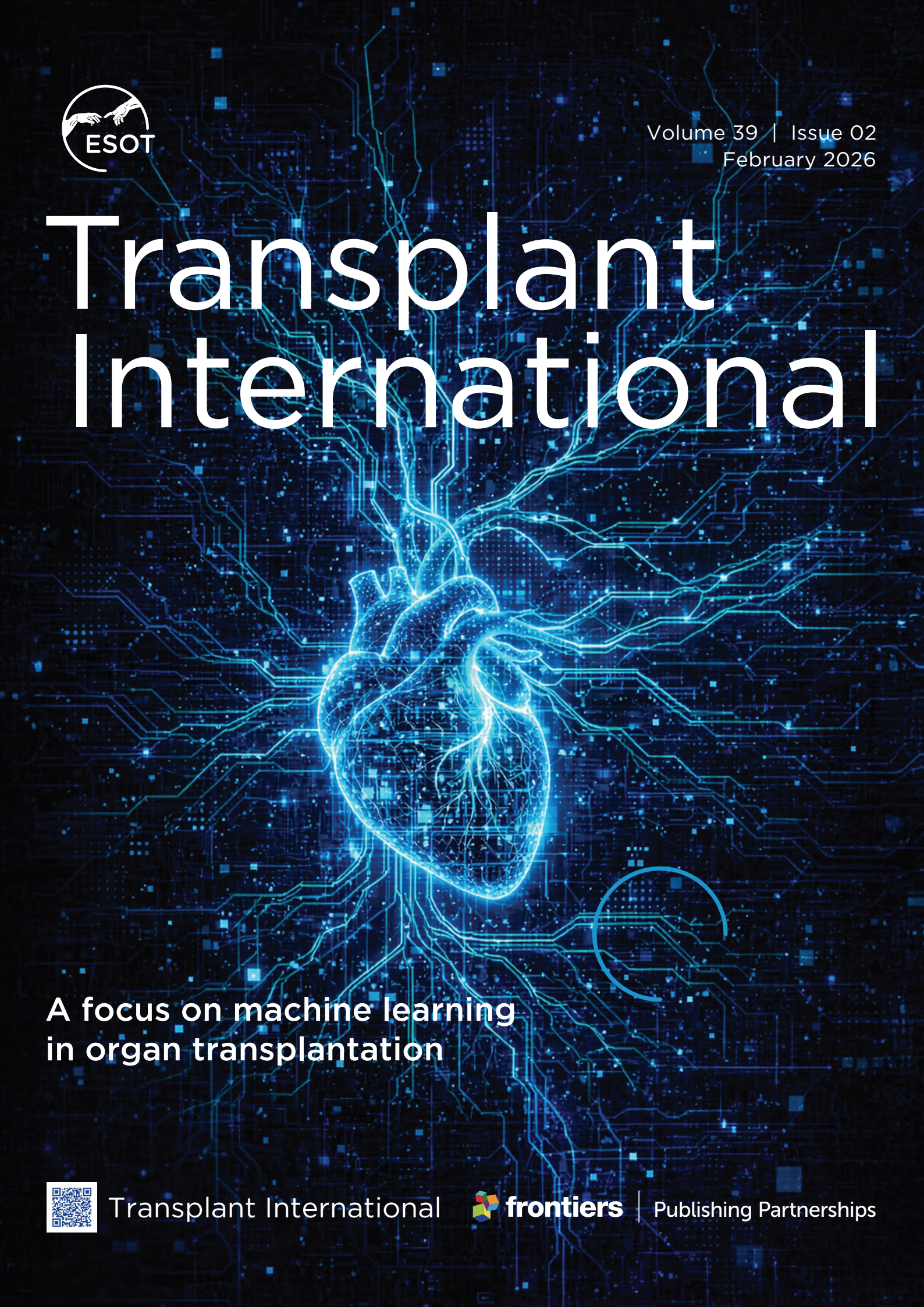
ESOT

Volume 39 | Issue 02
February 2026

Transplant International



A focus on machine learning in organ transplantation

Transplant International

frontiers | Publishing Partnerships

Transplant International

A focus on machine learning in organ transplantation

ISSN 1432-2277
ISBN 978-2-8325-7766-0
DOI 10.3389/978-2-8325-7766-0

Generative AI statement
Any alternative text (Alt text) provided alongside figures in the articles in this ebook has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Transplant International

Table of contents

Editorial

News and Views

Cover articles

Review

Original Research

Brief Research Reports

Letters to the Editor

ESOT®
Leading the way
in transplantation

Are You a Member?

Join us to help shape and nurture the future of ESOT!

Learn more about membership benefits, access, discounts, including reduced publishing fees in Transplant International

ESOT Membership

ESOT®
Leading the way
in transplantation

Transplant Science Summer School
11-13 June 2026
Krakow, Poland

7th Hepatologist in a Liver Transplant Program Course
29-30 October 2026
Padua, Italy

Advancing
METABOLIC DISORDERS
in Solid Organ Transplantation
1-3 December 2026
Barcelona, Spain

2026 CALENDAR OF EVENTS

Join us for a dynamic lineup of educational **hands-on workshops** and **in-person events**, dedicated to advancing transplantation through shared knowledge and collaboration.

For the full scope of events, visit:

www.esot.org/events

ESOT Congress

THE INTERNATIONAL TRANSPLANT CONGRESS®

PRAGUE | 26-29 SEPTEMBER 2027

”Mens et Machina”*

Human-centred transplantation in an AI world

#ESOTcongress

*Mind & Machine

ESOT | Transplant International

EDITORIAL
published: 11 February 2026
doi: 10.3389/ti.2026.16267

Transplant International: Looking Back at 2025, Looking Forward to 2026

Thierry Berney[1], Maria Irene Bellini[2], Oriol Bestard[2], Antonio Citro[3], Delphine Kervella[3], Nina Pilat[2], Stefan Schneeberger[2], Emilien Seizilles de Mazancourt[3], Arianna Trizzino[3] and Andrea Zajacova[3]*

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

Transplant International (TI) has had a busy year in 2025 and the start of a new year is an opportunity to thank our authors, reviewers and editors for the quality effort consented to make TI an ever improving and impactful journal. As an indicator of TI's growing attractivity, the impact factor has increased as have the numbers of manuscript submissions.

To our authors: Several articles published in TI in 2025, addressing various topics in the broad field of organ replacement, have been the object of considerable interest. Some papers have already been cited a few times despite the short time elapsed since publication. Congratulations to the authors who have submitted these papers and contributed to the high-quality standards of the journal (**Table 1**).

Measuring impact early after publication is not easy and requires other metrics than numbers of citations. Indeed, a rapid analysis on Web of Science shows that articles published in Transplant International reach their peak citation numbers the third year after publication. This is in concordance with what has been previously reported elsewhere [11, 12]. For this reason, capturing the impact early after publication requires considering other more immediate indices such as numbers of views and downloads. Expectedly, a look at the top 10 viewed or downloaded papers shows that the overlap is only partial and 9 additional articles can be identified among TI papers of highest interest (**Table 2**).

The topics that have generated the most interest are xenotransplantation and machine perfusion. Unsurprisingly, the same topics are found at the top of TI editors' selection of the most impactful papers published in 2025 in the field of clinical transplantation [22]. These 2 topics have been the focus of 2 special issues that just closed at the end of 2025 and titled "Current developments in artificial organs and engineered ex-situ perfused organs" and "Europeans and Xenotransplantation" [23, 24]. The collections are now complete and will be available as e-books in the coming months.

TI is extremely grateful to the reviewers who have spent their time and expertise to assess the papers submitted to our journal. We thank them for their voluntary participation, which is key to the scientific quality of the journal. The list of the reviewers who have contributed to TI in 2025 appears at the end of this editorial (**Appendix Table 3**).

The biennial ESOT Congress took place in London in 2025, from June 29 to July 2, and Transplant International participated actively to the congress activities. Sessions designed and organized by the editorial board and editorial fellows discussed 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, discussed in an interactive format several aspects of scientific publication that are not always obvious for young investigators, such as the good use of biostatistics, artificial intelligence or social media. Importantly, a collection of review and original articles based on invited lectures and best communications presented at the congress is getting ready to be completed in the first quarter of 2026 [25]. Most contributions are already published online. Highlights include reviews on immune monitoring by donor-derived cell-free DNA in heart/lung transplantation torque-

Editorial

OPEN ACCESS

***Correspondence**
Thierry Berney,
eic.ti@frontierspartnerships.org

Received: 19 January 2026
Revised: 19 January 2026
Accepted: 20 January 2026
Published: 11 February 2026

Citation:
Berney T, Bellini MI, Bestard O, Citro A, Kervella D, Pilat N, Schneeberger S, Seizilles de Mazancourt E, Trizzino A and Zajacova A (2026) Transplant International: Looking Back at 2025, Looking Forward to 2026. Transpl. Int. 39:16267. doi: 10.3389/ti.2026.16267

TABLE 1 | Top 10 2025 most cited TI artices (Dimensions scores).

The last mile in beta-cell replacement therapy for type 1 diabetes: Time to grow up	Piemonti [1]	8 citations
Donors with previous malignancy: When is it safe to proceed with organ transplantation?	Turra et al [2]	6 citations
The evolution of immunosuppressive therapy in pig-to-nonhuman primate organ transplantation	Sanatkar et al. [3]	6 citations
Ethical issues in uncontrolled donation after circulatory determination of death: A scoping review to reveal areas of broad consensus, and those for future research	Fritz et al. [4]	6 citations
Mono-HOPE versus Dual-HOPE in liver transplantation: A propensity score-matched evaluation of early graft outcome	Koch et al [5]	6 citations
Desensitization with imlifidase for HLA-incompatible deceased donor kidney transplantation: A delphi international expert consensus	Furian et al. [6]	5 citations
Heart transplantation and donation after circulatory death in children. A review of the technological, logistical and ethical framework	Kenny et al. [7]	5 citations
Targeting CD38 in antibody-mediated rejection	Mayer et al. [8]	4 citations
Progress in porcine kidney transplantation to non-human primates	Le bas-bernardet et al. [9]	4 citations
Multi-center outcome analysis of 16 face transplantations – a retrospective OPTN study	Knoedler et al. [10]	4 citations

TABLE 2 | Other TI articles with high views or downloads numbers (measured 8.1.26).

Liver transplantation in the context of acute-on-chronic liver failure (ACLF): Where do we stand in 2025?	L'Hermite et al [13]	8540 views 1530 downloads
Updates on donor-derived infection in solid organ transplantation, report from the 2024 GTI (infection and transplantation group) annual meeting	Eldin et al [14]	6122 views 872 downloads
The progress and challenges of implementing HLA molecular matching in clinical practice	Bezstarosti et al [15]	6100 views 684 downloads
Current techniques of gene editing in pigs for xenotransplantation	Galli [16]	5718 views 1456 downloads
State of art of dose individualization to support tacrolimus drug monitoring: What's next?	Lloberas et al [17]	5699 views 1310 downloads
Ex-Vivo heart perfusion machines in DCD heart transplantation model: The state of art	Tessari et al [18]	5639 views 398 downloads
Nurse-led self-management support after organ transplantation – a multicenter, stepped-wedge randomized controlled trial	Van zanten et al [19]	5400 views 1579 downloads
Development of non-HLA antibodies and their association with antibody-mediated rejection in pediatric kidney transplant recipients	Schmidt et al [20]	5369 views 704 downloads
Evaluating risk in kidney living donors	Ortiz et al [21]	4797 views 1284 downloads

tenovirus in kidney transplantation [26, 27] and on the evolution of our understanding of vascular lesions in the kidney graft biopsies [28].

The Transplant International editorial fellowship program is an ongoing success! In addition to their mentoring in all aspects of scientific editing and publishing, editorial fellows are also engaged in communication projects for the journal and have been instrumental in creating new products, such as video abstracts of TI papers and launching a TI podcast.

2025 has been a busy and productive year, but more will come the way of TI readership in 2026. From the beginning of our tenure, it has been our stated aim to strengthen the relationship with ESOT leadership and membership. We endeavor to make TI a journal close to ESOT's style and philosophy and integrate contents resulting from ESOT's educational and scientific activities, in the strict respect of editorial independence. Transplant International is the journal of ESOT and of the European transplant community of physicians, surgeons, scientists and allied healthcare professionals. We believe that our efforts will help increase the standing of the journal and its identification as the journal of this dynamic community!

FUNDING

The author(s) declared that financial support was not received for this work and/or its publication.

GENERATIVE AI STATEMENT

The author(s) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

REFERENCES

1. Piemonti L. The Last Mile in Beta-Cell Replacement Therapy for Type 1 Diabetes: Time to Grow up. *Transpl Int* (2025) 38:14565. doi:10.3389/ti.2025.14565
2. Turra V, Manzi J, Rombach S, Zaragoza S, Ferreira R, Guerra G, et al. Donors with Previous Malignancy: When Is It Safe to Proceed With Organ Transplantation? *Transpl Int* (2025) 38:13716. doi:10.3389/ti.2025.13716
3. Sanatkar SA, Kinoshita K, Maenaka A, Hara H, Cooper DKC. The Evolution of Immunosuppressive Therapy in Pig-to-Nonhuman Primate Organ Transplantation. *Transpl Int* (2025) 37:13942. doi:10.3389/ti.2024.13942
4. Georgiou A, Tan W, Ionescu MI, Kuhn IL, Fritz Z. Ethical Issues in Uncontrolled Donation After Circulatory Determination of Death: A Scoping Review to Reveal Areas of Broad Consensus, and Those for Future Research. *Transpl Int* (2025) 38:13992. doi:10.3389/ti.2025.13992
5. Koch DT, Tamai M, Schirren M, Drefs M, Jacobi S, Lange CM, et al. Mono-HOPE Versus Dual-HOPE in Liver Transplantation: A Propensity Score-Matched Evaluation of Early Graft Outcome. *Transpl Int* (2025) 38:13891. doi:10.3389/ti.2025.13891
6. Furian L, Heemann U, Bengtsson M, Bestard O, Binet I, Böhmig GA, et al. Desensitization With Imlifidase for HLA-Incompatible Deceased Donor Kidney Transplantation: A Delphi International Expert Consensus. *Transpl Int* (2025) 37:13886. doi:10.3389/ti.2024.13886
7. Kenny LA, Armstrong L, Berman M, Brierley J, Crossland D, Dark J, et al. Heart Transplantation and Donation After Circulatory Death in Children. A Review of the Technological, Logistical and Ethical Framework. *Transpl Int* (2025) 38:13801. doi:10.3389/ti.2025.13801
8. Mayer KA, Budde K, Diebold M, Halloran PF, Böhmig GA. Targeting CD38 in Antibody-Mediated Rejection. *Transpl Int* (2025) 38:14343. doi:10.3389/ti.2025.14343
9. Le Bas-Bernardet S, Blancho G. Progress in Porcine Kidney Transplantation to Non-Human Primates. *Transpl Int* (2025) 38:14003. doi:10.3389/ti.2025.14003
10. Knoedler L, Schaschinger T, Niederegger T, Hundeshagen G, Panayi AC, Cetrulo CL, Jr, et al. Multi-Center Outcome Analysis of 16 Face Transplantations - a Retrospective OPTN Study. *Transpl Int* (2025) 38:14107. doi:10.3389/ti.2025.14107
11. Finardi U. On the Time Evolution of Received Citations, in Different Scientific Fields: An Empirical Study. *J Infometrics* (2014) 8(1):13–24. doi:10.1016/j.joi.2013.10.003
12. Asknes DW. Characteristics of Highly Cited Papers. *Res Eval* (2003) 12(3):159–70. doi:10.3152/147154403781776645
13. L'Hermite S, Coirier V, Artru F. Liver Transplantation in the Context of Acute-on-Chronic Liver Failure (ACLF): Where Do We Stand in 2025? *Transpl Int* (2025) 38:14752. doi:10.3389/ti.2025.14752
14. Eldin C, Grossi PA, Manda V, Kamar N, Lortholary O, Hirsch HH, et al. Updates on Donor-Derived Infection in Solid Organ Transplantation, Report From the 2024 GTI (Infection and Transplantation Group) Annual Meeting. *Transpl Int* (2025) 38:14237. doi:10.3389/ti.2025.14237
15. Bezstarosti S, Heidt S. The Progress and Challenges of Implementing HLA Molecular Matching in Clinical Practice. *Transpl Int* (2025) 38:14716. doi:10.3389/ti.2025.14716
16. Galli C. Current Techniques of Gene Editing in Pigs for Xenotransplantation. *Transpl Int* (2025) 38:13807. doi:10.3389/ti.2025.13807
17. Lloberas N, Fernández-Alarcón B, Vidal-Alabró A, Colom H. State of Art of Dose Individualization to Support Tacrolimus Drug Monitoring: What's Next? *Transpl Int* (2025) 38:14201. doi:10.3389/ti.2025.14201
18. Tessari C, Lucertini G, Addonizio M, Geatti V, Bacich D, Pradegan N, et al. Ex-Vivo Heart Perfusion Machines in DCD Heart Transplantation Model: The State of Art. *Transpl Int* (2025) 38:12987. doi:10.3389/ti.2025.12987
19. van Zanten R, van Dijk M, van Rosmalen J, Beck DK, van Staa A, Van Hecke A, et al. Nurse-Led Self-Management Support After Organ Transplantation - A Multicenter, Stepped-Wedge Randomized Controlled Trial. *Transpl Int* (2025) 37:13175. doi:10.3389/ti.2024.13175
20. Schmidt F, Verboom M, Hallensleben M, Braumann A, Drube J, Brunkhorst L, et al. Development of Non-HLA Antibodies and Their Association With Antibody-Mediated Rejection in Pediatric Kidney Transplant Recipients. *Transpl Int* (2025) 38:14463. doi:10.3389/ti.2025.14463
21. Ortiz F, Marson L, Thomas R, Kousios A, Rista E, Lefaucheur C, et al. Evaluating Risk in Kidney Living Donors. *Transpl Int* (2025) 38:14024. doi:10.3389/ti.2025.14024
22. Berney T, Becchetti C, Bellini MI, Benning L, Berishvili E, Bestard O, et al. The Top 12 Most Impactful Papers in Clinical Transplantation in 2025: TI Editors' Choice. *Transpl Int* (2026) 39:16247. doi:10.3389/ti.2026.16247
23. Current Developments in Artificial Organs and Engineered Ex-Situ Perfused Organs (2026). Available online at: https://www.frontierspartnerships.org/research-topics/74/current-developments-in-artificial-organs-and-engineered-ex-situ-perfused-organs/articles. (Accessed January 16, 2026).
24. Europeans and Xenotransplantation (2026). Available online at: https://www.frontierspartnerships.org/research-topics/98/europeans-and-xenotransplantation/articles. (Accessed January 16, 2026).
25. Top Papers From the ESOT Congress 2025 (2026). Available online at: https://www.frontierspartnerships.org/research-topics/177/top-papers-from-the-esot-congress-2025/articles. (Accessed January 16, 2026).
26. Agbor-Enoh S, Fraser E, Nadella N, Andargie TE, Alnababteh M. dd-cfDNA: The New Frontier for Heart/Lung Transplant Surveillance? *Transpl Int* (2025) 38:15555. doi:10.3389/ti.2025.15555
27. Doberer K, Kapps S, Haupenthal F, Bond G. Immune Monitoring Goes Viral - Torque Teno Virus for Immunologic Risk Stratification After Kidney Transplantation. *Transpl Int* (2025) 38:15074. doi:10.3389/ti.2025.15074
28. Wellekens K, Koshy P, Roufosse C, Naesens M. From Banff 1991 to Today: The Changing Landscape of the v-Lesion in Kidney Transplant Rejection. *Transpl Int* (2025) 38:14818. doi:10.3389/ti.2025.14818

APPENDIX

APPENDIX TABLE 3 | Reviewers for Transplant International – 2025.

Anders Åsberg[a]
Olivier Aubert[a]
Alberto Benazzo[a]
Klemens Budde[a]
Nicos Kessaris[a]

Shadab Abadpour
Davide A. Abate
Fariba Abbassi
Amr Abdelaal
Rayid Abdulqawi
Maheen Abidi
Jose M. Aguado
Victoria Aguilera
Emin Baris Akin
Mohammad Aladaileh
Varuna Aluvihare
Amer Alzahrani
Antonio J. Amor
Dany Anglicheau
Corinne Antoine
Giuseppe Aresu
Miha Arnol
Emre Arpali
Florent Artru
Isa Ashoor
Alaa Atamna
Trinidad Serrano Aullo
Lissette Avilés
Alfonso Avolio
Omer Aziz
Andreina Baj
Chloe Balleste
David a Baran
Andrew S. Barbas
Louise Barbier
Yaniss Belaroussi
Caterina di Bella
Fabrizio di Benedetto
David Bennett
Marius Berman
Inigo Bermejo
Dominique Bertrand
Suzanne Bezstarosti
Luigi Biancone
Cecilia Bonazzetti
Caroline Bonner
Antoine Bouquegneau
Olivier Brugiere
Marcos Buj
Patrizia Burra
Roberto Cacciola
Rossana Caldara
Jasper Callemeyn
Diego Cantarovich
Lucio Careddu
Chiara Catelli
Miriam Cortés Cerisuelo
Matteo Cescon
Ernest G Chan
Xavier Charmetant
Efstratios Chatzixiros
Bertrand Chauveau
Yee Lee Cheah
Xiaomeng Chen

Mladen knotek[a]
La Salete Martins[a]
Johan Boble[a]
Axel Rahmel[a]
Juliano Riella[a]

Alexandra Geusau
Emmanouil Giorgakis
Laurent Godinas
Amanda Godoi
Petra Goldsmith
Nicolas Golse
Jeevan Prakash Gopal
Basu Gopal
Julien Gras
Paolo Antonio Grossi
Benoit Huery
René Hage
Franck Halary
Jerome Harambat
Takashi Harano
Hermien Hartog
Koichiro Haruki
Bulang He
Long He
Ilkka Helanterä
Merel Hellemons
Rosa Blanes Hernandez
Taizo Hibi
Jens Hillingsø
Toshihito Hirai
Takahisa Hiramitsu
Hans H Hirsch
Cédric Hirzel
Tomer Hoffman
Benson Hoffman
Are Holm
Luise Holzhauser
Min Hu
Volkert Huurman
Mahmoud Mohamed Elsayed Ibrahim
Franz Immer
Annika Ingvarsson
Georgina Irish
Michael Gerckens
Jasper Iske
Fabio Ius
Manhal Izzy
Spenser January
Erik Groot Jebbink
Jan Philipp Jonas
Yashutosh Joshi
Thomas Jouve
Wolfgang Jungraithmayr
Ivana Juric
Nassim Kamar
Hannah Kaminski
Nada Kanaan
Nikolaos Karydis (Karidis)
Martin Kauke-Navarro Md
Brendan Keating
Hussein Khambalia
Yoshitaka Kinoshita
Simon Knight
Takaaki Kobayashi

Elisa Ruiz-Arabi[a]
Navdeep Singh[a]
Dirk Van Raemdonck[a]
Lada Zibar[a]

René Novysedlák
Zhuldyz Nurmykhametova
Rupert Oberhuber
Jon Scott Odorico
Michael Oellerich
Grainne O'Kane
Valérie Olivier
Mihai Oltean
Vivian Lumi Onusic
Fernanda Ortiz
Balin Ozsoy
Alessandro Palleschi
Eduard Palou
María Paniagua-García
Christina Papachristou
Vassilios Papalois
Lars Pape
Stefano Partelli
Renato Pascale
Andrew R Pepper
David Pereyra
Richard Perez
Griffith Perkins
Marcelo Perosa
Quentin Perrier
Bjoern Petersen
Hessel Peters-Sengers
Palmina Petruzzo
Paul Phelan
Maheswaran Pitchaimuthu
Manuel Podesta
Robert Pol
Evgenia Preka
Francesco Procaccio
Gervasio Soler Pujol
Pankaj Puri
Marion Rabant
Mindaugas Rackauskas
Sarah Raevens
Rajesh Rajalingam
Sree Bhushan Raju
Pablo Ramírez
Raja Rashid
Joanna Raszeja-Wyszomirska
Tomas Reischig
Jose Otto Reusing Junior
Jinsoo Rhu
James Richards
Paul Ritschl
Valentin Ritschl
John Roberts
Manuel Rodríguez-Perálvarez
Gianluca Rompianesi
Alberto Rosati
Olivier Roux
Maria Jose Perez Saez
Berta Saez-Gimenez
Andrew Sage
Mustafa Kursat Sahin

(Continued on following page)

APPENDIX TABLE 3 | (*Continued*) Reviewers for Transplant International – 2025.

Mikael Chetboun
Oriana Ciacio
Umberto Cillo
Benjamin Coiffard
Nicolina Conti
Matthew Cooper
David Cooper
Alice Corbel
Emanuele Cozzi
David Cucchiari
Sílvia Gomes da Silva
Sunil Daga
Raja Dandamudi
José Davide
Luis Gustavo Modelli de Andrade
Marieke T de Boer
Sabina de Geest
Margriet de Jong
Jose Luis Campo-Cañaveral de La Cruz
Annelies de Weerd
Silvia Deaglio
Rebecca Deans
Arnaud Del Bello
Tommaso di Maira
Gonzalo Díaz-Cobacho
Laura Dichiacchio
Fritz Diekmann
Frank J.M.F. Dor
Didier Dorez
Pedro Augusto Reck dos Santos
Genady Drozdinsky
Geoffrey Dube
Halise Taşkın Duman
Richard Dumbill
Jerome Dumortier
Magdalena Durlik
Philipp Dutkowski
Janina Eden
Sofia el Hajji
Michelle Elias
Andreas Elmer
Mohamed Eltemamy
Juliet Emamaullee
Eric Epailly
Olivier Epaulard
Pauline Erpicum
Dilmurodjon Eshmuminov
Letizia lo Faro
Mario Fernández-Ruiz
Carla Ferrandiz-Pulido
Alberto Ferrarese
Paolo Ferrari
Joana Ferrer-Fábrega
Constanca Figueiredo
Konrad Fischer
Yohann Foucher
Helene Francois
Ilaria Gandolfini
Dale Gardiner
Brad Gardiner
Cyril Garrouste
Philippe Gatault
Milo Gatti
Alice Koenig
Dionysios Koliogiannis
Konstantinos Koutroutsos
Philipp Dominique Kron
Aleksandra Kukla
Vinay Kumaran
Nicholas Küng
Chitaru Kurihara
Niclas Kvarnstrom
Yong Kyong Kwon
Marie-Camille Lafargue
Rita Leal
Jong Soo Lee
Per Lindner
Sandra Lindstedt
Guangxiang Liu
Francesco Locatelli
Andrea Lombardi
Ibai Los-Arcos
Jessica Lum
John MacArthur
Umberto Maggiore
Manuel Maglione
Christian T. J. Magyar
Beatriz Mahillo
Angeles Maillo-Nieto
Rahul Mainra
Paolo Malvezzi
Nizam Mamode
Wenjun Mao
Lorenzo Marconi
Christian Margreiter
Marco Masetti
Emma Massey
Marie Matignon
Xavier Matillon
Katharina A. Mayer
Micheal Mcinnis
Cely Medeiros
Francisco lópez Medrano
Zhongcheng Mei
Raphael Meier
Madhav C Menon
Shaheed Merani
Marco Merli
Benoît Mesnard
Alberto Molina-Pérez
Michele Molinari
Jose Morales
Alfonso Morcillo
Francesc J Moreso
Midas Berend Mulder
Elmi Muller
William Mulley
Sarwa Darwish Murad
Silvio Nadalin
Nalu Navarro-Alvarez
Bashoo Naziruddin
Flavia Neri
Arne Neyrinck
Jan Niesing
Tom Nieto
Michiel Nijhoff
Faouzi Saliba
Jadranka Pavičić Šarić
Marco Schiavon
Roland Schmitt
Sameep Sehgal
Rajeevan Selvaratnam
Alp Sener
Rakesh Sethapati
Sadhana Shankar
Adnan Sharif
Ankit Sharma
Lena Sibulesky
Farjad Siddiqui
Alberto Costa Silva
Ksenija Vucur Simic
Rajesh Sivaprakasam
Renaud Snanoudj
Mario Spaggiari
Peter Stock
Robert Stratta
Yiliam Fundora Suárez
Harikesh Subramanian
Diana Sukackiene
Kalathil Sureshkumar
Caner Süsal
Andrew Sutherland
Anat R Tambur
Yakup Tanriver
Helen Te
Marta Tejedor
Inga Strand Thorsen
Claire Tinel
Samuel Tingle
Francesca Tinti
Giusy Tiseo
Pierluigi Toniutto
Burkhard Tönshoff
Lada Trajceska
Vasiliki Tsarpali
Cynthia Tsien
Andreas Tzakis
David Rodriguez-Arias Vailhen
Joris Van De Klundert
Lorena Van Den Bogaart
Teun Van Gelder
Deborah Verran
Ondrej Viklicky
Carmen Vinaixa
Julien Vionnet
Jonathan Visentin
Fabio Vistoli
Ashley Vo
Robin Vos
Jennifer Wainright
Charles-Henri Wassmer
Bettina Wiegmann
Felicity Williams
Colin Wilson
Tiffany Wong
Germaine Wong
Zachary Yetmar
Kenneth Yong
Bo Yu

[a]*Special thanks to our 14 top reviewers.*

ESOT | Transplant International

NEWS AND VIEWS
published: 23 February 2026
doi: 10.3389/ti.2026.16247

The Top 12 Most Impactful Papers in Clinical Transplantation in 2025: TI Editors' Choice

Thierry Berney[1,2,3], Chiara Becchetti[4,5], Maria Irene Bellini[6,7], Louise Benning[8,9], Ekaterine Berishvili[3,4,10], Oriol Bestard[6,11], Saskia Bos[4,12], Sophie Brouard[4,13], Federica Casiraghi[14,15], Antonio Citro[8,16], Lionel Couzi[4,17], Marta Jimenez-Blanco[4,18], Delphine Kervella[8,11], Wai H. Lim[4,19], Oriol Manuel[4,20], Nina Pilat[6,21], Stefan Schneeberger[6,22], Emilien Seizilles de Mazancourt[8,23], Nazia Selzner[14,24], Olivier Thaunat[2,14], Christian Toso[4,25], Arianna Trizzino[8,26], Dafna Yahav[4,27] and Andrea Zajacova[8,28] on behalf of the Transplant International Editorial Board*

[1]Editor-in-Chief, Transplant International, [2]Division of Transplantation, Immunology and Nephrology, Hospices Civils de Lyon, Lyon, France, [3]Ilia State University, Tbilisi, Georgia, [4]Associate Editor, Transplant International, [5]ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy, [6]Deputy Editor-in-Chief, Transplant International, [7]La Sapienza Universita di Roma, Rome, Italy, [8]Assistant Editor, Transplant International, [9]Heidelberg University Hospital, Heidelberg, Germany, [10]University of Geneva Medical University Center, Geneva, Switzerland, [11]University Hospital Vall d'Hebron, Barcelona, Spain, [12]University Hospital Leuven, Leuven, Belgium, [13]INSERM U1064, University of Nantes, Nantes, France, [14]Executive Editor, Transplant International, [15]Mario Negri Institute for Pharmacological Research, Bergamo, Italy, [16]Diabetes Research Institute, IRCCS Ospedale San Raffaele, Milan, Italy, [17]Bordeaux University Hospital, Bordeaux, France, [18]University Hospital Ramon y Cajal, Madrid, Spain, [19]University of Western Australia, Perth, WA, Australia, [20]Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland, [21]Medical University of Vienna, Vienna, Austria, [22]Medical University of Innsbruck, Innsbruck, Austria, [23]Department of Urology, Saint-Louis Hospital, Paris, France, [24]Ajmera Transplant Center, Toronto General Hospital, Toronto, ON, Canada, [25]University of Geneva Hospitals, Geneva, Switzerland, [26]Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy, [27]Sheba Medical Center, Ramat-Gan, Israel, [28]BREATHE, KU Leuven, Leuven, Belgium

Keywords: clinical transplantation, editors selection, game changers, impact, landmarks

News & Views

OPEN ACCESS

***Correspondence**
Thierry Berney,
eic.ti@frontierspartnerships.org

Received: 14 January 2026
Revised: 14 January 2026
Accepted: 21 January 2026
Published: 23 February 2026

Citation:
Berney T, Becchetti C, Bellini MI, Benning L, Berishvili E, Bestard O, Bos S, Brouard S, Casiraghi F, Citro A, Couzi L, Jimenez-Blanco M, Kervella D, Lim WH, Manuel O, Pilat N, Schneeberger S, Seizilles de Mazancourt E, Selzner N, Thaunat O, Toso C, Trizzino A, Yahav D and Zajacova A (2026) The Top 12 Most Impactful Papers in Clinical Transplantation in 2025: TI Editors' Choice. Transpl. Int. 39:16247. doi: 10.3389/ti.2026.16247

The New Year is a perfect opportunity, not only for resolutions (that are unlikely to last very long), but also to reflect on the events of the past year and how they are likely to impact the future. In this piece, the editorial board of Transplant International is presenting what we think are the original articles in clinical transplantation published in 2025 that are the most likely to be game changers in our field in the near or not-so-near future. As the saying goes, "it is difficult to make predictions, especially about the future", but despite the (very limited) risk, we are hopeful that we have not succumbed to hype in our selection, which was based on insights from members of the editorial board, but also on how well cited these papers already were. So, here are the top 12 papers, listed in **Table 1**, grouped by the topic they address. Selection is obviously a tricky task and for this reason, we will also mention other significant 2025 papers to provide the readers with a somewhat broader picture of what has happened in each of the selected topics (**Supplementary Table S1**).

XENOTRANSPLANTATION

Xenotransplantation has repeatedly made the headlines in recent years since the near-simultaneous transplantation in the fall of 2021 of genetically modified porcine kidneys into human brain-dead recipients, or what is called the human decedent model [13]. The availability of genome-edited pigs has been the game changer in the field, allowing to significantly lower the immune barriers met in xenotransplantation [14]. The first clinical transplants using this resource were performed compassionately in 2022 in patients with no access to deceased donor organs and a compromised survival prognosis in the short term. Although the heart and the kidney

TABLE 1 | The top 12 most impactful papers in clinical transplantation in 2025.

Title	Topics	Journal	Corresponding author institution	Brief description
Transplantation of a genetically modified porcine heart into a live human [1]	Xenotransplantation Heart transplantation	Nature medicine	University of Maryland school of Medicine, Baltimore, MD, USA	Comprehensive report of the first pig-to-human heart xenotransplant using multi gene-edited porcine donors
Xenotransplantation of a porcine kidney for end-stage kidney disease [2]	Xenotransplantation Kidney transplantation	New England journal of medicine	Massachussets general Hospital, Boston, MA, USA	Comprehensive report of the first pig-to-human kidney xenotransplant using multi gene-edited porcine donors
Enzyme-converted O kidneys allow ABO-incompatible transplantation without hyperacute rejection in a human decedent model [3]	ABO incompatible tx Kidney transplantation	Nature biomedical Engineering	West China hospital, sichuan university, chengdu, China	Comprehensive report of the successful transplantation into a human brain-dead recipient of an A blood group kidney enzymatically converted into O blood group
Survival of transplanted allogeneic beta cells with No immunosuppression [4]	Immune tolerance Islet transplantation	New England journal of medicine	University of uppsala, uppsala, Sweden	Comprehensive report of the transplantation of gene-edited immune-evasive islet constructs into a type 1 diabetic patient
Stem cell-derived, fully differentiated islets for type 1 diabetes [5]	Stem cells Islet transplantation	New England journal of medicine	Vertex Pharmaceuticals, Boston, MA, USA	Report of a successful industry-led clinical trial of transplantation of embryonic stem cell-derived islet constructs into type 1 diabetic recipients
Cold perfusion vs. Static cold storage of deceased-donor kidneys — at 10 Years [6]	Machine perfusion Kidney transplantation	New England journal of medicine	University of groningen, groningen, The Netherlands	Ten-year data of a large randomized controlled trial of normothermic machine perfusion versus cold storage in kidney transplantation
Clinical outcomes with normothermic pulsatile organ perfusion in heart transplantation: A report from the OCS heart perfusion registry [7]	Machine perfusion Heart transplantation	Circulation	University of Utah, Salt Lake City, UT, USA	Registry data report on the feasibility and benefits of normothermic machine perfusion for DCD heart transplantation
Determining safe washout period for immune checkpoint inhibitors prior to liver transplantation: An international retrospective cohort study [8]	Transplant oncology Liver transplantation	Hepatology	University of Geneva Hospital, Geneva, Switzerland	Multicenter study providing safety data on the optimal cut-off for the wash-out period before liver transplantation after checkpoint inhibitor treatment
Insights from the BKEVER trial comparing everolimus versus mycophenolate mofetil for BK polyomavirus infection in kidney [9]	Infectious diseases Kidney transplantation	Kidney international	Strasbourg university hospital center, strasbourg, France	Randomized controlled trial exploring the impact of a switch from CNI to mTOR inhibitors in BK virus nephropathy
Donor-recipient mismatch at the SIRPA locus adversely affects kidney allograft outcomes [10]	Transplant immunology Kidney transplantation	Science translational medicine	University of Pittsburgh Medical school, piitsburgh, PA, USA	Exploration of the impact of SIRPA mismatch and of the innate immune system on kidney allograft rejection in two human cohorts and a mouse model
Continuous indices to assess the phenotypic spectrum of kidney transplant rejection [11]	Transplant pathology Kidney transplantation	Nature communications	Catholic university of Leuven, Leuven, Belgium	A banff paper providing strong evidence to replace threshold-based rejection categories with robust continuous indices
Extracorporeal photopheresis for the prevention of rejection after lung transplantation: a Prospective randomised controlled trial [12]	Photopheresis Lung transplantation	European respiratory journal	Medical university of Vienna, Vienna, Austria	Randomized controlled trial showing the benefit of adding extracorporeal photopheresis to standard immunosuppression for the prevention of CLAD

xenotransplant recipients did not survive more than a few months, their causes of death not being fully clear, these first 2 cases provided evidence that the early phases of immune xenorejection had been harnessed [1, 2] thanks to the gene modification strategy, in particular the knocking off of 3 carbohydrate antigen genes known to cause hyperacute rejection (α 1-3 Gal, β 1-4 Gal, CMAH). The two featured articles provide a comprehensive narrative of the clinical course of the first heart recipient [1] and the first kidney recipient [2], from transplantation to death. While these cases cannot be considered successes from the patient perspective, they will provide invaluable information on the unforeseen hurdles met and how to overcome them [15]. In this regard, and despite the limitations of the decedent model [16], the in-depth analysis of the physiology, immunology and pathology of the first pig-to-human decedent kidney xenotransplant

(single gene modification), using multi-omics analysis techniques [17, 18], and in particular the novel patterns of immune rejection unveiled and characterized, will also contribute to the better understanding of the physiological processes that will have to be tackled by the next-generation of protocols and gene-edited animals.

