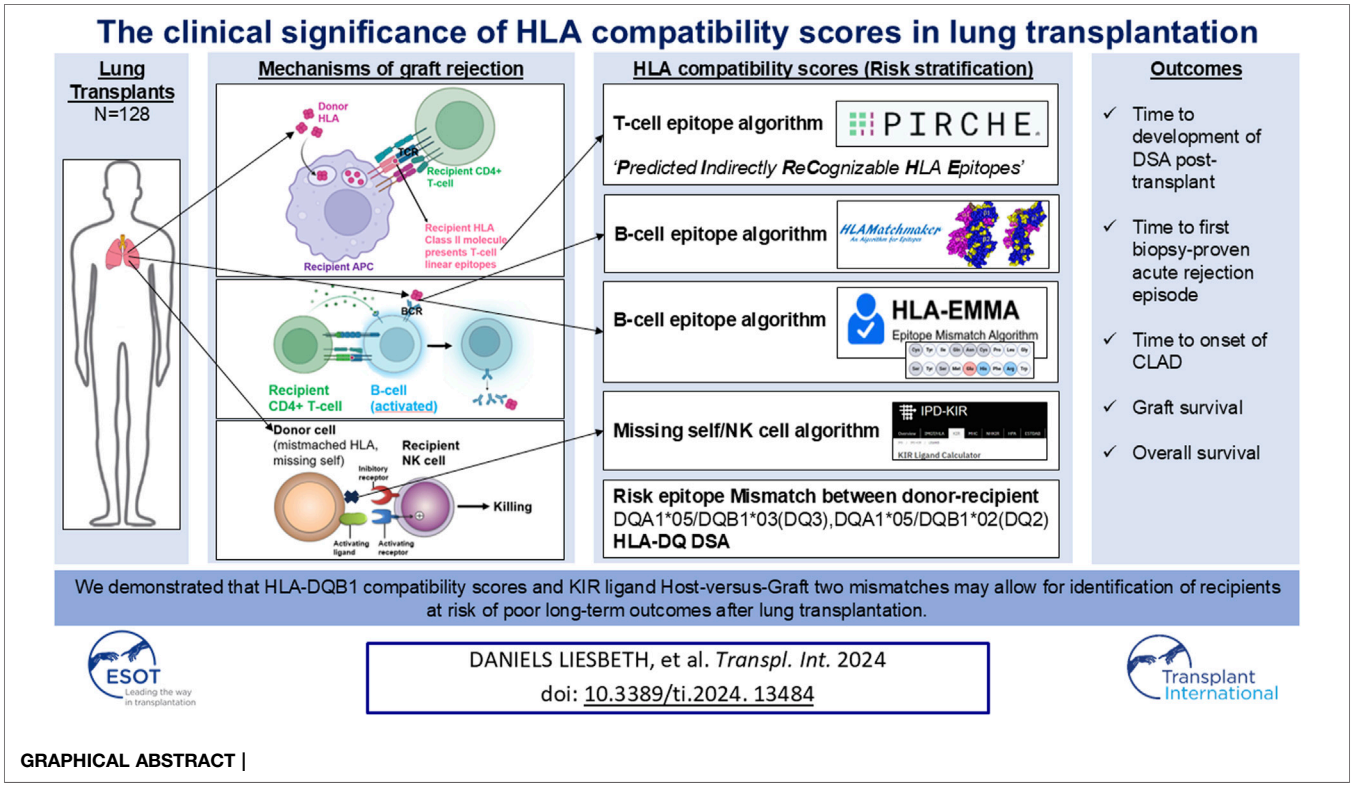
Abbreviations: CLAD, Chronic lung allograft dysfunction; HLA, Human leukocyte antigen; DSA, Donor-specific anti-HLA antibodies; PIRCHE-II, Predicted Indirectly ReCognizable HLA epitopes presented by HLA class II molecules; dn, *De novo*; ADCC, Antibody-dependent cell-mediated cytotoxicity; CDC, Complement-dependent cytotoxicity; AMR, Antibody-mediated rejection; ACR, Acute cellular rejection; NK cell, Natural killer cell; KIRs, Killer-cell immunoglobulin-like receptors; EFI, European Federation for Immunogenetics; CMV, Cytomegalovirus; HR, Hazard ratio; CI, Confidence interval; REM, Risk epitope mismatch; MM, Mismatch; HvG, Host-versus-Graft; MFI, Median Fluorescence Intensity; ISHLT, International Society for Heart and Lung Transplantation.



GRAPHICAL ABSTRACT |

INTRODUCTION

Lung transplantation is a life-saving therapeutic option for many chronic end-stage pulmonary diseases. However, long-term survival after lung transplantation is the worst of all solid organ transplantations and is, in large part, limited by chronic rejection, or so-called chronic lung allograft dysfunction (CLAD) [1]. CLAD encompasses a range of pathologies causing a transplanted lung allograft to not achieve or maintain its normal function, which clinically manifests as airflow obstruction and/or restriction [2].

Human leukocyte antigen (HLA) disparity between donor and recipient affects the alloimmune response and consequently has an impact on graft outcome [3]. The foreign HLA antigens of the donor are recognized by the adaptive immune system of the recipient, which - when activated - can lead to organ injury by rejection; and finally, the failure of the transplanted organ [4]. Immunogenicity is the ability to induce an antibody response while antigenicity is based on the actual interaction between antibody and an antigen and varies according to the recipient's self HLA and the mismatched donor HLA [5]. The portion of the HLA molecule that interacts with anti-HLA antibodies, the binding site, is called "epitope." An "eplet" represents the smallest functional unit contributing to the antibody specificity and forms a smaller portion (~ 3 Å diameter) of the larger overall epitope (~ 15 Å diameter) [6].

Besides B cell epitopes, T cell epitopes may also play a role in antibody formation, since donor-specific anti-HLA antibodies (DSA) production occurs via the indirect allorecognition pathway in which foreign HLA is processed by the recipient's antigen-presenting cells and presented by HLA class II to $CD4^+$ T cells, followed by B cell activation, plasma cell formation and antibody production. As such, HLA-derived T cell epitopes, designated as PIRCHE-II (Predicted Indirectly ReCognizable HLA epitopes presented by HLA class II molecules), also play a role in generation of *de novo* (dn)DSA and graft failure [7–9]. Circulating DSA bind to allogeneic HLA on donor cells' surface (e.g., endothelial cells), inducing endothelial cell activation, and subsequent recruitment of innate immune cells and complement factors. Next, recruited innate immune cells bind to the HLA-DSA and release cytotoxic granules (a process called antibody-dependent cell-mediated cytotoxicity/ADCC), and/or complement fixation and activation occurs, leading to formation of a membrane attack complex (a process called complement-dependent cytotoxicity/CDC). Both these pathways in the process of antibody-mediated rejection (AMR) result in cytolysis (cell death) of the targeted "non-self" cells. Moreover, T cells within the draining pulmonary lymph nodes are also activated after binding with membrane-bound allogeneic HLA on antigen-presenting cells, either donor- or recipient-derived, that have migrated from the lung allograft. Activated T cells then enter the blood circulation and may infiltrate the allograft inducing a local inflammatory response termed acute (T cell-mediated) cellular rejection (ACR).

In addition to antibody-mediated and T cell-mediated rejection, as described above, Koenig et al. [4] demonstrated in

kidney transplants that missing self-induced natural killer (NK) cell activation promotes the development of graft microvascular inflammation that has exactly the same harmful impact on organ survival as non-complement activating anti-HLA DSA, the principal cause of late transplant loss. In steady state, the interaction of inhibitory Killer-cell immunoglobulin-like receptors (KIRs) with self-HLA class I molecules of surrounding healthy cells provides a negative signal. On the contrary, the downregulated expression of HLA class I molecules associated with tumoral transformation or viral infection triggers NK cell activation, which results in destruction of the target cell, a process called response to 'missing self'. In clinical transplantation, however, graft endothelial cells are unable to deliver inhibitory signals to recipient NK cells because of different (mismatched) HLA class I molecules. This imitates the 'missing self' for NK cells.

We assume that primed NK cells in the lung transplant recipient's circulation (due to ischemia/reperfusion injuries and/or prior (viral) infections) may also promote endothelial damage in lung allografts, and that "missing self" thus should also be considered as a risk factor in the process of rejection after lung transplantation. Patients with missing self-induced rejection will not respond to the costly and tedious treatment of AMR [4]. Missing self-induced NK cell activation is mTORC1- dependent, and mTOR inhibitors may prevent development of this type of chronic vascular rejection [4]. Therefore, it is critically important to clinically identify this process in lung transplant patients at risk for/with rejection, to accordingly adjust treatment (i.e., pathway-directed therapy) in these patients.

Since HLA disparity between donor and recipient plays a major role in rejection, as evidenced by complement activating anti-HLA antibodies (CDC), ADCC caused by anti-HLA DSA, T cell-mediated cellular rejection and missing self-induced rejection by NK cells, it is important to explore which HLA software tools can be used to calculate HLA compatibility scores, in order to identify high-risk patients, fine-tune each patient's immunosuppressive regimen (personalized treatment) and further improve lung transplantation outcomes [10].

As data regarding HLA software-based risk identification are scarce in lung transplantation, we performed a single center, retrospective observational cohort analysis in our lung transplant cohort.

MATERIALS AND METHODS

Cohort

All consecutive adult lung transplant recipients at the University Hospitals Leuven between 1 January 2015 and 31 December 2021 with written informed consent, clinical/histopathological data and donor/recipient DNA samples available for high-resolution HLA typing, were eligible for this observational cohort study. Recipients of combined transplantation (i.e., heart-lung, lung-liver, lung-kidney transplant) or lung transplantation after another transplantation were excluded. Following induction treatment with rabbit anti-thymocyte globulin, baseline immunosuppression consisted of a standard triple regimen consisting of tacrolimus, mycophenolic acid, and corticosteroids. No desensitization therapies for pretransplant anti-HLA antibodies were used. Patients at risk for cytomegalovirus (CMV) primo infection or reactivation (donor positive or recipient positive status) received prophylaxis with ganciclovir and valganciclovir for 3–6 months. During the first year post-transplant, all participants were followed clinically at monthly intervals and thereafter at three monthly intervals. Protocol-bronchoscopy with biopsies is routinely performed at 1, 3, 6, 12, 18, and 24 months, and in addition, indication-bronchoscopy with biopsies is performed upon clinical suspicion of graft rejection. Follow-up was censored at death or the censor date 31 December 2021. The study was approved by the Ethics Committee of the University Hospitals Leuven (BREATHE, KU Leuven) (S66760).

HLA Typing

Until recently, high-resolution HLA typing was not routinely performed at the University Hospitals Leuven. Therefore, donor and recipient DNA samples obtained from blood were retrospectively genotyped at the EFI accredited HLA laboratory CHU UCL Namur Site Godinne using next-generation sequencing (GenDx NGSgo-MX11-3 on Illumina Miseq) for all loci (HLA-A, -B, -C, -DRB1, -DRB345, -DQB1, -DQA1, -DPB1, and DPA1). The HLA types of donor and recipient were reported as 2-field alleles for mismatch analysis, since it has been show that minor differences in one or more epitopes between donors and recipients at either locus are sufficient to generate an immune response [11].

HLA Antibody Testing

HLA antibody results were retrospectively retrieved from the routine clinical database. Venous blood samples were collected routinely on day 0 and after transplantation on days 1–30–90–180–360–540–730, and annually thereafter as well as at intermediate time-points (i.e., when an indication-bronchoscopy with biopsies was performed or in case of suspected graft rejection). HLA antibody evaluation of all patient samples was performed with Immucor LIFECODES® Lifescreen Deluxe kits. A positive screening for the presence of circulating HLA antibodies was followed by HLA antibody identification with Immucor LIFECODES® LSA (Luminex Single Antigen) kits. All tests were performed and interpreted according to the manufacturer's instructions. A Median Fluorescence Intensity (MFI) of ≥500 was used for assignment of HLA DSA positivity. All serum samples were treated with EDTA to eliminate the prozone effect.

Bronchoscopic Surveillance

Patients underwent surveillance bronchoscopy with bronchoalveolar lavage and transbronchial biopsy as per our hospital protocol. ACR was diagnosed and graded according to the International Society for Heart and Lung Transplantation (ISHLT) Rejection Working Group with A- and B-grade component [12, 13]. Rejection of a severity of A1 or B1 or above was identified as ACR. AMR was diagnosed according to the 2016 ISHLT consensus [14] and

include the presence of DSA and characteristic lung histology with or without evidence of complement 4d (C4d) within the graft. AMR was categorized into 3 mutually exclusive possibilities (definite, probable and possible). These categories were based on the degree of certainty related to the presence or absence of a number of pathologic, serologic, clinical and immunologic criteria (allograft dysfunction, other causes excluded, lung histology, lung biopsy C4d, DSA).

HLA Compatibility Scores

For evaluation of the differential immunogenicity of HLA mismatches in lung transplantation we used the publicly available software tools based i.e., for B-cell epitopes "HLAMatchmaker v4.0 (HLA class I)," "HLAMatchmaker v3.1 (HLA class II)"[1] [15] and "HLA-EMMA v1.06"[2] [16], for T-cell epitopes "PIRCHE-II v3.3"[3], and for missing self-induced NK cell activation [KIR ligand mismatch Host-versus-Graft (HvG)] "KIR Ligand Calculator" IPD-KIR Database (ebi.ac.uk) [17–19].

Clinical Outcomes

The outcomes of interest we assessed were overall survival, time to onset of CLAD (freedom from CLAD), graft survival (defined as death or CLAD onset), time to development of dnDSA and time to biopsy-proven acute rejection (either cellular/ACR or antibody-mediated/AMR). CLAD was defined as a substantial and persistent decline in graft function (≥20%) in measured forced expiratory volume in 1 s value (FEV_1) from the reference (baseline) value according to the latest ISHLT consensus [1]. Freedom from CLAD was calculated as the time between transplantation and the date of diagnosis of CLAD. Patients without CLAD were censored at the end of study follow-up or at the date of death. No CLAD patients included in our study underwent a retransplantation.