This paragraph would not be complete without mentioning the reports of gene-edited pig-to-human auxiliary liver transplantation in a living recipient, as a bridge to transplant [19], and lung transplantation using the decedent model [20]. Although the cell therapy procedure of islet transplantation would seem to have been an easier clinical model to explore in xenotransplantation, no attempt at transplanting genetically modified porcine islets has been reported to date [21].

ABO-INCOMPATIBLE ORGAN TRANSPLANTATION

ABO-incompatible living-donor kidney transplantation has evolved into a standard option to expand the donor pool, but it usually requires desensitization to mitigate the risk posed by preformed anti-A/B antibodies. Protocols commonly include antibody removal (immunoadsorption/antibody adsorption and/or plasma exchange) alongside immunomodulatory therapy, adding complexity, cost, and potential morbidity despite outcomes approaching those of ABO-compatible living-donor transplantation. Kidney paired exchange is an alternative, although limited O-compatible availability may lead to extended waiting times for patients bearing the O blood group.

This paper reports the successful conversion of an A blood group kidney into O blood group, using *α-galactosidase* from *Bacteroides fragilis* during hypothermic machine perfusion. Three hours of machine perfusion were sufficient to remove >95% of blood group A antigens and allowed successful transplantation of the kidney in a decedent model without experiencing hyperacute rejection [3]. After A-antigen regeneration, antibody-mediated lesions and complement deposition were found starting 3 days post-transplant, but single-cell sequencing confirms the elevated expression of accommodation-related genes, suggesting the potential for longer-term tolerance [3]. This paper was published a few months after a previous similar report in the less common B-to-O combination, with similar outcomes [22]. These two papers provide compelling proof of concept that *ex vivo* antigen modification can safely expand the donor pool in ABO-incompatible living kidney donation and may therefore fundamentally reshape access in kidney transplantation. This strategy could also be employed in a deceased donor setting in the most urgent cases. While the validity of this method requires confirmation in the setting of clinical live donor kidney transplantation, it could also be explored in the more challenging model of liver transplantation, in which traditional techniques of pre-transplant ABO antibody clearance can be associated with deleterious outcomes [23].

IMMUNE TOLERANCE

The field of islet of Langerhans transplantation has seen 2 landmark clinical papers published last year. They represent a major step forward in beta-cell replacement therapies. The studies show that immune rejection and limited cell availability can be addressed. They set new benchmarks for safety, efficacy, and translational feasibility.

Importantly, they provide insights to guide the next-generation of durable islet replacement strategies. They also provide strategies that have the potential to be applied in the bioengineering of other organs.

The first is a proof-of-concept article in which the authors have applied their hypoimmune platform (HIP) approach to human primary islet cells. For the first time, they demonstrated the feasibility of gene-editing dissociated human islets using CRISPR-Cas12b and lentiviral transduction, to knock out HLA class I and II genes and overexpress the CD47 transmembrane molecule, which delivers a "*don't eat me*" signal to cells of the innate immune system. These modified islet cells were then re-aggregated and transplanted into the forearm muscle of a patient with long-standing type 1 diabetes. In this first-in-human study, HIP allogeneic islet cells transplanted without immunosuppression (IS) were not rejected, remained functional up to 12 weeks and achieved functional glucose-responsive insulin secretion. This provides a landmark proof of concept that immune evasive cells can be generated by gene modification and could overcome one of the central barriers to curative cell therapy for type 1 diabetes. By design, the functional mass of endocrine cells implanted was insufficient to reverse diabetes, and difficulties in upscaling the technique to a sufficient islet mass can be anticipated. It should be mentioned that, before going to the clinical setting, the authors had reported successful reversal of diabetes, using the same islet modification methodology, in a humanized mouse model transplanted with human HIP stem cell islets [24] and remarkably in an allogeneic non-human primate model [25].

STEM CELLS

The second landmark paper in the islet transplantation field reported the results of transplantation of stem cell-derived islets in 14 patients with type 1 diabetes [5]. This trial was led by the Vertex company and utilized a cell product baptized zimislecel, obtained by an *in vitro* differentiation protocol able to obtain large quantities of fully differentiated and glucose-responsive islets [26]. The trial was designed as phase 1-2 to determine safety and efficacy of the product, and the paper is an unplanned interim analysis of the first 12 patients to have received a full dose of the product and completed a 12-month follow-up. The primary efficacy endpoint was freedom from severe hypoglycemic events until day 365 after infusion, with a glycated hemoglobin level <7%. Remarkably, all patients met the primary endpoint and 10/12 patients were insulin-independent at 1 year. All patients who came off insulin did so after a period of several months, suggesting that further differentiation may have been taking place *in vivo* after

transplantation [5]. These data demonstrate a formidable technological and clinical achievement and advance and provide the first evidence that stem cell-derived tissues can be successfully used as an organ replacement therapy. However, zimislecel administration was done similarly to islet transplantation and used an identical IS protocol; accordingly, the clinical outcomes were similar to those achieved with the landmark "Edmonton protocol" [27]. While zimislecel may become a solution in the USA, where allogeneic islet transplantation is essentially unavailable [28], several issues will have to be solved before it can become a real solution for bringing a cure to all type 1 diabetic patients [21]. The application of the immune evasiveness strategy described above [4] to stem cell-derived islets may become a solution to that end.

MACHINE PERFUSION

Ex vivo machine perfusion has rapidly developed in the last decade, driven by the ever-increasing gap between the numbers of donor organs and patients on the waiting list concomitantly with the increase in the proportion of extended criteria and marginal donors (older age, DCD, fatty livers, . . .). Hypothermic (HMP), hypothermic oxygenated and later, normothermic machine perfusion (NMP) have become a standard-of-care solution for the reconditioning of marginal organs [29, 30]. Machine perfusion also offers the possibility of assessing physical or biological parameters in the perfusate to provide information about organ quality, and thus transplantability [31].

The seminal multicenter European randomized controlled trial (RCT), comparing hypothermic kidney perfusion, using the Organ Recovery Systems machine, to cold storage enrolled >800 patients and its results were published already in 2009. The primary endpoint of the trial, i.e., occurrence of delayed graft function, was verified, thus demonstrating a significantly lower rate of delayed graft function in the perfusion group. Superior 1-year graft survival was also reported [32]. Better graft survival was still observed in a 3-year follow-up paper [33]. The article selected presents the long-awaited 10-year data [6]. Remarkably, the graft survival advantage was still present, and the long term observation revealed that this advantage was only conferred to expanded criteria donors [6]. This paper provides compelling evidence to recommend HMP for all kidneys procured from expanded criteria donors, notably DCDs.

Advances and utilization of machine perfusion technology have largely been driven by the kidney and liver transplantation fields. The amount of evidence gathered for thoracic organs has been less extensive and has mostly addressed lung transplantation [34, 35]. The rapid development of DCD heart transplantation in the past decade has provided an incentive for maximizing utilization of DCD hearts using machine perfusion technology. In contrast to kidneys and livers, NMP has been the preferred, and indeed sole, approach employed in this setting [36]. Unfortunately, and perhaps for understandable reasons, no prospective randomized trial is available to compare NMP to static cold storage (SCS) in DCD hearts.

The OCS NMP machine (Transmedics, Andover, MA, USA) is the only one approved for clinical use in the USA. It allows normothermic pulsatile perfusion during transportation of the heart from recovery to implantation. The selected paper is a Transmedics-led study combining data from the OPTN and OCS Heart Perfusion (OHP) registries and providing real-world data on the largest cohort (854 patients in 56 US centers) of NMP-preserved heart transplants ever reported [7]. The large numbers (>3,000 subjects, including OPTN data) and a rigorous methodology have allowed meaningful comparisons of DCD versus DBD cohorts, and NMP versus SCS strategies. The most striking result is the similar 1-year patient survival data in SCS-preserved DBD hearts and NMP-preserved DCD hearts (>90%), observed in spite of 2-3 times longer travelling distances and times. It is remarkable that >25% of hearts transplanted in the USA over the study period were preserved with NMP and <4% of procured NMP hearts were finally rejected [7]. In summary the study convincingly shows that NMP allows the recovery of hearts with longer shipping distances and from extended-criteria donors. This is likely to lead to the rapid normalization, in the USA and beyond, of NMP for the preservation of DCD and other marginal donor hearts.

TRANSPLANT ONCOLOGY

Hepatocellular carcinoma (HCC) is the third most important indication for liver transplantation after alcohol-associated cirrhosis and metabolic dysfunction–associated steatohepatitis [37]. Recently, Immune checkpoint inhibitors (ICIs) have revolutionized the management of HCC and become standard-of-care as part of the treatment of advanced HCC [38]. This has allowed not only prolonged patient survival, but also tumour downstaging, bringing patients within Milan or other liver transplantability criteria. Unfortunately, the mechanism of action of ICIs, which essentially boosts the adaptive immune system against the tumour, has resulted in the early experience in a high risk of acute rejection episodes in ICI-treated patients receiving a liver transplant [38, 39]. A consensus has evolved to suggest that ICIs were a powerful neoadjuvant therapy for downstaging or bridging purposes, but that the optimal "washout" period (free interval between end of ICI treatment and liver transplantation) still had to be determined [40].

The selected paper is a multi-center retrospective study of 119 liver transplant patients treated with ICIs before liver transplantation in 29 transplant centers worldwide [8]. The authors analyzed the relationship between the washout period and several outcome measures, including occurrence and type of rejection, tumour recurrence and survival. They reported a 20% rejection rate, occurring early after transplantation, with a linear reverse relationship between rejection risk and washout period between 3 and 50 days. Beyond 50 days an increased rejection risk was no longer observed. Importantly, with a median follow-up of 18 months, patients with a longer washout period did not present a higher risk for HCC recurrence. There is a caveat with the fact that 9 different ICIs were used in these 119 patients, making it

difficult to determine, due to low numbers, whether the 50-day cut-off was applicable equally to each of these molecules. Nonetheless, this is the first study to provide convincing evidence about the optimal washout period to observe and the uselessness of waiting times >50 days before performing liver transplantation in ICI-treated HCC patients.

The field of liver transplantation oncology is not limited to HCC, as unresectable colorectal liver metastases are increasingly becoming a valid indication for liver transplantation in selected cases. The ARTx-Onc study [41] provides a US perspective and favourable outcome results that slightly differ from those of the recent European Transmet RCT [42].

TRANSPLANT INFECTIOUS DISEASE

BK polyomavirus nephropathy is a significant challenge for the transplant nephrologist, which can have a serious impact on kidney graft function and compromise graft survival in the most serious cases. The standard IS regimen associating tacrolimus, mycophenolate (MMF) and steroids is the primary risk factor for BK viral replication, with a high risk of developing BK nephropathy. The primary strategy in the management of BK virus infection is reduction of the IS at the risk of triggering graft rejection. Due to their antireplicative properties, mTOR inhibitors, well established IS drugs, have demonstrated potent *in vitro* antiviral activity, including against BK polyomavirus. Accordingly, RCTs exploring the efficacy of everolimus-based versus MMF-based IS regimens in kidney transplantation have also looked at the occurrence of BK infection and nephropathy. It was found that subjects receiving everolimus had experienced a lower rate of BK infection at 12 months, but none of these studies consistently monitored BK DNA levels [43, 44]. Therefore, the real role of a switch from MMF to everolimus remains undetermined and prompted the authors of this paper to launch a RCT comparing the efficacy of reducing MMF dosage versus switching from MMF to everolimus, alongside reduced CNIs, in kidney transplant recipients with BK DNAemia [9]. To their surprise, the authors found that BK virus clearance was achieved significantly more often and more rapidly in patients with MMF dosage reduction alone, compared to those switched to everolimus. Parameters of kidney function at the end of follow-up were identical in both groups [9]. These data challenge the notion that a switch to mTOR inhibitors will lead to faster clearance of BK viremia. On the contrary, it appears ineffective for managing BK DNAemia in kidney transplant recipients and cannot be recommended as a management strategy for BK replication control.

TRANSPLANT IMMUNOLOGY

Minimization of donor-recipient HLA mismatches has been utilized as an allocation criterion for years as a strategy to avoid antibody and T cell–mediated rejection for most solid organ transplants. A role for the innate immune system in the pathogenesis of graft rejection has been unravelling in recent years [45, 46]. The main pathways thought to be involved are natural killer (NK) cell activation via missing self signals and monocyte activation through the signal regulatory protein α (SIRPα)-CD47 interaction. In the selected paper, the authors have studied the impact of mismatches in SIRPA, the gene encoding signal regulatory protein α (SIRPα), on the risk of renal allograft rejection [10]. They first determined that only 2 haplotype categories were present in >90% of the human population. In two independent cohorts of patients representing >700 subjects, they found that SIRPA mismatches were associated with an increased risk of renal allograft rejection, graft fibrosis and allograft survival. Differences in rejection-free and overall graft survivals were not only statistically significant, but also clinically relevant. For the most impactful mismatch type, acute rejection-free survival was 81% vs. 94%, and death-censored graft survival was 75% vs. 92%, both assessed at 7 years post-transplant [10]. In a murine model, they were able to show that SIRPα variants binding to CD47 elicit monocyte activation implicated in chronic allograft pathology. These results may impact both on matching strategies and on the therapeutic targeting of innate immunity and not only of T-cell activation. More specifically, with the simplicity of having only 2 haplotypes to consider and the high potential impact on kidney graft prognosis, the inclusion of SIRPA genotyping and matching could be an easy and efficient strategy to apply as a component of kidney graft allocation algorithms.

TRANSPLANT PATHOLOGY

Since 1991, the Banff classification has been the international, consensus system that standardizes the biopsy-based diagnosis, classification and grading of kidney graft rejection. It provides a universal language for pathologists and clinicians that characterizes specific types of lesions (inflammation, tubulitis, arteritis, glomerulitis, ...), and scores their severity to guide patient care and graft monitoring and to standardize reporting of outcomes [47].

With the reflection that the Banff classification is a dichotomic classification into discrete categories of a phenomenon that is a continuum in essence, the authors of this paper are proposing four continuous indices, easy to implement and interpret, for the global evaluation of kidney transplant histology [11]. Indices were developed from the analysis of nearly 20'000 biopsies from 10 centers worldwide. The formulas for these indices can be found in the article. The first 2 indices (AMR/MVI and TCMR/TI index) derived from routinely assessed histological lesion scores were designed to enable the quantification of the global spectrum of kidney transplant rejection, and the last 2 indices (Activity and Chronicity index) to replace the Banff diagnostic subcategories of rejection by more continuous activity and chronicity measures. The indices developed from a derivation cohort of >10'000 biopsies were verified in 2 separate validation cohorts of >5'000 and >1'000 biopsies respectively.

The four continuous measures of kidney transplant rejection capture much of the histological spectrum and severity of rejection while closely aligning with the current Banff

classification. A significant interest of these indices is that it allows to better define, on a continuum basis, the nature, and thus the necessary intervention, for lesions characterized as "borderline" or "probable" rejection in the Banff system. Overall, this paper offers a refreshing replacement of rigid, threshold-based rejection categories with robust continuous indices capturing the true biological continuum of kidney allograft rejection. It provides a scalable framework that can directly refine clinical decision-making and trial endpoints in transplant nephrology. It has the potential of being a true game-changer in the classification of kidney allograft rejection in the very near future.

PHOTOPHERESIS

Chronic lung allograft dysfunction (CLAD) is an umbrella term coined to cover the different manifestations of irreversible lung allorejection, its most common form being bronchiolitis obliterans syndrome (BOS). Despite advances in diagnosis, little progress has been achieved in the prevention and treatment of this crippling condition, and CLAD remains the main cause for late pulmonary graft loss [48]. Recently, extracorporeal photopheresis (ECP) has emerged as a promising supportive treatment for the prevention and management of rejection in heart and lung transplants, with growing evidence supporting its use in kidney and liver transplants [49]. ECP is an extracorporeal therapy, combining leukapheresis with photoactivation. It consists in the incubation of the recipient's mononuclear cells with a DNA-crosslinking molecule activated by UVA radiation, causing T-cell apoptosis, before reinfusing the cells into the patient. ECP was first developed for the treatment of T cell lymphomas and later used in other indications including graft-versus-host disease and organ cell-mediated rejection [49]. A recent multicenter study of lung transplant recipients started on ECP for a diagnosis of CLAD reported a response to treatment (stabilization or improvement) in about 50% of study subjects [50].

This article reports the outcomes of a RCT in which 62 patients with chronic obstructive pulmonary disease were randomized to receive ECP from day 2 to week 11 after lung transplant, in addition to conventional IS, or IS alone. The composite primary outcome measure was the occurrence of acute cellular rejection (ACR), cytomegalovirus infection, or CLAD within 2 years of transplantation [12]. ECP addition to IS resulted in a 3-fold decrease in meeting the primary endpoint (20% vs. 60%), mostly driven by a lower incidence of ACR or CLAD. Importantly, counting and phenotyping of circulating lymphocytes were similar in the treated and the control group, indicating that ECP had not led to overimmunosuppression, accounting for the absence of an increase in CMV infection.

This trial represents a potential game changer for the survival and quality of life of lung transplant recipients. It could redefine the standard IS protocol for lung transplantation if these results are confirmed in patients transplanted for other indications and in larger cohorts.

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

FUNDING

The author(s) declared that financial support was not received for this work and/or its publication.

CONFLICT OF INTEREST

The authors(s) declared that this work 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) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2026.16247/full#supplementary-material

REFERENCES

1. Griffith BP, Grazioli A, Singh AK, Tully A, Galindo J, Saharia KK, et al. Transplantation of a Genetically Modified Porcine Heart Into a Live Human. *Nat Med* (2025) 31:589–98. doi:10.1038/s41591-024-03429-1
2. Kawai T, Williams WW, Elias N, Fishman JA, Crisalli K, Longchamp A, et al. Xenotransplantation of a Porcine Kidney for End-Stage Kidney Disease. *N Engl J Med* (2025) 392:1933–40. doi:10.1056/NEJMoa2412747
3. Zeng J, Ma M, Tao Z, Rao Z, Wu C, Yin S, et al. Enzyme-Converted O Kidneys Allow ABO-Incompatible Transplantation Without Hyperacute Rejection in a Human Decedent Model. *Nat Biomed Eng* (2025). doi:10.1038/s41551-025-01513-6

4. Carlsson PO, Hu X, Scholz H, Ingvast S, Lundgren T, Scholz T, et al. Survival of Transplanted Allogeneic Beta Cells with No Immunosuppression. *N Engl J Med* (2025) 393:887–94. doi:10.1056/NEJMoa2503822
5. Reichman TW, Markmann JF, Odorico J, Witkowski P, Fung JJ, Wijkstrom M, et al. Stem Cell-Derived, Fully Differentiated Islets for Type 1 Diabetes. *N Engl J Med* (2025) 393:858–68. doi:10.1056/NEJMoa2506549
6. Schutter R, Moers C, Jochmans I, Gallinat A, Paul A, Treckmann J, et al. Machine Preservation Trial Study Group. Cold Perfusion Vs. Static Cold Storage of Deceased-Donor Kidneys - at 10 Years. *N Engl J Med* (2025) 393:1859–61. doi:10.1056/NEJMc2406608
7. Stehlik J, Farr MA, Mehra MR, Schroder JN, D'Alessandro DA, Pal JD, et al. Clinical Outcomes With Normothermic Pulsatile Organ Perfusion in Heart Transplantation: A Report from the OCS Heart Perfusion Registry. *Circulation* (2025) 151:896–909. doi:10.1161/CIRCULATIONAHA.124.071743
8. Moeckli B, Wassmer CH, El Hajji S, Kumar R, Rodrigues Ribeiro J, Tabrizian P, et al. Determining Safe Washout Period for Immune Checkpoint Inhibitors Prior to Liver Transplantation: An International Retrospective Cohort Study. *Hepatology* (2025) 82:1122–37. doi:10.1097/HEP.0000000000001289
9. Caillard S, Meyer N, Solis M, Bertrand D, Jaureguy M, Anglicheau D, et al. Insights from the BKEVER Trial Comparing Everolimus Versus Mycophenolate Mofetil for BK Polyomavirus Infection in Kidney Transplant Recipients. *Kidney Int* (2025) 107:338–47. doi:10.1016/j.kint.2024.09.018
10. Zhao D, Dai H, Macedo C, Sanders SM, Elias C, Friday AJ, et al. Donor-Recipient Mismatch at the SIRPA Locus Adversely Affects Kidney Allograft Outcomes. *Sci Transl Med* (2025) 17:eady1135. doi:10.1126/scitranslmed.ady1135
11. Vaulet T, Koshy P, Wellekens K, Aubert O, Bottomley C, Callemeyn J, et al. Continuous Indices to Assess the Phenotypic Spectrum of Kidney Transplant Rejection. *Nat Commun* (2025) 16:10417. doi:10.1038/s41467-025-65153-9
12. Benazzo A, Cho A, Auner S, Schwarz S, Kovacs Z, Ramazanova D, et al. Extracorporeal Photopheresis for the Prevention of Rejection After Lung Transplantation: A Prospective Randomised Controlled Trial. *Eur Respir J* (2025) 65:2400733. doi:10.1183/13993003.00733-2024
13. Berney T, Naesens M, Schneeberger S. Xenotransplantion: Defeating the "Shumway Curse" an Interview With Drs. Bartley Griffith, Jayme Locke, Robert Montgomery, and Bruno Reichart. *Transpl Int* (2022) 35:10439. doi:10.3389/ti.2022.10439
14. Ali A, Kurome M, Kessler B, Kemter E, Wolf E. What Genetic Modifications of Source Pigs Are Essential and Sufficient for Cell, Tissue, and Organ Xenotransplantation? *Transpl Int* (2024) 37:13681. doi:10.3389/ti.2024.13681
15. Cooper DKC, Cozzi E. Clinical Pig Heart Xenotransplantation-Where Do We Go from Here? *Transpl Int* (2024) 37:12592. doi:10.3389/ti.2024.12592
16. Cooper DKC, Mou L, Cleveland JD, Simmons JH, Cleveland DC. Xenotransplantation Research -the Nonhuman Primate Model Is Preferable to the Human Decedent Model. *Transpl Int* (2025) 38:14452. doi:10.3389/ti.2025.14452
17. Montgomery RA, Stern JM, Fathi F, Suek N, Kim JI, Khalil K, et al. Physiology and Immunology of a Pig-To-Human Decedent Kidney Xenotransplant. *Nature* (2025). doi:10.1038/s41586-025-09847-6
18. Schmauch E, Piening BD, Dowdell AK, Mohebnasab M, Williams SH, Stukalov A, et al. Multi-Omics Analysis of a Pig-To-Human Decedent Kidney Xenotransplant. *Nature* (2025). doi:10.1038/s41586-025-09846-7
19. Tao KS, Yang ZX, Zhang X, Zhang HT, Yue SQ, Yang YL, et al. Gene-Modified pig-to-human Liver Xenotransplantation. *Nature* (2025) 641:1029–36. doi:10.1038/s41586-025-08799-1
20. He J, Shi J, Yang C, Peng G, Ju C, Zhao Y, et al. Pig-To-Human Lung Xenotransplantation into a Brain-Dead Recipient. *Nat Med* (2025) 31:3388–93. doi:10.1038/s41591-025-03861-x
21. Berney T, Thaunat O, Berishvili E. Allogeneic Islet Transplantation: Chronicle of a Death Foretold? *Transpl Int* (2025) 38:14598. doi:10.3389/ti.2025.14598
22. Zeng J, Ma M, Jiang X, Rao Z, Huang D, Zhang H, et al. Enzymatic Conversion of Blood Group B Kidney Prevents Hyperacute Antibody-Mediated Injuries in ABO-Incompatible Transplantation. *Nat Commun* (2025) 16:1506. doi:10.1038/s41467-025-56563-w
23. Yoo YJ, Kim D-G, Min E-K, Yim SH, Choi MC, Koh H-H, et al. Number of Pretransplant Therapeutic Plasma Exchange Sessions Increase the Recurrence Risk of Hepatocellular Carcinoma in ABO-Incompatible Living Donor Liver Transplantation. *Transpl Int* (2025) 38:14304. doi:10.3389/ti.2025.14304
24. Hu X, Gattis C, Olroyd AG, Friera AM, White K, Young C, et al. Human Hypoimmune Primary Pancreatic Islets Avoid Rejection and Autoimmunity and Alleviate Diabetes in Allogeneic Humanized Mice. *Sci Transl Med* (2023) 15:eadg5794. doi:10.1126/scitranslmed.adg5794
25. Hu X, White K, Olroyd AG, DeJesus R, Dominguez AA, Dowdle WE, et al. Hypoimmune Induced Pluripotent Stem Cells Survive Long Term in Fully Immunocompetent, Allogeneic Rhesus Macaques. *Nat Biotechnol* (2024) 42:413–23. doi:10.1038/s41587-023-01784-x
26. Pagliuca FW, Millman JR, Gürtler M, Segel M, Van Dervort A, Ryu JH, et al. Generation of Functional Human Pancreatic B Cells *In Vitro*. *Cell* (2014) 159:428–39. doi:10.1016/j.cell.2014.09.040
27. Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, et al. Islet Transplantation in Seven Patients with Type 1 Diabetes Mellitus Using a Glucocorticoid-Free Immunosuppressive Regimen. *N Engl J Med* (2000) 343:230–8. doi:10.1056/NEJM200007273430401
28. Witkowski P, Wojcik N, Appelbaum N, Fung JJ, Barth RN, Ricordi C. Demise of Cadaveric Islet Transplantation in the USA: Quo Vadis, 1 Year After BLA Approval and 24 Years After the Edmonton Breakthrough? *Front Transpl* (2025) 4:1491568. doi:10.3389/frtra.2025.1491568
29. Fallon J, Sagar A, Elzawahry M, Sadik H, Gyoten K, Abbas SH, et al. The Hitchhiker's Guide to Isolated Organ Perfusion: A Journey to 2040. *Front Transpl* (2025) 4:1642724. doi:10.3389/frtra.2025.1642724
30. Ibrahim M, Dechantsreiter J, Müller PC, Finotti M, Kowal MR, Ngjelina J, et al. Machine Perfusion in an Ageing Population - Results From a German, National Survey Among Transplant Centers. *Transpl Int* (2025) 38:15681. doi:10.3389/ti.2025.15681
31. Verstraeten L, Jochmans I. Sense and Sensibilities of Organ Perfusion as a Kidney and Liver Viability Assessment Platform. *Transpl Int* (2022) 35(31):10312. doi:10.3389/ti.2022.10312
32. Moers C, Smits JM, Maathuis MH, Treckmann J, van Gelder F, Napieralski BP, et al. Machine Perfusion or Cold Storage in deceased-donor Kidney Transplantation. *N Engl J Med* (2009) 360:7–19. doi:10.1056/NEJMoa0802289
33. Moers C, Pirenne J, Paul A, Ploeg RJ, Machine Preservation Trial Study Group. Machine Perfusion or Cold Storage in Deceased-Donor Kidney Transplantation. *N Engl J Med* (2012) 366:770–1. doi:10.1056/NEJMc1111038
34. Amarelli C, Bello I, Aigner C, Berman M, Boffini M, Clark S, et al. European Society of Organ Transplantation (ESOT) Consensus Statement on Machine Perfusion in Cardiothoracic Transplant. *Transpl Int* (2024) 37:13112. doi:10.3389/ti.2024.13112
35. Schiavon M, Bennett D, Boffini M, Carillo C, Dell'Amore A, Fumagalli J, et al. Use of Normothermic Perfusion Machines in Lung Transplantation: Consensus Statement of the Italian Society of Organ and Tissues Transplantation Group with DELPHI Method. *Transpl Int* (2025) 38:14762. doi:10.3389/ti.2025.14762
36. Tessari C, Lucertini G, Addonizio M, Geatti V, Bacich D, Pradegan N, et al. *Ex-Vivo* Heart Perfusion Machines in DCD Heart Transplantation Model: The State of Art. *Transpl Int* (2025) 38:12987. doi:10.3389/ti.2025.12987
37. Tabrizian P, Chan A, Marino R, Héloïse G, Lam A, Manon A. The Role of Immunotherapy Prior to Liver Transplantation in Advanced or Down-Staged HCC - Timing of Transplants, Selection of Patients, and Hurdles to Overcome With Immunosuppression. *Int J Surg* (2025) 112:1–17. doi:10.1097/JS9.0000000000003520
38. Kwong AJ, Kim WR, Lake JR, Schladt DP, Handarova D, Howell J, et al. OPTN/SRTR 2023 Annual Data Report: Liver. *Am J Transpl* (2025) 25(2S1):S193–S287. doi:10.1016/j.ajt.2025.01.022
39. Tabrizian P, Holzner ML, Ajmera V, Kim AK, Zhou K, Schnickel GT, et al. Intention-To-Treat Outcomes of Patients with Hepatocellular Carcinoma Receiving Immunotherapy Before Liver Transplant: The Multicenter VITALITY Study. *J Hepatol* (2025) 82:512–22. doi:10.1016/j.jhep.2024.09.003
40. Claasen MPAW, Sneiders D, Rakké YS, Adam R, Bhoori S, Cillo U, et al. European Society of Organ Transplantation (ESOT) Consensus Report on Downstaging, Bridging and Immunotherapy in Liver Transplantation for

Hepatocellular Carcinoma. *Transpl Int* (2023) 36:11648. doi:10.3389/ti.2023.11648

41. Byrne MM, Baker TB, Chapman WC, Ruffolo LI, Hernandez-Alejandro R, American Registry for Transplant Oncology. Liver Transplant for Upfront Unresectable Colorectal Metastases. *JAMA Surg* (2025) 16:1159–62. doi:10.1001/jamasurg.2025.2879
42. Adam R, Piedvache C, Chiche L, Adam JP, Salamé E, Bucur P, et al. Collaborative Transmet Group. 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. *Lancet* (2024) 404:1107–18. doi:10.1016/S0140-6736(24)01595-2
43. Tedesco-Silva H, Pascual J, Viklicky O, Basic-Jukic N, Cassuto E, Kim DY, et al. TRANSFORM Investigators. Safety of Everolimus with Reduced Calcineurin Inhibitor Exposure in De Novo Kidney Transplants: An Analysis from the Randomized TRANSFORM Study. *Transplantation* (2019) 103:1953–63. doi:10.1097/TP.0000000000002626
44. Sommerer C, Suwelack B, Dragun D, Schenker P, Hauser IA, Witzke O, et al. An open-label, Randomized Trial Indicates that Everolimus with Tacrolimus or Cyclosporine Is Comparable to Standard Immunosuppression in De Novo Kidney Transplant Patients. *Kidney Int* (2019) 96:231–44. doi:10.1016/j.kint.2019.01.041
45. Thaunat O, Lakkis FG, Kosmoliaptsis V, Schinstock C, Tambur A, Heidt S, et al. Sensitization in Transplantation Assessment of Risk 2025 Innate Working Group: The Potential Role of Innate Allorecognition in Kidney Allograft Damage. *Am J Transpl* (2025) 25:2038–47. doi:10.1016/j.ajt.2025.06.030
46. Chambon M, Koenig A. NK Cells: Not Just Followers but Also Initiators of Chronic Vascular Rejection. *Transpl Int* (2024) 37:13318. doi:10.3389/ti.2024.13318
47. Naesens M, Roufosse C, Haas M, Lefaucheur C, Mannon RB, Adam BA, et al. The Banff 2022 Kidney Meeting Report: Reappraisal of Microvascular Inflammation and the Role of Biopsy-based Transcript Diagnostics. *Am J Transpl* (2024) 24(3):338–49. doi:10.1016/j.ajt.2023.10.016
48. Zajacova A, Dupont LJ, De Leyn P, Ceulemans LJ, Vos R, Leuven Lung Transplant Group. Characteristics and Outcomes of 1500 Lung Transplantations in the Leuven Lung Transplant Program: Turning past Lessons into Tomorrow's Foundations. *Transpl Int* (2025) 38:15495. doi:10.3389/ti.2025.15495
49. Cashmore SJ, Barten MJ, Diekmann F, Epailly E, Fisher AJ, Gennery AR, et al. Current Usage of Extracorporeal Photopheresis in Solid Organ Transplantations in Europe: A Narrative Review. *Transpl Int* (2025) 38:14906. doi:10.3389/ti.2025.14906
50. Benazzo A, Bagnera C, Ius F, Del Fante C, Gottlieb J, Hoetzenecker K, et al. A European Multi-Center Analysis of Extracorporeal Photopheresis as Therapy for Chronic Lung Allograft Dysfunction. *Transpl Int* (2024) 36:11551. doi:10.3389/ti.2023.11551

ESOT | Transplant International

REVIEW
published: 02 February 2026
doi: 10.3389/ti.2025.15640

Understanding Machine Learning Applications in Lung Transplantation: A Narrative Review

Bieke Vercauteren[1,2†], Balin Özsoy[1,3,4†], Jasper Gielen[2†], Meixing Liao[2†], Ewout Muylle[1,3†], Jan Van Slambrouck[1,3†], Bart M. Vanaudenaerde[1†], Robin Vos[1,5†], Pieterjan Kerckhof[1†], Saskia Bos[1,5†], Jean-Marie Aerts[2†] and Laurens J. Ceulemans[1,3†]*

[1]Department of Chronic Diseases and Metabolism, Laboratory of Respiratory Diseases and Thoracic Surgery (BREATHE), KU Leuven, Leuven, Belgium, [2]Department of Biosystems, M3-BIORES, KU Leuven, Leuven, Belgium, [3]Department of Thoracic Surgery, University Hospitals Leuven, Leuven, Belgium, [4]Department of Oncology, Laboratory of Angiogenesis and Vascular Metabolism, Center for Cancer Biology, VIB, KU Leuven, Leuven, Belgium, [5]Department of Respiratory Diseases, University Hospitals Leuven, Leuven, Belgium

OPEN ACCESS

***Correspondence**
Laurens J. Ceulemans,
laurens.ceulemans@uzleuven.be

†ORCID:
Bieke Vercauteren
orcid.org/0009-0004-8674-6168
Balin Özsoy
orcid.org/0000-0002-3976-2286
Jasper Gielen
orcid.org/0000-0002-9459-836X
Meixing Liao
orcid.org/0000-0002-9715-4844
Ewout Muylle
orcid.org/0000-0002-0646-2615
Jan Van Slambrouck
orcid.org/0000-0002-7069-1535
Bart M. Vanaudenaerde
orcid.org/0000-0001-6435-6901
Robin Vos
orcid.org/0000-0002-3468-9251
Pieterjan Kerckhof
orcid.org/0000-0002-3806-4478
Saskia Bos
orcid.org/0000-0002-5336-5914
Jean-Marie Aerts
orcid.org/0000-0001-5548-9163
Laurens J. Ceulemans
orcid.org/0000-0002-4261-7100

Received: 24 September 2025
Revised: 24 September 2025
Accepted: 22 December 2025
Published: 02 February 2026

Citation:
Vercauteren B, Özsoy B, Gielen J, Liao M, Muylle E, Van Slambrouck J, Vanaudenaerde BM, Vos R, Kerckhof P, Bos S, Aerts J-M and Ceulemans LJ (2026) Understanding Machine Learning Applications in Lung Transplantation: A Narrative Review. Transpl. Int. 38:15640. doi: 10.3389/ti.2025.15640

Lung transplantation (LTx) offers life-saving therapy for patients with end-stage lung disease but remains limited by donor shortages, complex postoperative management and graft failure. Machine learning (ML) enables opportunities to address these challenges by identifying patterns in complex, high-dimensional data, thereby providing novel insights and improving outcomes. This review outlines ML studies in LTx and explains the methodologies. ML has demonstrated promising results in organ allocation and outcome prediction. Techniques such as support vector machines, and deep learning are useful in risk stratification, while methods like random forests improve interpretability and transfer learning supports model development in data-scarce settings. ML has a growing role in multi-omics data and imaging-based diagnostics. Despite promising results, barriers such as small datasets, cross-center inconsistency, poor interpretability, and limited external validation, hinder clinical adoption. Future progress requires multicenter collaborations, transparent methodologies, and integration within clinical workflows. ML should serve as complementary tool that enhances decision-making, rather than replacing clinical judgement. With careful implementation, it holds the potential to improve transplant outcomes.

Keywords: machine learning, artificial intelligence, transplantation, lung transplantation (LTx), review of literature

INTRODUCTION

Lung transplantation (LTx) is a life-saving treatment for end-stage lung disease. Despite surgical and perioperative advances, challenges remain, including donor shortage, primary graft dysfunction (PGD), and chronic lung allograft dysfunction (CLAD). As clinical data expand and pathophysiology is better understood, these challenges also increase in complexity. Traditional decision-making and predictive modelling is therefore limited.

Machine learning (ML), can identify complex, non-linear patterns, supporting outcome prediction and personalized care [1–5]. In solid organ transplantation, ML is increasingly used to predict survival and improve organ allocation [6]. Nonetheless, integration in LTx lags behind due to small, heterogeneous datasets and complex pathways [7].

The aim of this narrative review is twofold. First, to provide clinicians with a conceptual foundation that fosters understanding of ML. Second, to explore ML applications in LTx, covering outcome prediction, organ allocation, imaging, omics, and other applications.

PRINCIPLES OF MACHINE LEARNING

ML enables mathematical models to learn from data, identify patterns, and make predictions with minimal human intervention. By leveraging algorithms, ML models extract insights and predict outcomes [1]. ML is a central component of artificial intelligence (AI) and closely connected to data science and computer science. These domains overlap (**Figure 1**) in methodologies, applications, and objectives, making clear distinction difficult [1, 3–5].

ML employs datasets specific for the task. In medical datasets, clinical factors (e.g., age, smoking) serve as *dimensions* (features), while individual observations (e.g., patients, images) represent *samples* (data points). Based on whether labeled data (samples with known outputs) are used, ML approaches can be classified as supervised, unsupervised, and semi-supervised [1–5, 8].

Supervised ML uses *labeled data* to train predictive models [1–5, 8]. To ensure generalizability, datasets are divided into *training, validation, and testing subsets*. Models first learn patterns from the *training set*. The *validation set* aids in hyperparameter tuning (e.g., batch size, learning rate). It detects underfitting and overfitting, meaning that the model is too simple to capture the true patterns, or learns the noise in the data, respectively (**Figure 2**) [1–3, 8]. *Cross-validation* is used to ensure generalizability by partitioning the dataset into training and validation subsets. An approach is *k-fold cross-validation,* which divides data randomly into *k* (a number) folds. The model is trained on *k-1* folds and validated on the remaining one, repeating this process *k* times so each subset serves as validation once [1, 2, 5, 8]. Cross-validation ensures the model outcomes are robust and not dependent on a single random split of the dataset [1, 2, 5, 8]. Finally, the *test set*, an unseen portion of data, is used to evaluate the final model performance [1, 2, 8].

Supervised ML is used for *classification* and *regression*. Both utilize labeled datasets, but differ in output: *classification* predicts categories, *regression* predicts continuous values [1, 2, 5, 8].

Conversely, unsupervised ML analyzes *unlabeled data* to identify patterns [1–3, 5, 8]. Choosing between supervised and unsupervised learning can be difficult, particularly when labeled data are scarce. Semi-supervised ML bridges this gap by combining limited labeled data alongside many unlabeled samples, useful in medical research where data annotation is resource-intensive [1, 2, 8]. Commonly used ML methods, shown in **Figure 3**, are evaluated and compared using diverse metrics (**Table 1**).

STATE-OF-THE-ART OF MACHINE LEARNING IN LUNG TRANSPLANTATION

LTx involves a heterogeneous, limited patient population with extensive data. LTx recipients have worse outcome than other solid organ transplant recipients, highlighting persistent gaps. ML could contribute to personalized treatment and improved outcomes, as seen in other transplants [5, 9, 10].

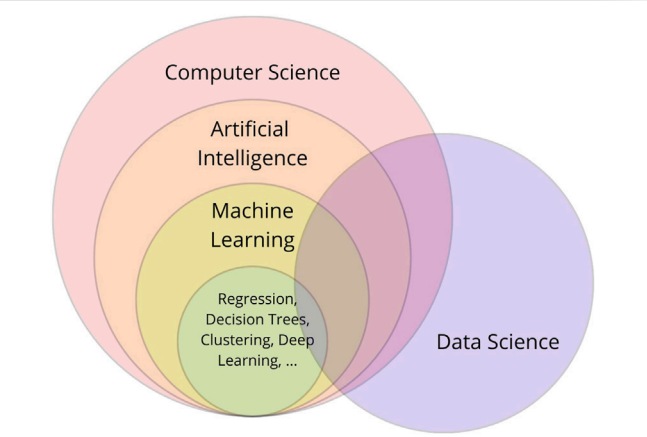


FIGURE 1 | Interrelationship between computer science, artificial intelligence, machine learning, and data science: a conceptual overview.

The following section reviews key studies, as far as we know (2004–2025), organized into: (1) outcome prediction, (2) organ allocation, and (3) imaging, omics, and other applications. A summary is presented in **Table 2**. Studies using simpler, borderline-ML methods are excluded from the main text but included in **Table 2** and **Figure 3**.

Outcome Prediction

Survival and Quality of Life

In a series of studies, Oztekin, Delen, Amini and colleagues demonstrated the value of ML for outcome prediction. Initially, they showed that ML outperformed expert–selected variables and traditional statistical models in predicting 9-year graft survival after heart–lung transplantation, identifying more relevant variables and relationships [12]. They applied logistic regression (**Supplementary Text**, **Figure 3A.2**), decision trees (DTs), and artificial neural networks (ANNs). *DTs* (**Figure 3A.6**) are interpretable models that recursively split data to form rule-based trees. They are sensitive to noise and require pruning (removing unnecessary parts) to improve generalizability [1, 2, 4, 5, 8]. *ANNs* (**Figure 3A.8**) are algorithms inspired by the brain (**Figures 4A–D**). The simplest form, a single-layer perceptron, mimics a biological neuron. Adding hidden layers, referring to synaptic connections creates a multilayer perceptron (MLP) [1, 2, 4, 5, 8]. Unlike DTs, ANNs lack interpretability and rely on large datasets, therefore, the United Network for Organ Sharing (UNOS) cohort of 16,604 patients was crucial for this approach [5, 8].

Later, their work was extended to survival estimation, again comparing ML with expert-selected and literature-based variables. ML outperformed both approaches by retaining important predictors overlooked in traditional methods. They applied DTs and ANNs, and additionally introduced support vector machines (SVMs) [13]. *SVMs* (**Figure 3A.5**) are algorithms that maximize the margin between classes (distance between the decision boundary and the nearest data points from

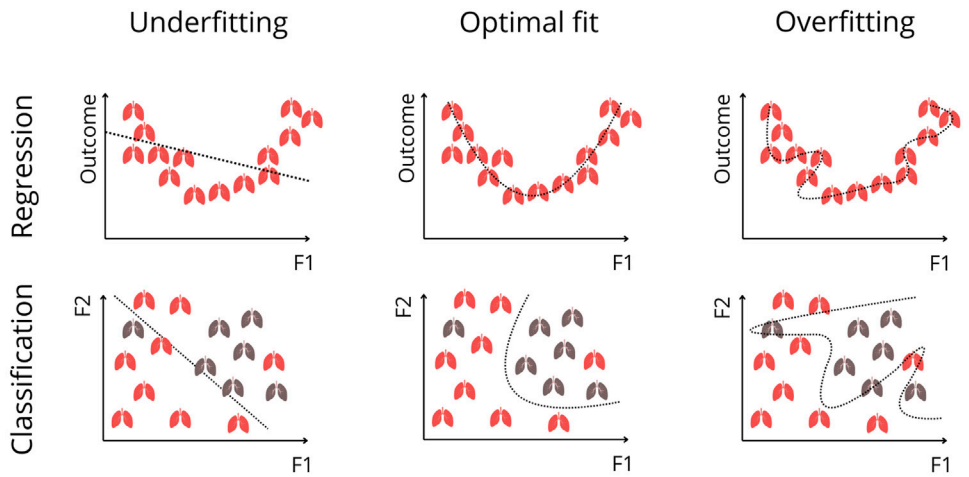


FIGURE 2 | Visualization of Underfitting, Optimal Fitting, and Overfitting in Regression and Classification. The top row illustrates regression settings, where the Outcome axis represents a continuous clinical measure (e.g., survival probability, biomarker level), and F1 represents a predictive feature. Every depicted lung represents a sample (e.g., patient). Underfitting occurs when the model is too simple to capture the true nonlinear relationship, whereas overfitting occurs when the model follows noise instead of the underlying trend. The optimal fit captures the true pattern without modeling random fluctuations. The bottom row shows these concepts in classification, where F1 and F2 represent two predictive features, and each lung corresponds to an individual patient belonging to one of two outcome classes (e.g., favorable vs. poor outcome). The model's decision boundary is shown as a dotted line. A linear boundary underfits when classes are not linearly separable. An overly complex boundary overfits by tailoring itself to noise and outliers. The optimal fit provides a smooth, generalizable separation between classes.

each class). An innovation is the kernel trick, which enables SVMs to classify nonlinearly separable data by mapping it into higher-dimensional space (**Figure 5**) [1–5, 8]. Model performance was compared using Cox regression (**Supplementary Text**, **Figure 3A.3**). Subsequently, k-means clustering, two-step cluster analysis, and conventional heuristic approaches were used to determine the optimal number of patient risk groups. *Unsupervised k-means clustering* (**Figure 3B.1**) groups data into a predefined number of clusters based on feature similarity by iteratively assigning samples to the nearest centroid (center of a cluster) and updating centroids as the mean of assigned samples. It offers an unbiased way to explore risk groups [1–5, 8]. In this study, three clusters were optimal [13].

In 2011, a DT–based hybrid model was designed to provide an interpretable ML approach. However, its accuracy remained low. Moreover, using variables predefined from previous studies biased the model, potentially missing important interactions [14]. To predict quality of life, Genetic Algorithm (GA)-based approaches for feature selection were introduced [16], particularly useful for complex, feature-rich domains with limited samples as in LTx. *GAs* (**Figure 3C.1**) are optimization techniques inspired by biological evolution, using selection, crossover, and mutation to find optimal solutions, e.g., determining representative variables [5, 59]. The GA was combined with three classification algorithms: SVM, ANN and k-Nearest Neighbors (kNN) (**Figure 3A.7**). Unlike other algorithms, *kNN* predicts without training, by averaging outcomes of the k most similar samples to unseen input. Performance depends on data quality, choice of distance metric, and k. In high-dimensional data, kNN's accuracy can degrade [1, 2, 5, 8], therefore, combining it with GA is appropriate.

Subsequent research performed classification of post-LTx survival (≤1 year vs. ≥10 years), incorporating additional methods, namely ensemble models such as random forests (RF) and gradient boosting trees [21]. *Ensemble learning* combines multiple models to improve predictive accuracy, reduce overfitting, and enhance robustness [1, 2, 5, 8]. *Bagging* (bootstrap aggregating) (**Figure 3A.10.1**) improves stability by training on different data subsets [1, 2, 5, 8]. *RF* is a common bagging method that aggregates DTs [1, 5, 8]. *Boosting* (**Figure 3A.10.2**) builds models sequentially, each correcting errors of the previous one [1, 5, 8]. Among all models, RF achieved the best performance. To improve model transparency, the authors employed an explainable AI (XAI) method: *SHapley Additive Explanations* (SHAP), a model-agnostic framework that quantifies each feature's contribution to a prediction by considering all possible feature combinations [60]. SHAP identified Hepatitis B surface antibody and forced expiratory volume in one second (FEV1) as predictors of long-term survival. However, methodological limitations warrant consideration. The use of binary classification (≤1 year vs. ≥10 years) excluded nearly half of the cohort [21]. This neglects intermediate survival, arguably the most challenging to predict, which makes the modest performance noticeable.

Moro et al. created a DT for survival predictions. Using UNOS data, 47 features were identified via stepwise logistic regression, assuming linear relationships. Consequently, meaningful nonlinear interactions may have been missed, and reducing 60 to 47 variables offered minimal dimensional or computational benefit. The final DT used six key predictors, including three postoperative variables, limiting the model's preoperative prognostic utility, despite its interpretability.

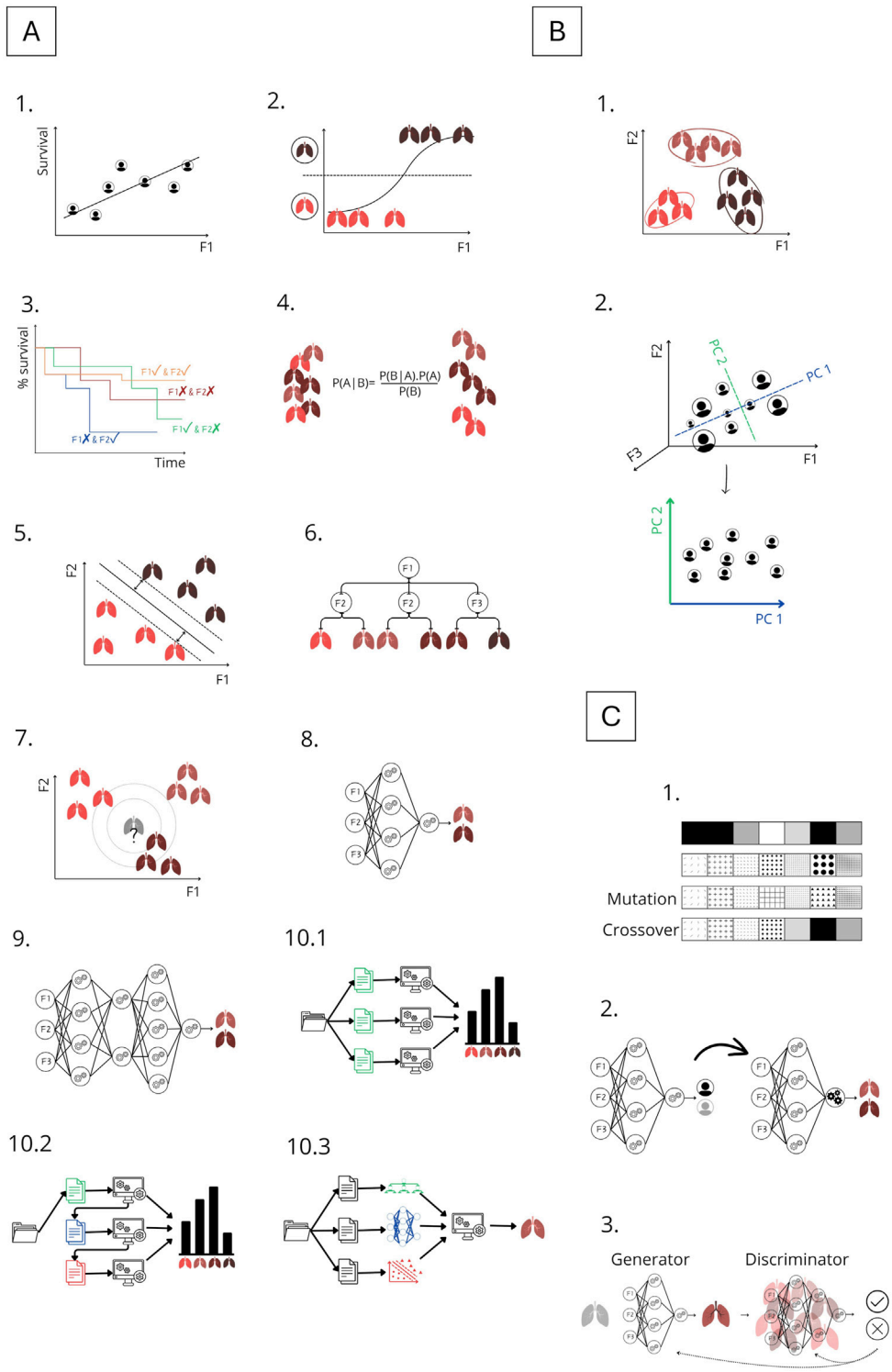


FIGURE 3 | Overview of Machine Learning Methods Explained in Chapter 2. Panel **(A)** Supervised learning methods: **A.1** Linear regression; **A.2** Logistic regression; **A.3** Cox regression; **A.4** Naive Bayes; **A.5** Support vector machine; **A.6** Decision tree; **A.7** k-Nearest Neighbors; **A.8** Artificial neural network; **A.9** Deep learning; **A.10** Ensemble methods: **A.10.1** Bagging, **A.10.2** Boosting, **A.10.3** Stacking. Panel **(B)** Unsupervised learning: **B.1** K-means clustering; **B.2** Principle component analysis. Panel **(C)** Advanced methods: **C.1** Genetic algorithm; **C.2** Transfer learning; **C.3** Generative adversarial network (GAN). F1-F3: represents features; P1-PC2 represents principle components.

TABLE 1 | Common metrics used in machine learning.

Number	Metric	ML type	Description	Common use case
1	Accuracy	Classification	Proportion of correct predictions among total samples	General performance for balanced binary/multiclass classification
2	Mean squared error (MSE)	Regression	Average of squared differences between predicted and true values	Penalize large errors
3	Root mean squared error (RMSE)	Regression	Square root of MSE	Interpretability with penalties
4	Precision	Classification	Proportion of true positives among predicted positives	When false positives are costly (e.g., spam filter)
5	Recall/sensitivity	Classification	Proportion of true positives among actual positives	When false negatives are costly (e.g., disease detection)
6	Specificity	Classification	Proportion of true negatives among actual negatives	When false positives must be avoided (e.g., excluding innocent suspects)
7	Area under the receiver operating characteristic curve (AUROC)	Classification	Area under the receiver operating characteristic curve, combination recall and false positive rate (sometimes interchanged with AUC)	Binary classification, model comparison
8	F1-score	Classification	Harmonic mean of precision and recall	Imbalanced classification
9	Confusion matrix	Classification	Table showing true positives, false positives, true negatives and false negatives	Detailed prediction breakdown
10	Gini index	Classification	Measure of impurity used in splits	Decision tree splitting criterion
11	C-statistic (concordance)	Classification	Probability that the model correctly ranks outcomes	Ranking in survival analysis
12	R^2 score	Regression	Explained variance ratio	Model fit evaluation
13	Silhouette score	Clustering	Cohesion and separation of clusters	Cluster validation
14	Intraclass inertia	Clustering	Compactness of the clusters, average of the distances between the centroids and the datapoints	Cluster validation

Eight subgroups (decision nodes) showed distinct survival curves. As expected, best outcomes occurred in younger recipients with short hospital stays, limited ventilation support, and no reintubation [25].

To compare survival between increased risk for disease transmission (IRD) organ recipients versus non-IRD organ recipients, Mark et al. applied RF and Cox regression. As Cox regression performed best, it was selected for further analysis, which somewhat diminished the novelty of ML implementation. Nevertheless, the study offered a data-driven perspective to expand the donor pool, demonstrating a 7.2% improvement in 5-year survival for IRD lung transplant recipients [17].

Unlike the prior study, Tian et al. demonstrated that RF can outperform Cox regression, for survival prediction under standard conditions, achieving high predictive accuracy. Generalizability across subgroups with different diagnoses and treatments was reported. However, the single-center design and limited sample size may question this [22].

The effectiveness of RF, combined DTs, was also shown by Fessler et al., analyzing 284 variables across 12 perioperative stages to predict one-year mortality. As presumed, the accuracy went up by including information of later stages. Lung allocation score (LAS) emerged as top predictor [18].

Primary Graft Dysfunction

A subsequent study by Fessler et al. used gradient boosting to predict PGD3, a syndrome linked to adverse outcomes [61]. Extracorporeal membrane oxygenation use, along with recipient factors, were revealed as top predictors [20]. Due to the short length of these papers [18, 20], the information provided on the ML implementation is limited. In their most recent paper [28], predicting PGD3 at 72h, they offer more information about logistic regression and *XGBoost*, an efficient gradient boosting variant, that improves computational memory usage, well-suited for large datasets [62]. Fessler's studies introduce an innovative approach by progressively incorporating data from successive transplant phases, allowing the prognosis to be refined at each stage.

Michelson et al. similarly predicted PGD3 using pretransplant data, enabling potential application in patient selection and pretransplant counseling. From 100 features, Least Absolute Shrinkage and Selection Operator (LASSO) (**Supplementary Text**) selected 11 predictors. Among four models, kNN performed best and was released as open-access risk calculator [26].

With data from 802 patients, Xia et al. evaluated nine algorithms. RF classified PGD3 best. SHAP identified blood loss as important, but prior feature selection, based on linear relation assumption, may have introduced selection bias [27].

Other Outcome Parameters

Using a small, unbalanced dataset, Tian et al. developed eight ML models combined with seven feature selection methods to predict airway stenosis requiring clinical intervention. Key predictors in RF included postoperative 6-minute walk test and indication for LTx. This model could guide postoperative follow-up [24].

Braccioni et al. assessed how clinical parameters relate to symptom severity during exercise testing after LTx. *Boruta*, a feature selection method based on RF [63], revealed associations for limited exercise capacity: dyspnea correlating with peak ventilation and work rate, muscle effort with breathing reserve, and muscle pain with VO_2 peaks. These findings

TABLE 2 | Overview of Studies about machine learning in lung transplantation.

Autor(s) (Year)	Study population	Input	Output	Model(s)	Metrics	Train/Validation/ Test and validation method	Transparency and explanations of ML (mathematical background, architecture, ...)
Outcome prediction							
Troiani and Carlin [11]*	30 LTx recipients (over 60 subject-years)	2-week epochs of daily/biweekly FEV1 and symptom data	Prediction of acute bronchopulmonary disease events	Heuristic rule-based, classical linear-logistic regression, Bayesian models	Bayesian model AUROC = 0.882 Sensitivity = 0.886 Specificity = 0.955	2-fold cross-validation	Detailed model descriptions, Bayesian priors disclosed, transparency limited in heuristic model
Oztekin et al. (2009) [12]	16604 heart-LTx patients (UNOS)	283 features (demographics, health-related and transplant-related)	9-year graft survival	DTs, ANNs, *logistic regression, Cox regression*	MLP Accuracy = 0.859 Sensitivity = 0.847 Specificity = 0.869	10-fold cross-validation	Hazard function, metrics, k-fold cross-validation, no insight in ML models (brief explantation)
Delen et al. [13]	106398 thoracic patients (UNOS)	565 features (demographics, health-related and transplant-related)	Graft survival time, risk groups	SVM, ANN,DTs, Cox regression and k-means, 2-step, heuristic clustering	SVM MSE = 0.023 R^2 = 0.879 k-means clustering 3 risk groups intraclass intertia = $1{,}68 \times 10^{-8}$	10-fold cross-validation	Hazard function, metrics, k-fold cross-validation, no insight in ML models (brief explantation)
Oztekin et al. [14]	6512 LTx records (UNOS)	25 features	Predict LTx success (graft survival and quality of life)	Structural equation modeling (meaning: Statistical method showing how different factors are related to each other, including hidden (latent) ones) DT	R^2 = 0.68	10-fold cross-validation	Mathematical methodology: Structural equation modeling and composite scores, metrics, k-fold cross-validation
Pande et al. [15]	509 LTx patients (9471 FEV1 evaluations over time)	Time-series FEV1, demographic and clinical features	Predict FEV1 over time and key feature-time interactions	Boosted DTs	RMSE = 0.115–0.421	In sample cross-validation	Models, algorithms, cross-validation, metrics
Oztekin et al. [16]	3684 LTx records (UNOS)	147 features	Predict quality of life post LTx	GA-kNN, GA-SVM, and GA-ANN	GA-SVM Accuracy = 0.994 Precision = 0.991–0.997 Sensitivity = 0.992–0.998 Specificity = 0.996–0.998 F1 = 0.991–0.995	5-fold cross-validation	Normalization, GA, k-fold cross-validation, metrics
Mark et al. [17]	LTx candidates: 1010 IRD, 12013 non-IRD and 19217 waitlist (UNOS)	Top 5 (out of >100 features): recipient and donor characteristics, IRD status, time on waitlist (UNOS)	Compare 5-year survival for IRD vs. non-IRD organ offers	Cox Proportional Hazards, random forests (500 DTs)	7.2% 5-year survival with IRD lung vs. non-IRD 69.9% of simulations favored IRD lung RMSE = 5.3	5-fold cross-validation	RF details
Fessler et al. [18]	410 double LTx recipients	284 patient, donor, and surgical variables in 12 stages	Predict one-year post-transplant mortality	RF	AUROC = 0.65–0.75	Train/test (80/20), 40 repetitions	Limited

(Continued on following page)

TABLE 2 | (*Continued*) Overview of Studies about machine learning in lung transplantation.

Autor(s) (Year)	Study population	Input	Output	Model(s)	Metrics	Train/Validation/ Test and validation method	Transparency and explanations of ML (mathematical background, architecture, . . .)
Braccioni et al. [19]	24 bilateral LTx recipients	24 recipients variables, incremental cardio-pulmonary exercise testing	Associations between the severity of symptoms (dyspnea, muscle effort, muscle pain) and exercise testing parameters	RF/Boruta	-	5-fold cross-validation (10 resamples)	Limited but short explanation RF/ Boruta
Fessler et al. [20]	478 double LTx recipients	6 recipient, donor, intraoperative features in 9 stages	Predict PGD3	Gradient boosting algorithm, SHAP	AUROC = 0.7–0.87	Train/test (80/20)	Limited
Amini et al. [21]	9864 adult US LTx recipients	171 features (demogragics, clincal, transplant)	Classify short-term (≤1 year) vs. long-term (≥10 years) survival after LTx	RF, DT, gradient boosted trees, kNN, ANN, SVM, logistic regression, SHAP	RF Accuracy = 0.7792 Sensitivity = 0.7626 Specificity = 0.7958 AUROC = 0.79	10-fold cross-validation	SHAP
Tian et al. (2023) [22]	504 adult LTx recipients	16 out of 22 clinical variables: recipient, donor, surgical and post-op factors	Predict overall survival	RF, Cox regression	RF integrated AUROC = 0.879 (better than Cox: Integrated AUROC = 0.658)	Train/test split (70/30), bootstrapping (1000 resamples)	Variable importance, overal limited
Melnyk et al. [23]*	369 patients, 125 cases	11 significant out of all preoperative recipient characterstics, procedural variables, perioperative blood product transfusions, and donor charactersitics	Relation between blood transfusion and morbitity (6 endpoints)	Elastic Net regression	Accuracy = 0.765 Sensitivity: 0.80 Specificity: 0.69	Cross-validation (500 repeats)	Limited
Tian et al. [24]	381 LTx patients	15 features: recipient and postoperative	Prediction of airway stenosis requiring clinical intervention	56 models: 7 features selection methods combined with 8 ML models	RF + determination coefficient AUROC = 0.760 Sensitivity = 0.782 Specificity = 0.689	Bootstrap validation (1000 resamples)	Limited
Moro et al. [25]	27296 LTx recipients (UNOS)	60 recipient and donor data	1-, 5-, 10-year survival propabilities	DT; stepwise logistic regression for variable selection	Logisitic regression Accuracy = 0.653 8 subgroups (DT)	Train/test split (70/30), 10-fold cross-validation	Logistic model, DT given, training explantation limited
Michelson et al. [26]	576 bilateral LTx recipients (UNOS, Unet, local)	11 out of 100 donor, recipient pretransplant features	Prediction of PGD3 within 72 h after LTx	LASSO + kNN, logistic regression, XGBoost, SVM, SHAP	kNN AUROC = 0.65 F1 = 0.62	Train/test split (75/25), 5-fold cross-validation (training set 50 resamples)	TRIPOD, preprocessing but limited info about ML, model hosted at pgdcalc.wustl.edu
Xia et al. [27]	802 LTx recipients	9 out of 37 features: Clinical	Predict PGD3 within 72 h post-transplant	9 models (DT, kNN, MLP,RF, SVM, . . .), SHAP, LASSO	RF: Internal validation AUROC = 0.7975 Sensitivity = 0.7520 Specificity = 0.7313	Train/validate/test split (56/24/20), 5-fold cross-validation	Limited, but visualizations and some information about RF

(Continued on following page)

TABLE 2 | (*Continued*) Overview of Studies about machine learning in lung transplantation.

Autor(s) (Year)	Study population	Input	Output	Model(s)	Metrics	Train/Validation/ Test and validation method	Transparency and explanations of ML (mathematical background, architecture, ...)
Fessler et a. [28]	477 LTx patients	66 features in 9 stages	Predict PGD3 at 72h	XGBoost, logistic regression, SHAP	XGBoost: AUROC = 0.84 Sensitivity = 0.81 Specificity = 0.68	Train/test split (80/20) (500 resamples), grid search approach, 5-fold cross-validation	XGBoost model hyperparameter tuning
Organ allocation							
Dueñas-Jurado et al. [29]	404 LTx cases	36 donor-recipient variables (clinical, surgical, functional)	Predict 6-month graft survival; optimize donor-recipient matching	Linear regression initial covariates and product units neural networks (LRIPU) model	-	Train/test1/test2 (70/13/17)	Model and coefficients
Zafar et al. [30]	15124 double LTx recipients (UNOS)	19 out of 42 recipient, donor, and transplant variables	Predict 1-, 5-, 10-year survival and half-life; and classify into risk clusters	Cox-LASSO, backward Cox and RF-Cox, clustering via expectation-maximization (LAPT)	Cox-LASSO C statistic for 1-year survival = 0.67 C statistic for 5-year survival = 0.64 C statistic for 10-year survival = 0.72	Train/test (70/30)	Limited
Brahmbhatt et al. [31]	19900 adult LTx patients (UNOS)	Pre-transplant recipient data	Prediction of 1- and 3-year post-transplant mortality	LAS, Houston Methodist model, clinician model, LASSO, RF	RF AUROC = 0.62 Specificity = 0.76 Sensitivity = 0.44 (similar to all other models)	Train/test split (85/15)	Limited
Sage et al. [32]	725 EVLP donor lung assessments	Recipient, donor and 24 EVLP variables	Predict transplant suitability/ extubation <72h	XGBoost (InsighTx model), RF	AUROC: 0.75–0.85	Train/test (80/20), 5-fold cross-validation	Code shared
Pu et al. [33]	4610 subjects	Demographics and computed tomography scans	Prediction of left/right/ total lung volume, thoracic cavity volume, and heart volume to improve size matching	CNN, 8 ML models (Incl. RF, kNN, DTs)	MLP right and left lung, thoracic cavity R^2 = 0.501–0.628 XGBoost heart and total lungs R^2 = 0.430–0.514	Train/validate/test (80/10/10), 10-fold cross-validation	10-Fold cross-validation, visualisations, hyperparameters
Dalton et al. [34]	13204 LTx candidates and 20763 recipients (SRTR)	Demographics and clinical features	Prediction of waitlist mortality at 1, 3, 6 months and post-transplant survival at 1, 3, and 5 years	Cox regression (LAS/lung Composite allocation score), re-estimated models, RF, linear discriminant analysis, logistic regression, boosted DT	Waitlist AUROC = 0.85–0.93 Transplant survival AUROC = 0.56–0.62	10-fold cross-validation	Model explanation in the authors' Supplementary Material
Imaging, omics and other applications							
Bartholmai et al. [35]	119 subjects with interstitial lung disease	High-resolution computed tomography, pulmonary function tests, clinical data	Quantitative classification of interstitial lung disease patterns (emphysema, ground glass, honeycombing, normal and reticular) with correlation to physiology and clinical outcomes	Computer aided lung Informatics for pathology evaluation and rating (CALIPER), ANN, Bayes, SVM, kNN	Analysis of similarity within a cluster R = 0.962	-	Limited, feature extraction

(Continued on following page)

TABLE 2 | (*Continued*) Overview of Studies about machine learning in lung transplantation.

Autor(s) (Year)	Study population	Input	Output	Model(s)	Metrics	Train/Validation/ Test and validation method	Transparency and explanations of ML (mathematical background, architecture, . . .)
Barbosa et al. (2017) [36]	176 LTx patients	Quantitative Computed tomography scans, PFT, semi-quantitative Computed tomography scores	Diagnose BOS	Multivariate logistic regression, SVM, PCA	Quantitative Computed tomography SVM PCA AUROC = 0.817	10-fold cross-validation (90%–10%)	Limited
Weigt et al. [37]	17 LTx recipients, 1 year post-LTx BAL samples	BAL cell pellet transcriptome (microarray); 40 genes with differential expression (immune-related)	Prediction of incipient CLAD within 2 years post-BAL	Unsupervised hierarchial clustering, SVM, PCA	SVM Accuracy = 0.941	Leave-one-out cross-validation	Limited
Barbosa et al. [38]	71 LTx recipients	Quantitative Computed tomography scans, PFT	Predict eventual onset of BOS	SVM	Accuracy = 85% (3 features); sensitivity = 73.3%; specificity = 92.3%	Train/test (80/ 20 or 90/10) with 500 or 100 random combinations	Limited
Halloran et al. [39]	242 single-piece LTx biopsies (transbronchial biopsies)	Gene expression (microarrays), 453 rejection-associated transcripts	Identify disease states/ phenotypes: normal, T cell mediated rejection, antibody mediated rejection, injury	Unsupervised archetypal analysis, PCA	-	-	Limited, sum of scores
Cantu et al. [40]	113 LTx patients	Clinical, recipient, donor and transplant features, preprocurement donor lung biopsies (gene expression of innate immunity: Toll-like receptor and nod-like receptor pathways)	Prediction of PGD3 at 48–72h post-transplant	Feed-forward deep learning	Toll-like receptor AUROC = 0.776 Sensitivity = 0.786 Specificity = 0.706	5-fold cross-validation	Architecture DL model
Halloran et al. [41]	243 mucosal biopsies from 214 LTx patients	Gene expression (microarrays), 315 rejection-associated transcripts (RATs), 11 pathogenesis based transcripts	Classification into molecular phenotypes: normal, rejection, late inflammation, injury	Unsupervised archetypal analysis, PCA	-	-	Limited, metrics in the authors' Supplementary Material
Halloran et al. [42]	457 transbronchiale and 314 mucosale biopsies	Gene expression (microarray), rejection-associated transcripts	Prediction of graft survival based on molecular T cell mediated rejection phenotype	Unsupervised archetypal analysis, PCA, RF	-	-	Limited, metrics in the authors' Supplementary Material
Dugger et al. [43]	49 LTx recipients (small airway brushes and transbronchial biopsies)	RNAseq and digital RNA counts	Diagnosis of CLAD and prediction of graft survival	LASSO logistic regression, RF	RF airway brushing AUROC = 0.84 Transbronchial biopsies AUROC = 0.62	Leave-one-out cross-validation	Limited
Berra et al. [44]	40 LTx patients (BAL)	Protein expession (incl. Angiotensin II-related)	CLAD development	Linear discriminant analysis, SVM, Bayes, quadratic discriminant analysis	CLAD vs. no-CLAD AUROC = 0.86 CLAD development AUROC = 0.97	Leave-one-out cross-validation	Limited

(Continued on following page)

TABLE 2 | (*Continued*) Overview of Studies about machine learning in lung transplantation.