In a second part of the study, we investigated the detection of dnDSA occurrence post-transplant and the significance of specific HLA-DQ mismatches, since not all mismatches equally contribute to generation of donor-specific immune responses and mismatches of HLA-DQ likely exhibit the highest immunogenicity, specifically the DQA1*05/DQB1*02 and DQA1*05/DQB1*03 [20–22]. For this purpose, the University Hospitals Leuven clinical database was consulted retrospectively to evaluate whether and which HLA antibodies had been detected by Luminex technology, and risk-epitope mismatches (DQA1*05/DQB1*02 and DQA1*05/DQB1*03) were also evaluated in the current cohort.

During the analyses, known risk factors at transplantation, namely, pretransplant HLA sensitization, donor and recipient CMV status, recipient sex and age, were taken into account.

Statistical Analysis

Patient statistics are presented as median and range or percentage, as appropriate. Cox proportional hazards model was used to investigate the association between mismatched scores and onset of first DSA post-transplant, time to first biopsy-proven acute rejection episode, survival and freedom from CLAD. Hazard ratios (HRs) (95% confidence interval (CI)) were used to define associations with scores and outcome variables of interest. Adjustment for known risk factors at transplantation were performed (sex, age, HLA sensitization and CMV status). In all models, a p-value of <0.05 was considered significant. RStudio version 4.3.1 was used for all statistical analyses and Kaplan-Meier survival curves.

RESULTS

Cohort

The study cohort comprised 128 lung transplants with a median age of 59 (range 18–66) in whom pretransplant DSA were detectable in 7 cases (5%). Chronic obstructive pulmonary disease (emphysema) (63%) was the most common indication for lung transplantation. Nineteen percent of patients (n = 24) developed dnDSA post-transplant with anti-HLA-DQ as the predominant dnDSA (n = 20, 83%), after a median detection time of 271 days (range 10–1847). A total of 30 patients (23%) developed CLAD (n = 24 bronchiolitis obliterans syndrome, n = 5 restrictive allograft syndrome, n = 1 mixed). Patient cohort characteristics and parameters are summarised in **Table 1**.

HLA Compatibility Scores

Recipients without detectable pre-transplant DSA received a transplant with a median cumulative number of HLA-A, -B, -DR antigen mismatches of 5 (range 3–6) and HLA-A, -B, -DR, -DQ, -DP allele mismatches of 13 (range 6–17). HLAMatchmaker scores ranged from 11 to 41 with a median of 24, HLA-EMMA scores ranged from 23 to 131 with a median of 75, and PIRCHE-II scores ranged from 32 to 189 with a median of 91. Fifty-four percent of patients (n = 65) presented a KIR ligand mismatch in the Host-versus-Graft direction, of which 18 with 2 mismatches (15%).

Given the dominance of anti-HLA DQ antibodies in the *de novo* occurrence of HLA antibodies, we then focused on mismatches in the HLA-DQB1 locus. HLAMatchmaker scores ranged from 0 to 9 with a median of 3, HLA-EMMA scores ranged from 0 to 32 with a median of 12, and PIRCHE-II scores ranged from 0 to 82 with a median of 27.

Association of HLA Compatibility Scores With Overall Survival, CLAD, and Graft Survival

Adjusted Cox proportional hazards models (adjusted for covariates sex, age, HLA sensitization and CMV status) regarding the outcomes of interest are summarized in **Table 2**.

For overall survival, only HLA-EMMA DQB1 score (HR, 2.49; 95% CI, 1.11–5.59; P, 0.0273), was significantly associated with worse survival. **Figure 1** shows the Kaplan-Meier analysis of HLA-EMMA DQB1 to overall survival using the median of 12 as cutoff. For CLAD, no association was seen between HLA

[1]http://www.epitopes.net
[2]https://hla-emma.com/
[3]https://www.pirche.com

TABLE 1 | Patient characteristics (n = 128).

Parameter	Median (range or percentage)
Age at time of transplant, y (range)	59 (18–66)
Female sex, n (%)	67 (52%)
DSA positivity prior to transplant (HLA sensitization), n (%)	7 (5%)
Time between transplantation and death/end of study, y (range)	4.9 (0.4–7.0)
Time between transplantation and CLAD (n = 30), y (range)	3.9 (0.3–5.9)
De novo DSA positivity, n (%)	24 (19%)
HLA class I, n (%)	3 (13%)
HLA class II, n (%)	20 (83%)
HLA class I + II, n (%)	1 (4%)
HLA-DQ, n (%)	20 (83%)
Subcohort without pre-transplant DSA (n = 121)	
HLA antigen mismatches (A-B-DR), median (range)	5 (3–6)
HLA allele mismatches (A-B-C-DR-DQ-DP), median (range)	13 (6–17)
B-cell epitopes	
HLAMatchmaker total score, median (range)	24 (11–41)
HLAMatchmaker DQB1 score, median (range)	3 (0–9)
HLA-EMMA total score, median (range)	75 (23–131)
HLA-EMMA DQB1 score, median (range)	12 (0–32)
T-cell epitopes	
PIRCHE-II total score, median (range)	91 (32–189)
PIRCHE-II DQB1 score, median (range)	27 (0–82)
Missing self/NK cell	
KIR ligand HvG mismatch	
1 MM, n (%)	65 (54%)
2 MM, n (%)	18 (15%)
Risk Epitope Mismatch (REM)	
DQA1*05/DQB1*03:01 (DQ7) MM, n (%)	31 (26%)
DQA1*05/DQB1*03 (DQ3)/DQB1*02 (DQ2) MM, n (%)	46 (38%)
DQA1*05/DQB1*03:01 (DQ7)/DQB1*02 (DQ2) MM, n (%)	47 (39%)

Legend: Data are presented as median and range or percentage, as appropriate. CLAD, chronic lung allograft dysfunction; DSA, donor-specific anti-HLA antibodies; HLA, Human Leukocyte Antigen; HvG, Host-versus-Graft; KIR, Killer-cell immunoglobulin-like receptors; MM, mismatch; PIRCHE-II, Predicted Indirectly ReCognizable HLA epitopes presented by HLA class II molecules; Y, years.

compatibility scores and freedom from CLAD. For graft survival, only KIR ligand HvG when 2 mismatches were present (HR, 2.13; 95% CI, 1.00–4.54): P, 0.0496) was significantly associated with CLAD or death.

Association of HLA Compatibility Scores With Time to *De Novo* DSA and Biopsy-Proven Acute Rejection

For the 120 patients in whom no DSA were detected pre-transplant, post-transplant anti-HLA antibody data were available (i.e. 1 patient had no post-transplant HLA data available). Of these, there were 24 patients (20%) in whom post-transplant DSA were detected. Three patients (13%) developed only HLA class I DSA, 1 patient (4%) developed only anti-HLA-DR DSA, and 20 patients (83%) developed anti-HLA-DQ DSA. Only 5 of the 20 patients (25%) with anti-HLA-DQ DSA developed CLAD by the end of the study and 1 patient (5%) deceased. However, we observed that these antibodies are mostly undetectable over time. Three of the 5 patients with HLA-DQ antibodies who developed CLAD (60%) had anti-HLA-DQ antibodies that were permanently detectable with an MFI value >7000 once in the follow-up period.

For time to dnDSA, HLA-EMMA DQB1 score and PIRCHE-II DQB1 score were associated with more rapid development of anti-HLA-DQ antibodies (HLA-EMMA DQB1 scores HR, 2.34; 95% CI, 1.13–4.84; P, 0.0215) (PIRCHE-II DQB1 scores HR, 2.17; 95% CI, 1.11–4.24; P, 0.0233). Regarding the specific HLA-DQ mismatches, we noticed a higher association with HLA-DQA1*05/DQ7 mismatch (HR, 2.31; 95% CI, 0.92–5.78; P, 0.0737) than with DQA1*05/DQ7/DQ2 (HR, 0.94; 95% CI 0.43–2.05; P, 0.8686) and DQA1*05/DQ3/DQ2 (HR, 0.90; 95% CI, 0.41–1.96; P, 0.7887) mismatches.

For time to first biopsy-proven rejection episode, only KIR ligand HvG when 2 mismatches were present (HR, 2.53; 95% CI, 1.05–6.08): P, 0.0383) was significantly associated with either cellular/ACR or antibody-mediated/AMR. Among which, 8 patients showed AMR (definite, n = 0; probable, n = 4; possible, n = 4), and 24 patients showed ACR (A0B1, n = 5; A0B2, n = 1; A0B3, n = 1; A1B0, n = 8; A1B1, n = 1; A1B2, n = 1; A1Bx, n = 1; A2B0, n = 3; A2Bx, n = 1; A3B1, n = 1; AxB2, n = 1).

DISCUSSION

In this single-center lung transplant cohort we demonstrated that HLA-EMMA DQB1 score was significantly associated with worse survival and more rapidly developing anti-HLA-DQ antibodies after lung transplantation. Also, the PIRCHE-II DQB1 score was significantly associated with time to *de novo* anti-HLA-DQ DSA. Although other results with B- and T-cell epitope mismatch scores were not significant, we observed higher hazard ratios regarding overall survival and time to *de novo* anti-HLA-DQ DSA when scores were calculated considering only the HLA-DQB1 locus. This is in line with the finding that 83% of included patients developing dnDSA presented with anti-HLA-DQ DSA.

A potential rationale why HLA-EMMA DQB1 score gave a significant result and not HLAMatchmaker DQB1, two different software tools for calculating the HLA B-cell epitope mismatch score, is that HLAMatchmaker postulates that eplets as defined by the HLA Eplet Registry[4] have immunogenic significance and are distinct from the 'structural epitope' which refers to the full footprint of the area recognized by an antibody [23, 24]. HLA-EMMA, on the contrary, does the calculation at the solvent accessible amino acid level, so potential bias of these eplets is excluded [16].

Previous research has demonstrated that not all molecular mismatches equally contribute to the generation of donor-specific immune responses and that immunogenicity is not merely a quantitative issue, but that one or only a few epitope mismatches are sufficient to induce an antibody response. We therefore also

[4]https://epregistry.com.br

TABLE 2 | HLA compatibility scores and outcomes of interest.

Outcome	Covariates/HLA compatibility score	HR	95% CI	p
Overall survival				
	Age	1.08	0.70–1.68	0.7164
	Sex	0.63	0.23–1.71	0.3647
	CMV	1.19	0.33–4.27	0.7986
	HLAMatchmaker total score	1.07	0.57–2.01	0.8281
	HLAMatchmaker DQB1 score	1.70	0.87–3.31	0.1196
	HLA-EMMA total score	1.29	0.67–2.48	0.4461
	HLA-EMMA DQB1 score	2.49	1.11–5.59	0.0273
	PIRCHE-II total score	0.95	0.45–2.01	0.8842
	PIRCHE-II DQB1 score	1.88	0.90–3.90	0.0920
	KIR ligand HvG mismatch			
	1 MM	2.02	0.69–5.91	0.1985
	2 MM	2.79	0.95–8.17	0.0616
	DSA anti-HLA-DQB1	1.90	0.60–6.00	0.2729
	DQA1*05/DQB1*03:01 (DQ7) MM	0.75	0.21–2.67	0.6521
	DQA1*05/DQB1*03:01 (DQ7)/DQB1*02 (DQ2) MM	0.61	0.19–0.93	0.4007
	DQA1*05/DQB1*03 (DQ3)/DQB1*02 (DQ2) MM	0.59	0.19–1.86	0.3673
CLAD				
	Age	1.28	0.87–1.87	0.2112
	Sex	0.74	0.35–1.56	0.4292
	CMV	1.20	0.48–2.98	0.6979
	HLAMatchmaker total score	1.00	0.61–1.65	0.9979
	HLAMatchmaker DQB1 score	0.74	0.43–1.28	0.2856
	HLA-EMMA total score	1.05	0.63–1.76	0.8571
	HLA-EMMA DQB1 score	0.77	0.41–1.45	0.4200
	PIRCHE-II total score	1.03	0.59–1.78	0.9199
	PIRCHE-II DQB1 score	0.97	0.54–1.73	0.9228
	KIR ligand HvG mismatch			
	1 MM	1.03	0.49–2.19	0.9323
	2 MM	2.16	0.91–5.10	0.0799
	DSA anti-HLA-DQ	1.21	0.46–3.21	0.7012
	DQA1*05/DQB1*03:01 (DQ7) MM	1.47	0.65–3.29	0.3527
	DQA1*05/DQB1*03:01 (DQ7)/DQB1*02 (DQ2)	0.94	0.43–2.05	0.8686
	DQA1*05/DQB1*03 (DQ3)/DQB1*02 (DQ2) MM	0.90	0.41–1.96	0.7887
Graft loss (CLAD or death)				
	Age	1.33	0.94–1.88	0.1021
	Sex	0.65	0.34–1.25	0.1991
	CMV	1.07	0.46–2.46	0.8777
	HLAMatchmaker total score	1.05	0.68–1.61	0.8389
	HLAMatchmaker DQB1 score	0.99	0.62–1.56	0.9499
	HLA-EMMA total score	1.18	0.75–1.84	0.4768
	HLA-EMMA DQB1 score	1.16	0.68–1.97	0.5915
	PIRCHE-II total score	0.98	0.61–1.59	0.9457
	PIRCHE-II DQB1 score	1.12	0.46–2.72	0.6284
	KIR ligand HvG mismatch			
	1 MM	1.18	0.61–2.26	0.6284
	2 MM	2.13	1.00–4.54	0.0496
	DSA anti-HLA-DQ			0.7975
	DQA1*05/DQB1*03:01 (DQ7) MM	1.36	0.66–2.78	0.4031
	DQA1*05/DQB1*03:01 (DQ7)/DQB1*02 (DQ2)	0.94	0.48–1.86	0.8601
	DQA1*05/DQB1*03 (DQ3)/DQB1*02 (DQ2) MM	0.90	0.46–1.78	0.7669
Time to first anti-HLA-DQ DSA				
	Age	0.91	0.64–1.31	0.6434
	Sex	1.11	0.45–2.69	0.8255
	CMV	1.03	0.34–3.10	0.9594
	HLAMatchmaker DQB1 score	1.44	0.77–2.67	0.2534
	HLA-EMMA DQB1 score	2.34	1.13–4.84	0.0215
	PIRCHE-II DQB1 score	2.17	1.11–4.24	0.0233
	KIR ligand HvG mismatch			
	1 MM	0.43	0.17–1.09	0.0767
	2 MM	0.00	$1.88*10^{-}20$–$2.47*10^{13}$	0.7078
	DSA anti-HLA-DQ	$4.37*10^{5}$	$2.46*10^{-}27$–$7.76*10^{37}$	0.7317
	DQA1*05/DQB1*03:01 (DQ7) MM	2.31	0.92–5.78	0.0737
	DQA1*05/DQB1*03:01 (DQ7)/DQB1*02 (DQ2)	1.38	0.56–3.40	0.4823

(Continued on following page)

TABLE 2 | (*Continued*) HLA compatibility scores and outcomes of interest.