Autor(s) (Year)	Study population	Input	Output	Model(s)	Metrics	Train/Validation/ Test and validation method	Transparency and explanations of ML (mathematical background, architecture, . . .)
McInnis et al. [45]	88 CLAD patients post-LTx	Computed tomography scans	CLAD phenotype prediction and graft survival prognosis based on lung texture (ML and radiologist scores): Normal, hyperlucent, reticular, ground-glass, honeycomb	Computer-aided lung Informatics for pathology evaluation and rating, Cox regression	Sensitivity: 0.90 Specificity: 0.71 Accuracy: 0.75 AUROC: 0.851	-	Limited
Tran-Dinh et al. [46]	40 LTx recipients	Plasma levels of soluble CD31, oxygenation ratio and respiratory sequential organ failure assement score at 24h/ 48h/72h	Predict acute cellular rejection within 1 year after LTx	Deep convolutional neural network using time series of biomarkers and multivariate modeling	AUROC = 0.85 Accuracy = 0.87 precision = 0.93 Recall = 0.33–1 (depending on class)	Stratified k-fold cross-validation and external test set with class weighting	Network architecture, modeling methods, time series handling and statistical background
Zhang et al. [47]	243 LTx patients (mucosal biopsies)	Gene expression profiles (19420 genes)	Prediction of 4 clinical response subtypes post-LTx: no rejection, rejection, late inflammation–atrophy, recent injury	Feature selection: boruta and others Classifiers: SVM, RF, kNN, DT	SVM Accuracy = 0.992 (247 genes used)	10-fold cross-validation	Metrics
Su et al. [48]	59 LTx recipients, 181 sputum samples	16S rRNA microbiota sequencing and clinical biomarkers (procalcitonin, T-lymphocyte levels)	Differentiate infection vs. acute rejection vs. event-free	RF, linear discriminant analysis	Infection vs. event-free AUROC = 0.898 Rejection vs. event-free AUROC = 0.919 Infection vs. rejection AUROC = 0.895	10-fold crossvalidation	Limited
Watzenboeck et al. [49]*	19 LTx recipients (BAL)	Microbiome (16S rRNA), metabolome, lipidome, BAL cell composition, clinical data, lung function tests	Predict FEV1 changes at 30/60/90 days (lung function trajectory)	ridge regression models	30 days r = 0.76 60 days r = 0.63 90 days r = 0.42	Nested cross-validation (train: 3-fold cross-validation, test: 4-fold cross-validation)	Limited
Stefanuto et al. [50]	35 LTx recipients, 58 BAL and blind bronchial aspirate samples	VOC profiles (386 features, reduced to 20 features)	Predict severe (PGD3) vs. mild/no PGD (PGD0–2)	SVM	AUROC = 0.90 Accuracy = 0.83 Sensitivity: 0.63 Specificity: 0.94	Train/test (50/50), leave-one-out cross-validation	Limited, visualisation of ML pipline
Qin et al. [51]	97 human LTx paired biopsies (pre/post-LTx)	Expression profiles (microarrays, incl. transcriptomics for cuproptosis-related genes)	Diagnosis of lung ischemia–reperfusion injury, identification of cuproptosis-related biomarkers	LASSO, SVM + recursive feature elimination, RF, logistic regression	15 biomarker, for each AUROC >0.8 Logisitic regression AUROC = 0.96	Train/test (53/47), validation in rat model	Limited
Wijbenga et al. [52]*	152 LTx recipients	Exhaled breath via SpiroNose (7-sensor eNose); patient and clinical characteristics	Diagnosis of CLAD and discrimination of phenotypes	Partial least squares discriminant analysis, logistic regression	AUROC = 0.94 Specificity = 0.78 Sensitivity = 1 Discrimination BOS vs. Restrictief allograft syndroom AUROC = 0.95	Train/test (67:33); 10-fold cross-validation	Limited

(Continued on following page)

TABLE 2 | (*Continued*) Overview of Studies about machine learning in lung transplantation.

Autor(s) (Year)	Study population	Input	Output	Model(s)	Metrics	Train/Validation/ Test and validation method	Transparency and explanations of ML (mathematical background, architecture, . . .)
Ram et al. [53]*	80 out of 100 donor lung pairs (Computed tomography-imaged *ex situ*)	*Ex vivo* CT scans, donors and recipient features	Donor lung suitability classification; prediction of ICU stay, PGD3 and 2-year CLAD	Dictionary learning (supervised ML) seen as a simpler technique	Accuracy = 0.727 AUROC = 0.743 F-score = 0.75 Precision = 0.78 Recall = 0.74	Train/test split (18/82)	In their Supplementary Material: explanation and formulas dictionary learning, sparse coding, classification, training
Chao et al. [54]	113 donor lungs evaluated with *ex vivo* lung perfusion	Chest radiographs, functional EVLP data	Predict transplant suitability and early post-transplant ventilation outcomes	Extreme gradient boosting (XGBoost)	Combined model AUROC = 0.807 Sensitivity = 0.76 Specificity = 0.89–0.94	75%–25% training-test split, repeated with 30 random seeds	Limited
Gouiaa et al. (2024) [55]	40 LTx patients	Plasma levels of soluble CD31, oxygenation ratio and respiratory sequential organ failure assement score at 24h/ 48h/72h	Predict acute cellular rejection within 1 year after LTx	Taelcore (topological autoencoder, ANN classifier) compared to other models (incl. RF, kNN)	MSE = 0.307 RMSE = 0.0.38	Stratified k-fold cross-validation; training/test split 75/25%	Topological loss function, persistence homology, entropy, rips filtration, metrics, short explanation other models, open-source code (GitHub)
Gao et al. [56]	113 + 97 lung graft biopsy samples	38 signature genes	Prediction of ischemia–reperfusion injury and PGD	Weighted gene coexpression network analysis, LASSO, RF and nomogram	AUROC >0.70 for all 4 genes	LASSO: 10-fold cross-validation	Limited, small explanations of models
Chen et al. [57]*	160 LTx patients	Demographics, LTx data and 69 lab indicators	Predict time to first rejection	LASSO regression, multivariate Cox model	1 year AUROC = 0.799 2 years AUROC = 0.757 3 years AUROC = 0.892	Train/test (70/30) 10-fold cross-validation	Limited
Choshi et al. [58]	117 + 6 LTx patients (87112 datapoints)	36 clinical factors, time series data of tacrolimus doses and route of administration	Predict tacrolimus trough levels	Multivariate long short-term memory: an improved RNN, SHAP	R^2 = 0.67 Tacrolimus trough levels within ±30% of actual = 88.5%	Train/validate/test (80/10/10)	Metrics

Partitioned in "outcome prediction," "organ allocation" and "Imaging, omics and other applications," in chronological order. If an article was not discussed in the text, an asterisk is placed next to it. If multiple models were tested, metrics were reported for best-performing ML methods. ANN, Artificial Neural Network; AUROC, Area Under the Receiver Operating Characteristic Curve; BAL, Bronchoalveolar Lavage; BOS, Bronchiolitis Obliterans Syndrome; CLAD, Chronic Lung Allograft Dysfunction; DL, Deep Learning; DT, Decision Tree; EVLP, Ex Vivo *Lung Perfusion; FEV1, Forced Expiratory Volume in one second; GA, Genetic Algorithm; IRD, Increased Risk for Disease Transmission; kNN, k-Nearest Neighbors; LAPT, Lung Transplantation Advanced Prediction Tool; LAS, Lung Allocation Score; LASSO, Least Absolute Shrinkage and Selection Operator; LTx, Lung Transplantation; ML, Machine Learning; MLP, Multilayer Perceptron; MSE, Mean Squared Error; PCA, Principal Component Analysis; PFT, Pulmonary Function Test; PGD, Primary Graft Dysfunction; RF, Random Forest; RMSE, Root Mean Squared Error; RNN, Recurrent Neural Network; SHAP, SHapley Additive Explanation; SVM, Support Vector Machine; UNOS, the United Network for Organ Sharing; VOC, Volatile Organic Compound.*

linked reduced aerobic capacity and high ventilatory cost to symptom severity. DT visualizations offered interpretable insights to guide exercise prescriptions [19]. Despite the small dataset (n = 24), the authors justified using ML, noting the method performs well in small, high-dimensional datasets without assuming normality or independence. Nonetheless, small cohorts increase overfitting risk and limit generalizability of the findings.

To analyze repeated FEV1 measurements after LTx, Pande et al. developed a longitudinal model, handling challenges as within-subject correlation, unequal time intervals, and unbalanced designs. Although FEV1 typically declines over

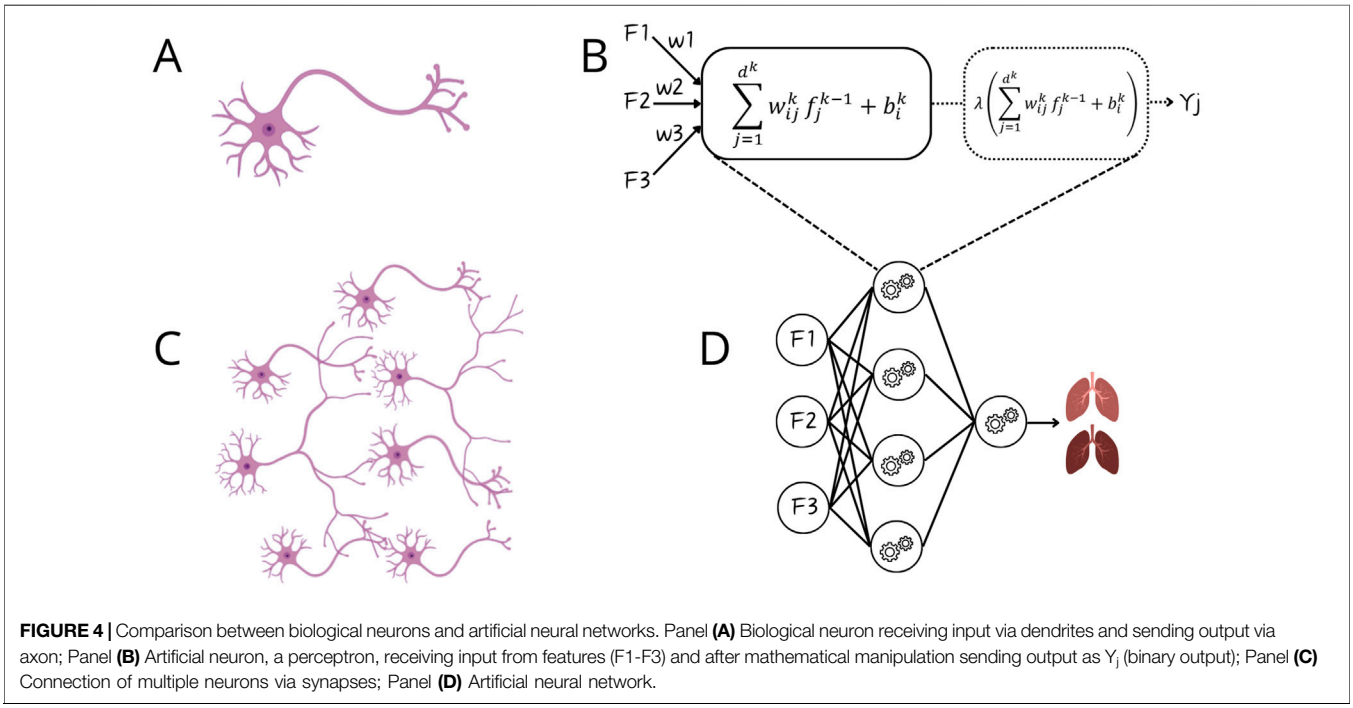


FIGURE 4 | Comparison between biological neurons and artificial neural networks. Panel **(A)** Biological neuron receiving input via dendrites and sending output via axon; Panel **(B)** Artificial neuron, a perceptron, receiving input from features (F1-F3) and after mathematical manipulation sending output as Y_j (binary output); Panel **(C)** Connection of multiple neurons via synapses; Panel **(D)** Artificial neural network.

time, patterns vary with individual factors. The method was clearly described and implemented in an R package [15].

Overall, the studies reviewed above show the potential of ML in LTx, but the applications stay rather limited. Stronger tools, e.g., deep learning (DL), could be implemented, as seen in section *Organ Allocation* [33].

Organ Allocation

LTx faces suboptimal organ allocation, causing long wait times and significant candidate mortality [64]. Varying donor selection criteria across centers limits organ availability. Allocation studies suffer from bias, as unaccepted organs are absent in training datasets. Unlike other transplants with comprehensive donor-recipient risk stratification, LTx allocation largely neglects the combined influence of factors [30].

To address these challenges, Zafar et al. developed the LTx Advanced Prediction Tool (LAPT). Based on 15,124 UNOS cases, LAPT grouped patients into low-, medium-, and high-risk subsets. LAPT outperformed LAS by predicting 1-, 5-, and 10-year survival and graft half-life for donor-recipient matches. This web-based tool enables data-driven allocation beyond recipient-centric systems [30].

Dueñas-Jurado et al. combined logistic regression with ANNs for donor-recipient matching. They incorporated donor, recipient and perioperative variables to predict 6-month graft survival, claiming to outperform traditional methods, although metrics were not reported. Key predictors included low pre-transplant CO_2, while prolonged donor ventilation, older donor and recipient age were linked to poorer outcomes [29].

To assess the suitability of donor lungs, Sage et al. created InsighTx, a RF model integrating *ex vivo* lung perfusion (EVLP) and other variables, offering a quantitative approach to evaluate and

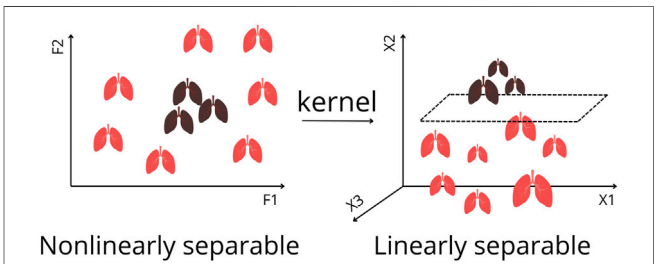


FIGURE 5 | Kernel Trick for Nonlinearly Separable Data in Feature Space to Linearly Separable Data. By applying a kernel function, the data are transformed into a higher dimension, where a separating hyperplane can be found. This enables Support Vector Machines to classify complex patterns that cannot be separated in the original feature space. F1, F2 represents features. X1, X2, X3 represents axis of projections in a higher dimension.

improve lung utilization [32]. However, its primary endpoint, extubation time, serves only as a short-term proxy for success and does not fully capture longer-term outcomes.

Pu et al. developed eight ML models using donor demographics to predict lung, heart, and thoracic cavity volumes, to improve donor-recipient size matching [33]. The performance of these approaches was benchmarked against convolutional neural network (CNN)-based image segmentation models, which were used to generate the volumetric ground truth. *CNNs* are a class of *DL* (**Figure 3A.9**), referring to ANNs with multiple hidden layers, designed to process structured grid-like data like images. They use filters to detect local structures (e.g., edges) and combine them to recognize shapes. Like other DL models, it requires large

labeled datasets and significant processing power [1–3, 8]. The best-performing model was a MLP for individual lungs and thoracic cavity estimates. These non-imaging-based volume predictions may enhance allocation [33].

In contrast to these optimistic findings, Brahmbhatt et al. concluded that LAS, clinician-based models, LASSO, and RF are not sufficiently accurate to predict post-LTx survival. LAS overestimated mortality in high-risk patients and the AUROC of the Houston Methodist model was not achieved, highlighting challenges of reproducibility and possible overfitting in earlier literature. Predictive performance was not improved by ML, disease-specific models, or donor variables [31].

Similarly, Dalton et al. reported that LAS refinement and advanced techniques did not improve performance. Seven models were evaluated with waitlist and post-transplant data to predict waitlist mortality or post-transplant survival. While waitlist models showed strong discrimination, all post-transplant models performed poorly [34]. A possible solution is integrating images or biological markers. Studies employing these approaches are examined in section *Imaging, Omics and Other Applications*.

Imaging, Omics and Other Applications

Barbosa et al. investigated quantitative CT (qCT) to diagnose bronchiolitis obliterans syndrome (BOS), a form of CLAD. Logistic regression and SVM were used to compare qCT metrics, pulmonary function tests (PFT), and semi-quantitative imaging scores as input. To reduce qCT dimensionality, principal component analysis (PCA) (**Figure 3B.2**) was applied, projecting the data onto components capturing the highest variance while minimizing information loss [1, 2, 5, 8]. PCA of qCT together with PFT outperformed all models. However, BOS diagnosis relied solely on chart-reviewed PFT decline, creating circularity, lacking pathological confirmation, and potentially biasing comparisons between qCT- and PFT-based models [36]. In a subsequent study, qCT features including lobar volumes, airway volumes, and airway resistance differed significantly in BOS patients, even at baseline. Using SVM, they constructed classifiers in one-, two-, and three-dimensional feature spaces. Remarkably, with only three qCT parameters, the model achieved 85% accuracy in predicting BOS [38]. Bartholmai et al. also used qCTs, to develop the CALIPER platform for interstitial lung diseases. They applied different ML methods to categorize lung parenchyma into five patterns, challenging even for expert readers to distinguish. CALIPER provided 3D visualizations for tracking of disease burden [35]. Later, McInnis et al. tested CALIPER to distinguish CLAD phenotypes and predict graft survival. Both CALIPER and radiologist scores independently predicted graft failure, with CALIPER enabling reproducible phenotyping and early prognostication without requiring expiratory CT [45]. An XGBoost model based on X-rays and perfusion data from EVLP was developed to predict transplant suitability and ventilation duration post-LTx. Abnormalities were scored per lobe and correlated with oxygenation, compliance and edema. SHAP ranked consolidation and infiltrates as strongest associated with function and transplantability [54]. These studies illustrate how ML-driven imaging analysis can overcome interobserver variability, provide objective and reproducible quantification, reduce human workload, and enable more accurate, scalable assessment of graft injury.

Tran-Dinh et al. developed a model to predict acute cellular rejection using soluble CD31 (sCD31) as biomarker. From only forty recipients, sCD31 levels were combined with recipient haematosis in a CNN model [46]. The authors claim their model uses concepts similar to *transfer learning* (**Figure 3C.2**), where a model trained on one task is adapted to another, valuable in data-scarce settings [1, 2]. However, this is questionable, as their network was trained from scratch rather than optimized from a pretrained model. In another study, a topological autoencoder (Taelcore) was created to improve these predictions by capturing underlying data structures. Applied to the same dataset, dimensionality reduction with Taelcore achieved more accurate predictions than methods like PCA [55]. Likewise, features extracted by Taelcore lack biological interpretability.

To predict tacrolimus trough levels (TTLs) in LTx patients, Choshi et al. developed a long short-term memory–based *Recurrent Neural Network* (RNN), a DL model handling sequential data. This approach relied on clinical inputs identified by SHAP, including previous TTLs and tacrolimus doses. The model captured temporal patterns in dosing and drug response, enabling individualized immunosuppressant management [58]. Yet, its accuracy may diminish in real-world patient settings where missed doses and irregular timing are common.

A gene expression–based DL classifier by Cantu et al. used preprocurement donor lung biopsies to predict PGD3. Their Toll-like receptor model outperformed clinical covariates [40], demonstrating strong discriminative ability and indicating donor innate immune activation as a key driver of PGD, though the analysis was limited to two pathways. Gao et al. also used transcriptomic data in different algorithms. Four neutrophil extracellular traps-related hub genes were identified as drivers of ischemia-reperfusion injury. Three of these were validated in clinical samples, related with PGD development [56]. Furthermore, transcriptomic data were used to explore cuproptosis, a form of cell death, as a potential mechanism in ischemia-reperfusion injury. Three methods (LASSO, SVM, RF) recognized critical biomarkers, with good performance. Functional enrichment linked these genes to immune regulation and cell death, while immune infiltration analysis revealed associations with distinct immune cell subsets [51].

Using unsupervised ML on LTx transbronchial biopsies, Halloran et al. defined four rejection archetypes. PCA linked T-cell mediated rejection (TCMR) and injury to T cell and macrophage transcripts, and antibody-mediated rejection-like to endothelial markers [39]. They also showed that this method worked for mucosal biopsies [41]. However, because mucosal biopsies were obtained only during protocol or clinically indicated bronchoscopies, the sampling may be biased toward unwell patients, limiting generalizability to asymptomatic recipients. Molecular TCMR was associated with future graft loss. Molecular scores outperformed clinical variables in RF and remained robust even in low-surfactant or mucosal samples [42]. Across these studies, Halloran et al. demonstrate that molecular profiling of biopsies provides a more biologically coherent assessment of rejection than histology, although the work remains limited by sampling bias, nonspecific injury signals, and small sample size. Using previously reported mucosal biopsy data

[41], Zhang et al. classified recipients into four rejection-related subgroups. Supervised classification achieved high accuracies (likely overfitted: more features than samples) and lacked external validation. Predictive genes were linked to T cell signaling and innate immunity [47].

In another study, lymphocytic bronchitis gene signature in transbronchial biopsies and small airway brushings were used to predict graft failure and differentiate CLAD from controls. Gene expression profiling with RF showed superior diagnostic performance for brushings over biopsies, but because brushings contain mixed epithelial and leukocyte populations, cell-type–specific interpretation remains limited. The lymphocytic bronchitis score was elevated in CLAD and associated with 2.4-fold increased risk of graft loss [43].

Su et al. analyzed 181 sputum samples from 59 recipients using 16S rRNA sequencing, classifying samples into "stable", "infection", and "rejection". Differences in microbial composition appeared, with six genera enriched during acute rejection, suggesting immune-modulatory roles. Integrating these genera and clinical data in a RF classified well, though repeated samples per patient may cause biased results [48]. A study by Weigt described that gene expression profiling of cells in bronchoalveolar lavage (BAL) revealed an immune activation signature preceding clinical CLAD diagnosis. Forty genes were differentially expressed in incipient CLAD versus CLAD-free samples, enriched for cytotoxic lymphocyte markers. SVM achieved 94.1% accuracy in distinguishing only seventeen cases [37]. Berra et al. also used BAL samples to predict CLAD and investigate the association with the renin–angiotensin system. Although single proteins could not discriminate, combinations in ML classifiers can, reflecting ML's strength in modelling beyond human assessment [44].

Another study predicted PGD using volatile organic compounds (VOCs) from BAL fluid and bronchial aspirate samples. VOC profiling with SVM modeling achieved 83% accuracy in distinguishing PGD3 from lower grades. Twenty VOCs, associated with lipid peroxidation and oxidative stress, were top predictors. Additional analyses linked VOC patterns to clinical variables, including donor BMI and Organ Care System, indicating potential confounding. Recipient and intraoperative factors did not significantly influence VOC profiles [50].

KEY INSIGHTS, FUTURE DIRECTIONS AND CONCLUSION

A consistent strength of ML is its ability to integrate many weak or noisy features into a meaningful signal, where human interpretation or single-variable analyses fail. ML can capture complex, nonlinear interactions, reveal hidden patterns, and offer early risk stratification that traditional clinical or statistical methods miss. Yet, the limitations across studies are strikingly uniform. Most studies are small, single-center, only internally validated, and based on imbalanced datasets. Sampling bias, missing confounders, and heterogeneous data quality further reduce generalizability. Compared with kidney, liver, and heart transplantation, where ML-based tools are more mature, ML approaches in LTx research remains largely underexplored [65–74]. Reporting is often insufficient: many papers provide limited mathematical detail about model design, preprocessing, hyperparameter tuning, or validation, making replication difficult and hindering fair comparison across studies. More transparent, standardized reporting following frameworks like MI-CLAIM (Minimum Information about Clinical Artificial Intelligence Modeling) and TRIPOD-AI (Transparent Reporting of a Multivariable prediction model for individual Prognosis Or Diagnosis) should be strongly encouraged.

Future Directions & Underused Advanced Methods

Future directions should include more multimodal datasets, true external validation, and the careful use of advanced ML methods. Stacking, an ensemble model, could improve performance by combining diverse base learners and a meta learner. Generative Adversarial Networks (GANs) could augment datasets. They consist of a generator that creates synthetic data and a discriminator that evaluates authenticity. Through adversarial training, based on unlabeled data, both networks iteratively improve, allowing to generate realistic data. Although the information content does not increase, it enhances model flexibility and generalization. These are only two examples of underused ML methods, that could strengthen model performance. Post-hoc explanation tools such as Local Interpretable Model-agnostic Explanations [5] and SHAP will remain essential to ensure that predictions are clinically interpretable.

Conclusion

ML holds major potential in LTx, from improving outcome prediction and organ allocation, to imaging and omics-based insights. Yet, clinical adoption remains limited due to small, single-center datasets and insufficient external validation. Enhancing generalizability and building trust requires large multicenter studies, XAI, and standardized reporting. Additionally, ethical considerations remain important when using ML in medicine [2]. Progress in other solid organ transplants highlights opportunities for LTx, with techniques still unexplored, offering room for future innovation. Crucially, ML should complement clinical decision-making, and not replace clinical judgement. Its success relies on collaboration among clinicians, data scientists, ethicists, and regulators. Overcoming current barriers will enable ML to meaningfully improve transplant outcomes.

AUTHOR CONTRIBUTIONS

BV conceived and drafted the review, prepared all figures, and compiled the tables. All authors contributed to the article and approved the submitted version.

FUNDING

The author(s) declared that financial support was received for this work and/or its publication. LC is supported by a University

Chair from Medtronic and IGL and a senior clinical research mandate from Research Foundation Flanders FWO (18E2B24N) and philanthropic grants by Mr. Broere. RV is supported by a research mandate from Research Foundation Flanders FWO (1803521N). PK is supported by a research grant from Research Foundation Flanders FWO (1120425N).

CONFLICT OF INTEREST

The author(s) declared that this work 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) declared that generative AI was used in the creation of this manuscript. During the preparation of this work the author(s) used ChatGPT (OpenAI) and Gemini (Google) in order to enhance the readability of the manuscript. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2025.15640/full#supplementary-material

REFERENCES

1. Burkov A. *The Hundred Page Machine Learning Book*.
2. Goodfellow I, Bengio Y, Courville A. *Deep Learning*. The MIT Press (2016).
3. Lim CP, Vaidya A, Chen YW, Jain V, Jain LC. *Artificial Intelligence and Machine Learning for Healthcare: Emerging Methodologies and Trends*, 2. Springer (2023).
4. Lim CP, Vaidya A, Chen YW, Jain T, Jain LC, editors. *Artificial Intelligence and Machine Learning for Healthcare: Vol. 1: Image and Data Analytics*, 228. Springer International Publishing (2023). doi:10.1007/978-3-031-11154-9
5. Simon GJ, Aliferis C, editors. *Artificial Intelligence and Machine Learning in Health Care and Medical Sciences: Best Practices and Pitfalls*. Springer International Publishing (2024). doi:10.1007/978-3-031-39355-6
6. Gholamzadeh M, Abtahi H, Safdari R. Machine Learning-Based Techniques to Improve Lung Transplantation Outcomes and Complications: A Systematic Review. *BMC Med Res Methodol* (2022) 22(1):331. doi:10.1186/s12874-022-01823-2
7. Rampolla R. Lung Transplantation: An Overview of Candidacy and Outcomes. *Ochsner J* (2014) 14(4):641–8.
8. James G, Witten D, Hastie T, Tibshirani R, Taylor J. *An Introduction to Statistical Learning: With Applications in Python*. Springer International Publishing (2023). doi:10.1007/978-3-031-38747-0
9. Connor KL, O'Sullivan ED, Marson LP, Wigmore SJ, Harrison EM. The Future Role of Machine Learning in Clinical Transplantation. *Transplantation* (2021) 105(4):723–35. doi:10.1097/TP.0000000000003424
10. He J, Baxter SL, Xu J, Xu J, Zhou X, Zhang K. The Practical Implementation of Artificial Intelligence Technologies in Medicine. *Nat Med* (2019) 25(1):30–6. doi:10.1038/s41591-018-0307-0
11. Troiani JS, Carlin BP. Comparison of Bayesian, Classical, and Heuristic Approaches in Identifying Acute Disease Events in Lung Transplant Recipients. *Stat Med* (2004) 23(5):803–24. doi:10.1002/sim.1651
12. Oztekin A, Delen D, Kong Z. Predicting the Graft Survival for Heart–Lung Transplantation Patients: An Integrated Data Mining Methodology. *Int J Med Inf* (2009) 78(12):e84–e96. doi:10.1016/j.ijmedinf.2009.04.007
13. Delen D, Oztekin A, Kong Z. A Machine Learning-Based Approach to Prognostic Analysis of Thoracic Transplantations. *Artif Intell Med* (2010) 49(1):33–42. doi:10.1016/j.artmed.2010.01.002
14. Oztekin A, Kong ZJ, Delen D. Development of a Structural Equation Modeling-Based Decision Tree Methodology for the Analysis of Lung Transplantations. *Decis Support Syst* (2011) 51(1):155–66. doi:10.1016/j.dss.2010.12.004
15. Pande A, Li L, Rajeswaran J, Ehrlinger J, Kogalur UB, Blackstone EH, et al. Boosted Multivariate Trees for Longitudinal Data. *Mach Learn* (2017) 106(2):277–305. doi:10.1007/s10994-016-5597-1
16. Oztekin A, Al-Ebbini L, Sevkli Z, Delen D. A Decision Analytic Approach to Predicting Quality of Life for Lung Transplant Recipients: A Hybrid Genetic Algorithms-Based Methodology. *Eur J Oper Res* (2018) 266(2):639–51. doi:10.1016/j.ejor.2017.09.034
17. Mark E, Goldsman D, Keskinocak P, Sokol J. Using Machine Learning to Estimate Survival Curves for Patients Receiving an Increased Risk for Disease Transmission Heart, Liver, or Lung Versus Waiting for a Standard Organ. *Transpl Infect Dis* (2019) 21(6):e13181. doi:10.1111/tid.13181
18. Fessler J, Gouy-Pailler C, Fischler M, Guen ML. Machine Learning in Lung Transplantation. *J Heart Lung Transpl* (2020) 39(4):S385. doi:10.1016/j.healun.2020.01.497
19. Braccioni F, Bottigliengo D, Ermolao A, Schiavon M, Loy M, Marchi MR, et al. Dyspnea, Effort and Muscle Pain During Exercise in Lung Transplant Recipients: An Analysis of Their Association with Cardiopulmonary Function Parameters Using Machine Learning. *Respir Res* (2020) 21(1):267. doi:10.1186/s12931-020-01535-5
20. Fessler J, Vallee A, Gouy-Pailler C, Davignon M, Fischler M, Guen ML. Machine-Learning for Primary Graft Dysfunction in Lung Transplantation. *J Heart Lung Transpl* (2021) 40(4):S380. doi:10.1016/j.healun.2021.01.1069
21. Amini M, Bagheri A, Delen D. An Explanatory Analytics Model for Identifying Factors Indicative of Long-Versus Short-Term Survival After Lung Transplantation. *Decis Anal J* (2022) 3:100058. doi:10.1016/j.dajour.2022.100058
22. Tian D, Yan HJ, Huang H, Zuo YJ, Liu MZ, Zhao J, et al. Machine Learning–Based Prognostic Model for Patients After Lung Transplantation. *JAMA Netw Open* (2023) 6(5):e2312022. doi:10.1001/jamanetworkopen.2023.12022
23. Melnyk V, Xu W, Ryan JP, Karim HT, Chan EG, Mahajan A, et al. Utilization of Machine Learning to Model the Effect of Blood Product Transfusion on Short-Term Lung Transplant Outcomes. *Clin Transpl* (2023) 37(6):e14961. doi:10.1111/ctr.14961
24. Tian D, Zuo YJ, Yan HJ, Huang H, Liu MZ, Yang H, et al. Machine Learning Model Predicts Airway Stenosis Requiring Clinical Intervention in Patients After Lung Transplantation: A Retrospective Case-Controlled Study. *BMC Med Inform Decis Mak* (2024) 24(1):229. doi:10.1186/s12911-024-02635-8
25. Moro A, Janjua HM, Rogers MP, Kundu MG, Pietrobon R, Read MD, et al. Survival Tree Provides Individualized Estimates of Survival After Lung Transplant. *J Surg Res* (2024) 299:195–204. doi:10.1016/j.jss.2024.04.017

26. Michelson AP, Oh I, Gupta A, Puri V, Kreisel D, Gelman AE, et al. Developing Machine Learning Models to Predict Primary Graft Dysfunction After Lung Transplantation. *Am J Transpl* (2024) 24(3):458–67. doi:10.1016/j.ajt.2023.07.008
27. Xia W, Liu W, He Z, Song C, Liu J, Chen R, et al. Machine Learning for Predicting Primary Graft Dysfunction After Lung Transplantation: An Interpretable Model Study. *Transplantation* (2025) 109:1458–70. doi:10.1097/TP.0000000000005326
28. Fessler J, Gouy-Pailler C, Ma W, Devaquet J, Messika J, Glorion M, et al. Machine Learning for Predicting Pulmonary Graft Dysfunction After Double-Lung Transplantation: A Single-Center Study Using Donor, Recipient, and Intraoperative Variables. *Transpl Int* (2025) 38:14965. doi:10.3389/ti.2025.14965
29. Dueñas-Jurado JM, Gutiérrez PA, Casado-Adam A, Santos-Luna F, Salvatierra-Velázquez A, Cárcel S, et al. New Models for Donor-Recipient Matching in Lung Transplantations. *Plos One* (2021) 16(6):e0252148. doi:10.1371/journal.pone.0252148
30. Zafar F, Hossain MM, Zhang Y, Dani A, Schecter M, Hayes D, Jr, et al. Lung Transplantation Advanced Prediction Tool: Determining Recipient's Outcome for a Certain Donor. *Transplantation* (2022) 106(10):2019–30. doi:10.1097/TP.0000000000004131
31. Brahmbhatt JM, Hee Wai T, Goss CH, Lease ED, Merlo CA, Kapnadak SG, et al. The Lung Allocation Score and Other Available Models Lack Predictive Accuracy for Post-Lung Transplant Survival. *J Heart Lung Transpl* (2022) 41(8):1063–74. doi:10.1016/j.healun.2022.05.008
32. Sage AT, Donahoe LL, Shamandy AA, Mousavi SH, Chao BT, Zhou X, et al. A machine-Learning Approach to Human Ex Vivo Lung Perfusion Predicts Transplantation Outcomes and Promotes Organ Utilization. *Nat Commun* (2023) 14(1):4810. doi:10.1038/s41467-023-40468-7
33. Pu L, Leader JK, Ali A, Geng Z, Wilson D. Predicting left/right Lung Volumes, Thoracic Cavity Volume, and Heart Volume from Subject Demographics to Improve Lung Transplant. *J Med Imaging* (2023) 10(05):051806. doi:10.1117/1.JMI.10.5.051806
34. Dalton JE, Lehr CJ, Gunsalus PR, Mourany L, Valapour M. Refining the Lung Allocation Score Models Fails to Improve Discrimination Performance. *CHEST* (2023) 163(1):152–63. doi:10.1016/j.chest.2022.08.2217
35. Bartholmai BJ, Raghunath S, Karwoski RA, Moua T, Rajagopalan S, Maldonado F, et al. Quantitative Computed Tomography Imaging of Interstitial Lung Diseases. *J Thorac Imaging* (2013) 28(5):298–307. doi:10.1097/RTI.0b013e3182a21969
36. Barbosa EM, Simpson S, Lee JC, Tustison N, Gee J, Shou H. Multivariate Modeling Using Quantitative CT Metrics May Improve Accuracy of Diagnosis of Bronchiolitis Obliterans Syndrome After Lung Transplantation. *Comput Biol Med* (2017) 89:275–81. doi:10.1016/j.compbiomed.2017.08.016
37. Weigt SS, Wang X, Palchevskiy V, Gregson AL, Patel N, DerHovanessian A, et al. Gene Expression Profiling of Bronchoalveolar Lavage Cells Preceding a Clinical Diagnosis of Chronic Lung Allograft Dysfunction. *Plos One* (2017) 12(1):e0169894. doi:10.1371/journal.pone.0169894
38. Barbosa EJM, Lanclus M, Vos W, Van Holsbeke C, De Backer W, De Backer J, et al. Machine Learning Algorithms Utilizing Quantitative CT Features May Predict Eventual Onset of Bronchiolitis Obliterans Syndrome After Lung Transplantation. *Acad Radiol* (2018) 25(9):1201–12. doi:10.1016/j.acra.2018.01.013
39. Halloran KM, Parkes MD, Chang J, Timofte IL, Snell GI, Westall GP, et al. Molecular Assessment of Rejection and Injury in Lung Transplant Biopsies. *J Heart Lung Transpl* (2019) 38(5):504–13. doi:10.1016/j.healun.2019.01.1317
40. Cantu E, Yan M, Suzuki Y, Buckley T, Galati V, Majeti N, et al. Preprocurement *in Situ* Donor Lung Tissue Gene Expression Classifies Primary Graft Dysfunction Risk. *Am J Respir Crit Care Med* (2020) 202(7):1046–8. doi:10.1164/rccm.201912-2436LE
41. Halloran K, Parkes MD, Timofte IL, Snell GI, Westall GP, Hachem R, et al. Molecular Phenotyping of Rejection-Related Changes in Mucosal Biopsies from Lung Transplants. *Am J Transpl* (2020) 20(4):954–66. doi:10.1111/ajt.15685
42. Halloran K, Parkes MD, Timofte I, Snell G, Westall G, Havlin J, et al. Molecular T-Cell–Mediated Rejection in Transbronchial and Mucosal Lung Transplant Biopsies Is Associated with Future Risk of Graft Loss. *J Heart Lung Transpl* (2020) 39(12):1327–37. doi:10.1016/j.healun.2020.08.013
43. Dugger DT, Fung M, Hays SR, Singer JP, Kleinhenz ME, Leard LE, et al. Chronic Lung Allograft Dysfunction Small Airways Reveal a Lymphocytic Inflammation Gene Signature. *Am J Transpl* (2021) 21(1):362–71. doi:10.1111/ajt.16293
44. Berra G, Farkona S, Mohammed-Ali Z, Kotlyar M, Levy L, Clotet-Freixas S, et al. Association Between the Renin–Angiotensin System and Chronic Lung Allograft Dysfunction. *Eur Respir J* (2021) 58(4):2002975. doi:10.1183/13993003.02975-2020
45. McInnis MC, Ma J, Karur GR, Houbois C, Levy L, Havlin J, et al. Chronic Lung Allograft Dysfunction Phenotype and Prognosis by Machine Learning CT Analysis. *Eur Respir J* (2022) 60(1):2101652. doi:10.1183/13993003.01652-2021
46. Tran-Dinh A, Laurent Q, Even G, Tanaka S, Lortat-Jacob B, Castier Y, et al. Personalized Risk Predictor for Acute Cellular Rejection in Lung Transplant Using Soluble CD31. *Sci Rep* (2022) 12(1):17628. doi:10.1038/s41598-022-21070-1
47. Zhang YH, Li ZD, Zeng T, Chen L, Huang T, Cai YD. Screening Gene Signatures for Clinical Response Subtypes of Lung Transplantation. *Mol Genet Genomics* (2022) 297(5):1301–13. doi:10.1007/s00438-022-01918-x
48. Su J, xi LC, yue LH, et al. The Airway Microbiota Signatures of Infection and Rejection in Lung Transplant Recipients. *Microbiol Spectr* (2022) 10 (2): e00344–21. doi:10.1128/spectrum.00344-21
49. Watzenboeck ML, Gorki AD, Quattrone F, Gawish R, Schwarz S, Lambers C, et al. Multi-Omics Profiling Predicts Allograft Function After Lung Transplantation. *Eur Respir J* (2022) 59(2):2003292. doi:10.1183/13993003.03292-2020
50. Stefanuto PH, Romano R, Rees CA, Nasir M, Thakuria L, Simon A, et al. Volatile Organic Compound Profiling to Explore Primary Graft Dysfunction After Lung Transplantation. *Sci Rep* (2022) 12(1):2053. doi:10.1038/s41598-022-05994-2
51. Qin J, Xiao X, Li S, Wen N, Qin K, Li H, et al. Identification of Cuproptosis-Related Biomarkers and Analysis of Immune Infiltration in Allograft Lung Ischemia-Reperfusion Injury. *Front Mol Biosci* (2023) 10:1269478. doi:10.3389/fmolb.2023.1269478
52. Wijbenga N, Hoek RAS, Mathot BJ, Seghers L, Moor CC, Aerts JGJV, et al. Diagnostic Performance of Electronic Nose Technology in Chronic Lung Allograft Dysfunction. *J Heart Lung Transpl* (2023) 42(2):236–45. doi:10.1016/j.healun.2022.09.009
53. Ram S, Verleden SE, Kumar M, Bell AJ, Pal R, Ordies S, et al. Computed Tomography–Based Machine Learning for Donor Lung Screening Before Transplantation. *J Heart Lung Transpl* (2024) 43(3):394–402. doi:10.1016/j.healun.2023.09.018
54. Chao BT, McInnis MC, Sage AT, Yeung JC, Cypel M, Liu M, et al. A Radiographic Score for Human Donor Lungs on Ex Vivo Lung Perfusion Predicts Transplant Outcomes. *J Heart Lung Transpl* (2024) 43(5):797–805. doi:10.1016/j.healun.2024.01.004
55. Gouiaa F, Vomo-Donfack KL, Tran-Dinh A, Morilla I. Novel Dimensionality Reduction Method, Taelcore, Enhances Lung Transplantation Risk Prediction. *Comput Biol Med* (2024) 169:107969. doi:10.1016/j.compbiomed.2024.107969
56. Gao J, Zhang Z, Yu J, Zhang N, Fu Y, Jiang X, et al. Identification of Neutrophil Extracellular Trap-Related Gene Expression Signatures in Ischemia Reperfusion Injury During Lung Transplantation: A Transcriptome Analysis and Clinical Validation. *J Inflamm Res* (2024) 17:981–1001. doi:10.2147/JIR.S444774
57. Chen Y, Li E, Yang Q, Chang Z, Yu B, Lu J, et al. Predicting Time to First Rejection Episode in Lung Transplant Patients Using a Comprehensive Multi-Indicator Model. *J Inflamm Res* (2025) 18:477–91. doi:10.2147/JIR.S495515
58. Choshi H, Miyoshi K, Tanioka M, Arai H, Tanaka S, Shien K, et al. Long Short-Term Memory Algorithm for Personalized Tacrolimus Dosing: A Simple and Effective Time Series Forecasting Approach Post-Lung Transplantation. *J Heart Lung Transpl* (2025) 44(3):351–61. doi:10.1016/j.healun.2024.10.026
59. Ahmad F, Mat-Isa NA, Hussain Z, Boudville R, Osman MK. Genetic Algorithm-Artificial Neural Network (GA-ANN) Hybrid Intelligence for Cancer Diagnosis. In: *2010 2nd International Conference on Computational Intelligence, Communication Systems and Networks* (2010). p. 78–83. doi:10.1109/CICSyN.2010.46
60. Štrumbelj E, Kononenko I. Explaining Prediction Models and Individual Predictions with Feature Contributions. *Knowl Inf Syst* (2014) 41(3):647–65. doi:10.1007/s10115-013-0679-x

61. Van Slambrouck J, Van Raemdonck D, Vos R, Vanluyten C, Vanstapel A, Prisciandaro E, et al. A Focused Review on Primary Graft Dysfunction After Clinical Lung Transplantation: A Multilevel Syndrome. *Cells* (2022) 11(4):745. doi:10.3390/cells11040745
62. Hakkal S, Lahcen AA. XGBoost to Enhance Learner Performance Prediction. *Comput Educ Artif Intell* (2024) 7:100254. doi:10.1016/j.caeai.2024.100254
63. Kursa MB, Jankowski A, Rudnicki WR. Boruta – A System for Feature Selection. *Fundam Informaticae* (2010) 101(4):271–85. doi:10.3233/FI-2010-288
64. Pierson RN, Barr ML, McCullough KP, Egan T, Garrity E, Jessup M, et al. Thoracic Organ Transplantation. *Am J Transpl* (2004) 4:93–105. doi:10.1111/j.1600-6135.2004.00401.x
65. Yoon J, Zame WR, Banerjee A, Cadeiras M, Alaa AM, Schaar M. Van Der. Personalized Survival Predictions via Trees of Predictors: An Application to Cardiac Transplantation. *PLOS ONE* (2018) 13(3):e0194985. doi:10.1371/journal.pone.0194985
66. Kampaktsis PN, Moustakidis S, Tzani A, Doulamis IP, Drosou A, Tzoumas A, et al. State-Of-The-Art Machine Learning Improves Predictive Accuracy of 1-Year Survival After Heart Transplantation. *ESC Heart Fail* (2021) 8(4):3433–6. doi:10.1002/ehf2.13425
67. Ayers B, Sandholm T, Gosev I, Prasad S, Kilic A. Using Machine Learning to Improve Survival Prediction After Heart Transplantation. *J Card Surg* (2021) 36(11):4113–20. doi:10.1111/jocs.15917
68. Hsich EM, Thuita L, McNamara DM, Rogers JG, Valapour M, Goldberg LR, et al. Variables of Importance in the Scientific Registry of Transplant Recipients Database Predictive of Heart Transplant Waitlist Mortality. *Am J Transpl* (2019) 19(7):2067–76. doi:10.1111/ajt.15265
69. Seraphin TP, Luedde M, Roderburg C, van Treeck M, Scheider P, Buelow RD, et al. Prediction of Heart Transplant Rejection from Routine Pathology Slides with Self-Supervised Deep Learning. *Eur Heart J - Digit Health* (2023) 4(3):265–74. doi:10.1093/ehjdh/ztad016
70. Esteban C, Staeck O, Yang Y, Tresp V. Predicting Clinical Events by Combining Static and Dynamic Information Using Recurrent Neural Networks. Arxiv *Preprint Posted Online* (2016) 93–101. doi:10.48550/arXiv.1602.02685
71. Marsh JN, Matlock MK, Kudose S, Liu TC, Stappenbeck TS, Gaut JP, et al. Deep Learning Global Glomerulosclerosis in Transplant Kidney Frozen Sections. *IEEE Trans Med Imaging* (2018) 37(12):2718–28. doi:10.1109/TMI.2018.2851150
72. Yoo D, Divard G, Raynaud M, Cohen A, Mone TD, Rosenthal JT, et al. A Machine Learning-Driven Virtual Biopsy System for Kidney Transplant Patients. *Nat Commun* (2024) 15(1):554. doi:10.1038/s41467-023-44595-z
73. Bertsimas D, Kung J, Trichakis N, Wang Y, Hirose R, Vagefi PA. Development and Validation of an Optimized Prediction of Mortality for Candidates Awaiting Liver Transplantation. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2019) 19(4):1109–18. doi:10.1111/ajt.15172
74. Shao W, Ding H, Wang Y, Shi Z, Zhang H, Meng F, et al. Key Genes and Immune Pathways in T-Cell Mediated Rejection Post-Liver Transplantation Identified via Integrated RNA-Seq and Machine Learning. *Sci Rep* (2024) 14(1):24315. doi:10.1038/s41598-024-74874-8

GLOSSARY

AI Artificial Intelligence
ANN Artificial Neural Network
AUROC Area Under the Receiver Operating Characteristic Curve
Bagging Bootstrap Aggregating
BAL Bronchoalveolar Lavage
BMI Body Mass Index
BOS Bronchiolitis Obliterans Syndrome
CLAD Chronic Lung Allograft Dysfunction
CNN Convolutional Neural Network
DL Deep Learning
DT Decision Tree
EVLP *Ex Vivo* Lung Perfusion
FEV1 Forced Expiratory Volume in one second
GA Genetic Algorithm
GAN Generative Adversarial Network
IRD Increased Risk for Disease Transmission
kNN k-Nearest Neighbors
LAPT Lung Transplantation Advanced Prediction Tool
LAS Lung Allocation Score
LASSO Least Absolute Shrinkage and Selection Operator
LTx Lung Transplantation
ML Machine Learning
MLP Multilayer Perceptron
MSE Mean Squared Error
PCA Principal Component Analysis
PFT Pulmonary Function Test
PGD Primary Graft Dysfunction
qCT quantitative Computed Tomography
RF Random Forest
RMSE Root Mean Squared Error
RNN Recurrent Neural Network
sCD31 soluble CD31
SHAP SHapley Additive Explanation
SVM Support Vector Machine
TCMR T-cell-mediated Rejection
TTLs Tacrolimus Trough Levels
UNOS the United Network for Organ Sharing
VO_2 Volume of Oxygen Consumption
VOC Volatile Organic Compound
XAI explainable artificial intelligence

ESOT | Transplant International

ORIGINAL RESEARCH
published: 11 February 2026
doi: 10.3389/ti.2026.15781

Machine Learning–Based Evaluation of Combined EBV and CMV Serostatus as Predictors of Post-Transplant Lymphoproliferative Disorder

Ghazal Azarfar[1], *Muath A. M. Alotaibi*[1,2,3], *Yingji Sun*[1], *Shahid Husain*[1,2,4], *Aman Sidhu*[1,4], *Mamatha Bhat*[1,5] *and Seyed M. Hosseini-Moghaddam*[1,2,4]*

[1]*Ajmera Transplant Centre, University Health Network, Toronto, ON, Canada,* [2]*Division of Infectious Diseases, Department of Medicine, University Health Network, University of Toronto, Toronto, ON, Canada,* [3]*Department of Medicine, King Faisal Specialist Hospital and Research Centre, Madinah, Saudi Arabia,* [4]*Toronto General Hospital Research Institute (TGHRI), University Health Network, Toronto, ON, Canada,* [5]*Division of Gastroenterology and Hepatology, Department of Medicine, University of Toronto, Toronto, ON, Canada*

Post-transplant lymphoproliferative disorder (PTLD) is a major complication of solid organ transplantation (SOT), with the greatest risk in Epstein–Barr virus (EBV) donor-positive/recipient-negative (D+/R–) pairs. The contribution of cytomegalovirus (CMV) serostatus is less well defined. We conducted a population-based study of 47,333 abdominal SOT recipients in the United States (1995–2015) using linked SRTR data. Donor–recipient EBV/CMV serostatus was evaluated as a compound variable. The primary outcome was PTLD incidence, with secondary analyses assessing predictors of PTLD and impact on survival. Overall, 716 patients (1.5%) developed PTLD at a median of 6.1 years (IQR 2.9–9.7) after transplant. EBV D+/R- recipients had the highest incidence (3.2%), and those with compound [EBV D+/R–, CMV D–/R–] serostatus had more than double the PTLD risk compared with [EBV D+/R–, CMV D+/R–] (5.3% vs. 2.5%, $p < 0.001$). Logistic regression and random forest models consistently identified [EBV D+/R–, CMV D-/R-] serostatus, age, and race as leading predictors, though discrimination was modest (test AUC ~0.61). In a matched survival analysis, PTLD was not associated with increased all-cause mortality (aHR ~1.0). Our findings demonstrate that combined EBV/CMV serostatus improves PTLD risk prediction compared with EBV alone and emphasize the need for targeted preventive strategies.

Keywords: cytomegalovirus (CMV), Epstein–barr virus (EBV), machine learning, post-transplant lymphoproliferative disorder (PTLD), solid organ transplantation

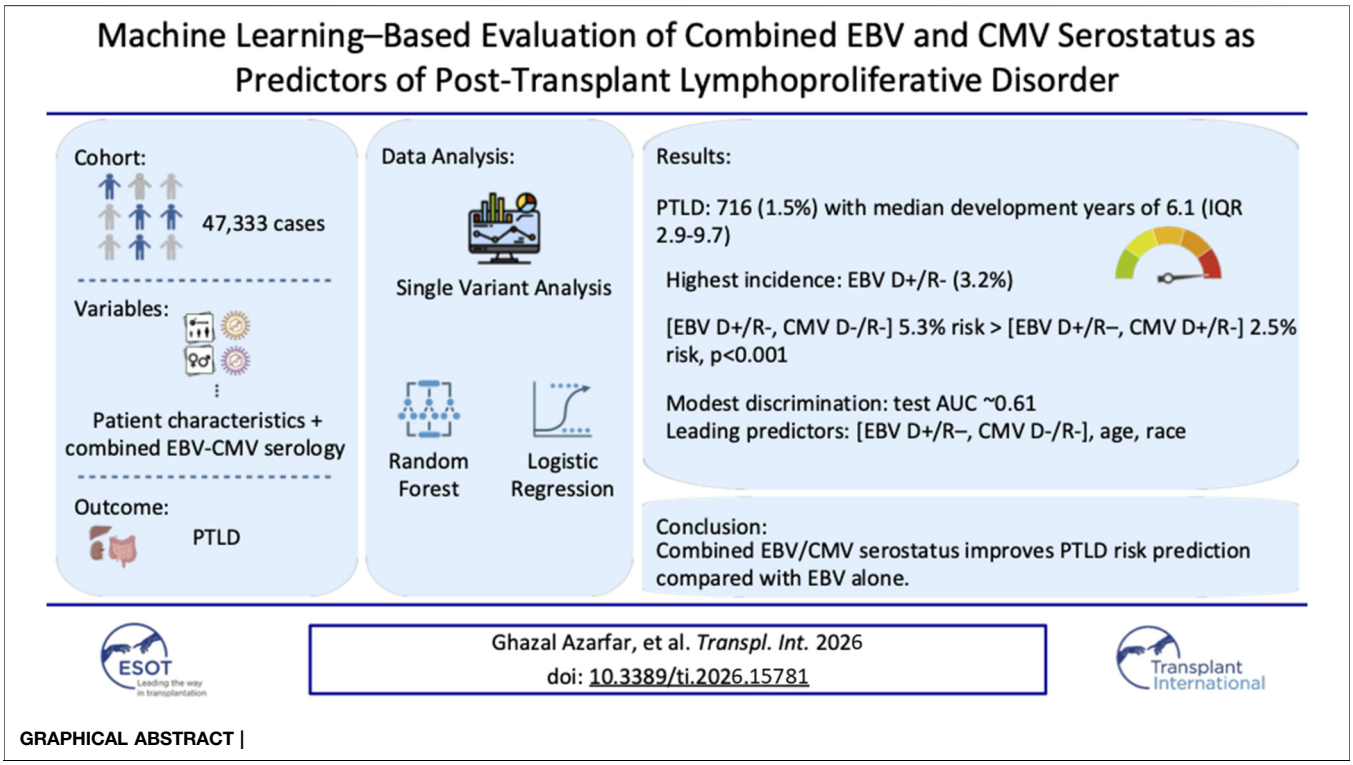
OPEN ACCESS

***Correspondence**
Seyed M. Hosseini-Moghaddam,
sasan.hosseini@uhn.ca

Received: *21 October 2025*
Revised: *12 December 2025*
Accepted: *22 January 2026*
Published: *11 February 2026*

Citation:
Azarfar G, Alotaibi MAM, Sun Y, Husain S, Sidhu A, Bhat M and Hosseini-Moghaddam SM (2026) Machine Learning–Based Evaluation of Combined EBV and CMV Serostatus as Predictors of Post-Transplant Lymphoproliferative Disorder. Transpl. Int. 39:15781. doi: 10.3389/ti.2026.15781

Abbreviations: AUC, area under the curve; aHR, adjusted hazard ratio; aOR, adjusted odds ratio; C-index, concordance index; CMV, cytomegalovirus; D/R, donor/recipient serostatus; DLBCL, diffuse large B-cell lymphoma; DSA, donor-specific antibodies; EBV, Epstein–Barr virus; LR, logistic regression; OPTN, Organ Procurement and Transplantation Network; PTLD, post-transplant lymphoproliferative disorder; rATG, rabbit antithymocyte globulin; RFC, random forest classifier; SHAP, Shapley Additive Explanations; SOT, solid organ transplantation; SRTR, Scientific Registry of Transplant Recipients; UNOS, United Network for Organ Sharing.



GRAPHICAL ABSTRACT |

INTRODUCTION

Post-transplant lymphoproliferative disorder (PTLD) is a potentially life-threatening complication that occurs in approximately 1%–16% of solid organ transplant (SOT) recipients [1–4]. The intensity of immunosuppression and Epstein–Barr virus (EBV) replication are key drivers in the development of PTLD [1, 5]. The risk of PTLD is particularly high in EBV-seronegative recipients who acquire primary EBV infection post-transplant, especially when the donor is EBV-seropositive, highlighting the pivotal role of EBV serostatus in PTLD development [6]. The association between cytomegalovirus (CMV) infection and PTLD remains inconclusive, with inconsistent findings across studies. Some studies have identified CMV disease and CMV-EBV coinfection as risk factors for PTLD, while others found no consistent association after adjusting for EBV serostatus and immunosuppression intensity [7–12]. The association between CMV infection and post-transplant outcomes, including PTLD, may be confounded or modified by antiviral prophylaxis [13, 14]. Although some data showed antiviral agents such as valganciclovir may delay EBV viremia, the role of antiviral prophylaxis in preventing PTLD remains debated [15, 16]. A systematic review found insufficient evidence to support this conclusion [17–19]. However, a recent meta-analysis suggests antiviral prophylaxis may reduce PTLD risk and EBV viremia, especially in patients receiving intensive immunosuppression [20].

Inconsistencies in prior observational studies evaluating PTLD risk likely reflect methodological limitations, including small sample sizes, short follow-up durations, single-center designs, and lack of adjustment for CMV serostatus. In this study, we applied machine learning (ML) to estimate PTLD risk in SOT recipients, incorporating EBV-CMV serostatus as a compound variable to explore whether patients with [EBV (D+/R–), CMV (D–/R–)] serostatus are at elevated risk of PTLD.

MATERIALS AND METHODS

Study Design and Population

In this large-scale, population-based study, we included all recipients of abdominal organ transplants (liver, kidney, kidney-pancreas, and intestine) between May 1995 and March 2015, for whom both the recipient's and the donor's EBV and CMV serostatus were documented. We used the linked health-related data included in the Scientific Registry of Transplant Recipients (SRTR), which comprises information from every transplant and organ donation that has taken place in the United States since October 1987.[1] The SRTR provides information on donor-recipient matching and transplant recipients' demographics, clinical data, and outcomes, as supplied by the Organ Procurement and Transplantation Network (OPTN) and managed by the United Network for Organ Sharing (UNOS). We linked various variables and datasets in the SRTR, including transplant recipient information, registration records, follow-up data,

[1]https://www.srtr.org/about-the-data/the-srtr-database/

immunosuppressive therapies, and malignancy reports. To minimize bias, we excluded variables with >20% missingness and thoracic transplant recipients, as EBV/CMV serostatus was frequently unreported in this group.

Exposure and Outcomes

The exposure of interest was the combined EBV and CMV donor/recipient (D/R) serostatus at the time of transplantation, treated as a compound variable. The primary outcome was the development of PTLD, and the secondary outcome was all-cause post-transplant mortality. The observation period extended through 30 November 2023.

Statistical Analysis

Categorical variables were presented as proportions, and continuous variables as medians with interquartile ranges (IQR). We calculated the cumulative incidence of PTLD across 16 EBV/CMV compound variable categories. Associations between PTLD and categorical variables were assessed using the Chi-square test, while the Mann-Whitney U test was used for continuous variables. A p-value <0.05 was considered statistically significant. Variables significantly associated with PTLD were used as input features for two machine learning algorithms—logistic regression (LR) and random forest classifier (RFC)—to predict PTLD development. Due to the low incidence of PTLD, random undersampling was used to achieve a balanced 1:1 ratio of patients with and without PTLD [21]. The resulting dataset was then randomly split into 80% for training and 20% for testing. This process was repeated 20 times, and the mean and standard deviation of the area under the curve (AUC) and accuracy were calculated for both models on the training and test sets. The results of the LR analysis were reported as adjusted odds ratios (aOR). To interpret model performance and identify the relative importance of predictors, Shapley Additive Explanations (SHAP) analysis was performed for both the LR and RFC models [22, 23]. We estimated the confidence intervals using 200 bootstrap resamples.

Using survival analysis, we determined the all-cause post-transplant mortality risk as the secondary outcome. To account for immortal time bias, we matched each patient diagnosed with PTLD with four patients who did not develop PTLD and survived until the time of PTLD diagnosis in the corresponding case. Matching variables included transplant year (±2.5 years), age (±5 years), sex, race, organ transplanted, and the presence of comorbidities [24]. Matching was achieved by filtering patients based on predetermined matching variables, followed by a random selection of non-PTLD patients for each PTLD case. Patients were censored at the time they were lost to follow-up (i.e., right-censoring). We used the log-rank test and the Kaplan-Meier analysis to compare survival differences between patients with PTLD and patients without PTLD. Cox proportional hazards regression analysis was performed to identify risk factors associated with mortality. All analyses were conducted using Python (version 3.12.4, packaged by Anaconda, Inc.) with the Scikit-learn (sklearn) and scikit-survival (sksurv) libraries.

RESULTS

During the study period, a total of 47,333 patients underwent abdominal organ transplants (median [IQR] age: 49 [37–58] years, male: 28,718 [60.6%]). Cohort characteristics are provided in **Table 1**.

PTLD

A total of 716 patients (1.5%) developed PTLD. The median time to PTLD diagnosis was 6.1 years (IQR 2.9–9.7 years), with 86 patients (12%) developing PTLD within the first year post-transplant. The predominant pathological PTLD subtype was monomorphic PTLD (n = 279 [39.0%]). **Supplementary Table 1** provides disease characteristics in SOT recipients with PTLD. Among patients with early-onset PTLD (i.e., diagnosed within 2 years post-transplant), 62 of 142 (42.2%) had extranodal involvement, compared to 112 of 573 (19.5%) among those with late-onset PTLD (P < 0.0001).

The incidence of PTLD in the EBV D+/R− group (137/4,236 [3.23%]) was significantly higher than in the other serogroups (EBV D-/R-: 11/1,073 [1.02%]; EBV D−/R+: 35/2,905 [1.20%]; EBV D+/R+: 533/38,979 [1.36%]; p < 0.001) regardless of the timing of the PTLD development (see **Supplementary Tables 4, 5**). **Table 2** presents the PTLD incidence considering compound variable of [EBV (D/R), CMV (D/R)]. Among EBV mismatch recipients, those with CMV mismatch serostatus (CMV D+/R−) had a significantly lower incidence of PTLD compared to those with CMV D−/R− status [23/938 (2.45%) vs. 67/1,332 (5.3%), p < 0.001]. The lowest PTLD cumulative incidence was in the [EBV (D-/R+), CMV (D+/R-)] recipients (0.57%).

In multivariable LR model, we estimated the training accuracy of 61.172 %± 0.010% and the training AUC of 0.658 ± 0.001. The test accuracy and test AUC for the LR were 57.17% ± 0.022% and 0.605 ± 0.023, respectively. **Supplementary Table 2** presents the adjusted OR in LR analysis. In the multivariable RFC model, we estimated a training accuracy of 60.658 ± 0.009, with a training AUC of 0.653 ± 0.006. The RFC test accuracy and AUC were 58.104 %± 0.018% and 0.615 ± 0.018, respectively (**Table 3**).

SHAP Analysis

Figure 1 presents the SHAP rankings of the variables used in each model, highlighting the top 20 predictors of PTLD. In both models, age, white race, and combined serostatus—specifically [EBV (D+/R-), CMV (D-/R-)]—consistently emerged as the most influential predictors. The variables incorporated into the two machine learning algorithms are presented in **Supplementary Table 3**. The rankings of other predictors varied between the LR and RFC models. Male sex and the use of immunosuppressive agents such as mycophenolate compounds, azathioprine, cyclosporine or tacrolimus were associated with PTLD development, while treatment with mTOR inhibitors showed an inverse relationship. **Figure 2** provides Bees worm plots of the shapely valued for the LR and RFC analyses.

TABLE 1 | Patients' characteristics in the cohort.

	Patients without PTLD (n = 46,617)	Patients with PTLD (n = 716)	p Value
Sex, male, *n* (%)	28,262 (60.6)	456 (63.7)	**0.096**
Age (years), median (IQR)	49 (37–58)	52 (42–61)	<**0.001**
BMI, median (IQR)	27 (23–31)	26.5 (23–30)	0.115
Recipient ethnicity (no. [%])			
White	31,918 (68.5)	559 (78.1)	<**0.001**
Black	6,481 (13.9)	54 (7.6)	<**0.001**
Hispanic	5,659 (12.1)	55 (7.7)	<**0.001**
Asian	1,940 (4.2)	26 (3.6)	0.969
Other	619 (1.3)	21 (2.9)	0.026
Previous malignancy (no. [%])	378 (0.8)	6 (0.8)	0.135
Drug treated COPD	537 (1.2)	7 (1.0)	0.670
Drug treated systemic hypertension	34,984 (75.0)	454 (63.4)	0.478
Symptomatic peripheral vascular disease	2,046 (4.4)	33 (4.6)	0.762
Symptomatic cerebrovascular disease	983 (2.1)	21 (2.9)	0.148
Peptic ulcer disease	1,349 (2.9)	23 (3.2)	0.637
Organ transplanted			
Intestine	3 (0.0)	0 (0.0)	-
Kidney	43,215 (92.7)	630 (87.9)	<**0.001**
Liver	2,082 (4.5)	55 (7.7)	<**0.001**
Kidney-pancreas	1,317 (2.8)	31 (4.3)	**0.016**
Post-transplant immunosuppression			
Cyclosporine, N (%)	5,310 (11.4%)	77 (10.7)	0.263
mTOR inhibitor, N (%)	3,953 (8.5%)	52 (7.3)	0.407
Tacrolimus, N (%)	38,523 (82.6%)	611 (85.3)	**0.035**
Corticosteroid, N (%)	32,171 (69.0%)	480 (67.0)	0.510
Azathioprine, N (%)	631 (1.4%)	20 (2.8)	<**0.001**
Mycophenolate mofetil, N (%)	40,377 (86.6%)	615 (85.9)	0.772
Any anti lymphocyte exposure in the first year, N (%)	898 (1.9%)	9 (1.3)	0.051

Bold values: statistically significant.

TABLE 2 | EBV-CMV serology status of the recipients (R) and the donors (D).

	EBV D−/R−			EBV D+/R−		
	Patients with PTLD (11)	**Patients without PTLD (1062)**	**Cumulative incidence**	**Patients with PTLD (137)**	**Patients without PTLD (4239)**	**Cumulative incidence**
CMV D−/R−	5	457	1.08%	67	1265	5.03%
CMV D+/R−	2	145	1.36%	23	915	2.45%
CMV D-/R+	2	280	0.71%	21	758	2.69%
CMV D+/R+	2	180	1.10%	26	1301	1.96%
	EBV D−/R+			EBV D+/R+		
	Patients with PTLD (35)	Patients without PTLD (2870)	Cumulative incidence	Patients with PTLD (533)	Patients without PTLD (38446)	Cumulative incidence
CMV D−/R−	15	853	1.73%	140	9089	1.52%
CMV D+/R−	2	350	0.57%	82	6414	1.26%
CMV D−/R+	9	838	1.06%	114	8441	1.33%
CMV D+/R+	9	829	1.07%	197	14502	1.34%

The Chi-square test yielded a global p-value <0.001, demonstrating a significant association between combined EBV–CMV serostatus and PTLD.

All-cause Post-transplant Mortality

In this analysis, 570 patients with PTLD were matched to 2,280 controls without PTLD. Using Cox proportional hazards models, including stratified analyses by age, sex, and race, PTLD was not associated with increased all-cause mortality. Adjusted hazard ratios ranged from 0.991 to 1.008, with 95% confidence intervals consistently crossing unity (e.g., aHR 1.004 [95% CI, 0.900–1.120]; aHR 0.991 [95% CI, 0.883–1.113]). White

TABLE 3 | Comparison of models' performances. The average of 20 under sampling.

Model	Training set		Test set	
	AUC (95% CI)	Accuracy (95% CI)	AUC (95% CI)	Accuracy (95% CI)
Logistic regression	0.658 (0.657–0.659)	61.172 (61.162–61.182)	0.605 (0.582–0.628)	57.170 (57.148–57.192)
Random forest	0.653 (0.647–0.659)	60.658 (60.649–60.667)	0.615 (0.597–0.633)	58.104 (58.086–58.122)

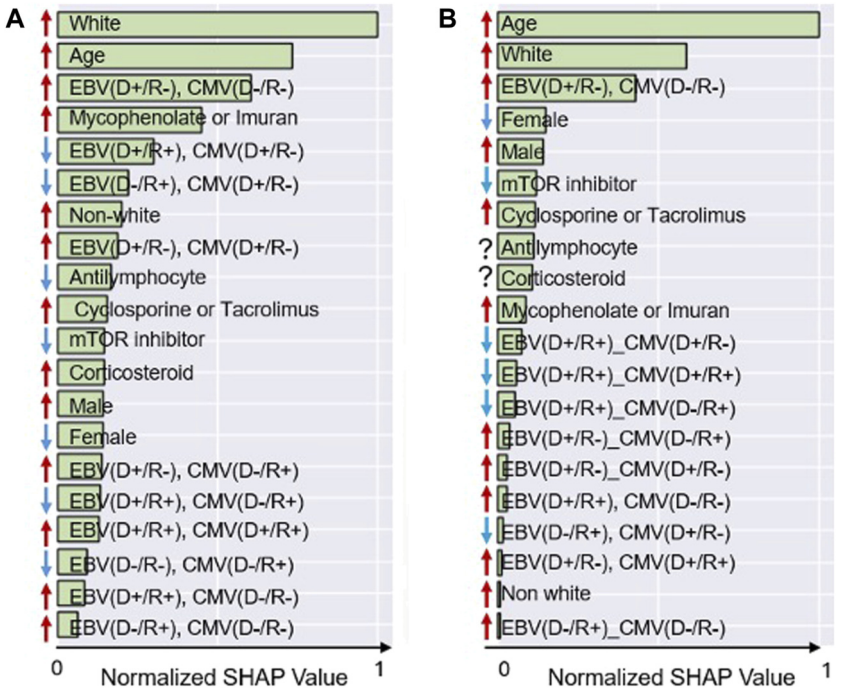


FIGURE 1 | SHAP Analysis, impact of the features on model output. **(A)** Logistic regression. **(B)** Random forest. This figure illustrates the variable importance for the top 20 variables and the association with PTLD. Higher average SHAP values indicate a greater contribution of the variable to the model. The arrows indicate the direction of the association: red arrows represent positive associations, while blue arrows represent negative associations.

recipients had a higher risk of mortality compared to non-White recipients (aHR 1.153 [95% CI, 1.027–1.295]). Model discrimination was limited (C-index ~0.50), indicating no strong mortality signal associated with PTLD in this matched cohort. Kaplan–Meier analysis and log-rank testing showed no significant survival difference between patients with and without PTLD (P = 0.261; **Supplementary Figure 1**).

DISCUSSION

EBV-naïve recipients are exposed to donor-derived EBV infection and are vulnerable to PTLD due to unregulated viral replication under immunosuppressive conditions [1]. Previous studies consistently showed patients with EBV D+/R− serostatus are at the greatest risk of PTLD development [25–27]. However, the relationship between CMV serostatus and PTLD has not been thoroughly investigated. Using two ML models, we found a consistent association between [EBV [(D+/R−), CMV (D−/R−)] serostatus and increased PTLD risk. Among EBV mismatch recipients, those with CMV D+/R− had a significantly lower risk of PTLD compared to those with CMV D−/R−. Although single-center studies have suggested a potential link between PTLD and CMV infection, the association between PTLD and CMV serostatus has not been systematically investigated [10, 28, 29]. An early study by Walker et al. in 1995 examined a cohort of 381 non-renal SOT recipients at the Mayo Clinic and proposed a possible role for CMV serostatus in PTLD pathogenesis. However, the interpretation of these results is constrained by confounding from concurrent use of potent immunosuppressive therapy, notably OKT3, which substantially elevates PTLD risk [9]. Opelz et al. conducted a large SRTR-based cohort study (1999–2007) of 23,340 SOT recipients and found no significant association between recipient CMV serostatus and non-Hodgkin lymphoma; however, donor CMV serostatus was not included in their analyses [30]. In a separate cohort study, Geris et al. found a relationship between CMV D-/R- serostatus and the development of PTLD. However, the study outcome was

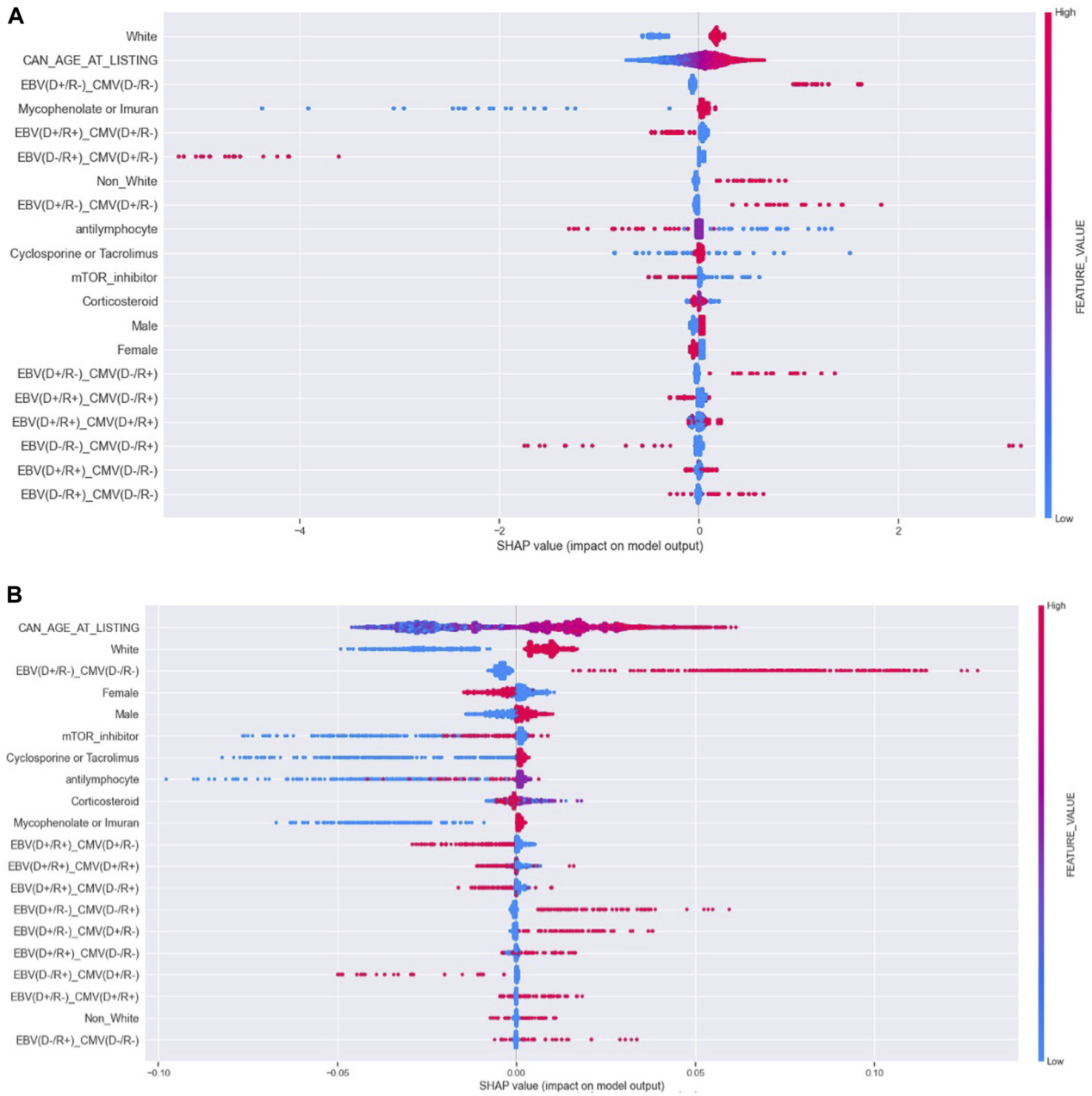


FIGURE 2 | Bees worm Plot of the shapely valued for the LR and RFC analyses. **(A)** Bees worm Plot of the shapely valued for the LR model, **(B)** Bees worm Plot of the shapely valued for the RFC model.

limited to diffuse large B-cell lymphoma (DLBCL) and did not investigate the simultaneous roles of EBV (D/R) and CMV (D/R) as a compound variable [7]. Ali et al., in a cohort of 10,947 pediatric renal transplant recipients, found that recipient CMV seropositivity was associated with a protective effect against PTLD (HR = 0.82; 95% CI: 0.73–0.94) [11]. To our knowledge, no prior study has evaluated the association between PTLD and combined EBV/CMV donor–recipient serostatus as a single compound variable.