Outcome	Covariates/HLA compatibility score	HR	95% CI	p
	DQA1*05/DQB1*03 (DQ3)/DQB1*02 (DQ2) MM	1.32	0.54–3.25	0.5436
Time to first biopsy-proven acute rejection				
	Age	0.90	0.67–1.19	0.4566
	Sex	1.23	0.57–2.67	0.5941
	CMV	1.05	0.39–2.79	0.9260
	HLAMatchmaker total score	1.45	0.88–2.39	0.1413
	HLAMatchmaker DQB1 score	0.88	0.50–1.53	0.6515
	HLA-EMMA total score	1.08	0.63–1.84	0.7835
	HLA-EMMA DQB1 score	0.84	0.44–1.58	0.5879
	PIRCHE-II total score	1.17	0.67–2.05	0.5570
	PIRCHE-II DQB1 score	0.90	0.49–1.64	0.7320
	KIR ligand HvG			
	1 MM	1.18	0.54–2.57	0.6717
	2 MM	2.53	1.05–6.08	0.0383
	DSA DQ	0.86	0.30–2.51	0.7839
	DQA1*05/DQB1*03:01 (DQ7) MM	1.34	0.58–3.09	0.4926
	DQA1*05/DQB1*03:01 (DQ7)/DQB1*02 (DQ2)	1.19	0.55–2.61	0.6561
	DQA1*05/DQB1*03 (DQ3)/DQB1*02 (DQ2) MM	1.35	0.62–2.93	0.4450

Legend: Adjusted Cox proportional hazards models (adjusted for covariates sex, age, HLA sensitization and CMV status) regarding the outcomes of interest. CI, confidence interval; CLAD, chronic lung allograft dysfunction; DSA, donor-specific anti-HLA antibodies; HLA, human leukocyte antigen; HR, hazard ratio; HvG, Host-versus-Graft; KIR, Killer-cell immunoglobulin-like receptors, MM, mismatch; PIRCHE-II, Predicted Indirectly ReCognizable HLA epitopes presented by HLA class II molecules.

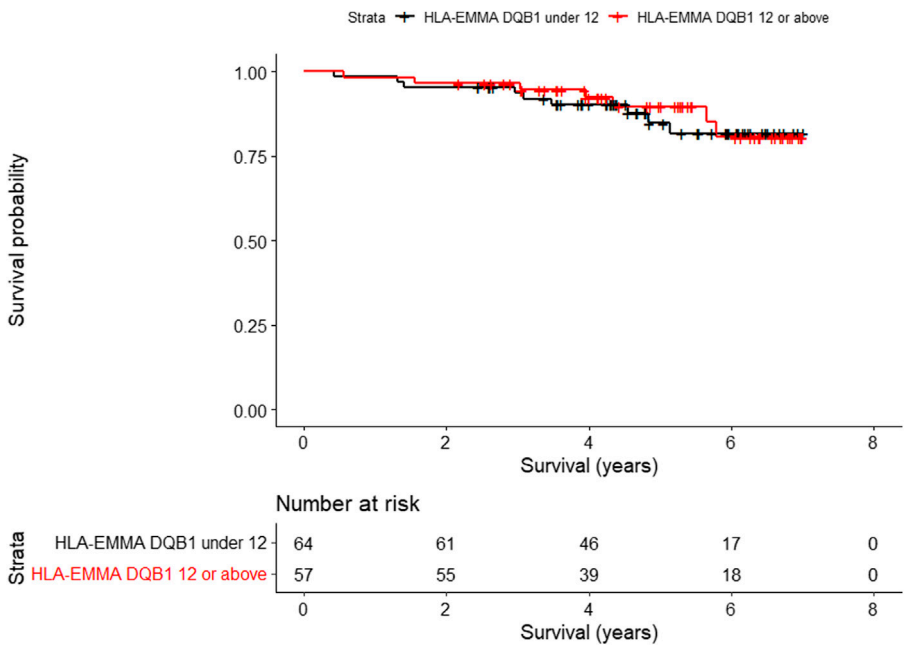


FIGURE 1 | Kaplan-Meier analysis of HLA-EMMA DQB1 to overall survival (p = 0.0273) using the median of 12 as cutoff.

looked specifically at the mismatches considered in the literature as so-called high-risk epitope mismatches (REMs) [20–22, 25]. For overall survival, CLAD, graft survival and time to biopsy-proven acute rejection, no significant associations with REMs were found. For time to *de novo* anti-DQ-HLA DSA, we observed a trend for an association with HLA-DQA1*05/DQ7 mismatch (HR, 2.3; 95% CI, 0.92–5.78; P, 0.0737), more than with DQA1*05/DQ7/DQ2 (HR, 0.94; 95% CI 0.43–2.05; P, 0.8686) and DQA1*05/DQ3/DQ2 (HR, 0.90; 95% CI, 0.41–1.96; P, 0.7887) mismatches.

Our results partly align with similar observations in the kidney/lung transplant literature, identifying HLA-DQ mismatches and HLA-DQ mismatch load as risk factors for dnDSA development and poor allograft outcome [20–22]. The study on lung transplant recipients from Hiho et al. [26] showed that a lower number of HLA class II mismatches (specifically

HLA-DR and -DQ) for all approaches (HLAMatchmaker, HLA-EMMA, PIRCHE-II) was associated with a reduced risk of restrictive allograft syndrome (restrictive phenotype of CLAD), DSA development, and improved overall survival. The lung transplant studies from Bedford et al. [27], Kleid et al [28]. and Lobashevsky et al. [29] showed an association between a higher epitope mismatch load and an increased risk of dnDSA development. These results were more pronounced with HLA class II [28] and HLA-DQ (HLA-DQA1*05 + HLA-DQB1*02/03:01) mismatches [27]. Further studies with larger cohorts are needed to further unravel the importance of these HLA-DQ compatibility scores and specific HLA-DQ mismatches.

A limitation of our study, which may affect the strength of our observations and may explain why some of the reported statistical differences are marginal, is the limited number of included patients (n = 128) which may hinder the analysis of subtle outcome differences (low event numbers for some endpoints) in multi-confounding endpoints like graft survival. Lack of inclusion of other competing risk factors (levels of immunosuppression, competing immune events such as infection, etc.), and HLA expression of HLA molecules on the donor lung influenced by the degree of inflammation and T-cell activation upon transplantation [30], may influence the observed transplant outcome and may hinder analysis of HLA compatibility. DSA may also not be detected because of phasic release and DSA adsorption/precipitation in the graft due to the 'sponge effect' related to the higher capillary surface in the lung [31, 32] or the DSA may be antibodies to self-antigens or non-HLA antigens, which can also lead to CLAD after lung transplantation [33–35].

Regarding missing self-induced rejection by NK cells (KIR ligand Host-versus-Graft mismatch), we saw only a significant association for graft survival (CLAD or death) and for time to first biopsy-proven rejection episode when 2 mismatches were present. We also observed a higher hazard ratio for overall survival (HR, 2.79; 95% CI, 0.95–8.17; P, 0.0616) and CLAD (HR, 2.16; 95% CI, 0.91–5.10; P, 0.0799) when 2 mismatches were present. In addition to the limitations described above, insufficient priming events and insufficient number of NK cells may affect our results. Recent experimental evidence has demonstrated that educated NK cells need to undergo priming such as ischaemia/reperfusion injuries and viral infections to acquire their full effector functions, in addition to individual heterogeneity of the NK cell population [4]. In contrast to previous research in kidney transplantation [4, 36], we did not perform any KIR gene sequencing and expression testing, which would be necessary for accurate determination of mismatch scores. The KIR ligand calculation we used was based on KIR ligands grouped into 3 major categories based on the KIR-binding epitope in HLA-C and HLA-B [17–19]. The impact of missing self-induced rejection by NK cells warrants further investigation.

In summary, despite the limitations related to its retrospective design, our study suggests that HLA-DQB1 compatibility scores and KIR ligand HvG 2 mismatches at the time of transplant may allow for identifying recipients at risk of poor long-term outcomes after lung transplantation. These data indicate that HLA-DQB1 compatibility scores and KIR ligand HvG two mismatches could become useful for risk stratification after lung transplantation, which could potentially translate into the recommendation of close surveillance and/or fine-tuning of immunosuppressive regimens in this immunologically high-risk population to improve survival, but further validation in independent cohorts is necessary.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by the Ethics Committee of the University Hospitals Leuven (BREATHE, KU Leuven) (S66760). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

RV, HB, BV, and LD participated in the design, interpretation of the studies and analysis of the data. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

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No Emergence of Colistin Resistance in the Respiratory Tract of Lung Transplant Patients Treated With Inhaled Colistin

*Nathalie Grall[1,2], Maksud Assadi[3], Marina Esposito-Farese[4], Brice Lortat-Jacob[3], Sébastien Tanaka[3,5], Enora Atchade[3], Jonathan Messika[6,7,8], Vincent Bunel[6], Hervé Mal[6,7], Pierre Mordant[7,8,9], Yves Castier[7,9], Bastien Garnier[3], Signara Gueye[1], Marie Petitjean[2], Erick Denamur[2], Laurence Armand-Lefevre[1,2], Charles Burdet[4], Philippe Montravers[3,7] and Alexy Tran-Dinh[3,10]**

[1]Université Paris Cité, AP-HP, Hôpital Bichat Claude Bernard, Service de Bactériologie, Paris, France, [2]INSERM UMR 1137 IAME, Université Paris Cité, Paris, France, [3]Université Paris Cité, AP-HP, Hôpital Bichat Claude Bernard, Département d'Anesthésie-Réanimation, Paris, France, [4]Université Paris Cité, AP-HP, Hôpital Bichat, Département d'Epidémiologie et Recherche Clinique, Paris, France, [5]Réunion Island University, INSERM U1188 Diabetes Atherothrombosis Réunion Indian OCean (DéTROI), CYROI Plateform, Saint-Denis de La Réunion, France, [6]Université Paris Cité, AP-HP, Hôpital Bichat Claude Bernard, Pneumologie B et Transplantation Pulmonaire, Paris, France, [7]INSERM UMR 1152 PHERE, Université Paris Cité, Paris, France, [8]Paris Transplant Group, Paris, France, [9]Université Paris Cité, AP-HP, Hôpital Bichat Claude Bernard, Service de Chirurgie Vasculaire, Thoracique et Transplantation Pulmonaire, Paris, France, [10]INSERM UMR 1148 LVTS, Université Paris Cité, Paris, France

Secondary prophylaxis using inhaled colistin (IC) was implemented to prevent recurrences of Pseudomonas aeruginosa or extended-spectrum β-lactamase-producing Enterobacterales (ESBL-PE) pneumonia during the postoperative intensive care unit (ICU) stay after lung transplantation (LT). We evaluated the risk of emergence of colistin resistance in the respiratory tract during secondary IC prophylaxis. We conducted a prospective, single-centre, observational study of all adult patients who underwent LT between 1 July 2018 and 30 June 2019. IC was started and continued for at least 90 days for *P. aeruginosa* or ESBL-PE pneumonia. During the 90 days following LT, all respiratory samples were routinely tested for the presence of GNB of reduced susceptibility to colistin. Twenty-seven (38.6%) of the 70 included patients received IC. Among the 867 respiratory samples tested, IC did not promote the emergence of bacterial species with natural or acquired resistance to colistin (incidence-rate ratio of 0.21 [0.03–1.58], $p = 0.13$ and 1.68 [0.55–5.12], $p = 0.37$, respectively). Our study suggests no association between the use of IC and an increased risk of colistin resistance in the respiratory tract within 90 days of LT.