We evaluated PTLD risk using combined [EBV (D/R), CMV (D/R)] serostatus and found that EBV D+/R- recipients with CMV D−/R− serostatus were at increased risk of PTLD. This observation does not inherently indicate a protective effect of valganciclovir, which is typically not administered to CMV D-/R- recipients. Although they may receive acyclovir or valacyclovir, these agents have negligible activities against CMV and no proven benefit in preventing EBV-related PTLD. Aldabbagh et al. conducted a metanalysis

in 2017 including 9 studies and 2,366 SOT recipients and concluded that existing data are insufficient to support the routine use of antivirals to prevent PTLD in EBV-naive SOT recipients [19]. However, a recent meta-analysis by Moghadamnia et al., including 22 studies and 13,498 SOT recipients, found that PTLD incidence was significantly lower among those who received antiviral prophylaxis (RR 0.77; 95% CI, 0.63–0.94) [20]. In heart and kidney transplant recipients, Albatati et al. found that valganciclovir delayed viremia onset compared to no antiviral use (143 vs. 90 days; $p = 0.008$), with each additional day of prophylaxis increasing viremia-free survival by 1.4% ($p < 0.001$) [15]. In a case-control study of 100 PTLD cases and 375 controls, ganciclovir use was associated with a 38% reduction in early PTLD risk for each 30-day period during the first posttransplant year [17]. Lytic replication during primary EBV infection promotes dissemination via infected B cells and remains susceptible to antiviral suppression [31]. However, these findings do not necessarily support the use of nucleoside analogs in EBV D+/R- recipients, who are at the highest risk of primary infection. Antiviral prophylaxis is not currently recommended by current guidelines, as evidence remains limited [1]. In the absence of interventional studies evaluating preventive strategies, further research is warranted to identify approaches that reduce PTLD risk.

In our study, 60% of PTLD cases occurred in male transplant recipients, consistent with prior research indicating the disproportionate impact of PTLD, particularly late-onset disease, on males [30]. This disparity may stem from differential immune responses and increased susceptibility to EBV-associated complications [14, 32]. We also observed that patients with PTLD were older than those without, likely reflecting increased risk due to immunosenescence [33]. Most PTLD cases in our cohort were late-onset (median time from transplant to diagnosis: 6.1 years), consistent with prior studies linking late-onset PTLD to older age and early-onset PTLD to younger recipients [30].

We found an inconsistent association between PTLD and rabbit antithymocyte globulin (rATG) use, despite previous studies showing a possible link [34, 35]. This inconsistency may be partly explained by selective survival bias: recipients at a higher risk for PTLD are less likely to receive rATG, as clinicians frequently avoid its use in these patients due to its known association with PTLD [36, 37]. Additionally, 88% of patients with PTLD in this cohort had a late-onset disease, typically many months or even years after rATG exposure.

The application of newer ML models underscores the evolving methodology in estimating PTLD risk. RFC particularly aggregates predictions from multiple decision trees to mitigate overfitting and enhance generalizability [38, 39]. These strengths make RFC particularly suitable for analyzing highly imbalanced datasets and predicting rare events [40]. Previous studies have demonstrated that RFC often outperforms LR in terms of predictive accuracy and interpretability in various medical domains, including organ transplantation [41, 42]. In our study, RFC and LR models produced comparable AUC values of approximately 0.65 in the training set and 0.61 in the testing set. Model performance was likely impacted by extreme class imbalance with PTLD occurring in only 1.5% of patients. To address this, we employed random undersampling repeating the process 20 times to balance the dataset and improve model sensitivity to minority-class predictions [21]. Although random undersampling risks excluding valuable majority-class data, this iterative approach helped achieve a balance between data retention and model focus [43]. We could not include granular clinical, dynamic, or omics data—such as medication dosages, viral load, or immune markers—which likely limited model performance. These individual-level variables are unavailable in population-based sources like the SRTR. The modest overall performance may also reflect the limited granularity of the feature set, which may not adequately capture individual-level variation in PTLD risk. Incorporating personalized variables, such as pharmacogenetic or molecular data, could improve future model performance.

Our study had some limitations. First, the retrospective cohort design is subject to inherent constraints, including the potential for selection bias. Due to considerable missing data on EBV and CMV serostatus among thoracic transplant recipients, we excluded this subgroup from the cohort. Another limitation is the small number of PTLD cases, which reduced model performance; as such, our analysis is intended to generate hypotheses and highlight potential risk patterns rather than serve as a clinical prediction tool. The predictive performance of both logistic regression and random forest models was modest (test AUC ~0.61), which likely reflects several methodological challenges: the low incidence of PTLD, the resulting class imbalance despite undersampling, and the absence of granular clinical variables such as viral load, dynamic immune monitoring, heterogeneity in immunosuppressive regimens, donor-recipient characteristics, and center-specific protocols in the SRTR database. These limitations may have reduced the ability of our models to achieve higher discrimination.

This study demonstrates the utility of ML models in predicting the rare post-transplant outcomes such as PTLD in the SOT population. Our study may challenge traditional reliance on linear models. By bridging established research with cutting-edge methodologies, this study paves the way for improved clinical decision-making and personalized patient care. Our findings have potential implications in the PTLD risk assessment and suggest using compound [EBV (D/R), CMV (D/R)] serostatus rather than simple EBV (D/R) for PTLD prediction.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: The data will be available upon request and approval.

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

GA: data analysis and interpretation, MA: study design, data interpretation, drafting of the manuscript, S-HM: study concept and design, data interpretation, critical revision of the manuscript; MB: study design, data interpretation, critical revision of the manuscript, SH: study design, data interpretation, critical revision of the manuscript, YS: study design, data analysis, AS: data.

FUNDING

The author(s) declared that financial support was received for this work and/or its publication. This work was supported by the University Health Network Ajmera Transplant AI Initiative. The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

CONFLICT OF INTEREST

The author(s) declared that this work 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) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2026.15781/full#supplementary-material

REFERENCES

1. Allen UD, Preiksaitis JK. Post-Transplant Lymphoproliferative Disorders, Epstein-Barr Virus Infection, and Disease in Solid Organ Transplantation: Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. *Clin Transpl* (2019) 33(9):1–22. doi:10.1111/ctr.13652
2. San-Juan R, Comoli P, Caillard S, Moulin B, Hirsch HH, Meylan P, et al. Epstein-Barr virus-Related Post-Transplant Lymphoproliferative Disorder in Solid Organ Transplant Recipients. *Clin Microbiol Infect* (2014) 20(s7):109–18. doi:10.1111/1469-0691.12534
3. Kamdar KY, Rooney CM, Heslop HE. Post-Transplant Lymphoproliferative Disease Following Liver Transplantation. *Curr Opin Organ Transpl* (2011) 16(3):274–80. doi:10.1097/mot.0b013e3283465715
4. Lee TC, Savoldo B, Rooney CM, Heslop HE, Gee AP, Caldwell Y, et al. Quantitative EBV Viral Loads and Immunosuppression Alterations can Decrease PTLD Incidence in Pediatric Liver Transplant Recipients. *Am J Transpl* (2005) 5(9):2222–8. doi:10.1111/j.1600-6143.2005.01002.x
5. Green M, Michaels MG. Epstein-Barr Virus Infection and Posttransplant Lymphoproliferative Disorder. *Am J Transpl* (2013) 13(s3):41–54. doi:10.1111/ajt.12004
6. Allen UD, Preiksaitis JK. Epstein-Barr Virus and Posttransplant Lymphoproliferative Disorder in Solid Organ Transplantation. *Am J Transpl* (2013) 13(Suppl. 4):107–20. doi:10.1111/ajt.12104
7. Geris JM, Spector LG, Pfeiffer RM, Limaye AP, Yu KJ, Engels EA. Cancer Risk Associated with Cytomegalovirus Infection Among Solid Organ Transplant Recipients in the United States. *Cancer* (2022) 128(22):3985–94. doi:10.1002/cncr.34462
8. Aucejo F, Rofaiel G, Miller C. Who Is at Risk for Post-Transplant Lymphoproliferative Disorders (PTLD) After Liver Transplantation? *J Hepatol* (2006) 44(1):19–23. doi:10.1016/j.jhep.2005.10.008
9. Walker RC, Marshall WF, Strickler JG, Wiesner RH, Velosa JA, Habermann TM, et al. Pretransplantation Assessment of the Risk of Lymphoproliferative Disorder. *Clin Infect Dis* (1995) 20(5):1346–53. doi:10.1093/clinids/20.5.1346
10. Manez R, Breinig MC, Linden P, Wilson J, Torre-Cisneros J, Kusne S, et al. Posttransplant Lymphoproliferative Disease in Primary Epstein-Barr Virus Infection After Liver Transplantation: The Role of Cytomegalovirus Disease. *J Infect Dis* (1997) 176(6):1462–7. doi:10.1086/514142
11. Kaies H, Ali IE, Mohamed M, Briggs D, Krishnan N. MO993: Incidence of Ptld and Its Relationship with Cmv-Serostatus Positivity Among Childhood and Adulthood Population: A Registry Data Study. *Nephrol Dial Transpl* (2022) 37(Suppl. ment_3). doi:10.1093/ndt/gfac087.051
12. Kim JM, Lee SK, Kim SJ, Joh JW, Kwon CHD, Choe YH, et al. Risk Factors for Posttransplant Lymphoproliferative Disorder in Pediatric Liver Transplant Recipients with Cytomegalovirus Antigenemia. *Transpl Proc* (2010) 42(3):895–9. doi:10.1016/j.transproceed.2010.02.042
13. Höcker B, Böhm S, Fickenscher H, Küsters U, Schnitzler P, Pohl M, et al. (Val-)ganciclovir Prophylaxis Reduces Epstein-Barr Virus Primary Infection in Pediatric Renal Transplantation. *Transpl Int* (2012) 25(7):723–31. doi:10.1111/j.1432-2277.2012.01485.x
14. Ville S, Imbert-Marcille BM, Coste-Burel M, Garandeau C, Meurette A, Cantarovitch D, et al. Impact of Antiviral Prophylaxis in Adults Epstein–Barr Virus-Seronegative Kidney Recipients on Early and Late Post-Transplantation Lymphoproliferative Disorder Onset: A Retrospective Cohort Study. *Transpl Int* (2018) 31(5):484–94. doi:10.1111/tri.13085
15. Albatati S, Sharma A, Haubrich K, Wright A, Gantt S, Blydt-Hansen TD. Valganciclovir Prophylaxis Delays Onset of EBV Viremia in High-Risk Pediatric Solid Organ Transplant Recipients. *Pediatr Res* (2020) 87(5):892–6. doi:10.1038/s41390-019-0523-4
16. Holmes RD, Orban-Eller K, Karrer FR, Rowe DT, Narkewicz MR, Sokol RJ. Response of Elevated Epstein-Barr Virus DNA Levels to Therapeutic Changes in Pediatric Liver Transplant Patients: 56-Month Follow up and Outcome. *Transplantation* (2002) 74(3):367–72. doi:10.1097/00007890-200208150-00013
17. Funch DP, Walker AM, Schneider G, Ziyadeh NJ, Pescovitz MD. Ganciclovir and Acyclovir Reduce the Risk of Post-Transplant Lymphoproliferative Disorder in Renal Transplant Recipients. *Am J Transpl* (2005) 5(12):2894–900. doi:10.1111/j.1600-6143.2005.01115.x

18. Darenkov IA, Marcarelli MA, Basadonna GP, Friedman AL, Lorber KM, Howe JG, et al. Reduced Incidence of Epstein-Barr Virus-Associated Posttransplant Lymphoproliferative Disorder Using Preemptive Antiviral Therapy. *Transplantation* (1997) 64(6):848–52. doi:10.1097/00007890-199709270-00010
19. AlDabbagh MA, Gitman MR, Kumar D, Humar A, Rotstein C, Husain S. The Role of Antiviral Prophylaxis for the Prevention of Epstein-Barr Virus-Associated Posttransplant Lymphoproliferative Disease in Solid Organ Transplant Recipients: A Systematic Review. *Am J Transpl* (2017) 17(3): 770–81. doi:10.1111/ajt.14020
20. Moghadamnia M, Delroba K, Heidari S, Rezaie Z, Dashti-Khavidaki S. Impact of Antiviral Prophylaxis on EBV Viremia and Posttransplant Lymphoproliferative Disorders in Solid Organ Transplant Recipients: A Systematic Review and meta-analysis. *Virol J* (2025) 22(1):11. doi:10.1186/s12985-025-02623-y
21. Haixiang G, Yijing L, Shang J, Mingyun G, Yuanyue H, Bing G. Learning from Class-Imbalanced Data: Review of Methods and Applications. *Expert Syst Appl* (2017) 73:220–39. doi:10.1016/j.eswa.2016.12.035
22. Lundberg SM, Lee SI. A Unified Approach to Interpreting Model Predictions. *Adv Neural Inf Process Syst* (2017) 4766–75. doi:10.5555/3295222.3295230
23. Ladbury C, Zarinshenas R, Semwal H, Tam A, Vaidehi N, Rodin AS, et al. Utilization of Model-Agnostic Explainable Artificial Intelligence Frameworks in Oncology: A Narrative Review. *Transl Cancer Res* (2022) 11(10):3853–68. doi:10.21037/tcr-22-1626
24. Francis A, Johnson DW, Craig J, Teixeira-Pinto A, Wong G. Post-Transplant Lymphoproliferative Disease May Be an Adverse Risk Factor for Patient Survival but Not Graft Loss in Kidney Transplant Recipients. *Kidney Int* (2018) 94(4):809–17. doi:10.1016/j.kint.2018.06.009
25. Dierickx D, Habermann TM. Post-Transplantation Lymphoproliferative Disorders in Adults. *N Engl J Med* (2018) 378(6):549–62. doi:10.1056/NEJMra1702693
26. Sampaio MS, Cho YW, Shah T, Bunnapradist S, Hutchinson IV. Impact of Epsteinbarr Virus Donor and Recipient Serostatus on the Incidence of Post-Transplant Lymphoproliferative Disorder in Kidney Transplant Recipients. *Nephrol Dial Transpl* (2012) 27(7):2971–9. doi:10.1093/ndt/gfr769
27. Taylor AL, Marcus R, Bradley JA. Post-Transplant Lymphoproliferative Disorders (PTLD) After Solid Organ Transplantation. *Crit Rev Oncol Hematol* (2005) 56(1 SPEC. ISS.):155–67. doi:10.1016/j.critrevonc.2005.03.015
28. Mattila PS, Aalto SM, Heikkilä L, Mattila S, Nieminen M, Auvinen E, et al. Malignancies After Heart Transplantation: Presence of Epstein-Barr Virus and Cytomegalovirus. *Clin Transpl* (2001) 15(5):337–42. doi:10.1034/j.1399-0012.2001.150506.x
29. Paraskevas S, Coad JE, Gruessner A, Kandaswamy R, Humar A, Sutherland DER, et al. Posttransplant Lymphoproliferative Disorder in Pancreas Transplantation: A Single-Center Experience. *Transplantation* (2005) 80(5): 613–22. doi:10.1097/01.tp.0000168366.07896.d7
30. Quinlan SC, Pfeiffer RM, Morton LM, Engels EA. Risk Factors for Early-Onset and Late-Onset Post-Transplant Lymphoproliferative Disorder in Kidney Recipients in the United States. *Am J Hematol* (2011) 86(2):206–9. doi:10.1002/ajh.21911
31. Yager JE, Magaret AS, Kuntz SR, Selke S, Huang ML, Corey L, et al. Valganciclovir for the Suppression of Epstein-Barr Virus Replication. *J Infect Dis* (2017) 216(2):198–202. doi:10.1093/infdis/jix263
32. Preiksaitis JK, Keay S. Diagnosis and Management of Posttransplant Lymphoproliferative Disorder in Solid-Organ Transplant Recipients. *Clin Infect Dis* (2001) 33:S38–S46. doi:10.1086/320903
33. Murukesan V, Mukherjee S. Managing Post-Transplant Lymphoproliferative Disorders in Solid-Organ Transplant Recipients: A Review of Immunosuppressant Regimens. *Drugs* (2012) 72(12):1631–43. doi:10.2165/11635690-000000000-00000
34. Kinch A, Baecklund E, Molin D, Pauksens K, Sundström C, Tufveson G, et al. Prior Antithymocyte Globulin Therapy and Survival in Post-Transplant Lymphoproliferative Disorders. *Acta Oncol (Madr)* (2021) 60(6):771–8. doi:10.1080/0284186X.2021.1904520
35. Dharnidharka VR, Stevens G. Risk for Post-Transplant Lymphoproliferative Disorder After Polyclonal Antibody Induction in Kidney Transplantation. *Pediatr Transpl* (2005) 9(5):622–6. doi:10.1111/j.1399-3046.2005.00361.x
36. Shaw C, Hayes-Larson E, Glymour MM, Dufouil C, Hohman TJ, Whitmer RA, et al. Evaluation of Selective Survival and Sex/Gender Differences in Dementia Incidence Using a Simulation Model. *JAMA Netw Open* (2021) 4(3):e211001. doi:10.1001/jamanetworkopen.2021.1001
37. Howe CJ, Robinson WR. Survival-Related Selection Bias in Studies of Racial Health Disparities. *Epidemiology* (2018) 29(4):521–4. doi:10.1097/EDE.0000000000000849
38. Nugroho DR, Idris N, Kurniawan K, Fitrianah D, Irwansyah E, Kusuma GP. Logistic Regression and Random Forest Comparison in Predicting Students' Qualification Based on Students' Half-Semester Performance. *2023 11th Int Conf Inf Commun Technol ICoICT 2023* (2023) 2023:214–9. doi:10.1109/ICoICT58202.2023.10262783
39. Reddy Thummala GS, Baskar R, RimlonShibi S. Prediction of Heart Disease Using Random Forest in Comparison with Logistic Regression to Measure Accuracy. *Proc 2nd IEEE Int Conf Adv Comput Commun Appl Informatics, ACCAI* (2023):2023. doi:10.1109/ACCAI58221.2023.10199851
40. Zhao X, Sole-Casals J, Sugano H, - al Laiq F, Al-Obeidat F, et al. Exploring Clusters of Rare Events Using Unsupervised Random Forests. *J Phys Conf Ser* (2022) 2314(1):012019. doi:10.1088/1742-6596/2314/1/012019
41. He ZL, Zhou JB, Liu ZK, Dong SY, Zhang YT, Shen T, et al. Application of Machine Learning Models for Predicting Acute Kidney Injury Following Donation After Cardiac Death Liver Transplantation. *Hepatobiliary Pancreat Dis Int* (2021) 20(3):222–31. doi:10.1016/j.hbpd.2021.02.001
42. Wu X, Zhang H, Cai M, Zhang Y, Xu A. Predicting the Risk of Pulmonary Infection After Kidney Transplantation Using Machine Learning Methods: A Retrospective Cohort Study. *Int Urol Nephrol* (2024) 57(3):947–55. doi:10.1007/s11255-024-04264-6
43. Liu XY, Wu J, Zhou ZH. Exploratory Undersampling for Class-Imbalance Learning. *IEEE Trans Syst Man Cybern B Cybern* (2009) 39(2):539–50. doi:10.1109/TSMCB.2008.2007853

ESOT | Transplant International

REVIEW
published: 25 February 2026
doi: 10.3389/ti.2026.15366

Achievements, Challenges and Promises of Minimally Invasive Liver Transplantation

Clara Gomez[1,2‡], *Ismail Labgaa*[1,2,3,4†‡], *Elias Karam*[1,2,5†], *Federica Dondero*[1,2†], *Nassiba Beghdadi*[1,2†], *Christian Hobeika*[1,2†], *Safi Dokmak*[1,2†§] *and Mickaël Lesurtel*[1,2,6*†§]

[1]*Department of HPB Surgery and Liver Transplantation, APHP-Nord, Beaujon Hospital, Département médico-universitaire (DMU) DIGEST, Clichy, France,* [2]*Université Paris Cité, Paris, France,* [3]*Department of Visceral Surgery, Lausanne University Hospital (CHUV), Lausanne, Switzerland,* [4]*Faculty of Biology and Medicine (FBM), University of Lausanne (UNIL), Lausanne, Switzerland,* [5]*Institut national de la recherche et la santé médicale (Inserm) UMR1327 ISCHEMIA Membrane Signaling and Inflammation in reperfusion injuries, Université de Tours, Tours, France,* [6]*Institut national de la recherche et la santé médicale (Inserm), UMR-S1149, Centre de Recherche sur l'Inflammation (CRI), Université Paris Cité, Paris, France*

The integration of minimal invasive (MIS) techniques in liver transplantation (LT) emerged as a natural progression following advances in laparoscopic and robotic hepato-pancreato-biliary surgery. However, it poses specific challenges that are inherent to LT. Chronologically, it is a recent topic that only emerged 2 decades ago in donors and recently in recipients, but it has showed a meteoric rise with tremendous progress over the last years. This review aimed to provide a comprehensive yet synthetic overview of the available data on minimal invasive liver transplantation (MILT), for both donor hepatectomy (DH), recipient hepatectomy and graft implantation. Developments were numerous: top-notch technical skills have not only been reported but have foremost been performed worldwide by an increasing number of groups. Technology also played a central role, as exemplified by the integration of 3D visualization techniques, the utilization of indocyanine green (ICG) near-infrared fluorescence camera system or the use of robotic technology. Research efforts finally illustrated this progress with a rapid rise of number of publications and adoption. The present analysis of the available data permitted to identify gaps that may be valuable to explore by future research projects.

Keywords: laparoscopy, minimally invasive surgery, minimally invasive liver transplantation, robotic surgery, liver transplantation

OPEN ACCESS

***Correspondence**
Mickaël Lesurtel,
mickael.lesurtel@aphp.fr

†ORCID:
Ismail Labgaa
orcid.org/0000-0003-4286-2170
Elias Karam
orcid.org/0000-0002-6603-3804
Federica Dondero
orcid.org/0000-0002-3671-6962
Nassiba Beghdadi
orcid.org/0000-0002-5649-0702
Christian Hobeika
orcid.org/0000-0002-9592-2520
Safi Dokmak
orcid.org/0000-0002-3335-4388
Mickaël Lesurtel
orcid.org/0000-0003-2397-4599

‡*These authors share first authorship*

§*These authors share senior authorship*

Received: *02 August 2025*
Revised: *30 December 2025*
Accepted: *13 February 2026*
Published: *25 February 2026*

Citation:
Gomez C, Labgaa I, Karam E, Dondero F, Beghdadi N, Hobeika C, Dokmak S and Lesurtel M (2026) Achievements, Challenges and Promises of Minimally Invasive Liver Transplantation. Transpl. Int. 39:15366. doi: 10.3389/ti.2026.15366

INTRODUCTION

Liver transplantation (LT) is the best therapeutical option for a wide range of end-stage liver diseases, acute liver failure, and some liver malignancies. LT has been increasingly performed with approximately 41,000 procedures worldwide in 2023 [1].

Over the past decades, hepatic minimally invasive surgery (MIS) has been developed, with both laparoscopic and robotic approaches [2, 3]. The main reported benefits of these

Abbreviations: AI, artificial Intelligence; BMI, body mass index; CUSA, cavitron ultrasonic suction aspirator; CUSUM, cumulative sum method; DH, donor hepatectomy; ICG, indocyanine green; L-DH, laparoscopic donor hepatectomy; L-RH, laparoscopic right hepatectomy; LDLT, living donor liver transplantation; LH, left hepatectomy; LLS, left lateral sectionectomy; LoS, length of stay; LT, liver transplantation; MIDH, minimal invasive donor hepatectomy; MILT, minimal invasive liver transplantation; MIS, minimal invasive surgery; MIRH, minimally invasive recipient hepatectomy; O-DH, open donor hepatectomy; PSM, propensity score matching; R-DH, robotic donor hepatectomy; RH, right hepatectomy.

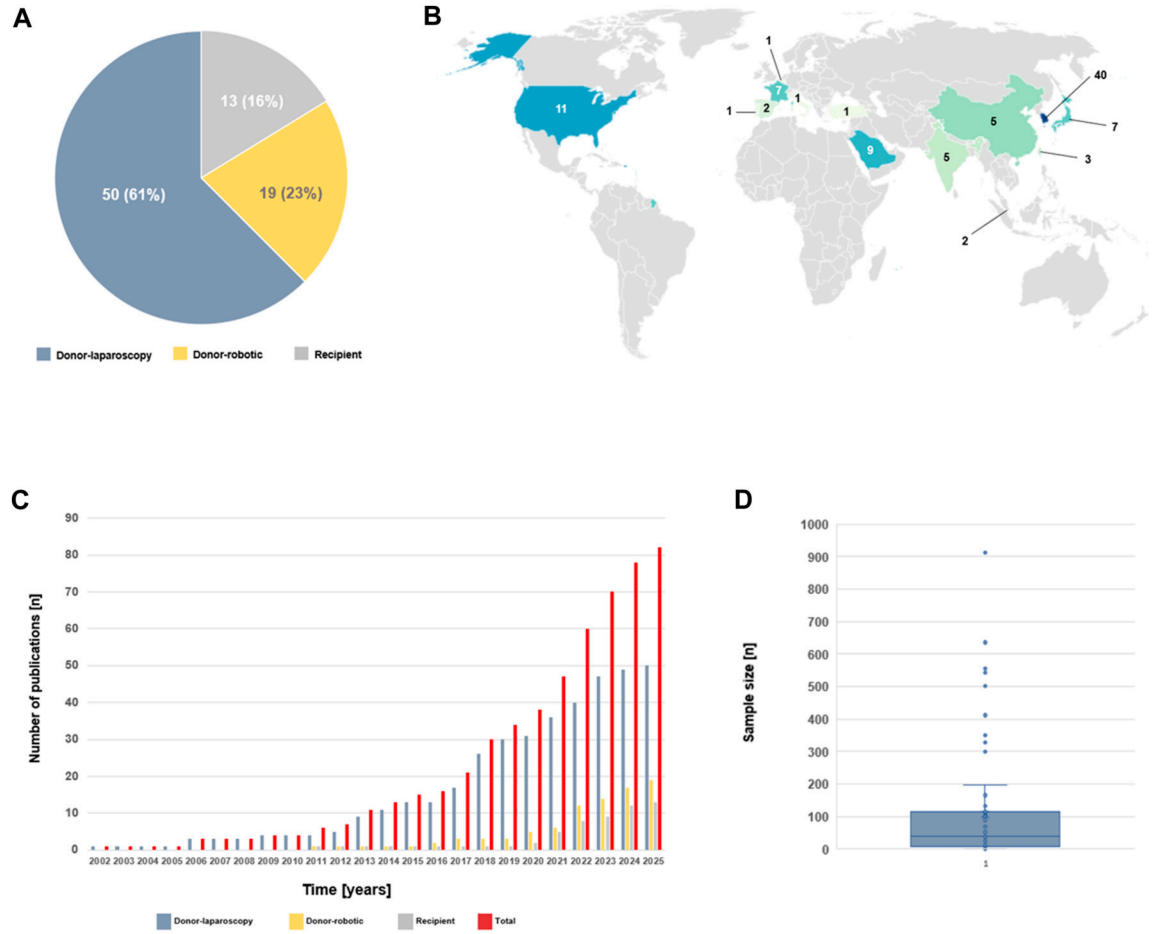


FIGURE 1 | Minimally Invasive Liver Transplantation. Overview of the available studies on MILT (n = 82). **(A)** Pie chart illustrating the distribution between laparoscopic donor hepatectomy (L-DH), robotic DH (R-DH) and minimal invasive techniques in the recipients. **(B)** Global map illustrating the number of publications per country. **(C)** Bar plots showing the chronological evolution of publications in the field. **(D)** Box plot illustrating sample size of the available studies.

techniques include reduced bleeding, a lower inflammatory response to trauma, decreased postoperative pain, improved cosmetic outcomes, and faster postoperative recovery [4]. The first laparoscopic liver resection was reported by H. Reich in 1991 [5]. Since then, MIS indications have expanded to include increasingly complex procedures. The first laparoscopic left lateral sectionectomy (LLS - segments II and III) in a living donor was reported by Cherqui et al. in [6] and 10 years later the first laparoscopic living donor right hepatectomy was described by Soubrane et al. [7]. These techniques then spread to Asia (South Korea) in particular where living donor liver transplantation (LDLT) is much more developed, and since 2016 attention has shifted toward the robotic approach [8]. However, partial liver resection from a living donor has been controversial, as it exposes a healthy individual to surgical morbidity and mortality and may impact long-term quality of life. Recent studies have shown that laparoscopic donor hepatectomy (L-DH) is feasible and safe when performed in an experienced liver transplant centre on selected donors [9–11].

Even though MIS was developed in living donors, it was only later applied to recipients. In 2011 Eguchi et al. described a hand assisted laparoscopic approach using MIS for liver mobilization, but a short midline incision was required for the subsequent explantation and implantation [12]. In 2019, the first laparoscopic total explant hepatectomy was reported by Dokmak et al. at Beaujon Hospital in France [13].

Although MIS in LT only implicates highly specialized hospital centers, it is considered a significant LT breakthrough. The present article aims to provide a thorough but synthetic overview of minimally invasive liver transplantation (MILT) and its different subdomains. It first focuses on the different aspects of the procedures and results in the donor, followed by a state-of-the art in the recipient.

MATERIALS AND METHODS

A detailed description of the methods is available in Supplementary Methods.

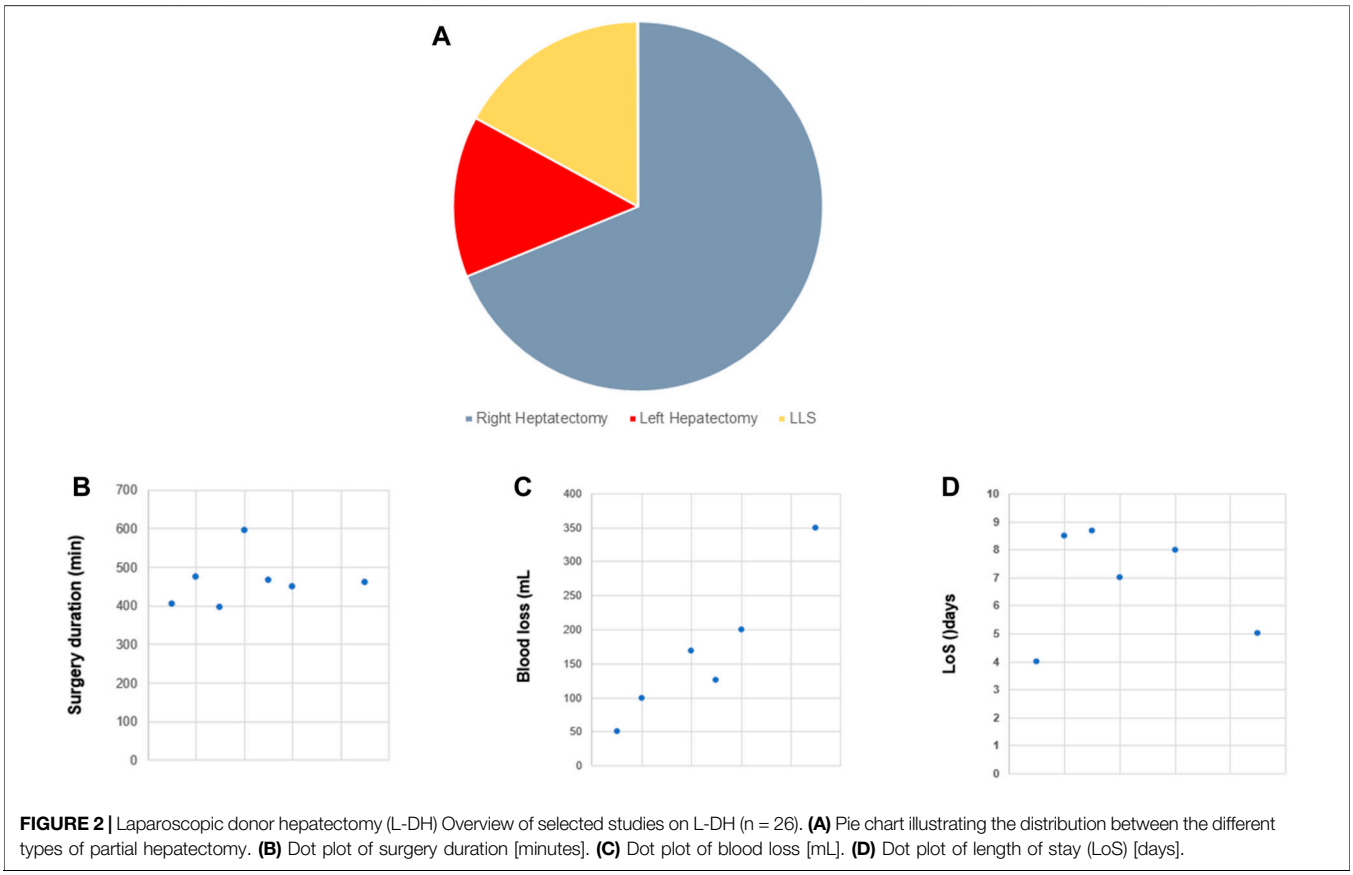


FIGURE 2 | Laparoscopic donor hepatectomy (L-DH) Overview of selected studies on L-DH (n = 26). **(A)** Pie chart illustrating the distribution between the different types of partial hepatectomy. **(B)** Dot plot of surgery duration [minutes]. **(C)** Dot plot of blood loss [mL]. **(D)** Dot plot of length of stay (LoS) [days].

RESULTS

Minimally Invasive Liver Transplantation at a Glance

Our review of the literature identified a total of 82 publications on MILT [6–8], [10–88]. Most of them (50 studies, 61%) reported laparoscopic donor hepatectomy (L-DH) whereas reports on robotic donor hepatectomy (R-DH) and MIS techniques in recipients represented 19 (23%) and 13 (16%) articles, respectively (**Figure 1A**). In term of scientific contributions, Republic of South Korea (40 contributions), United States of America (11 contributions), Saudi Arabia (9 contributions), Japan and France (7 contributions, each) appeared as the leading countries (**Figure 1B**). While the first report on MILT was published in 2002, the number of publications remained relatively constant during the following decade and started rising upon 2017 (**Figure 1C**). Likewise, the purport of these articles has progressively increased, partly illustrated by larger sample sizes over the years (**Figure 1D**).

Minimal Invasive Donor Hepatectomy (MIDH)

Laparoscopic Donor Hepatectomy (L-DH)

Laparoscopic donor hepatectomy (L-DH) was first reported in 2002, performed in two young parents in whom a left lateral sectionectomy (LLS) was performed and transplanted to their 1-year old sons suffering from biliary atresia [6]. Both donors and recipients recovered uneventfully and liver grafts showed excellent function. A decade later, striking progress were achieved to develop L-DH in pediatrics and adults, in particular in Asian countries such as Republic of South Korea. Literature on L-DH entails >50 peer-reviewed articles, detailed in **Supplementary Table S1**.

Overview of Laparoscopic Donor Hepatectomy (L-DH) Results

Twenty-six studies were selected for analysis [7, 11, 15, 19, 20, 22–24, 28, 31, 38, 42, 56, 60, 61, 64, 66, 68, 69, 71, 74, 77–79, 81, 82], yielding a total of 2404 patients. Most studies reported experiences of pure L-DH whereas a hybrid approach was also used. Right hepatectomy (RH) represented most procedures (**Figure 2A**). Conversion was requested in 30 patients (1.3%) (**Table 1**). Duration of surgery averaged 400 min (**Figure 2B**) and blood loss ranged from 100–600 mL (**Figure 2C**). No case of mortality was reported but 266/2404 (11.1%) and 95 (4%) patients developed overall and severe complications, respectively (**Table 1**). Most patients stayed 6–10 days at hospital after surgery (**Figure 2D**). Overall, these results demonstrate safety of L-DH.

These data provide an overview on the outcomes of patients undergoing L-DH but it must obviously be stratified for each specific procedures (e.g., RH vs. LLS). Unfortunately, data comparing outcomes after RH, left hepatectomy (LH) and LLS

TABLE 1 | Conversion rates and incidence of adverse events in minimally invasive liver transplantation in the 82 listed studies.

	Conversion	Overall complications	Major complications	Mortality
Laparoscopic DH	30/2404 (1.3%)	266/2404 (11.1%)	95/2404 (4%)	0/2404
Robotic DH	22/1629 (1.4%)	145/1629 (8.9%)	38/1629 (2.3%)	0/1629
Recipient	10/39 (25.6%)	5/39 (12.8%)	2/39 (5.1%)	1/39 (2.6%)
MILT	62/4072 (1.5%)	416/4072 (10.2%)	135/4072 (3.3%)	1/4072 (0.02%)

are lacking, because most studies reported series of a specific procedure for which the authors gained sufficient experience. Rare studies included different procedures; although outcomes were excellent for each specific procedures, data reasonably showed a trend toward higher complications rates after RH as opposed to LH or LLS [48].

The added value of L-DH on cosmetic and patients' satisfaction was also reported by several studies, as opposed to open donor hepatectomy (O-DH) [77, 82].

Patients' Selection and Predictors of Adverse Outcomes

Although patients' selection is paramount, most studies did not detail their selection criteria and/or did not precise whether specific conditions should be considered as contraindications for L-DH. Of note, the selection criteria of certain groups varied overtime, as exemplified by two groups that excluded donors with vascular or biliary anatomical variants in the initial phase of their experience with RH L-DH but thereafter extended their criteria and also included patients with anatomical variants [53, 63].

Important efforts were pursued to conduct research to assess safety and eventual benefits of L-DH. As an example, Rhu et al. thoroughly analyzed a monocentric cohort of 636 donors undergoing L-DH in South Korea [11]. Not only providing classical endpoints such as overall/major complications, mortality, and biliary complications, they also assessed postoperative bleeding, reoperation, and readmission rates that reached 6%, 2.2% and 5.2%, respectively. Furthermore, they identified risk factors of specific types of complications in donors: the presence of 2 hepatic arteries was associated with an increased risk of biliary leakage, whilst the Pringle maneuver appeared to be protective against this complication. Similarly, a multicentric Korean study including 543 patients aimed to identify factors associated with adverse events in to predict safety and thereby to facilitate patient selection [29]. BMI >30 kg/m2 was a predictor of higher conversion rate whereas graft weight >700 g and surgery duration >400 min predicted higher risk of overall- and major complications. In a recent study comparing L-DH and O-DH, multiple portal veins were identified as an independent predictor of major- (OR, 5.75; 95% CI, 1.28-25.79; p = 0.022) and biliary (OR, 3.84; 95% CI, 1.71-8.69; p = 0.001) complications, in donors [15].

Comparison With Open Approach

Subsequently, authors naturally aimed to determine whether L-DH was comparable or superior to O-DH. A cohort study reviewed 894 donors and conducted propensity score matching (PSM) for a head-to-head comparison of 198 donor-recipient pairs [42]. No case of mortality was observed. Compared to O-DH, L-DH was associated with longer duration of surgery (290 vs. 271 min, $p < 0.001$), longer time to remove the liver from the abdomen (211 vs. 166 min, $p < 0.001$) and longer warm ischemia time (12 vs. 4 min, $p < 0.001$), but reduced length of stay (LoS) (8 vs. 9 days, $p < 0.001$) and comparable overall complication rates (6.1% vs. 10.6%, p = 0.102); no difference in recipient survival was highlighted (p = 0.935). Another recent study also used PSM to compared both laparoscopic (n = 329) and open (n = 3019) approaches in living donors, and showed similar results [15].

Outcomes After Laparoscopic Donor Hepatectomy (L-DH)

Reporting their initial experience on L-DH in a cohort of 54 patients, Kwon et al. also analyzed recipients' outcomes [63]: biliary and arterial complications occurred in 31.5% and 2.7%, respectively whereas graft failure was reported in 5 (9.3%) patients. A PSM analysis comparing L-DH and O-DH in 220 pediatric transplantations showed similar outcomes for recipients [67]. Park et al. also conducted a PSM analysis comparing 72 recipients from O-DH and L-DH, showing no difference for major complications (40.3% vs. 47.2%, p = 0.397), graft failure (4.2% vs. 5.6%, p = 0.699) and mortality (2.8% vs. 4.2%, p = 0.657) [57].

Cho et al. compared outcomes in both donors and recipients after laparoscopic RH *versus* laparoscopic right posterior sectionectomy [32]. Overall outcomes for recipients showed major complications and mortality rates of 36.5% and 2.3%, respectively, and comparison further detected higher rates of major complications after laparoscopic right posterior sectionectomy as opposed to laparoscopic RH (62.5% versus 35.2%, p = 0.034). Kim et al. identified multiple bile ducts as a predictor of bile leakage and biliary stricture in the recipients [15].

Technical Considerations

One may reasonably question the feasibility of implementing L-DH, particularly in Western countries. Encouraging data demonstrated the feasibility to develop programs dedicated to L-DH in Western countries with good outcomes [10, 31]. This raises the question of the learning curve, unfortunately barely investigated. Cumulative sum method (CUSUM) of the operative time of a single surgeon who performed 100 L-RH, showed a continuous fall after 43 operations, which was used as a cut-off to split the retrospective cohort in two groups (i.e., initial n = 43, and recent n = 57) [49]. In comparison to the initial group, surgery

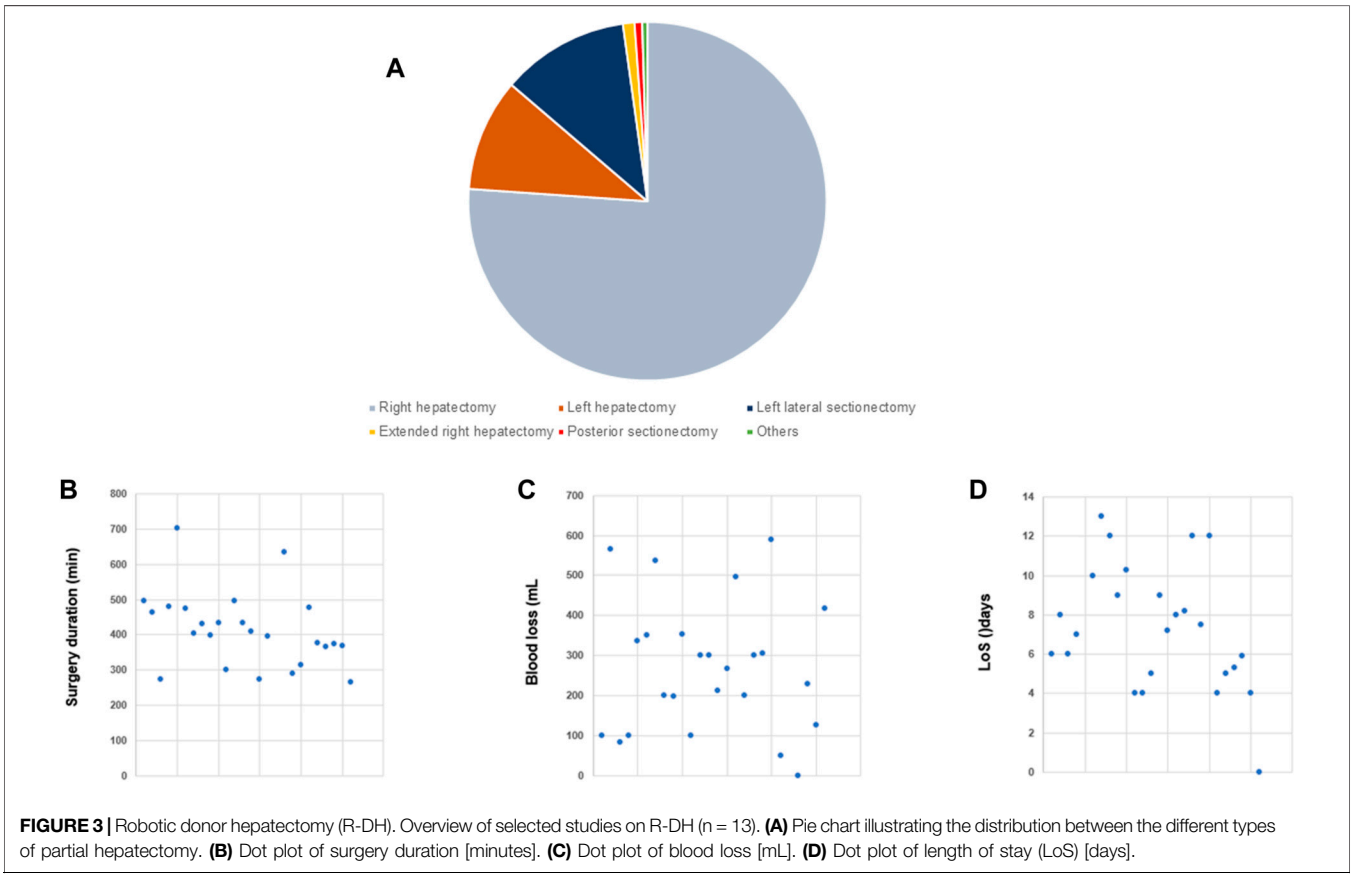


FIGURE 3 | Robotic donor hepatectomy (R-DH). Overview of selected studies on R-DH (n = 13). **(A)** Pie chart illustrating the distribution between the different types of partial hepatectomy. **(B)** Dot plot of surgery duration [minutes]. **(C)** Dot plot of blood loss [mL]. **(D)** Dot plot of length of stay (LoS) [days].

duration (282 vs. 181 min, $p < 0.01$) and length of stay (7.1 vs. 5.8 days, $p < 0.01$) were shorter in the recent group while overall complications rate was comparable (1.8% vs. 9.3%, $p = 0.1$). Following a similar approach, another group established that 1 year including 115 patients was sufficient to standardize the procedure [62].

Visualization techniques is also an important point. Although data comparing 2D versus 3D technologies are not yet available, recent studies mostly used 3D techniques. As an example, Kwon et al. reported switching from 2D to 3D during the study period [63], and rapidly recognized the advantages offered by 3D vision.

Likewise, indocyanine green (ICG) near-infrared fluorescence camera system has gained important interest and is more and more often utilized to facilitate the visualization of bile duct division and/or to demarcate the exact midplane [42]. As energy-sealing devices are more likely to be used in MIS, and they are presumably at higher risk of causing thermal damages to the microvasculature surrounding bile ducts. Offering the option to accurately delineate the biliary tree before transection, ICG may be particularly valuable to prevent biliary injuries.

Robotic Donor Hepatectomy (R-DH)

Robotic donor hepatectomy (R-DH) remains restraint to the experience of a small number of centers and surgeons that have developed the specific skills and expertise. Consequently, reports on the topic are scant, with only 20 publications [8, 14, 16–18, 20, 25, 26, 34, 36, 40, 41, 44, 46, 54, 55, 70, 80, 87, 88] retrieved from the literature (**Supplementary Table S2**). Those included 3 case reports, 2 case series, 11 cohort studies and 4 case-match studies. Five and 6 studies were conducted in South Korea and Saudi Arabia, respectively. Median sample size was 64 (12-116), heterogeneously varying from 1 to 913 patients.

In 2011, Giulianotti et al. reported the first case of robotic right hepatectomy for LDLT [80]. The procedure was exclusively performed with a minimal invasive technique and the specimen was extracted through a small lower midline incision. Cold and warm ischemia were limited to 25 and 35 min, respectively, and both the donor and the recipient showed an uneventful postoperative course. Subsequently, publications on the topic showed a meteoric rise.

Overview of Robotic Donor Hepatectomy (R-DH) Results

Thirteen studies including 1629 patients undergoing robotic DH were reviewed [8, 14, 16–18, 20, 26, 41, 44, 70, 80, 87, 88]. Distribution of the types of partial hepatectomies is illustrated in **Figure 3A**, showing a majority of RH (69%). Conversion was indicated in 22/1629 (0.7%) patients (**Table 1**). Duration of surgery was typically between 400 and 500 min (**Figure 3B**), with blood loss essentially approximating 200 mL (**Figure 3C**). In term of postoperative outcomes, overall and major complications appeared in 145 (8.9%) and 38 (2.3%) patients, respectively. No case of postoperative mortality was reported (**Table 1**). LoS varied

from 4 to 9 days (**Figure 3D**). In summary, R-DH appears as a safe procedure with low incidences of adverse events and no reported mortality, to date, given it is performed in centers with high expertise in MIS.

Patients' Selection and Predictors of Adverse Outcomes

Like in L-DH, exclusion criteria essentially included high BMI, large graft volume or anatomical variants [14, 41, 44]. While predictors of adverse outcomes have been identified for L-DH, it precisely represents an unmet need in the field of R-DH. Future studies should actively tackle this challenge.

Comparison With Open and Laparoscopic Approaches

Studies compared R-DH with O-DH and/or with L-DH, tackling the stake question: does robotic offer any advantage in DH [8, 14, 16, 18, 20, 36, 40, 44, 46, 55]. Most comparisons showed that R-DH was associated with longer surgery duration, lower blood loss and similar postoperative complications rates [16, 18, 36, 40, 44]. Associations with lower pain (visual analogue scale on POD 3 of 2.4 in R-DH vs. 3.1 in O-DH, $p < 0.001$) [18] and shorter LoS (8 vs. 9 days, $p < 0.001$) [44] were also reported. The group of Riyadh recently published a landmark study providing a comprehensive analysis of 1724 donor-recipient pairs, and comparing 913 R-DH with 646 O-DH and 165 L-DH [20]. R-DH showed lower rate of overall complications (R-DH = 4%, L-DH = 8%, O-DH = 16%; $p < 0.001$) but major complications (R-DH = 0.1%, L-DH = 0%, O-DH = 0.8%; $p = 0.065$) and mortality (no case of mortality reported) were similar among the three groups. A study applying PSM to compare R-DH to L-DH, including 71 donor-recipient pairs in each group, reported reduced biliary after R-DH (22.5% *versus* 42.3%, $p = 0.012$) [16].

Outcomes After R-DH

Raptis DA et al. also analyzed outcomes of the recipients: both adult (R-DH = 23%, L-DH = 44%, O-DH = 31%; $p = 0.001$) and pediatric (R-DH = 15%, L-DH = 25%, O-DH = 19%; $p = 0.033$) recipients showed lower incidence of major complications after R-DH, as opposed to O-DH and L-DH. In 2024, the same group performed a fully robotic donor total hepatectomy and recipient liver graft implantation and therewith established an important milestone in the development of R-DH [25]. Likewise, propensity score matching was applied to compare 71 donor-recipients pairs undergoing either R-DH or L-DH, and specifically soughing biliary complications [16]. In donors, outcomes were similar but recipients of robotic-procured grafts showed lower rates of biliary complications (22.5% vs. 42.3%, $p = 0.012$), compared to recipients from L-DH. The authors attributed this difference to the precision of robotics for dissection and for bile duct division, which presumably reduced the risk of bile duct openings.

In a multicentric retrospective study using PSM, 50 recipients of robotic-procured grafts were compared to 100 recipients of open- and laparoscopic-procured grafts. Rate of major complications and survival were comparable among the groups [18]; another study by Amma et al. including 102 R-DH and 152 O-DH showed consistent findings [44].

Technical Considerations

Analysis suggested that 17 procedures were required to achieve the learning curve for robotic right donor hepatectomy [17]. Descriptions of surgical techniques are quite concordant among the different reports, at least for living donor right hepatectomy. DaVinci® system was the most used platform and surgeons typically placed 5 trocars. Most groups used a Pfannenstiel incision to extract the graft [8, 14, 16, 17, 26, 35, 36, 40, 41, 44, 46, 54, 55, 70, 87, 88]. Variations included Pringle maneuver and the use of indocyanine green cholangiography. The former was inconstant, described in some reports (on for 15 min, off for 5 min) [41], but seemed to be avoided by a majority of teams while it does not appear deleterious when applied [8, 17, 44]. Regarding the latter, it has been integrated in some surgical protocols to facilitate the visualization of the bile ducts before dividing them and thus presumably reduce the risk of biliary complications [8, 14, 16, 17, 36, 40, 41, 46, 54, 55, 87, 88].

Like in conventional liver surgery, parenchymal transection techniques and devices highly varied. Most studies described using harmonic scalpel and Maryland bipolar forceps [8, 14, 16, 17, 26, 36, 40, 41, 44, 46, 54, 55, 70], whereas a combined laparoscopic Cavitron Ultrasonic Suction Aspirator (CUSA) was also utilized in some cases requiring a second liver surgeon at the sterile operating table [26, 44]. Likewise, multiple techniques exist to divide bile ducts, but "clip and cut" was the most frequently reported option [14, 26].

Minimally Invasive Liver Transplantation: Recipient's Perspective

The first reported use of a minimally invasive recipient hepatectomy (MIRH) was in a Japanese study from Eguchi et al. with nine cases, mostly for viral chronic liver disease patients with a median Child-Pugh score of 9 [12]. Surgical technique consisted in a hand-assisted liver mobilization with a Gelport device inserted through an 8-cm upper midline laparotomy which was eventually extended to 12–15 cm to finish the explantation and perform the anastomoses. Median blood loss was 3940 mL and operative duration was 74min with one postoperative death. Results were not different from the 13 patients operated through a Mercedes-Benz-type incision during the same period, except for a longer median operative duration (812 vs. 741 min, $p < 0.05$).

The first report of a full laparoscopic explantation was published by Dokmak et al. in France in 2020 [13] in a patient with liver metastases of a neuroendocrine tumor. Without any underlying liver disease hence no portal hypertension and associated portosystemic shunts, portal flow must be preserved until the very end of the explantation. Rapid dissection of the bile duct and hepatic artery was performed with no porto-caval shunt, and extensive caval dissection was eased by the early division of the left hepatic vein trunk, aiming the shortest anhepatic phase duration. A previous 12-cm midline incision helped retrieve the specimen and perform a lateral

clamping of the vena cava and anastomoses similar to the open approach. In this patient, a left lateral sectionectomy had to be performed. This report was later completed with a case series of 6 patients [43]. All patients had liver metastases from neuroendocrine tumors, all grafts were from brain death donors, midline incision length varied from 12 to 20 cm, blood loss from 250 to 600 mL, operative duration from 323 to 450 min and there was no postoperative death. Dokmak and colleagues emphasized the importance of small liver grafts of excellent quality, like in DH. Indications have been recently expended to selected cirrhotic patients with moderate liver volume and portosystemic venous shunts allowing early division of the portal vein with no portocaval anastomosis.

The first report of a full laparoscopic LDLT comes from Suh et al. in South Korea in 2021 [33]. The right liver graft from a living donor was inserted through a Pfannenstiel incision with laparoscopic implantation. Blood loss was 3300 mL, operative duration 960 min, warm ischemia time 84 min and portal clamping time 212 min. Left portal flow preservation technique was applied to shorten as much as possible the anhepatic phase. Laparoscopic anastomoses proved to be challenging, leading the same team to propose a hybrid approach, with robot-assisted arterial and biliary anastomoses, with blood loss of 11500 mL and operative duration of 1140 min [34]. In both cases there was no major complication and hospital stay were respectively 11 and 13 days.

In 2023, other pioneers pushed the envelope and published the first cases of fully robotic liver transplant, with R-DH followed by robotic graft implantation [25, 27]. Lee et al. reported blood loss of 6300 mL and operative duration of 850 min [27] while Broering et al. almost simultaneously reported a 3-case series with blood loss of 700–1000 mL and no major postoperative complication in both donors and recipients [25]. Eventually, Khan et al. performed a full robotic LT from a brain death donor with uneventful follow-up [85]. More recently, the groups from Lisbon and from Modena commonly reported their experience of robotic whole liver transplantation in 6 patients. Selection criteria were patients with hepatocellular carcinoma, small caudate lobe, low degree of portal hypertension, absence of porto-mesenteric thrombosis and low MELD score. Fully R-DH was followed by robotic implantation of the graft through a small midline incision. Reported outcomes were excellent: warm ischemia ranged from 55 to 90 min, surgery duration from 440 to 710 min. Altogether, 5/6 patients experienced no postoperative complication whereas one patient showed prolonged hyperbilirubinemia with no particular consequence [86].

Apart from these landmark publications, other reports were published between 2010 and 2025 representing a total of 35 patients (**Supplementary Table S3**) [21, 25, 30, 39, 47]. Procedures required five or six various size trocars, with pedicle dissection leaving long biliary and vascular stumps. Portal vein division was either performed during the pedicle dissection or at the latest point during the explantation (*i.e.*, left portal flow preserving dissection) [30, 33, 47]. Graft implantation was performed through a midline incision [12, 13, 21, 30, 43, 47] or a Pfannenstiel incision combined with a Gelport device [25, 27, 33, 34, 39]. Clamping of the inferior vena cava was lateral [13, 43], total with a Glover clamp (especially for minimally invasive implantation) [25] or with a combination of distal Chitwood clamp and proximal bulldog clamps [27, 39, 47]. In case of a right liver graft, iced gauze was put beneath the liver in the right upper abdominal quadrant [39, 47] and the graft portal vein was elongated during the backtable [39]. Minimally invasive anastomoses were robotic, hybrid or laparoscopic. Laparoscopy allows a larger range of movement and facilitates the presence of an assistant to position the iced gauze. Venous anastomoses are large enough to be performed laparoscopically [39, 47] whereas the robotic approach seems to be particularly adapted to the small diameter of the arterial and biliary anastomoses [27, 39, 47].

Throughout the literature, a total of 55 MIRH have already been performed. Operative time varied from 350 to 1065 min [13, 34], blood loss from 100 to 24200 mL [21, 30], intraoperative transfusion from 0 to 42 units of red blood cells [13, 30] and conversion rate from 0% to 60% during explantation [30]. Major complications (i.e., Clavien >2) occurred at most in 10% of patients [30]. Cold and warm ischemia times were not always reported but ranged respectively from 50 to 575 min and 21–117 min [30, 43, 47]. Operative and ischemia times as well as blood loss were greater in patients undergoing MIRH although postoperative outcomes did not seem to be worsened. This highlights the importance of the learning curve in such procedures, even considering that all surgeons involved are already highly skilled [39]. Coordination with the graft harvesting team is paramount to reduce ischemia time.

MIRH is feasible and challenges reside mostly in the implantation phase, where concerns can be raised about the necessity of vena cava total clamping, prolonged duration of the portal vein occlusion and its consequences especially in patients without portal hypertension. The hybrid laparoscopic/robotic approach seems to be a good alternative in the early experience with minimal risk for both recipients and grafts.

DISCUSSION

MILT is a rapidly emerging field, as exemplified by the rising number of publications during the last 5–10 years (**Figure 1B**). Tremendous progress has been made in a very short period of time as assessed by the number of publications and patients.

Obvious considerations and specificities render the use of minimal invasive techniques in LT much more complex which, given MILT controversial nature, limits its generalization. Conversely to conventional surgery that is typically performed in patients harboring diseases that indicate surgery, living donors are healthy by definition. Hence, safety becomes even more crucial in these patients. In addition, moderate or poor outcomes would likely discourage potential donors, which would ultimately accentuate the dramatic issue of organ shortage, particularly in Eastern countries where LDLT remains the main source of liver grafts. Therefore, most available studies previously discussed focused on safety. Recent studies provided valuable data that not only

addressing safety or technical aspects of MILT, but aiming to identify risk factors or tackling the difficult challenge of patients' selection. Improving patients' selection is precisely at the crossroad between challenges and promises. It is likely a game-changer in MILT. It is a pivotal stake as important in donors as in recipients. For the latter, on a more technical point of view, patients' selection must facilitate MILT procedures. Ideal recipients are those who need non complicated LT (e.g., no portal vein thrombosis) harboring small liver and small segment I, allowing easier manipulation and giving more space for instruments and cameras. Cirrhotic livers, stiff, are more difficult to retract and mobilize. Patients with ascites also were found to provide more workspace because of a dilated abdominal cavity. A left lateral sectionectomy can be performed to create space, minding a risk of disease dissemination in case of associated cancer disease. Presence of portal hypertension and collateral circulation can be beneficial by allowing rapid division of the portal vein without porto-caval shunt to ease caval dissection and increase tolerability of prolonged duration of portal and caval clamping. On the other hand, the absence of a porto-caval shunt increase mesenteric congestion and bleeding risk [25, 33, 43]. Along with the learning curve completion, indications are to be extended and future studies are needed to better understand how create the "bel-ensemble" and how pairing surgical approaches according to both donors' and recipients' characteristics. Presently, apart from feasibility, it is very early to conclude on the benefit of this approach regarding recovery, early and long-term complications.

Another challenge is the democratization of MILT. Although, certain groups have demonstrated the feasibility to start, develop and maintain MILT programs, achieving great results in short periods of time, it is a very demanding task. Again, MILT is essentially driven by a few groups, worldwide. In term of research, most articles provided data deriving from a single training cohort but lacked validation cohort. This is an important aspect that needs to be addressed by future studies in the field. Likewise, multicentric studies were quite uncommon.

A minimally invasive organ transplant consensus conference was held in Riyadh in December 2024. The aim was to develop consensus-driven recommendations for applying those techniques across various organ types (kidney, liver, pancreas, lung, heart, and uterus). The produced recommendations offer a guide for centers worldwide to implement MILT with ongoing evaluation and adaptation based on emerging evidence and technological advancements [89].

Drawing definitive conclusions about MILT from the literature is quite early. L-DH is the most studied field and the most performed procedure, with results backed by a sizable body of evidence. Recipient-related procedures are still confidential, with case reports or at best case series from highly-experienced surgeons. If one extrapolates the kinetics of MILT that occurred during the last 2-3 years, the field has a bright future. Promises rely on technological developments like the improvement of robotic platforms, for instance. The application of artificial intelligence is another important domain that has not yet been explored but that may offer pivotal options to overcome specific difficulties.

In summary, MILT is a rapidly emerging topic that gained a striking interest along the last years. Challenges and promises in MILT are closely related. Future studies may further tackle the challenge of patients' selection and new technologies such as the application of artificial intelligence may be of interest to moving the field forward.

AUTHOR CONTRIBUTIONS

CG, IL, and EK: Methodology, Investigation, Data Curation, Formal analysis, Writing Original draft, Writing – review and editing; FD, NB, and CH: Validation, Writing – review and editing; SD and ML: Conceptualization, Resources, Validation, Writing Original draft, Writing – review and editing, Visualization, Supervision.

FUNDING

The author(s) declared that financial support was not received for this work and/or its publication.

CONFLICT OF INTEREST

The authors(s) declared that this work 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) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2026.15366/full#supplementary-material

REFERENCES

1. Global Observatory on Donation and Transplantation (GODT). Organ Donation and Transplantation Activities 2023 Report (2024). Available online at: https://www.transplant-observatory.org/wp-content/uploads/2024/12/2023-data-global-report-17122024.pdf (Accessed March 14, 2025).
2. Abu Hilal M, Aldrighetti L, Dagher I, Edwin B, Troisi RI, Alikhanov R, et al. The Southampton Consensus Guidelines for Laparoscopic Liver Surgery: From Indication to Implementation. *Ann Surg* (2018) 268(1):11–8. doi:10.1097/SLA.0000000000002524
3. Hobeika C, Pfister M, Geller D, Tsung A, Chan ACY, Troisi RI, et al. Recommendations on Robotic Hepato-Pancreato-Biliary Surgery. The Paris Jury-Based Consensus Conference. *Ann Surg* (2025) 281(1):136–53. doi:10.1097/SLA.0000000000006365
4. Ciria R, Cherqui D, Geller DA, Briceno J, Wakabayashi G. Comparative Short-Term Benefits of Laparoscopic Liver Resection: 9000 Cases and Climbing. *Ann Surg* (2016) 263(4):761–77. doi:10.1097/SLA.0000000000001413
5. Reich H, McGlynn F, DeCaprio J, Budin R. Laparoscopic Excision of Benign Liver Lesions. *Obstet Gynecol* (1991) 78(5 Pt 2):956–8.
6. Cherqui D, Soubrane O, Husson E, Barshasz E, Vignaux O, Ghimouz M, et al. Laparoscopic Living Donor Hepatectomy for Liver Transplantation in Children. *Lancet Lond Engl* (2002) 359(9304):392–6. doi:10.1016/S0140-6736(02)07598-0
7. Soubrane O, Perdigao Cotta F, Scatton O. Pure Laparoscopic Right Hepatectomy in a Living Donor. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2013) 13(9):2467–71. doi:10.1111/ajt.12361
8. Chen PD, Wu CY, Hu RH, Ho CM, Lee PH, Lai HS, et al. Robotic Liver Donor Right Hepatectomy: A Pure, Minimally Invasive Approach. *Liver Transpl Off Publ Am Assoc Study Liver Dis Int Liver Transpl Soc* (2016) 22(11):1509–18. doi:10.1002/lt.24522
9. Cherqui D, Ciria R, Kwon CHD, Kim KH, Broering D, Wakabayashi G, et al. Expert Consensus Guidelines on Minimally Invasive Donor Hepatectomy for Living Donor Liver Transplantation from Innovation to Implementation: A Joint Initiative From the International Laparoscopic Liver Society (ILLS) and the Asian-Pacific Hepato-Pancreato-Biliary Association (A-PHPBA). *Ann Surg* (2021) 273(1):96–108. doi:10.1097/SLA.0000000000004475
10. Soubrane O, Eguchi S, Uemoto S, Kwon CHD, Wakabayashi G, Han HS, et al. Minimally Invasive Donor Hepatectomy for Adult Living Donor Liver Transplantation: An International, Multi-institutional Evaluation of Safety, Efficacy and Early Outcomes. *Ann Surg* (2022) 275(1):166–74. doi:10.1097/SLA.0000000000003852
11. Rhu J, Choi GS, Kim JM, Kwon CHD, Joh JW. Risk Factors Associated with Surgical Morbidities of Laparoscopic Living Liver Donors. *Ann Surg* (2023) 278(1):96–102. doi:10.1097/SLA.0000000000005851
12. Eguchi S, Takatsuki M, Soyama A, Hidaka M, Tomonaga T, Muraoka I, et al. Elective Living Donor Liver Transplantation by Hybrid hand-assisted Laparoscopic Surgery and Short Upper Midline Laparotomy. *Surgery* (2011) 150(5):1002–5. doi:10.1016/j.surg.2011.06.021
13. Dokmak S, Cauchy F, Sepulveda A, Choinier PM, Dondéro F, Aussilhou B, et al. Laparoscopic Liver Transplantation: Dream or Reality? The First Step with Laparoscopic Explant Hepatectomy. *Ann Surg* (2020) 272(6):889–93. doi:10.1097/SLA.0000000000003751
14. Sambommatsu Y, Kumaran V, Imai D, Savsani K, Khan AA, Sharma A, et al. Early Outcomes of Robotic Vs Open Living Donor Right Hepatectomy in a US Center. *Surg Endosc* (2025) 39(3):1643–52. doi:10.1007/s00464-024-11469-4
15. Kim SH, Kim KH, Yoon YI, Kang WH, Lee SK, Hwang S, et al. Feasibility of Pure Laparoscopic Donor Right Hepatectomy Compared With Open Donor Right Hepatectomy: A Large Single-center Cohort Study. *Ann Surg* (2025) 281(5):823–33. doi:10.1097/SLA.0000000000006633
16. Kim NR, Han DH, Joo DJ, Lee JG, Kim DG, Kim MS, et al. Propensity Score-Matched Donor and Recipient Outcomes: Robotic Versus Laparoscopic Donor Right Hepatectomy. *Transplantation* (2025) 109(3):e166–74. doi:10.1097/TP.0000000000005245
17. Cheah YL, Yang HY, Simon CJ, Akoad ME, Connor AA, Daskalaki D, et al. The Learning Curve for Robotic Living Donor Right Hepatectomy: Analysis of Outcomes in 2 Specialized Centers. *Liver Transpl Off Publ Am Assoc Study Liver Dis Int Liver Transpl Soc* (2025) 31(2):190–200. doi:10.1097/LVT.0000000000000480
18. Troisi RI, Cho HD, Giglio MC, Rhu J, Cho JY, Sasaki K, et al. Robotic and Laparoscopic Right Lobe Living Donation Compared to the Open Approach: A Multicenter Study on 1194 Donor Hepatectomies. *Liver Transpl Off Publ Am Assoc Study Liver Dis Int Liver Transpl Soc* (2024) 30(5):484–92. doi:10.1097/LVT.0000000000000304
19. Tırnova İ, Alim A, Karataş C, Akbulut A, Demir B, Alper A, et al. Complications of Laparoscopic and Open Donor Hepatectomy for Living Donor Liver Transplantation: Single Center Experience. *Exp Clin Transpl Off J Middle East Soc Organ Transpl* (2024) 22(8):629–35. doi:10.6002/ect.2023.0241
20. Raptis DA, Elsheikh Y, Alnemary Y, Marquez KAH, Bzeizi K, Alghamdi S, et al. Robotic Living Donor Hepatectomy Is Associated with Superior Outcomes for Both the Donor and the Recipient Compared with Laparoscopic or Open - A Single-Center Prospective Registry Study of 3448 Cases. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2024) 24(11):2080–91. doi:10.1016/j.ajt.2024.04.020
21. Liu XM, Li Y, Feng Z, Zhang XG, Wang SP, Xiang JX, et al. Laparoscopic-Assisted Full-Sized Liver Transplantation With Magnetically Fast Portal Vein Anastomosis: An Initial Cohort Study. *Int J Surg Lond Engl* (2024) 110(9):5483–8. doi:10.1097/JS9.0000000000001730
22. Linn YL, Chong Y, Tan EK, Koh YX, Cheow PC, Chung AYF, et al. Early Experience With Pure Laparoscopic Donor Hepatectomy: Comparison With Open Donor Hepatectomy and Non-Donor Laparoscopic Hepatectomy. *ANZ J Surg* (2024) 94(4):515–21. doi:10.1111/ans.18464
23. Ju MK, Yoo SH, Choi KH, Yoon DS, Lim JH. Selective Hanging Maneuver and Rubber Band Retraction Technique for Pure Laparoscopic Donor Right Hepatectomy. *Asian J Surg* (2024) 47(1):354–9. doi:10.1016/j.asjsur.2023.08.227
24. Hong SK, Kim JY, Lee J, Kim J, Choi HH, Lee S, et al. Pure Laparoscopic Donor Hepatectomy: Experience of 556 Cases at Seoul National University Hospital. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2024) 24(2):222–38. doi:10.1016/j.ajt.2023.06.007
25. Broering DC, Raptis DA, Elsheikh Y. Pioneering Fully Robotic Donor Hepatectomy and Robotic Recipient Liver Graft Implantation - A New Horizon in Liver Transplantation. *Int J Surg Lond Engl* (2024) 110(3):1333–6. doi:10.1097/JS9.0000000000001031
26. Rela M, Rajalingam R, Cherukuru R, Palaniappan K, Kumar SA, Kanagavelu R, et al. Experience with Establishing a Robotic Donor Hepatectomy Program for Pediatric Liver Transplantation. *Transplantation* (2023) 107(12):2554–60. doi:10.1097/TP.0000000000004649
27. Lee KW, Choi Y, Lee S, Hong SY, Suh S, Han ES, et al. Total Robot-Assisted Recipient's Surgery in Living Donor Liver Transplantation: First Step Towards the Future. *J Hepato-biliary-pancreat Sci* (2023) 30(10):1198–200. doi:10.1002/jhbp.1327
28. Law JH, Tan CHN, Tan KHJ, Gao Y, Pang NQ, Bonney GK, et al. Safely Implementing a Program of Pure Laparoscopic Donor Right Hepatectomy: The Experience From a Southeast Asian Center. *Transpl Direct* (2023) 9(6):e1486. doi:10.1097/TXD.0000000000001486
29. Kim SH, Kim KH, Cho HD, Suh KS, Hong SK, Lee KW, et al. Donor Safety and Risk Factors of Pure Laparoscopic Living Donor Right Hepatectomy: A Korean Multicenter Study. *Ann Surg* (2023) 278(6):e1198–203. doi:10.1097/SLA.0000000000005976
30. Kim JC, Hong SK, Lee KW, Lee S, Suh S, Hong SY, et al. Early Experiences With Developing Techniques for Pure Laparoscopic Explant Hepatectomy in Living Donor Liver Transplantation. *Liver Transpl Off Publ Am Assoc Study Liver Dis Int Liver Transpl Soc* (2023) 29(4):377–87. doi:10.1002/lt.26564
31. Fujiki M, Pita A, Kusakabe J, Sasaki K, You T, Tuul M, et al. Left Lobe First with Purely Laparoscopic Approach: A Novel Strategy to Maximize Donor Safety in Adult Living Donor Liver Transplantation. *Ann Surg* (2023) 278(4):479–88. doi:10.1097/SLA.0000000000005988
32. Cho CW, Choi GS, Lee DH, Kim HJ, Yun SS, Lee DS, et al. Comparison of Pure Laparoscopic Donor Right Posterior Sectionectomy Versus Right Hemihepatectomy for Living Donor Liver Transplantation. *Liver Transpl Off Publ Am Assoc Study Liver Dis Int Liver Transpl Soc* (2023) 29(11):1199–207. doi:10.1097/LVT.0000000000000181