Keywords: colistin, resistance, lung transplantation, prophylaxis, pneumonia

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***Correspondence**
Alexy Tran-Dinh,
alexy.trandinh@gmail.com

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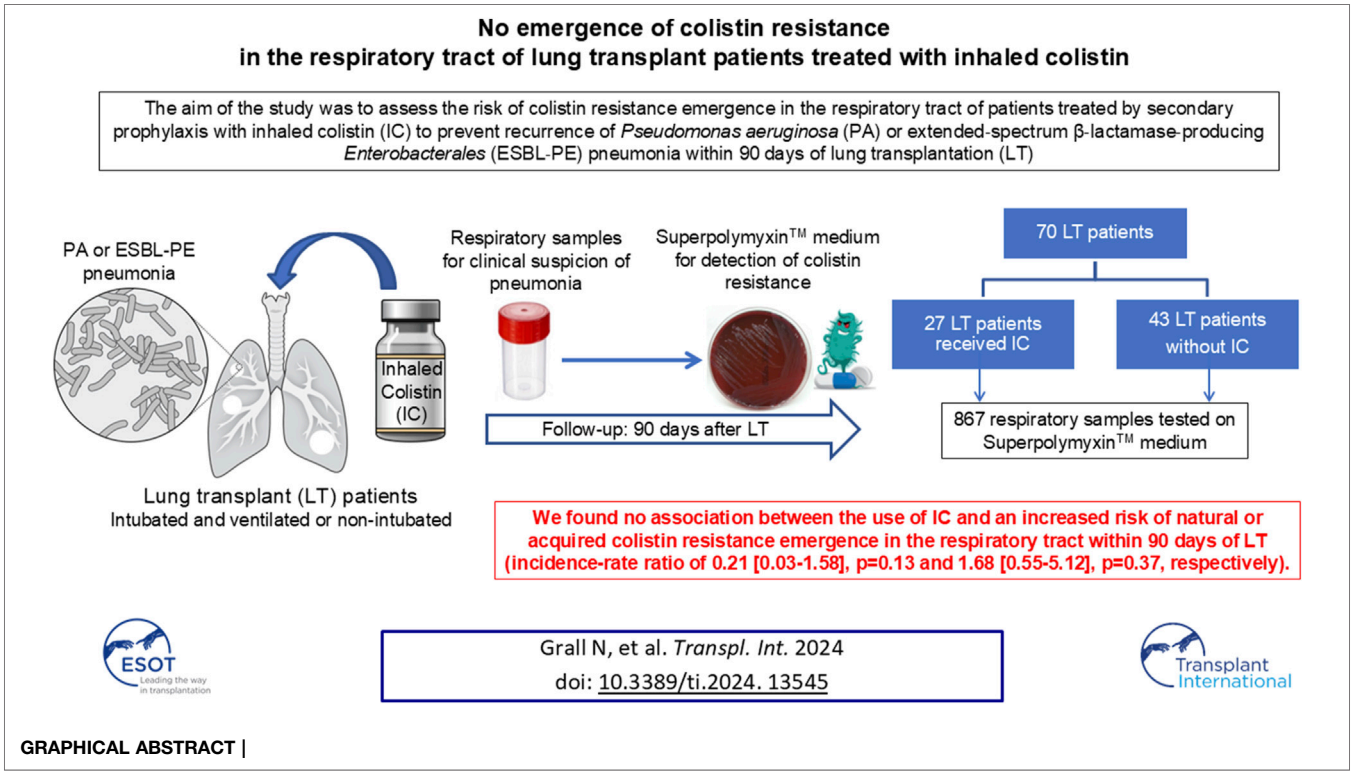
Abbreviations: BA, bronchial aspirate; BAL, bronchoalveolar lavage; BMI, body mass index; COPD, chronic obstructive pulmonary disease; ECMO, extracorporeal membrane oxygenation; ESBL-PE, extended-spectrum β-lactamase-producing *Enterobacterales*; GNB, Gram-negative bacilli; IC, inhaled colistin; ICU, intensive care unit; ILD, interstitial lung diseases; LT, lung transplantation; MIC, minimum inhibitory concentration; MIU, million international units; MPC, mutant prevention concentration; PTC, plugged telescoping catheter; ST, sequence type.



GRAPHICAL ABSTRACT |

INTRODUCTION

Lung transplantation (LT) is a last-resort therapy for patients with end-stage lung diseases. Nearly three-fourths of patients experience at least one episode of pneumonia within 1 year of LT, especially within the first month, and this complication is an independent risk factor for 1-year mortality [1]. Gram-negative bacilli (GNB), led by *Pseudomonas aeruginosa*, are the most common infectious agents causing pneumonia in lung transplant patients [1]. In addition, *P. aeruginosa* airway colonization increases the risk of chronic lung allograft dysfunction [2, 3]. Since January 2018, we implemented secondary prophylaxis using inhaled colistin (IC) at our institution to prevent recurrences of *P. aeruginosa* or extended-spectrum β-lactamase-producing *Enterobacterales* (ESBL-PE) pneumonia during the postoperative intensive care unit (ICU) stay after LT. In a before-and-after retrospective cohort analysis of 271 LT patients, including 125 recipients in the observation period before the use of secondary prophylaxis with IC, and 146 recipients in the intervention period with the use of secondary prophylaxis with IC, we showed that the use of IC as secondary prophylaxis decreased the proportion of patients who experienced at least one recurrence of *P. aeruginosa* or ESBL-PE pneumonia (7.2% during the observation period versus 0.7% during the intervention period, $p = 0.007$) [4]. Colistin belongs to the polymyxin family and has significant antibacterial activity against GNB by targeting and disrupting lipopolysaccharides in the outer cell membrane [5]. Because colistin is often used as a last line antibiotic in multidrug-resistant GNB infections [6], the risk of emergence of acquired resistance to colistin is of concern, especially since the identification of the first plasmid gene for colistin resistance, *mcr* [7]. The latest report from the French National Reference Center for Resistance to Antibiotics reported a 2%–4% prevalence of colistin-resistant *P. aeruginosa* strains in 2019 [8]. The present study evaluated the risk of emergence of colistin resistance in the respiratory tract during secondary IC prophylaxis introduced for *P. aeruginosa* or ESBL-PE pneumonia in the ICU after LT.

MATERIALS AND METHODS

Study Design

We performed a prospective, single-centre, observational study (Bichat-Claude Bernard Hospital, Paris, France). The studies involving humans were approved by Ethical authorizations were obtained from the National Ethics Committee for the Protection of Persons Nord-Ouest (N°034/2018). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. No animal studies are presented in this manuscript. No potentially identifiable images or data are presented in this study. The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

All adult patients who underwent LT between 1 July 2018 and 30 June 2019 were included.

The included patients were followed up for 90 days. Surgical transplantation procedures and perioperative care, including postoperative and immunosuppressive management, were

standardised for all patients according to our local protocol as previously described [9]. Cefazolin (or the antibiotic that was administered to the donor at harvest) was used as the standard antibiotic prophylaxis and was adapted to microbiological cultures obtained from bronchoalveolar lavage (BAL), which was systematically performed just after surgery. Antibiotic prophylaxis was stopped after 48 h in patients with negative cultures of postoperative BAL, as recommended [10]. IC was started [3 Million International Units (MIU) twice daily] in combination with intravenous antibiotic therapy in cases of *P. aeruginosa* or ESBL-PE pneumonia during the postoperative ICU stay, which were diagnosed from the recommendations for the standardisation of definitions of infections in cardiothoracic transplant recipients [11], as previously described elsewhere [4]. IC was used as secondary prophylaxis on the assumption that it could prevent recurrence of *P. aeruginosa* or ESBL-PE pneumonia, and intravenous antibiotic therapy was used to curatively treat the *P. aeruginosa* or ESBL-PE pneumonia episode according to the recommendations [12–14]. IC was continued for at least 90 days, regardless of whether the patient was still on mechanical ventilation. After 90 days, continuation of this treatment was left to the discretion of the physician in charge. The duration of intravenous antibiotic therapy was generally 7 days, but could be longer depending on the doctor's decision.

Data Collection

We recorded patient characteristics at baseline (age, sex, body mass index, and aetiology of pulmonary disease), type of LT (i.e., single or double LT), rate of pneumonia, duration of mechanical ventilation, length of ICU stay, tracheostomy, time from LT and initiation of IC, duration of IC treatment within 3 months of LT, IC-related side effects, exposure to antibiotics within 3 months, specific lung graft complications (acute cellular rejection confirmed by histopathological evidence after transbronchial lung biopsies performed only in cases of suspicion and not systematically [15]; definite, probable or possible antibody-mediated rejection according to Levine et al [16]); and airway complications that were severe bronchial stenosis requiring balloon dilation or insertion of endobronchial stent and bronchial anastomosis dehiscence [17], ICU and mortality rates at 28 days and 90 days.

Microbiological Analysis

During the 90 days following LT, all respiratory samples [plugged telescoping catheter (PTC), BAL, bronchial aspirate (BA), sputum] were only taken during usual care (i.e., when pneumonia was suspected) during the postoperative ICU stay, conventional pulmonology hospitalisation and day hospital and systematically plated on a selective medium (SuperPolymyxinTM, Ellitech, Puteaux, France). There was no systematic respiratory sampling protocol to detect tracheobronchial colonization. Samples were incubated at 37°C for 48 h, in addition to the standard cultures. This selective medium allows for the detection of GNBs with reduced susceptibility to polymyxins, including colistin, regardless of the mechanism or level of resistance. All distinct colonies were studied. Identification was performed using mass spectrometry (Maldi Biotyper®, Bruker Daltonics, Bremen, Germany). The susceptibility to antibiotics was determined using the disk diffusion method according to the recommendations of EUCAST.[1] ESBL production was confirmed using the double-disk synergy test [18]. We distinguished GNB with natural resistance to colistin (*Proteus* spp., *Providencia* spp., *Serratia* spp., *Morganella* spp., *Hafnia alvei*) from GNB with acquired resistance to colistin. The minimum inhibitory concentration (MIC) of colistin was determined using microdilution (Umic, Biocentric, Bandol, France) for all strains naturally susceptible to colistin. Resistance to colistin was defined as an MIC >2 mg/L (EUCAST).

Whole Genome Sequencing and Analysis

To determine the colistin resistance mechanism, whole genome sequencing (WGS) of *Enterobacterales* and *P. aeruginosa* isolates with acquired resistance to colistin was performed on each isolate of the same species with identical antibiotic susceptibility per patient. WGS was performed on a MiniSeq system (Illumina, San Diego, United States) with paired-end reads and read lengths of 150 bases. Libraries were prepared using the Nextera DNA Sample Preparation Kit from Illumina. Reads from Illumina sequencing were used for whole genome analyses. Read quality was assessed using FastQC v0.11.8. and Trim Galore v0.4.5 was used for quality and adapter trimming. Trim Galore was set up to trim basecalls with a Phred quality score inferior to 30, and reads less than 50 bases long were withdrawn. MetaPhlAn2 v2.6.0 [19] was used to verify the identifications of isolates and identify putative cross-contaminations. Reads were assembled using SPAdes v3.11.1 [20]. The quality of the assemblies was examined using QUAST v5.0.2 [21]. Gene annotation was performed using Prokka v1.13.3 [22].

The sequence type (ST) of the isolates was determined using CGE MLST software [23]. Diamond [24] was used to identify all of the antibiotic resistance genes by aligning all genomes against the AMRFinder database (version 2019-04-29). To obtain a reference genome, *Enterobacter cloacae* and *Klebsiella aerogenes* strains were downloaded from the Genome Taxonomy Database (GTDB) [25], and the closeness of the strain was tested using Mash [26]. For *Escherichia coli*, strains from the same phylogroup were downloaded from the EnteroBase database [27]. We used the closest strain to avoid SNPs linked to evolution. For *P. aeruginosa*, PAO1 genes were downloaded from NCBI. Using CD-HIT v4.7 [28], interesting genes (*pho*P, *pho*Q, *pmr*A, *pmr*B and *mgr*B/*yob*G for *Enterobacterales* and *pho*P, *pho*Q, *pmr*A, *pmr*B, *par*R, *par*S, *col*R, *col*S, *cpr*R and *cpr*S for *P. aeruginosa*) were searched in the genomes of our strains. Polymorphisms in these genes were determined using ClustalOmega v1.2.4 [29] for alignment against the reference genome downloaded from NCBI and a Python script for specific SNP detection. The impact of mutations detected was assessed using SIFT [30], PROVEAN [31] and Polyphen-3 [32]. We considered a deleterious effect for the

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TABLE 1 | Baseline characteristics of patients, early complications and mortality during the 3 months after lung transplantation.

	Overall (n = 70)	Patients with inhaled colistin (n = 27)	Patients without inhaled colistin (n = 43)	*p*-value
Age, years	59 [52: 63]	57 [50: 60.5]	60 [54: 64]	0.83
Female	25 (35.7)	12 (44.4)	13 (30.2)	0.30
LT aetiology				1.00
COPD/Emphysema	22 (31.4)	8 (29.6)	14 (32.6)	
Interstitial lung diseases	40 (57.1)	16 (59.3)	24 (55.8)	
Others	8 (11.4)	3 (11.1)	5 (11.6)	
Type of LT				0.61
Single LT	27 (38.6)	9 (33.3)	18 (41.9)	
Double LT	43 (61.4)	18 (66.7)	25 (58.1)	
BMI, kg/m^2	25 [22: 28]	26 [23: 29]	24 [21: 26]	0.036
Respiratory samples	11 [9: 16]	13 [11: 19]	11 [8: 14]	0.009
Tracheotomy	24 (34.3)	16 (59.3)	8 (18.6)	0.0007
Mechanical ventilation, days	3 [1: 25]	25 [4: 45]	2 [1: 5]	0.0004
Gram-negative pneumonia	51 (72.9)	27 (100)	24 (55.8)	0.00001
Gram-positive pneumonia	12 (17.1)	5 (18.5)	7 (16.3)	1
ICU length of stay, days	20 [12: 42]	41 [24: 68]	14 [11: 23]	0.0002
Time to IC initiation, days	NA	16 [10: 32]	NA	
Duration of IC exposure, days	NA	74 [60: 80]	NA	
Severe bronchial stenosis	17 (24.3)	8 (29.6)	9 (20.9)	0.57
Bronchial anastomosis dehiscence	6 (8.6)	2 (7.4)	4 (9.3)	1
Acute cellular rejection	19 (27.1)	11 (40.7)	8 (18.6)	0.06
Antibody-mediated rejection	38 (54.3)	17 (63)	21 (48.8)	0.33
ICU mortality	11 (15.7)	4 (14.8)	7 (16.3)	1
D28 mortality	7 (10)	1 (3.7)	4 (9.3)	0.64
D90 mortality	10 (14.3)	3 (11.1)	7 (16.3)	0.73

Categorical and continuous measures are represented as numbers (%) and medians, respectively [Q1: Q3]. LT, lung transplantation; COPD, chronic obstructive pulmonary disease; BMI, body mass index; ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit; NA, not applicable.

mutation if two of these software packages predicted a deleterious effect.

Statistical Methods

Data are presented as medians and interquartile ranges for continuous variables and as frequencies and percentages for categorical variables.

We compared the baseline characteristics of patients and outcomes according to their exposure to inhaled colistin using Fisher's exact or Wilcoxon tests, as appropriate.

The incidence rate of colistin-resistant GNB emergence in the respiratory tract in the 90 days following LT was estimated by pooling the GNB with acquired or natural resistance to colistin. To control for the immortal-time bias induced by direct comparison of exposed and unexposed patients to IC treatment, we applied the statistical methodology described and developed by Suissa [33, 34]. The immortal-time bias is the bias induced by the period before exposure to IC treatment in patients who will be exposed at a given time. During this unexposed period, a bias occurs because no events may be observed under exposure. According to this approach, comparisons are made between exposed and unexposed person-times, unlike subjects. Following the methods described in a previous study [33], we defined the observation period from LT to 90 days for surviving patients and death for the other patients. Exposure and non-exposure times to IC were identified for each patient, and the emergence of resistance to colistin, if applicable. Incidence rates under exposed and unexposed periods, their ratios and 95% confidence intervals (CI) were estimated using Poisson log-linear regressions.

All tests were 2-sieded, with a type-I error of 0.05. Analyses were performed using R software, version 4.0.5 (Copyright (C) 2021 The R Foundation for Statistical Computing).

RESULTS

Patient Demographics and Outcomes After Lung Transplantation

Seventy patients underwent LT during the study period and were included in the present study. No patient was lost to follow-up. The baseline characteristics of the patients are presented in **Table 1**. Patients were primarily transplanted for chronic obstructive pulmonary disease (COPD) (31.4%) and interstitial lung diseases (ILD) (57.1%), with a median age of 59 [52–63] years and a male/female ratio of 1.80. Three patients (two patients who did not receive IC and one patient who received IC) had been colonized by *P. aeruginosa* prior to lung transplantation. No patient had a history of IC treatment prior to lung transplantation.

Twenty-seven (38.6%) patients received IC (**Figure 1**). The median time between LT and the initiation of IC was 16 [10–32] days, with a median duration of exposure of 74 [60–80] days. Patients receiving IC experienced important morbidity during the postoperative course in the ICU with a longer duration of mechanical ventilation (25 [4–45] vs. 2 [1–5] days, $p = 0.0004$), more tracheostomies (59.3% vs. 18.6%, $p = 0.0007$), and longer ICU length of stay (41 [24–68] vs. 14 [11–23] days, $p = 0.0002$) than patients who did not receive IC. The rate of GNB

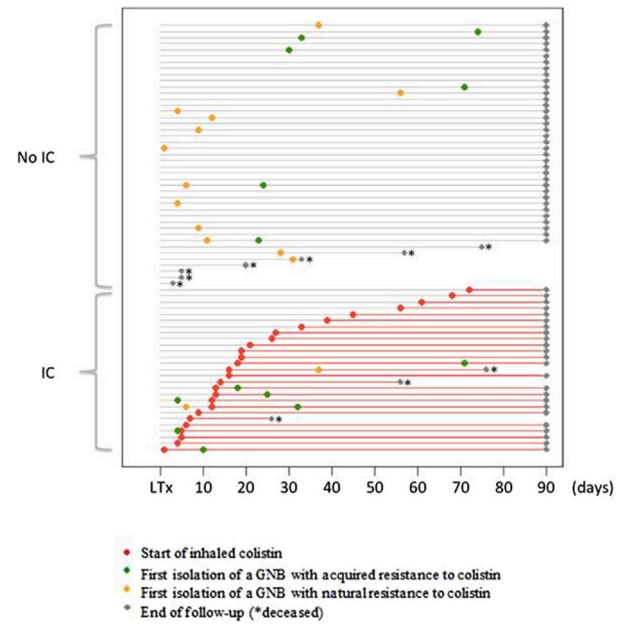

FIGURE 1 | Initiation of colistin treatment and dynamics of colonization by colistin-resistant GNB during the 90 days following lung transplantation. Each horizontal grey line represents the course of one patient, from lung transplantation (LT) to 3 months punctuated by a grey dot or before in case of death (*). The red lines represent the period of inhaled colistin (IC) treatment after initiation (red dot). The time of the first isolation of a Gram-negative bacilli (GNB) with acquired resistance to colistin is represented by a green dot. The time of the first isolation of a Gram-negative bacilli (GNB) with natural resistance to colistin is represented by a yellow dot. Grey dots indicate the time of the end of follow-up, and * indicates the time of death.

pneumonia was higher in patients who received IC than in those who did not (100% vs. 55.8%, $p = 0.00001$), whereas the rate of Gram-positive cocci pneumonia was similar (18.5% vs. 16.3%, $p = 1$). Patients who received IC had similar rates of acute cellular rejection (40.7% vs. 18.6%, $p = 0.06$), antibody-mediated rejection (63% vs. 48.8%, $p = 0.33$), severe bronchial stenosis (29.6% vs. 20.9%, $p = 0.57$), and bronchial anastomosis dehiscence (7.4% vs. 9.3%, $p = 1$) versus patients who did not receive IC. The mortality rates at Day 28 and Day 90 were not significantly different between the patients with and without IC (3.7% vs. 9.3%, $p = 0.64$ and 11.1% vs. 16.3%, $p = 0.73$) (**Table 1**).

No significant IC-related adverse events were observed, particularly no bronchospasm or acute kidney injury.

Phenotypic Analysis of the Respiratory Samples

A total of 867 respiratory samples were screened for the presence of GNB resistant to colistin, with 393 (45.3%) BAL, 38 (4.4%) PTC, 420 (48.4%) BA and 16 (1.9%) sputum samples.

The median number of samples collected per patient was 11 [9–16]. Patients receiving IC had more samples than patients without IC (13 [10.5–18.5] vs. 11 [8–14], $p = 0.009$).

Emergence of GNB With Natural Resistance to Colistin

A naturally colistin-resistant GNB was isolated in 54 (6.2%) samples (13 BAL, 39 BA and 2 sputum) from 14 (20%) patients. Among these 14 patients, 12 patients never received IC, one patient had his only positive sample before the introduction of IC, and one patient had his only positive sample during IC treatment (**Figure 1**). The species isolated were *Morganella morganii* (n = 18), *Serratia marcescens* (n = 17), *Proteus mirabilis* (n = 12) and *Hafnia alvei* (n = 7). Incidence rate ratio between exposed and unexposed patients to colistin was 0.21 [0.03–1.58] ($p = 0.13$) (**Table 2**).

Emergence of GNB With Acquired Resistance to Colistin

A GNB with acquired resistance to colistin was isolated in 28/867 (3.2%) samples (14 BAL, 11 BA and 3 sputum) from 13/70 (18.6%) patients. Among these 13 patients, 6 patients never received IC, 2 patients had their first positive sample before the introduction of IC, and 5 patients had their first positive sample during IC treatment. The time between IC introduction and the first isolation of a GNB with acquired colistin resistance ranged from 5 to 53 days. The species isolated were *E. cloacae* (n = 7), *E. coli* (n = 1), *K. aerogenes* (n = 1), *P. aeruginosa* (n = 13) and *Stenotrophomonas maltophilia* (n = 6).

Among the 13 patients with GNB with acquired colistin resistance, 7 patients (3 with IC and 4 without IC) had only one positive sample, and 4 patients (3 with IC and 1 without IC) had 2 positive samples. One patient who never received IC was colonized by an *E. cloacae* strain that was resistant to colistin, with 7 positive samples over a period of 53 days. The last patient had 6 positive samples during IC treatment. A colistin-resistant *K. aerogenes* strain was isolated in the first sample 12 days after IC introduction, and a colistin-resistant *P. aeruginosa* strain was isolated in the 5 other samples between 38 and 77 days after IC introduction.

IC did not promote the emergence of acquired colistin resistance in the respiratory samples, with an incidence-rate ratio of 1.68 [0.55–5.12] ($p = 0.37$) (**Table 2**).

Characteristics of Strains With Acquired Colistin Resistance

The MIC to colistin of the 28 GNB isolates with acquired resistance was between 4 and 16 mg/L.

WGS was performed on one isolate of the same species with identical antibiotic susceptibility per patient for *Enterobacterales* and *P. aeruginosa* strains. The results are described in **Supplementary Table S1**. The 7 isolates of *E. cloacae* isolated from the same patient were ESBL-producing and carried $bla_{\text{CTX-M-15}}$. No known *mcr* genes (*mcr*-1 to *mcr*-10) were detected. A missense mutation in the *pmr*A gene coding for a two-component system (PmrAB) associated with colistin resistance was identified in the *E. coli* and *K. aerogenes* strains. For both strains, the mutation was localized in G53, which is an amino acid hot spot in PmrA [35]. The mutation was G53C for *E. coli* and G53R for *K. aerogenes*. Both mutations

TABLE 2 | Incidence rates of acquired and natural colistin resistance among the 70 patients included.

Event	First colistin resistant GNB isolation during inhaled colistin treatment	Number of events	Person-days	Incidence rates	IRR [95% CI]	*p*-value
Acquired resistance	Yes	5	1,681	0.0030	1.68 [0.55–5.12]	0.37
	No	8	4,507	0.0020		
Natural resistance	Yes	1	1,681	0.0006	0.21 [0.03–1.58]	0.13
	No	13	4,507	0.0029		

GNB, Gram-negative bacilli; IRR, incidence-rate ratio; CI, confidence interval.

TABLE 3 | Exposure to antibiotics within 3 months in patients with or without inhaled colistin.

Antibiotic exposure (days)	Patients with inhaled colistin (n = 27)	Patients without inhaled colistin (n = 43)	*p*-value
Amoxicillin	119	45	<0.001
Amoxicillin-clavulanate	44	109	0.01
Cefazolin	148	59	<0.001
Ceftazidime	166	50	<0.001
Cefotaxime	35	43	0.25
Cefepim	132	128	<0.001
Piperacillin-tazobactam	3	35	<0.001
Ceftolozane-tazobactam	28	0	<0.001
Carbapenem	48	65	0.39
Levofloxacin/Ciprofloxacin	124	75	<0.001
Trimethoprim-sulfamethoxazole	59	28	<0.001
Aminoglycosides	12	2	<0.001
Linezolid	82	31	<0.001

were predicted to impact the protein and were described previously [35]. No mutation was found in the pmrB, phoP, phoQ or mgrB genes. No mutation was found in *pmr*A, pmrB, phoP, phoQ or mgrB genes in the *E. cloacae* strain. This strain belongs to cluster VIII, which is not known to have a heteroresistance phenotype to colistin [36]. Among the eight sequenced *P. aeruginosa* strains, a missense mutation was identified in the pmrB gene (P175S) in one strain and in the parS gene (V216A) in another strain. These mutations have not been described, but they were predicted to impact the protein, and both genes are associated with colistin resistance [37]. No mutation was found in the *pmr*A, phoP, phoQ, parR, colR, colS, cprR, or cprS genes. For the 6 remaining *P. aeruginosa* strains, no mutation was found in the 10 studied genes.

Exposure to Antibiotics Within 3 Months

Antibiotic exposure is represented as the total number of days over the 3-month follow-up period (**Table 3**). Overall, patients receiving inhaled colistin had greater exposure to antibiotics than patients without colistin, with 11/13 antibiotics more used in patients receiving IC.

DISCUSSION

Our study suggests that the use of IC as secondary prophylaxis to prevent recurrence of early *P. aeruginosa* or ESBL-PE pneumonia after LT did not promote the emergence of colistin-resistant GNB in respiratory samples via natural or acquired resistance. This report is the first prospective study to assess the risk of emergence of colistin resistance after LT. Our results are consistent with studies in non-transplanted patients, which did not observe an increased risk of colistin resistance acquisition [38–41].

Only one study evaluated the impact of IC on bronchial colonization with difficult-to-treat GNB in 70 cystic fibrosis transplant patients [42]. The authors showed that among the 15 patients who were not colonized by difficult-to-treat GNB in the immediate postoperative period, 3 of the 9 patients treated with IC prophylaxis did not develop colonization at 12 months. However, the 6 other patients who did not receive IC prophylaxis were all secondarily colonized with one or more difficult-to-treat GNB. IC did not eradicate this colonization in patients already colonized by difficult-to-treat GNB after LT. Acquired resistance to colistin was identified in only 2 of 33 patients colonized by *P. aeruginosa* and in 2 of 4 patients colonized by *Achromobacter* sp.

The lack of colistin resistance emergence when colistin is used by inhalation may be explained by the high concentrations of the antibiotic in the lung, which surpasses the MIC and the mutant prevention concentration (MPC) [43]. The MPC 90 for colistin is between 64 and 128 mg/L [44, 45]. Colistin concentrations obtained in BAL after IV administration are often below the limit of quantification, but they reach 150–180 mg/L in animals via inhalation [46, 47]. Boisson et al. showed that colistin concentrations in BAL were 100–1,000 times higher after inhalation than by IV in humans and ranged from 9.53 to 1,137 mg/L [48]. Yapa et al. also showed a higher concentration of colistin in the sputum of cystic fibrosis patients when colistin was administered via nebulization compared to the IV route [49].

We intended to determine the resistance mechanisms of colistin-resistant strains. Notably, none of the 11 sequenced strains with acquired resistance to colistin were *mcr*-positive. This result is consistent with the low prevalence of *mcr*-positive strains in France. Terveer et al. found that only two of 576 patients attending a tertiary care hospital (0.35%) were positive for *mcr*-1 in faecal samples [50]. We may have missed mcr-positive strains because of the use of a selective screening medium and the well-known existence of some colistin-susceptible mcr-positive strains [51]. We only found a mutation associated with colistin resistance in 4/11 sequenced strains (*E. coli*, K. aerogenes and 2/8 *P. aeruginosa*). Three of the 4 missense mutations found in our study concerned the two-component system PmrAB, which is largely responsible for colistin resistance via LPS modifications by the addition of cationic groups to the LPS membrane [37]. The last mutation found also concerned a two-component system, ParRS, which is also responsible for colistin resistance in *P. aeruginosa* [52]. The mechanisms of colistin resistance are primarily achieved by modification of lipid A of LPS and are not fully understood. The unexplained colistin resistance in our 7 strains may be related to mutations in other genes implicated in LPS biosynthesis [53–55] or the overexpression of efflux pumps. Some studies showed that efflux pumps contributed to colistin resistance in *E. cloacae* [56, 57], *Klebsiella pneumoniae* [58] or *Acinetobacter baumannii* [59]. To strengthen this hypothesis, Ni et al. showed that an efflux pump inhibitor, cyanide 3-chlorophenylhydrazone, suppressed and reversed colistin resistance in GNB [60].

Finally, patients receiving IC had higher morbidity during their ICU stay than patients not receiving IC. This is explained by the fact that patients were treated with IC as secondary prophylaxis after the onset of *P. aeruginosa* and ESBL-PE pneumonia in the ICU. In a retrospective before-and-after cohort analysis, we showed that patients with these pneumonias had higher morbidity in the ICU [4].

This study has several limitations. The main limitation is that it was a single-centre study with a limited number of patients. Our results primarily concerned transplant patients for COPD or ILD and cannot be generalised to patients with cystic fibrosis. The latter group are often treated with multiple lines of prolonged antibiotic therapy before LT, including colistin aerosols, for chronic colonization with *P. aeruginosa* or naturally colistin-resistant bacteria, such as *Burkholderia cepacia* [61]. The limited number of patients included in the study was compensated for by the prospective analysis of more than 800 respiratory samples. However, these samples were collected as part of the routine care for suspected pneumonia. There was no systematic respiratory sampling protocol to detect tracheobronchial colonization, thus we may have missed acquisition of resistance in asymptomatic patients. The 90-day follow-up time from LT to monitor the emergence of colistin resistance is also limited and may be evaluated more remotely in LT patients on long-term IC therapy.

CONCLUSION

Our study did not find an association between the use of IC as secondary prophylaxis to prevent recurrence of early *P. aeruginosa* or ESBL-PE pneumonia after LT and an increased risk of colistin resistance in the respiratory tract. However, the efficacy of secondary prophylaxis with IC should be evaluated in a specific study to confirm the value of its use policy.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by Ethical authorizations were obtained from the National Ethics Committee for the Protection of Persons Nord-Ouest (N°034/2018). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

NG: project design, data analysis, manuscript writing; MA: data collection, data analysis; ME-F: statistics; BL-J: data collection, data analysis; ST: data collection, data analysis; EA: data collection, data analysis; JM: data collection, data analysis; VB: data collection, data analysis; HM: data collection, data analysis; PiM: data collection, data analysis; YC: data collection, data analysis; BG: data collection, data analysis; SG: data collection, data analysis; MP: data collection, data analysis; DE: data analysis; LA-L: data collection, data analysis; CB: statistics; PhM: project design, data analysis, manuscript writing; AT-D: project design, data analysis, manuscript writing. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

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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.2024.13545/full#supplementary-material

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ORIGINAL RESEARCH
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Improved Results Over Time With Bridge-to-Lung Transplantation: A 10-Year Experience of a Single High-Volume Center

*Gyungah Kim, Jee Hwan Ahn, Tae Sun Shim, Pil-Je Kang, Geun Dong Lee, Sehoon Choi, Won Kim, Sung-Ho Jung, Dong Kwan Kim, Seung-Il Park and Sang-Bum Hong**

Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea

When donor scarcity limits timely lung transplantation (LTx), extracorporeal membrane oxygenation (ECMO) as a bridge to transplantation (BTT) can prolong survival and delay deconditioning until the donor lungs become available. We reviewed 10-year BTT experiences of a single high-volume center, where 99 (59%) were on ECMO BTT among 169 eligible adult LTx cases. Both 28-day and 2-year survivals did not differ between BTT and non-BTT. The BTT data was then divided into two periods, delineated by the most recent 3 years. The clinical outcomes of the earlier period ("Period 1") and the later period ("Period 2") were compared, and mortality within 28 days of LTx was significantly lower in Period 2 (n = 1, 1.7%) than in Period 1 (n = 6, 14.6%, $p < 0.01$). Improved survival was observed in the subgroup with BTT duration of 14 days or more. Taken together, more experiences in BTT and improved competence may contribute to better survival after LTx, especially in patients receiving ECMO for 14 days or more.

Keywords: lung transplantation, bridge to transplantation, extracorporeal membrane oxygenation, learning curve, ECMO duration

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***Correspondence**
Sang-Bum Hong,
sbhong@amc.seoul.kr

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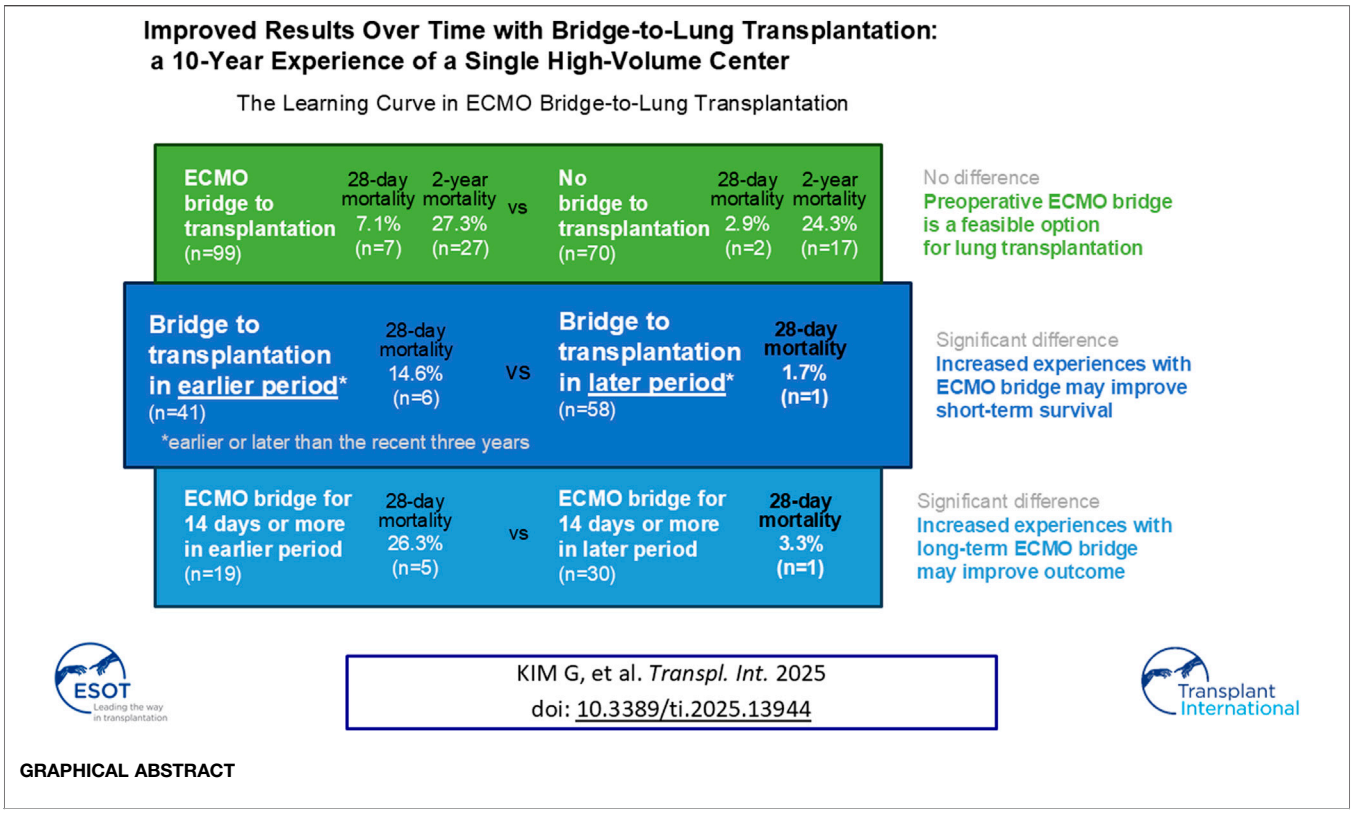
INTRODUCTION

Lung transplantation (LTx) is the treatment option for medically and surgically refractory lung conditions [1]. Despite various efforts and some improvement, waitlist mortality is a problem due to donor shortage [2, 3]. Bridge-to-transplantation (BTT) with extracorporeal membrane oxygenation (ECMO) is used preoperatively to maintain the best possible conditions for LTx by optimizing gas exchange and end-organ perfusion [4, 5]. Rehabilitation is implemented to overcome the deconditioning while on the waitlist [6], and ECMO in awake patients allows active rehabilitation which can improve patients' outcome [7]. Especially through the coronavirus disease 2019 pandemic, ECMO is increasingly used for severe respiratory failure [8]. However, BTT is frequently associated with complications, ranging from blood clotting-related embolism and serious ischemia of end-organs to bleeding complications and catheter site problems [9–12].

Abbreviations: BMI, Body mass index; BTT, Bridge-to-transplantation; CI, Confidence interval; CPB, Cardiopulmonary bypass; ECMO, Extracorporeal membrane oxygenation; V-V, Veno-venous; V-A, Veno-arterial; V-AV, Veno-arteriovenous; HR, Hazard ratio; ILD, Interstitial lung disease; MV, Mechanical ventilation; ICU, Intensive care unit; LTx, Lung transplantation; OxyRVAD, Right ventricular assist device with an oxygenator; PGD, Primary graft dysfunction; SAPS II, Simplified Acute Physiology Score II.



GRAPHICAL ABSTRACT

Clinical outcomes of BTT vary among centers, with 1-year survivals ranging from 29% to 93% [13]. One of the factors associated with the inter-center discrepancies is the annual number of BTT at the LTx center. A retrospective review of all LTx recipients in the United Network for Organ Sharing dataset from May 2005 to June 2011 revealed that survival of patients with high risk (high lung allocation scores, requiring mechanical ventilation (MV) or ECMO support) was better in high-volume centers compared to in low-volume centers [14]. Accumulated know-hows in BTT have been demonstrated to bring better clinical outcomes for the patients undergoing LTx [15, 16].

The learning curve of BTT in LTx with a large number of BTT cases has yet to be studied. We aimed to investigate whether BTT experiences over time in a high-volume center result in improved clinical outcomes. We hypothesized that accumulated experiences in BTT improve the survival of LTx patients. We further explored the factors associated with improved survival and the subgroup with the most improvement.

MATERIALS AND METHODS

Study Population

The data was retrieved retrospectively from patients over 19 years of age (legal age for adulthood in Korea) who received LTx at Asan Medical Center in Seoul, Republic of Korea during 2008 and 2021. Patients with liver-lung simultaneous transplantations were excluded. Patients were followed until death or December 2023. To estimate clinical severity, SAPS II was used in this study because it was validated in medical ICU patients and patients with respiratory failure on ECMO and employed in studies of BTT LTx patients [17–19].

LTx was achieved solely through strictly regulated process by the relevant legislation, and all organs used for transplantation were freely given with written informed consent by donors or family members through the government agency, the Korean Network for Organ Sharing. The study protocol was approved by the institutional review board of Asan Medical Center (approval number 2020–0209) and the requirement for informed consent was waived because of the retrospective nature of the study and the use of anonymized clinical data.

Study Design

LTx cases were divided into BTT and non-BTT cases and compared. BTT group was then divided into two period groups based on whether LTx was performed before ("Period 1") or within ("Period 2") the most recent 3 years (2019–2021) during the study period. The two periods were further categorized into subgroups according to the duration of BTT (short-term vs long-term), with the reference duration of 14 days based on previous studies [20, 21]. Clinical outcomes were compared.

Outcomes

The primary outcomes were 28-day and 2-year mortality, and the secondary outcomes were hospital and intensive care unit (ICU)

lengths of stay, primary graft dysfunction (PGD), postoperative MV duration and MV-free days, and requirement for postoperative tracheostomy.

Clinical Strategies

LTx Protocol

Patients with end-stage lung diseases except lung cancer were considered for LTx and selected according to the recommendations of the International Society for Heart and Lung Transplantation [22]. After the confirmation of the suitability for LTx by an institutional multidisciplinary committee comprised of pulmonologists, intensivists, cardiothoracic surgeons, infectious disease specialists, anesthesiologists, and radiologists, the candidate was listed through the Korean Network for Organ Sharing for donor lung allocation according to the urgency status, which gives the most urgency priority (status 0) only to the patients requiring MV or ECMO [23, 24]. The committee re-evaluate the condition of both donor and recipient at the time of donor lung availability to decide to proceed LTx, meticulously checking for the contraindications for LTx and the risk factors for poor post-transplant outcomes such as untreatable major organ dysfunction, uncorrectable bleeding diathesis, and limited functional status with poor rehabilitation potential [22]. Bilateral total lung transplantation rather than single or lobar lung transplantation and standard-criteria donor lungs rather than extended-criteria donor lungs were utilized as much as possible. Cardiopulmonary support during transplant surgery consisted of central veno-arterial (V-A) ECMO or cardiopulmonary bypass (CPB) with most patients weaned from the support at the end of LTx, although V-A or veno-venous (V-V) ECMO was applied postoperatively according to the recipient's conditions, which continued until recovery or death.

ECMO Protocol

ECMO as BTT was managed as recommended by the Extracorporeal Life Support Organization [25]. ECMO BTT was considered in LTx candidates with refractory hypoxemia, hypercarbia, or right heart failure despite optimal medical treatment. Patients on BTT were tracheostomized or extubated within a few days from the ECMO support to maximize mobilization, unless LTx was proceeded before tracheostomy or extubation. Patients with BTT were mobilized as soon as possible to preserve the skeletal muscle mass. BTT was not applied to patients who did not require MV or who were capable of rehabilitation without BTT despite the application of MV. Patients ineligible for LTx were not considered for BTT, and factors such as old age (older than 65 years of age), limitations in vascular access, uncontrolled sepsis, coagulopathy, and prolonged MV were also considered before starting ECMO.

Cannulation was performed and configuration was carefully selected based on individual patient conditions [5]. Intensive care physicians executed comprehensive evaluation of the cardiac function including right ventricular function by performing cardiac ultrasound, checking cardiac enzymes and brain natriuretic peptide, and assessing hemodynamic stability. Comprehensive echocardiography was performed by cardiologists if necessary. Intensivist routinely re-evaluate cardiac functions of bridged patients. The ECMO configuration was changed based on the clinical evaluation. V-V ECMO was primarily applied to patients with hypoxemic respiratory failure without hemodynamic instability, and V-A ECMO was applied to patients requiring hemodynamic support. Veno-arteriovenous (V-AV) ECMO was considered in differential hypoxia. Configuration changes to V-A, V-AV, or right ventricular assist device with an oxygenator (OxyRVAD) were considered in patients developing right ventricular dysfunction, as previously described [26]. To briefly describe OxyRVAD, the main pulmonary artery was approached by left anterior mini-thoracotomy and then a graft was anastomosed to the main pulmonary artery. Next, an arterial reinfusion cannula was connected to the graft, followed by the initiation of the RVAD. In this study, configuration changes only include changes during the preoperative BTT. Efforts were made to awaken all patients to participate in maximal rehabilitation while on BTT support, and central ECMO such as OxyRVAD was considered for further engagement in physical rehabilitation [27, 28].

The QUADROX PLS System (Maquet Cardiopulmonary AG, Rastatt, Germany) and the CAPIOX EBS System (Terumo Cardiovascular Systems Corporation, Tokyo, Japan) were used, each with its own oxygenator, pump, and console. Unfractionated heparin was predominantly used as intravenous anticoagulation during BTT, and argatroban was alternatively used for confirmed or suspected heparin-induced thrombocytopenia [29], with the dose titrated to achieve a target activated partial thromboplastin time of 40–60 s. A bolus of unfractionated heparin (50–70 units/kg) was infused at the start of ECMO support and usually 800 units/hour of unfractionated heparin was initiated, and then dosage was adjusted based on the activated partial thromboplastin time. Complications were monitored, and bleeding complications included major bleeding requiring surgical or radiological interventions as well as minor bleeding requiring close monitoring. Leg ischemia complication was defined as requiring decannulation, fasciotomy, amputation, or new distal perfusion cannulation.

Statistical Analysis

Categorical variables were presented as frequencies and proportions in percent and continuous variables were presented as medians and interquartile ranges. For intergroup comparison, the Student's *t* test or the Mann-Whitney U test were used for continuous variables, and the Chi-square test or the Fisher exact test were used for categorical variables. Survival curves were estimated using the Kaplan-Meier method and the log-rank test was performed for intergroup comparison. Univariate analysis was performed to identify risk factors for 28-day mortality, and a Cox proportional hazard regression model was used for multivariable analysis to assess the relationship between the independent variable and the post-transplantation mortality with $p < 0.10$ for inclusion of variables. The hazard ratios (HR) and 95% confidence intervals (CI) were presented. All statistical analyses were two-sided, and the level of significance was set to type I error rate of 0.05. Statistical analysis was performed using R version 3.4.4 (R Foundation for Statistical Computing, Vienna, Austria).

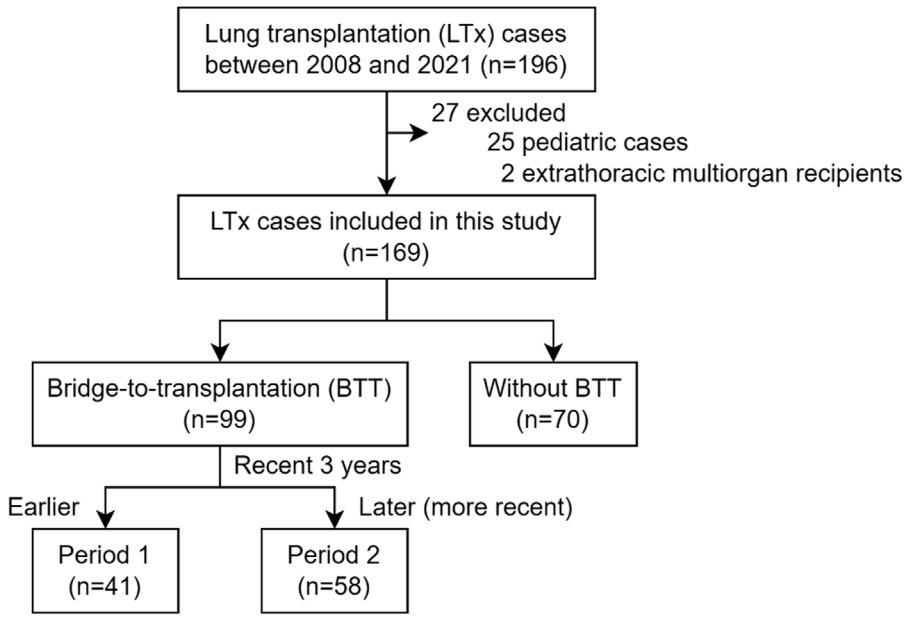


FIGURE 1 | Study population.

RESULTS

Study Population

From 2008 to 2021, 196 cases underwent LTx, and 169 cases were included in this study after excluding 25 pediatric cases and 2 cases receiving simultaneous liver transplantation (**Figure 1**). Based on the application of extracorporeal life support before LTx, 99 cases (58.6%) were bridged on ECMO (BTT group), and 70 cases (41.4%) were not on BTT (non-BTT group). The BTT group was further divided into earlier (Period 1, n = 41) and later (Period 2, n = 58) period groups, delineated by the most recent 3 years within the study period.

Basic Characteristics

The number of total LTx cases at our center increased over time since the beginning of LTx in 2008. While 77 cases received LTx during the first 10 years (2008–2018), 92 cases underwent LTx during the subsequent 3 years between 2019 and 2021 (**Supplementary Figure S1A**). The median age of the study population was 57 [44–63], and 63.3% (n = 107) were males. More than half (n = 112, 66.3%) were diagnosed with interstitial lung diseases (ILD) among whom idiopathic pulmonary fibrosis was the most common (n = 59), and 121 (71.6%) were Status 0 (i.e., on MV or ECMO) at the time of transplantation. There was no loss in follow-up during the 2-year post-transplant period for the investigated outcomes.

Our center began performing BTT in 2011. The number of BTT cases also rapidly expanded around the beginning of 2019, and cases per year exceeded 10 since then. The proportion of BTT has also increased over the years, and 75.0% were bridged on ECMO in 2021 (**Supplementary Figure S1B**). At the time of transplantation, 32 cases (32.3%) were on central ECMO, and 67 cases (67.7%) were on peripheral ECMO.

Comparison Between Earlier (Period 1) and Later (Period 2) Periods

The BTT group was further divided into earlier (Period 1, n = 41) and later (Period 2, n = 58) groups based on whether LTx was performed during the recent 3 years or earlier.

Epidemiologic and Clinical Characteristics

Compositions of the patient from two period groups were generally homogeneous, with unvaried sex, body mass index (BMI), diagnosis, preoperative hospital stay, BTT duration, and total hospital stay (**Table 1**). The median ages were 55 [42–62] years for Period 1 and 60 [54–64] years for Period 2 ($p = 0.04$). A substantial difference was noted in intraoperative circulatory support, as V-A ECMO was introduced and mostly replaced CPB (n = 3, 7.3% in Period 1 vs n = 57, 98.3% in Period 2, $p < 0.01$). SAPS II at the time of LTx was significantly higher in Period 1 [35 (31–45)] than in Period 2 [29 (26–35)] ($p < 0.01$).

Clinical Outcomes

Comparison of clinical outcomes between Period 1 and Period 2 among BTT group is shown in **Table 2**. Notably, 28-day mortality was significantly higher (HR = 0.11, 95% CI = 0.01–0.91, $p = 0.01$) in Period 1 (n = 6, 14.6%) compared to Period 2 (n = 1, 1.7%) (**Figure 2A**). No significant difference was observed in 2-year mortality (HR = 0.79, 95% CI = 0.37–1.7, $p = 0.55$) (**Figure 2B**), PGD, hospital or ICU lengths of stay, postoperative MV duration and MV-free days. Postoperative tracheostomy was required less frequently during Period 2 (n = 6, 10.3%) compared to Period 1 (n = 23, 60.5%, $p < 0.01$). Postoperative ECMO was required in 7.3% (n = 3) of Period 1% and 15.5% (n = 9) of Period 2 ($p = 0.36$).

TABLE 1 | Basic characteristics of BTT group in Period 1 and Period 2.

		Period 1 (n = 41)	Period 2 (n = 58)	*p*-value
Age		55 [42–62]	60 [54–64]	0.04
Sex	Female	15 (36.6%)	25 (43.1%)	0.66
	Male	26 (63.3%)	33 (56.9%)	
BMI		23.1 [20.3–25.3]	22.5 [19.8–25.3]	0.68
Diagnosis	ILD	30 (73.2%)	41 (70.7%)	0.66
	Bronchiolitis obliterans	2 (4.9%)	2 (3.4%)	
	Acute respiratory distress syndrome	6 (14.6%)	13 (22.4%)	
	Pulmonary hypertension	3 (7.3%)	2 (3.4%)	
Hospital days to LTx (days)		29 [14–41]	34 [17–48]	0.33
BTT duration (days)		13 [8–17]	15 [4–26]	0.57
Long term BTT	≥14 days	19 (46.3%)	30 (51.7%)	0.75
Configuration change		15 (36.6%)	29 (50.0%)	0.26
Configuration at LTx	V-V	29 (70.7%)	30 (51.7%)	<0.01
	V-A	10 (24.4%)	1 (1.7%)	
	V-AV	1 (2.4%)	2 (3.4%)	
	OxyRVAD	1 (2.4%)	25 (43.1%)	
Intraoperative support	CPB	38 (92.7%)	1 (1.4%)	<0.01
	V-A ECMO	3 (7.3%)	57 (98.3%)	
Preoperative rehabilitation	Rehabilitation	28 (75.6%)	44 (75.9%)	1.00
	Immobile	10 (24.4%)	14 (24.1%)	
Tracheostomy		10 (24.4%)	15 (25.9%)	0.26
Renal replacement therapy during BTT		5 (12.2%)	4 (6.9%)	0.58
ECMO complications	Total	15 (36.6%)	27 (46.6%)	0.43
	Pump clot	9 (22.0%)	6 (10.3%)	0.19
	Catheter site	7 (17.1%)	6 (10.3%)	0.50
	Bleeding	13 (31.7%)	26 (44.8%)	0.27
SAPS II		35 [31–45]	29 [26–35]	<0.01

TABLE 2 | Clinical outcomes of BTT group in Period 1 and Period 2.

	Period 1 (n = 41)	Period 2 (n = 58)	*p*-value
28-day mortality	6 (14.6%)	1 (1.7%)	0.04
2-year mortality	12 (29.3%)	15 (25.9%)	0.88
Hospital length of stay (days)	105 [58–146]	147 [81–197]	0.07
ICU length of stay (days)	40 [25–54]	50 [29–82]	0.10
PGD			0.30
Grade 1	6 (14.6%)	7 (12.1%)	
Grade 2	10 (24.4%)	7 (12.1%)	
Grade 3	6 (14.6%)	7 (12.1%)	
Postoperative MV duration (days)	11 [6–18]	13 [6–27]	0.37
Postoperative MV-free days (/30 days)	15 [0–24]	18 [3–24]	0.65
Postoperative tracheostomy	23 (60.5%)	6 (10.3%)	<0.01

Univariate analysis on 28-day mortality identified factors associated with surviving population as age at LTx, preoperative rehabilitation, ECMO site complications, intraoperative support, and SAPS II (**Supplementary Figure S2**). Multivariable analysis using logistic regression did not reveal any statistically significant factors associated with 28-day mortality (**Supplementary Figure S3**).

Subgroups Based on BTT Duration

The BTT group was further divided according to the duration of BTT (short-term vs long-term) and compared between Period 1 and Period 2 (**Figure 3**). The short-term BTT group (bridged for less than 14 days) showed similar 28-day survival rates between Period 1 (n = 21, 95.5%) and Period 2 (n = 28, 100.0%, $p = 0.26$) (**Figure 3A**). Long-term BTT group (bridged for 14 days or more) showed significantly improved 28-day survival in Period 2 (n = 29, 96.7%) compared to Period 1 (n = 14, 73.7%, $p = 0.01$) (**Figure 3B**). In the short-term BTT subgroup, 68.0% (n = 34) were able to participate in rehabilitation, compared to 83.7% (n = 41) in the long-term BTT subgroup ($p = 0.11$).

Configuration Change

Initial ECMO configuration was mostly V-V in both periods (n = 35, 85.4% in Period 1 vs n = 50, 86.2% in Period 2, $p = 0.21$). The frequency of configuration change was similar between Period 1 (n = 15, 36.6%) and Period 2 (n = 29, 50.0%, $p = 0.26$) (**Table 1**). The final configuration at the time of LTx was also mostly V-V (n = 29, 70.7% in Period 1 vs n = 30, 51.7% in Period 2), and OxyRVAD was more

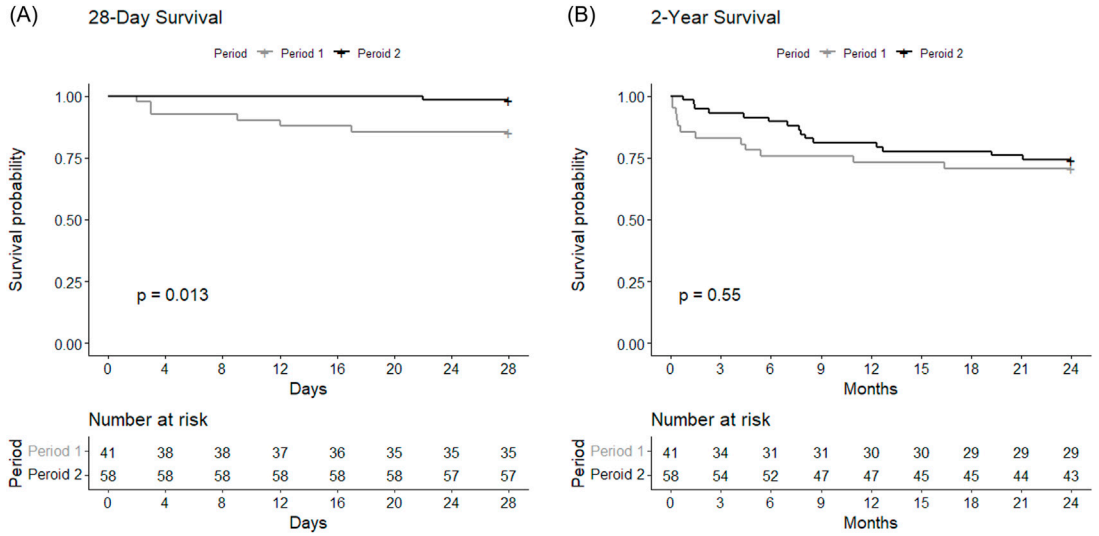


FIGURE 2 | Survival curves showing **(A)** 28-day and **(B)** 2-year postoperative survivals of Period 1 and Period 2 among BTT group. The curves were compared between the periods using log-rank test.

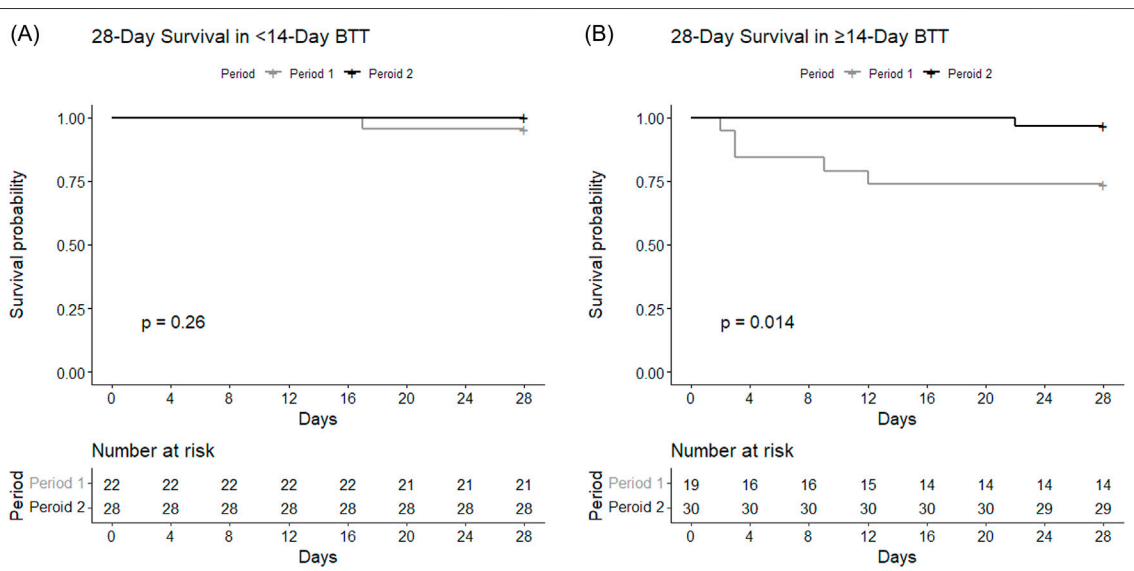


FIGURE 3 | Survival curves depicting 28-day survival according to the duration of BTT. Survival rates of subgroups within BTT group with BTT durations of ECMO for **(A)** less than 14 days and **(B)** 14 days or longer were each compared between Period 1 and Period 2.

frequently utilized during Period 2 (n = 25, 43.1%) compared to Period 1 (n = 1, 2.4%, $p < 0.01$). The 28-day mortality rates for V-V ECMO (n = 4, 6.8%), V-A and V-AV ECMO (n = 2, 14.3%), and OxyRVAD (n = 1, 3.8%) did not differ ($p = 0.47$).

Comparison Between BTT and Non-BTT

Compared with non-BTT group (n = 70), BTT group (n = 99) was associated with a higher BMI at operation [22.6 (19.9–25.3) vs. 21.0 (17.8–24.2) kg/m^2, $p = 0.01$], and a higher proportion of acute respiratory distress syndrome (n = 20, 20.2% vs. n = 5, 7.1%, $p < 0.01$) (**Supplementary Table S1**). BTT group included more Status 0 (n = 99, 100.0% vs. n = 22, 31.4%, $p < 0.01$) and more patients on MV (n = 99, 100.0% vs n = 22, 31.4%, $p < 0.01$). BTT group showed longer hospital days to transplantation [32 (16–43) vs. 0 (0–16) days, $p < 0.01$] and a higher SAPS II at LTx [33 (28–36) vs. 12 (10–22), $p < 0.01$] than non-BTT group.

Initial ECMO configuration was predominantly V-V (n = 85, 85.9%), followed by V-A (n = 9, 9.1%), V-AV (n = 2, 2.0%), and OxyRVAD (n = 2, 2.0%). The configuration was changed before LTx in 23 cases (23.2%), and the configuration at time of transplantation was mostly V-V (n = 59, 59.6%), followed by OxyRVAD (n = 26, 26.3%), V-A (n = 11, 11.1%), and V-AV (n = 3, 3.0%). No

statistical difference was found in clinical outcomes between those with and without configuration changes.

Post-transplantation mortality between BTT and non-BTT cases did not significantly differ at 28 days (n = 7, 7.1% vs. n = 2, 2.9%, p = 0.31) or 2 years (n = 27, 27.3% vs n = 17, 24.3%, p = 0.80) (**Supplementary Table S2**). PGD was also similar between the two groups. BTT group showed longer hospital and ICU lengths of stay and postoperative MV duration. Postoperative ECMO was applied in 12.1% (n = 12) of BTT and 5.7% (n = 4) of non-BTT groups (p = 0.26). Similar proportions required tracheostomy postoperatively between BTT and non-BTT groups. Survival analysis showed similar 28-day (HR = 2.57, 95% CI = 0.47–26.2, p = 0.31) and 2-year (HR = 1.17, 95% CI = 0.64–2.14, p = 0.62) mortality rates between the non-BTT and BTT groups. Re-transplantation did not differ between BTT and non-BTT groups, which occurred in one case from each group (1.0% vs. 1.4%, p = 1.00), and the reasons for re-transplantation were chronic lung allograft dysfunction for the BTT case and acute rejection for the non-BTT case. One case of leg ischemia occurred among those with peripheral arterial cannulas.

DISCUSSION

This high-volume single-center retrospective observational study showed BTT in later period was associated with better 28-day survival compared to earlier period. The improvement in 28-day survival was especially apparent in preoperative BTT for 14 days or longer. Also, 2-year mortality did not differ between BTT and non-BTT undergoing LTx, suggesting BTT is a feasible option for patients with end-stage lung diseases.

BTT outcomes are different among centers. For example, a systematic review showed 1-year survivals ranging from 29% to 93% [13]. Some studies previously showed compromised overall mortality for BTT [14, 30]. A report of 26 ECMO patients showed 27% survived until hospital discharge after LTx [31]. A recent study of 40,866 LTx patients showed worse 2-year survival for ECMO patients (53.8%) than for non-ECMO patients (61.8%) [32]. In this regard, preoperative ECMO was previously considered a contraindication to LTx due to unfavorable outcomes [33, 34]. Other studies, however, showed no difference in survival regarding BTT. A report of 71 patients with intention of BTT showed 89% survived through LTx, and 1-year, 3-year, and 5-year survival was 66%, 58%, and 48%, respectively [5]. Likewise, studies showed similar overall survival between BTT and non-BTT [16, 33]. Consistent with these latter studies, our study showed overall survival at 2 years of BTT cases similar to that of non-BTT cases. Notably, the 2-year survival for the BTT group in this study was 72.7% (n = 72, **Supplementary Table S2**), higher than most of aforementioned studies.

High-volume LTx centers may have better outcomes with BTT possibly due to protocolized institutional support that must be established over time through accumulation of clinical experiences. In one study, high-volume centers with more than 30 total LTx cases per year showed improved survival for BTT patients [14]. Another study of the United Network for Organ Sharing database investigated 342 BTT cases and showed better 1-year survival in high-volume centers, with "high-volume" defined as more than 15 BTT cases during the 15-year period [15]. In our center, the proportion of BTT (58.6%) and the number of BTT cases (99 cases in a 14-year duration) were much higher than those in high-volume centers of previous studies. The following factors may explain the increased use of BTT in our center (**Supplementary Figure S1**). First, the increased experience with BTT could lead to competence, which allowed our lung transplantation team to accommodate more BTT cases. Second, the globally increased experiences and advances in ECMO through the pandemics justified the choice of BTT [8, 35]. More literature reported benefits of ECMO including awake ECMO bridging in lung transplantation candidates [7, 36].

The donor lung allocation may explain the exceptionally high proportion of BTT in our center. Donor shortage is a problem especially in Korea because Korea only accepts donation after neurologic death and families are often unwilling to donate perhaps due to Korea's conservative culture. Moreover, donor lungs are vulnerable to damage, limiting their availability [37]. On average, there were 489 donors per year in Korea over the past 5 years, and only 159 out of 489 (32.5%) donor lungs were used for lung transplantation according to Korea Organ Donation Agency. The high proportion of BTT is also likely influenced by the urgency-based donor lung allocation system in Korea, which gives highest priority to Status 0 patients on invasive MV or ECMO [24]. Status 0 is responsible for 64% (n = 104) of annual lung transplantation (n = 162) in Korea according to the Center for Korean Network for Organ Sharing. This suggests that Korean patients with end-stage lung diseases on the waitlist often have to wait until they cannot go further without MV or ECMO before they are able to receive lung transplantation. Furthermore, our center is a tertiary referral center and patients with the most severe diseases are referred nationwide. These factors may have contributed to the high proportion of BTT in our center.

As more experiences lead to competence, we hypothesized that later period in a center's LTx history with acquired expertise would result in better clinical outcomes compared to earlier period. This was demonstrated in a recent study with the United Network for Organ Sharing database [32]. Learning curves in ECMO have been observed in previous studies, as centers experienced with more annual ECMO cases have better survival rates [38, 39]. Similarly, we found survival at 28 days was higher in Period 2 compared to Period 1 (**Figure 2A**), which might imply that the precedent 10-year period was a steppingstone for improvement. Experiences with around 40 BTT cases in our center may have equipped our team to maintain stable physiological states of the waitlist patients on ECMO, resulting in lower SAPS II at the time of LTx during Period 2 (**Table 1**).

We attempted to identify the factors associated with 28-day mortality following LTx. The univariate analysis identified age, the use of V-A ECMO or CPB for intraoperative support, ECMO site complications, operation of rehabilitation program, and SAPS II as factors associated with 28-day mortality (**Supplementary Figure S2**). Some of these factors have been identified as clinically important in previous reports [6, 25, 40]. However, multivariable analysis using a Cox proportional hazard regression model showed none of the factors associated with 28-day mortality. These discrepancies may be explained by the small number of mortality cases (n = 7), which was not sufficient to yield statistically significant results. Also, the factors identified in the univariate analysis may not be independent determinants of 28-day

mortality. Further studies may be required to determine the factors associated with 28-day mortality in BTT.

BTT cases were further divided into subgroups based on the duration of preoperative ECMO, and the earlier and later period groups showed differences in 28-day mortality only in the subgroup with BTT for 14 days or longer (**Figure 3**). Previous studies showed BTT for longer than 14 days was associated with poorer outcome [20, 21]. In this study, the improved 28-day mortality rate in the later period was mostly attributable to improvement in those with BTT for longer than 14 days. Increased duration of ECMO exposes patients to more complications which can make clinical management difficult [12]. Only the 28-day survival, not the 2-year survival, showed differences, which may suggest that increased BTT experiences particularly improves more immediate postoperative management, while long-term outcomes are affected by many different factors that are not entirely explained by the learning curve alone. Future studies should aim to improve long-term outcomes as well as short-term outcomes.

OxyRVAD was more frequently used in Period 2. We previously showed that OxyRVAD should be considered to support right heart dysfunction and to facilitate preoperative rehabilitation [26–28, 41]. In our cohort, the 28-day mortality with OxyRVAD (n = 1, 3.8%) was not higher compared to V-A or V-AV ECMO (n = 2, 14.3%), suggesting OxyRVAD is noninferior to other configurations. Our team recently showed proper configuration change from V-V ECMO in patients with increasing lactate levels and vasoactive inotropic scores may prevent clinical deterioration [26]. OxyRVAD is an option for patients on V-V ECMO developing right heart failure, stabilizing hemodynamics and enabling active rehabilitation to maintain the best fit for transplantation [41]. More investigations are needed to clarify the contributions of OxyRVAD during BTT.

This study examined changes in BTT outcomes over time in a large-volume single center with a high survival rate. The strength of our study is the inclusion of a large number of long-standing BTT cases compared to previous studies, as our cohort involved a large proportion of BTT (n = 99, 58.6%) and a long median duration of BTT (around 15 days). This study attempts to investigate factors and subgroups associated with improvement over time. There are limitations. Although the number of BTT cases was relatively large, only 7 cases died within 28 days past LTx, which makes further statistical analysis difficult to perform due to the small sample size. The retrospective nature of the study limits the interpretation of the results. This study shares experiences of a single center and the results cannot be generalized.

In conclusion, accumulation of experiences over time is associated with improved 28-day mortality in BTT for LTx, especially in BTT for 14 days or longer. BTT is a feasible option for LTx, with similar 28-day and 2-year survival rates compared to non-BTT.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by Institutional Review Board of Asan Medical Center (IRB number: 2020-0209). The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because of the observational nature of the study.

AUTHOR CONTRIBUTIONS

GK: Writing–original draft, Data curation, Formal analysis, Methodology, Writing–review and editing. JA: Resources, Investigation, Validation, Writing–review and editing. TS: Resources, Investigation, Validation, Writing–review and editing. P-JK: Resources, Investigation, Validation, Writing–review and editing. GL: Resources, Investigation, Validation, Writing–review and editing. SC: Resources, Investigation, Validation, Writing–review and editing. WK: Resources, Investigation, Validation, Writing–review and editing. S-HJ: Resources, Investigation, Validation, Writing–review and editing. DK: Resources, Investigation, Validation, Writing–review and editing. S-IP: Resources, Investigation, Validation, Writing–review and editing. S-BH: Conceptualization, Methodology, Resources, Investigation, Validation, Supervision, Writing–review and editing. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

GENERATIVE AI STATEMENT

The author(s) declare that no Generative AI was used in the creation of this manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2025.13944/full#supplementary-material

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Editorial Office

Avenue du Tribunal Fédéral 34
CH – 1005 Lausanne
Switzerland

Tel +41 (0)21 510 17 40
Fax +41 (0)21 510 17 01

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