33. Suh KS, Hong SK, Lee S, Hong SY, Suh S, Han ES, et al. Pure Laparoscopic Living Donor Liver Transplantation: Dreams Come True. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2022) 22(1):260–5. doi:10.1111/ajt.16782
34. Suh KS, Hong SK, Lee S, Hong SY, Suh S, Han ES, et al. Purely Laparoscopic Explant Hepatectomy and Hybrid laparoscopic/Robotic Graft Implantation in Living Donor Liver Transplantation. *Br J Surg* (2022) 109(2):162–4. doi:10.1093/bjs/znab322
35. Schulze M, Elsheikh Y, Boehnert MU, Alnemary Y, Alabbad S, Broering DC. Robotic Surgery and Liver Transplantation: A Single-Center Experience of 501 Robotic Donor Hepatectomies. *Hepatobiliary Pancreat Dis Int HBPD INT* (2022) 21(4):334–9. doi:10.1016/j.hbpd.2022.05.006
36. Rho SY, Lee JG, Joo DJ, Kim MS, Kim SI, Han DH, et al. Outcomes of Robotic Living Donor Right Hepatectomy From 52 Consecutive Cases: Comparison With Open and Laparoscopy-Assisted Donor Hepatectomy. *Ann Surg* (2022) 275(2):e433–42. doi:10.1097/SLA.0000000000004067
37. Park JH, Suh S, Hong SK, Lee S, Hong SY, Choi Y, et al. Pure Laparoscopic Versus Open Right Donor Hepatectomy Including the Middle Hepatic Vein: A Comparison of Outcomes and Safety. *Ann Surg Treat Res* (2022) 103(1):40–6. doi:10.4174/astr.2022.103.1.40
38. Moon HH, Jo JH, Choi YI, Shin DH. Outcomes of Pure Laparoscopic Living Donor Right Hepatectomy at a Small-Volume Center. *Exp Clin Transpl Off J Middle East Soc Organ Transpl* (2022) 20(4):402–7. doi:10.6002/ect.2022.0022
39. Lee KW, Choi Y, Hong SK, Lee S, Hong SY, Suh S, et al. Laparoscopic Donor and Recipient Hepatectomy Followed by Robot-Assisted Liver Graft Implantation in Living Donor Liver Transplantation. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2022) 22(4):1230–5. doi:10.1111/ajt.16943
40. Kim NR, Han DH, Choi GH, Lee JG, Joo DJ, Kim MS, et al. Comparison of Surgical Outcomes and Learning Curve for Robotic Versus Laparoscopic Living Donor Hepatectomy: A Retrospective Cohort Study. *Int J Surg Lond Engl* (2022) 108:107000. doi:10.1016/j.ijsu.2022.107000
41. Jang EJ, Kim KW, Kang SH. Early Experience of Pure Robotic Right Hepatectomy for Liver Donors in a Small-Volume Center. *JSLS* (2022) 26(4):e2022. doi:10.4293/JSLS.2022.00063
42. Hong SK, Tan MY, Worakitti L, Lee JM, Cho JH, Yi NJ, et al. Pure Laparoscopic Versus Open Right Hepatectomy in Live Liver Donors: A Propensity Score-Matched Analysis. *Ann Surg* (2022) 275(1):e206–12. doi:10.1097/SLA.0000000000003914
43. Dokmak S, Cauchy F, Aussilhou B, Dondero F, Sepulveda A, Roux O, et al. Laparoscopic-Assisted Liver Transplantation: A Realistic Perspective. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2022) 22(12):3069–77. doi:10.1111/ajt.17118
44. Amma BSPT, Mathew JS, Varghese CT, Nair K, Mallick S, Chandran B, et al. Open to Robotic Right Donor Hepatectomy: A Tectonic Shift in Surgical Technique. *Clin Transpl* (2022) 36(9):e14775. doi:10.1111/ctr.14775
45. Yang JD, Lee KW, Kim JM, Kim MS, Lee JG, Kang KJ, et al. A Comparative Study of Postoperative Outcomes Between Minimally Invasive Living Donor Hepatectomy and Open Living Donor Hepatectomy: The Korean Organ Transplantation Registry. *Surgery* (2021) 170(1):271–6. doi:10.1016/j.surg.2021.03.002
46. Troisi RI, Elsheikh Y, Alnemary Y, Zidan A, Sturdevant M, Alabbad S, et al. Safety and Feasibility Report of robotic-assisted Left Lateral Sectionectomy for Pediatric Living Donor Liver Transplantation: A Comparative Analysis of Learning Curves and Mastery Achieved With the Laparoscopic Approach. *Transplantation* (2021) 105(5):1044–51. doi:10.1097/TP.0000000000003332
47. Suh KS, Hong SK, Hong K, Han ES, Hong SY, Suh S, et al. Minimally Invasive Living Donor Liver Transplantation: Pure Laparoscopic Explant Hepatectomy and Graft Implantation Using Upper Midline Incision. *Liver Transpl Off Publ Am Assoc Study Liver Dis Int Liver Transpl Soc* (2021) 27(10):1493–7. doi:10.1002/lt.26066
48. Hong SK, Choi GS, Han J, Cho HD, Kim JM, Han YS, et al. Pure Laparoscopic Donor Hepatectomy: A Multicenter Experience. *Liver Transpl Off Publ Am Assoc Study Liver Dis Int Liver Transpl Soc* (2021) 27(1):67–76. doi:10.1002/lt.25848
49. Han ES, Suh KS, Lee KW, Yi NJ, Hong SK, Lee JM, et al. Advances in the Surgical Outcomes of 300 Cases of Pure Laparoscopic Living Donor Right Hemihepatectomy Divided into Three Periods of 100 Cases: A Single-Centre Case Series. *Ann Transl Med* (2021) 9(7):553. doi:10.21037/atm-20-6886
50. Han ES, Lee KW, Suh KS, Yi NJ, Choi Y, Hong SK, et al. Shorter Operation Time and Improved Surgical Outcomes in Laparoscopic Donor Right Hepatectomy Compared With Open Donor Right Hepatectomy. *Surgery* (2021) 170(6):1822–9. doi:10.1016/j.surg.2021.06.005
51. Cho HD, Kim KH, Yoon YI, Kang WH, Jung DH, Park GC, et al. Comparing Purely Laparoscopic Versus Open Living Donor Right Hepatectomy: Propensity Score-Matched Analysis. *Br J Surg* (2021) 108(7):e233–4. doi:10.1093/bjs/znab090
52. Rhu J, Choi GS, Kim JM, Joh JW, Kwon CHD. Feasibility of Total Laparoscopic Living Donor Right Hepatectomy Compared With Open Surgery: Comprehensive Review of 100 Cases of the Initial Stage. *J Hepato-biliary-pancreat Sci* (2020) 27(1):16–25. doi:10.1002/jhbp.653
53. Jeong JS, Wi W, Chung YJ, Kim JM, Choi GS, Kwon CHD, et al. Comparison of Perioperative Outcomes Between Pure Laparoscopic Surgery and Open Right Hepatectomy in Living Donor Hepatectomy: Propensity Score Matching Analysis. *Sci Rep* (2020) 10(1):5314. doi:10.1038/s41598-020-62289-0
54. Broering DC, Zidan A. Advancements in Robotic Living Donor Hepatectomy, Review of Literature and Single-Center Experience. *Curr Transpl Rep* (2020) 7(4):324–31. doi:10.1007/s40472-020-00311
55. Broering DC, Elsheikh Y, Alnemary Y, Zidan A, Elsarawy A, Saleh Y, et al. Robotic Versus Open Right Lobe Donor Hepatectomy for Adult Living Donor Liver Transplantation: A Propensity Score-Matched Analysis. *Liver Transpl Off Publ Am Assoc Study Liver Dis Int Liver Transpl Soc* (2020) 26(11):1455–64. doi:10.1002/lt.25820
56. Song JL, Wu H, Yang JY. Pure Three-Dimensional Laparoscopic Full Left Hepatectomy of a Living Donor for an Adolescent in China. *Chin Med J (Engl)* (2019) 132(2):242–4. doi:10.1097/CM9.0000000000000052
57. Park J, Kwon DCH, Choi GS, Kim SJ, Lee SK, Kim JM, et al. Safety and Risk Factors of Pure Laparoscopic Living Donor Right Hepatectomy: Comparison to Open Technique in Propensity Score-matched Analysis. *Transplantation* (2019) 103(10):e308–16. doi:10.1097/TP.0000000000002834
58. Lee B, Choi Y, Han HS, Yoon YS, Cho JY, Kim S, et al. Comparison of Pure Laparoscopic and Open Living Donor Right Hepatectomy After a Learning Curve. *Clin Transpl* (2019) 33(10):e13683. doi:10.1111/ctr.13683
59. Suh KS, Hong SK, Lee KW, Yi NJ, Kim HS, Ahn SW, et al. Pure Laparoscopic Living Donor Hepatectomy: Focus on 55 Donors Undergoing Right Hepatectomy. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2018) 18(2):434–43. doi:10.1111/ajt.14455
60. Song JL, Yang J, Wu H, Yan LN, Wen TF, Wei YG, et al. Pure Laparoscopic Right Hepatectomy of Living Donor Is Feasible and Safe: A Preliminary Comparative Study in China. *Surg Endosc* (2018) 32(11):4614–23. doi:10.1007/s00464-018-6214-0
61. Safwan M, Nagai S, Collins K, Rizzari M, Yoshida A, Abouljoud M. Impact of Abdominal Shape on Living Liver Donor Outcomes in Mini-Incision Right Hepatic Lobectomy: Comparison Among 3 Techniques. *Liver Transpl Off Publ Am Assoc Study Liver Dis Int Liver Transpl Soc* (2018) 24(4):516–27. doi:10.1002/lt.25001
62. Lee KW, Hong SK, Suh KS, Kim HS, Ahn SW, Yoon KC, et al. One Hundred Fifteen Cases of Pure Laparoscopic Living Donor Right Hepatectomy at a Single Center. *Transplantation* (2018) 102(11):1878–84. doi:10.1097/TP.0000000000002229
63. Kwon CHD, Choi GS, Kim JM, Cho CW, Rhu J, Soo Kim G, et al. Laparoscopic Donor Hepatectomy for Adult Living Donor Liver Transplantation Recipients. *Liver Transpl Off Publ Am Assoc Study Liver Dis Int Liver Transpl Soc* (2018) 24(11):1545–53. doi:10.1002/lt.25307
64. Kobayashi T, Miura K, Ishikawa H, Soma D, Ando T, Yuza K, et al. Long-Term Follow-Up of Laparoscope-Assisted Living Donor Hepatectomy. *Transpl Proc* (2018) 50(9):2597–600. doi:10.1016/j.transproceed.2018.03.035
65. Hong SK, Lee KW, Choi Y, Kim HS, Ahn SW, Yoon KC, et al. Initial Experience with Purely Laparoscopic Living-Donor Right Hepatectomy. *Br J Surg* (2018) 105(6):751–9. doi:10.1002/bjs.10777
66. Eguchi S, Soyama A, Hara T, Natsuda K, Okada S, Hamada T, et al. Standardized Hybrid Living Donor Hemihepatectomy in Adult-To-Adult Living Donor Liver Transplantation. *Liver Transpl Off Publ Am Assoc Study Liver Dis Int Liver Transpl Soc* (2018) 24(3):363–8. doi:10.1002/lt.24990

67. Broering DC, Elsheikh Y, Shagrani M, Abaalkhail F, Troisi RI. Pure Laparoscopic Living Donor Left Lateral Sectionectomy in Pediatric Transplantation: A Propensity Score Analysis on 220 Consecutive Patients. *Liver Transpl Off Publ Am Assoc Study Liver Dis Int Liver Transpl Soc* (2018) 24(8):1019–30. doi:10.1002/lt.25043
68. Takahara T, Wakabayashi G, Nitta H, Hasegawa Y, Katagiri H, Umemura A, et al. The First Comparative Study of the Perioperative Outcomes Between Pure Laparoscopic Donor Hepatectomy and Laparoscopy-Assisted Donor Hepatectomy in a Single Institution. *Transplantation* (2017) 101(7): 1628–36. doi:10.1097/TP.0000000000001675
69. Rotellar F, Pardo F, Benito A, Zozaya G, Martí-Cruchaga P, Hidalgo F, et al. Totally Laparoscopic Right Hepatectomy for Living Donor Liver Transplantation: Analysis of a Preliminary Experience on 5 Consecutive Cases. *Transplantation* (2017) 101(3):548–54. doi:10.1097/TP.0000000000001532
70. Liao MH, Yang JY, Wu H, Zeng Y. Robot-Assisted Living-Donor Left Lateral Sectionectomy. *Chin Med J (Engl)* (2017) 130(7):874–6. doi:10.4103/0366-6999.202745
71. Kitajima T, Kaido T, Iida T, Seo S, Taura K, Fujimoto Y, et al. Short-Term Outcomes of Laparoscopy-Assisted Hybrid Living Donor Hepatectomy: A Comparison With the Conventional Open Procedure. *Surg Endosc* (2017) 31(12):5101–10. doi:10.1007/s00464-017-5575-0
72. Kim KH, Kang SH, Jung DH, Yoon YI, Kim WJ, Shin MH, et al. Initial Outcomes of Pure Laparoscopic Living Donor Right Hepatectomy in an Experienced Adult Living Donor Liver Transplant Center. *Transplantation* (2017) 101(5):1106–10. doi:10.1097/TP.0000000000001637
73. Suh SW, Lee KW, Lee JM, Choi Y, Yi NJ, Suh KS. Clinical Outcomes of and Patient Satisfaction with Different Incision Methods for Donor Hepatectomy in Living Donor Liver Transplantation. *Liver Transpl Off Publ Am Assoc Study Liver Dis Int Liver Transpl Soc* (2015) 21(1):72–8. doi:10.1002/lt.24033
74. Scatton O, Katsanos G, Boillot O, Goumard C, Bernard D, Stenard F, et al. Pure Laparoscopic Left Lateral Sectionectomy in Living Donors: From Innovation to Development in France. *Ann Surg* (2015) 261(3):506–12. doi:10.1097/SLA.0000000000000642
75. Han HS, Cho JY, Yoon YS, Hwang DW, Kim YK, Shin HK, et al. Total Laparoscopic Living Donor Right Hepatectomy. *Surg Endosc* (2015) 29(1):184. doi:10.1007/s00464-014-3649-9
76. Zhang X, Yang J, Yan L, Li B, Wen T, Xu M, et al. Comparison of laparoscopy-assisted and Open Donor Right Hepatectomy: A Prospective Case-Matched Study From China. *J Gastrointest Surg Off J Soc Surg Aliment Tract* (2014) 18(4):744–50. doi:10.1007/s11605-013-2425-9
77. Makki K, Chorasiya VK, Sood G, Srivastava PK, Dargan P, Vij V. Laparoscopy-Assisted Hepatectomy Versus Conventional (Open) Hepatectomy for Living Donors: When You Know Better, You Do Better. *Liver Transpl Off Publ Am Assoc Study Liver Dis Int Liver Transpl Soc* (2014) 20(10):1229–36. doi:10.1002/lt.23940
78. Samstein B, Cherqui D, Rotellar F, Griesemer A, Halazun KJ, Kato T, et al. Totally Laparoscopic Full Left Hepatectomy for Living Donor Liver Transplantation in Adolescents and Adults. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2013) 13(9):2462–6. doi:10.1111/ajt.12360
79. Marubashi S, Wada H, Kawamoto K, Kobayashi S, Eguchi H, Doki Y, et al. Laparoscopy-Assisted Hybrid Left-Side Donor Hepatectomy. *World J Surg* (2013) 37(9):2202–10. doi:10.1007/s00268-013-2117-3
80. Giulianotti PC, Tzvetanov I, Jeon H, Bianco F, Spaggiari M, Oberholzer J, et al. Robot-Assisted Right Lobe Donor Hepatectomy. *Transpl Int Off J Eur Soc Organ Transpl* (2012) 25(1):e5–9. doi:10.1111/j.1432-2277.2011.01373.x
81. Choi HJ, You YK, Na GH, Hong TH, Shetty GS, Kim DG. Single-Port Laparoscopy-Assisted Donor Right Hepatectomy in Living Donor Liver Transplantation: Sensible Approach or Unnecessary Hindrance? *Transpl Proc* (2012) 44(2):347–52. doi:10.1016/j.transproceed.2012.01.018
82. Baker TB, Jay CL, Ladner DP, Preczewski LB, Clark L, Holl J, et al. Laparoscopy-Assisted and Open Living Donor Right Hepatectomy: A Comparative Study of Outcomes. *Surgery* (2009) 146(4):817–23. doi:10.1016/j.surg.2009.05.022
83. Soubrane O, Cherqui D, Scatton O, Stenard F, Bernard D, Branchereau S, et al. Laparoscopic Left Lateral Sectionectomy in Living Donors: Safety and Reproducibility of the Technique in a Single Center. *Ann Surg* (2006) 244(5):815–20. doi:10.1097/01.sla.0000218059.31231.b6
84. Koffron AJ, Kung R, Baker T, Fryer J, Clark L, Abecassis M. Laparoscopic-Assisted Right Lobe Donor Hepatectomy. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2006) 6(10):2522–5. doi:10.1111/j.1600-6143.2006.01498.x
85. Khan AS, Scherer M, Panni R, Cullinan D, Martens G, Kangarga I, et al. Total Robotic Liver Transplant: The Final Frontier of Minimally Invasive Surgery. *Am J Transpl* (2024) 24(8):1467–72. doi:10.1016/j.ajt.2024.03.030
86. Pinto-Marques H, Sobral M, Magistri P, Gomes da Silva S, Guerrini GP, Mega R, et al. Full Robotic Whole Graft Liver Transplantation: A Step Into the Future. *Ann Surg* (2025) 281(1):67–70. doi:10.1097/SLA.0000000000006420
87. Soin AS, Yadav KS, Valappil F, Shetty N, Bansal R, Chaudhary S, et al. Hepatic Duct Division During Robotic Living Donor Hepatectomy: A Comparison Between the Novel Triple C (Clip-Clamp-Cut) and the Cut-Suture Techniques. *J Transpl* (2024) 2024:8955970. doi:10.1155/2024/8955970
88. Varghese CT, Chandran B, Gopalakrishnan U, Nair K, Mallick S, Mathew JS, et al. Extended Criteria Donors for Robotic Right Hepatectomy: A Propensity Score Matched Analysis. *J Hepatobiliary Pancreat Sci* (2022) 29(8):874–83. doi:10.1002/jhbp.1145
89. Broering DC, Benedetti E. MIOT.CC Collaborative (Group Authorship, Supplementary Material, Pubmed Indexed). Recommendations From the 2024 Minimally Invasive Organ Transplant Consensus Conference - MIOT.CC. *Ann Surg* (2025). doi:10.1097/SLA.0000000000006804

ESOT | Transplant International

ORIGINAL RESEARCH
published: 19 February 2026
doi: 10.3389/ti.2026.15993

Institut Georges Lopez-2M as a Novel Lung Preservation Solution Attenuates Ischemia-Reperfusion Injury in a Rat *Ex Vivo* Lung Perfusion Model

Annalisa Barbarossa[1,2], *Jan Van Slambrouck*[1,2], *Cara Trivett*[3], *Phéline Kortleven*[1,4], *Cedric Vanluyten*[1,2], *Alberto Martin Medina*[3], *Xin Jin*[1], *Nicole Jannis*[1], *Balin Özsoy*[1], *Sandra Claes*[5], *Dominique Schols*[5], *Tine Wylin*[6], *Karen Moermans*[7], *Steve Stegen*[7], *Arnau Panisello Rosello*[8], *Ilhan Inci*[9], *Paul De Leyn*[1,2], *Bart Vanaudenaerde*[1], *Jacques Pirenne*[6], *Elizabeth A. V. Jones*[3,10] and *Laurens J. Ceulemans*[1,2]*

[1]*Laboratory for Respiratory Diseases and Thoracic Surgery, Katholieke Universiteit Leuven, Leuven, Belgium,* [2]*Department of Thoracic Surgery, University Hospitals Leuven, Leuven, Belgium,* [3]*Centrum voor Moleculaire en Vasculaire Biologie, Katholieke Universiteit Leuven, Leuven, Belgium,* [4]*Laboratory of Molecular Virology and Gene Therapy, Katholieke Universiteit Leuven, Leuven, Belgium,* [5]*Department of Microbiology, Immunology and Transplantation, Rega Institute for Medical Research, Molecular, Structural and Translational Virology Research Group, Katholieke Universiteit Leuven, Leuven, Belgium,* [6]*Laboratory of Abdominal Transplantation, Transplantation Research Group, Department of Microbiology, Immunology and Transplantation, Katholieke Universiteit Leuven, Leuven, Belgium,* [7]*Clinical and Experimental Endocrinology, Department of Chronic Diseases and Metabolism, Katholieke Universiteit Leuven, Leuven, Belgium,* [8]*Translational Research and Innovation in Surgery Group (GITIC), Department of General and Digestive Surgery, La Paz University Hospital - IdiPaz, Madrid, Spain,* [9]*Klinik Hirslanden Zurich, Thoracic Surgery Clinic, Zurich, Switzerland,* [10]*Universiteit Maastricht Cardiovascular Research Institute Maastricht, Maastricht, Netherlands*

OPEN ACCESS

***Correspondence**
Laurens J. Ceulemans,
laurens.ceulemans@uzleuven.be

Received: *03 December 2025*
Revised: *20 January 2026*
Accepted: *28 January 2026*
Published: *19 February 2026*

Citation:
Barbarossa A, Van Slambrouck J, Trivett C, Kortleven P, Vanluyten C, Medina AM, Jin X, Jannis N, Özsoy B, Claes S, Schols D, Wylin T, Moermans K, Stegen S, Rosello AP, Inci I, De Leyn P, Vanaudenaerde B, Pirenne J, Jones EAV and Ceulemans LJ (2026) Institut Georges Lopez-2M as a Novel Lung Preservation Solution Attenuates Ischemia-Reperfusion Injury in a Rat Ex Vivo Lung Perfusion Model. Transpl. Int. 39:15993. doi: 10.3389/ti.2026.15993

Institut Georges Lopez-2M (*IGL-2M*), a novel preservation solution containing polyethylene glycol (PEG 35kD, 5 g/L), preserves mitochondrial integrity and redox balance in liver grafts. This study assesses *IGL-2M*'s effect on lung preservation during prolonged cold ischemia. Rat's heart-lung blocks were procured and subjected to 18 h cold ischemia (4 °C). Lungs were flushed and preserved using one of these preservation solutions: *OCS*, *Perfadex Plus*, *IGL-2M* (n = 6/group). Following ischemia, lungs underwent up to 7 h normothermic *ex vivo* lung perfusion. Edema was quantified by weight gain. Lung physiological parameters were recorded. Perfusate, bronchoalveolar lavage (BAL), and tissue samples were collected. All lungs in *IGL-2M* group completed 7 h EVLP protocol. Compared to *OCS*, *IGL-2M* reduced edema formation ($p < 0.01$), preserved superior compliance ($p < 0.01$), and maintained lower pulmonary vascular resistance ($p < 0.01$). *IGL-2M* showed lower perfusate concentrations of IL-1β ($p < 0.05$), IL-6 ($p < 0.05$), and

Abbreviations: α-SMA, Smooth muscle actin; ATP, Adenosine triphosphate; BAL, Bronchoalveolar lavage; CHS, Controlled hypothermic storage; CO, Cardiac output; CXCL, Chemokine (C-X-C motif) ligand; EVLP, *Ex vivo* lung perfusion; FDA, Food and drug administration; FiO_2, Fraction of inspired oxygen; HABP, Hyaluronan binding protein; IGL-2M, Institut Georges Lopez-2 modified; IL, Interleukin; IRI, Ischemia-reperfusion injury; OCS, Organ Care System lung solution; PA, Pulmonary artery; PAP, Pulmonary arterial pressure; PCR, Polymerase chain reaction; PEEP, Positive end-expiratory pressure; PEG, Polyethylene glycol; PGD, Primary graft dysfunction; PVR, Pulmonary vascular resistance; RNA, Ribonucleic acid; ROS, Reactive oxygen species; RT-qPCR, Reverse transcription quantitative polymerase chain reaction; SD, Standard deviation; TEER, Transendothelial electrical resistance; TNF-α, Tumor necrosis factor; TV, Tidal volume; VR, Ventilatory rate.

TNF-α ($p = 0.08$). In BAL, *IGL-2M* reduced IL-1β ($p < 0.01$), IL-6 ($p < 0.05$), TNF-α ($p < 0.01$), and CXCL1 ($p < 0.01$). *IGL-2M* showed lower release of Syndecan-1 ($p < 0.05$). Compared to *Perfadex Plus*, *IGL-2M* was not inferior, with reduced expression of TNF-α in the perfusate ($p < 0.05$). *IGL-2M* effectively prevents edema development like *Perfadex Plus*. *IGL-2M* results in decreased inflammation and a stronger endothelial lining, making it a promising solution for lung preservation.

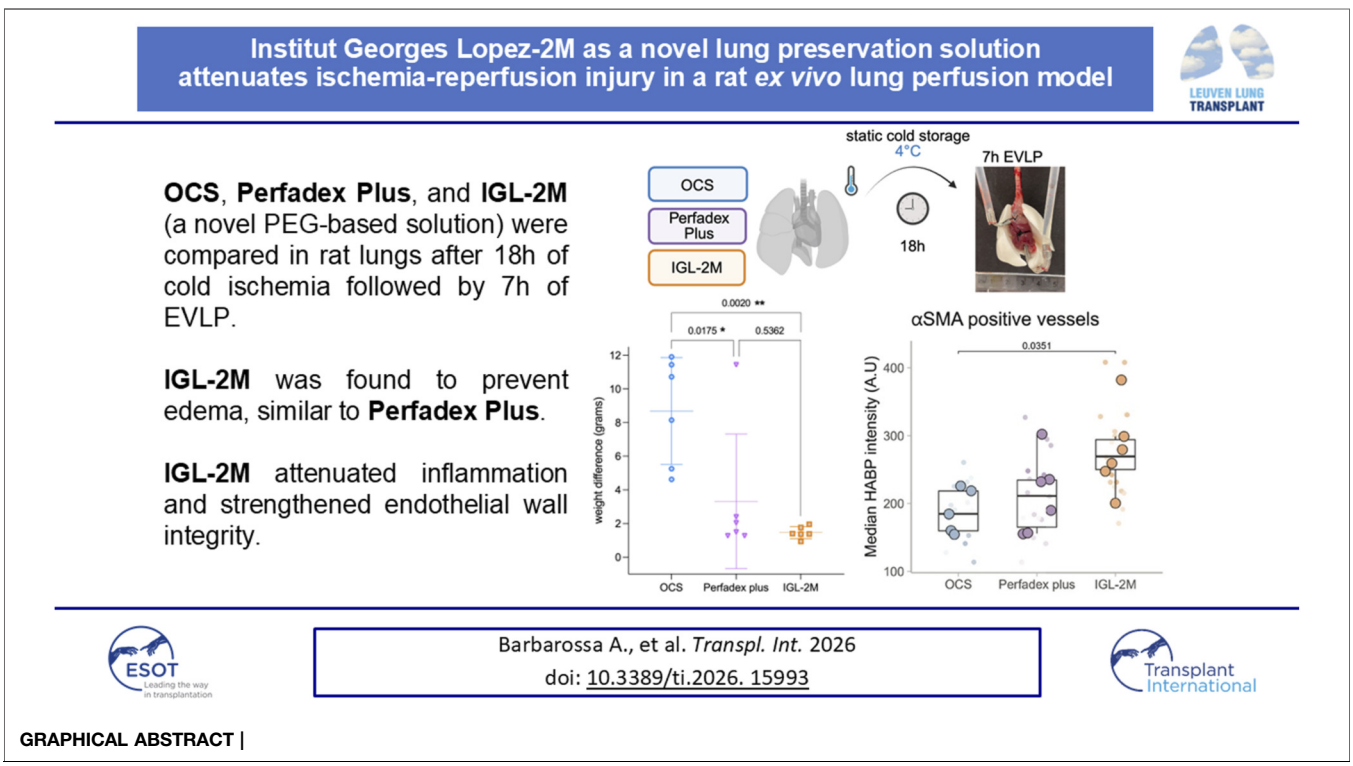
Keywords: endothelial glycocalyx, ischemia-reperfusion injury, lung preservation, polyethylene glycol (PEG), preservation solution

INTRODUCTION

Ischemia–reperfusion injury (IRI) is one of the key challenges in lung transplantation and plays a pivotal role in the development of primary graft dysfunction (PGD), leading to early morbidity and mortality [1]. The sequence of ischemia during organ procurement, static cold storage, and subsequent reperfusion after implantation triggers a cascade of cellular and molecular events that culminate in tissue injury [2]. At the onset of ischemia, an imbalance between metabolic supply and demand disrupts epithelial and endothelial homeostasis, alters mitochondrial function, and induces ionic disequilibrium. Cold preservation further amplifies this process through the generation of reactive oxygen species (ROS), inflammatory cytokine release, microvascular injury, and epithelial barrier disruption, ultimately resulting in lung edema and PGD following reperfusion [3, 4].

A central element in IRI is the inflammatory response, which is amplified upon reperfusion. Pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α, play a key role by activating endothelial cells, promoting leukocyte adhesion, and increasing vascular permeability [5, 6]. Chemokines like CXCL1 further drive neutrophil recruitment, while dysregulation of anti-inflammatory mediators such as IL-10 contributes to an imbalance between pro- and anti-inflammatory signaling [7, 8]. This cytokine storm accelerates endothelial dysfunction, microcirculatory failure, and edema formation, ultimately impairing graft function, leading to PGD [9].

Progress in optimizing lung preservation remains limited. Current preservation solutions differ across centers but share common design principles: colloids to counteract cellular edema, buffers to stabilize pH, antioxidants to scavenge ROS, and metabolic precursors to support ATP regeneration [10, 11]. However, clinical outcomes indicate that existing solutions provide partial protection, and novel approaches are needed to better preserve vascular integrity and attenuate IRI, especially in the context of evolving lung preservation practices toward controlled hypothermic storage and prolonged preservation time [12].



GRAPHICAL ABSTRACT |

One promising strategy involves the use of polyethylene glycol (PEG)-based preservation solutions. *Institut Georges Lopez-2* (*IGL-2*®), with PEG 35 kDa at 5 g/L, has been shown to stabilize mitochondrial function, preserve redox balance, and reduce endothelial injury in experimental liver transplantation [13, 14].

The aim of this study is to evaluate whether a further modified PEG-based preservation solution *IGL-2M* improves lung preservation during prolonged cold ischemia compared to clinically used Organ Care System lung solution (*OCS)* and *Perfadex Plus*. The primary endpoint of the study is to assess the success rates of 7 h of EVLP and edema formation. We analyzed the effect on edema, inflammation, and endothelial injury, using a rat *ex vivo* lung perfusion (EVLP) model to mimic IRI.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (350–400 g, Janvier Labs, France) received adequate care, and the study was performed after authorization by local Ethical Committee for Animal Experimentation (Ethische Commissie Dierproeven) (P128/2023).

Surgical Techniques

Rats were anesthetized with 5% isoflurane and maintained with 3% isoflurane during lung procurement. A tracheostomy was performed, followed by mechanical ventilation with rodent ventilator (R415VentStarSmallAnimalVentilator; RWD Life Science Co., Ltd. Guangdong, P.R. China). A tidal volume (TV) of 8 mL/kg, ventilatory rate (VR) of 70/min, positive end-expiratory pressure (PEEP) of 3cmH_2O, and a fraction of inspired oxygen (FiO_2) of 50% were applied. A laparotomy was performed, and 200U/kg heparin (LeoPharma, Denmark) was injected into abdominal caval vein followed by sternotomy with removal of thymus to gain access to the pulmonary artery (PA). Exsanguination was obtained by cutting abdominal caval vein. The PA (inflow) was cannulated through an incision in the right ventricular outflow tract, while left atrium (outflow) was cannulated through an incision in the apex of left ventricle and after dilatation of mitral valve. Cold pulmonary antegrade flush was performed using one of three preservation solutions (20cc at 4 °C): *OCS*, *Perfadex Plus*, *IGL-2M* and 500U heparin. After pulmonary flush, pneumonectomy was performed. Trachea was clamped at inspiration and transected. The heart-lung block was weighed, submerged in same cold preservation solution used for the flush, and stored for 18 h in a temperature-controlled cold room at 4 °C. A cold ischemic time of 18 h was chosen to establish a severe, robust and reproducible injury model that mimics ischemia reperfusion injury, thereby enabling discrimination between preservation solutions under extreme conditions, which would not be achievable with shorter cold ischemia times.

Ex-Vivo Lung Perfusion

Lungs were mounted on an isolated perfused lung system for rats (IPL-2 platform; Hugo-Sachs Elektronik, Germany). The *ex vivo* lung perfusion protocol used was inspired by the protocols employed in the research laboratories of Lausanne and Zurich [15, 16]. A 75 mL of acellular albumin-rich Steen solution (XVIVO, Sweden) at 7 °C was used as perfusate supplemented with 1 mL of diluted Tham Koler 3M. Flow-controlled perfusion was started at 1% of cardiac output (CO) and was increased to 7.5% CO at incremental steps within 20 min. Perfusate temperature was increased to 37 °C within 25 min. Volume-controlled ventilation was started after 20 min reperfusion time at a 3 mL/kg TV, 10/min VR, 21% FiO_2 and 3cmH_2O PEEP. TV was increased to 6 mL/kg and VR to 30/min after 30min. The perfusate was continuously deoxygenated through a membrane filter (D150, Medica, Italy) with a 12% CO_2/6% O_2/82% N_2 sweep gas. A recruitment maneuver with a TV of 10 mL/kg was performed every hour, followed by 3min of ventilation at 100% FiO_2 and subsequent gas analysis of inflow and outflow perfusate samples (ABL800 FLEX, Radiometer, Denmark). Differential perfusate pO_2 (outflow-inflow) (ΔpO_2) and outflow perfusate lactate levels were recorded.

Respiratory physiology data were measured and recorded using dedicated software (Pulmodyn HSE software, Germany). Pulmonary arterial pressure (PAP), pulmonary vascular resistance (PVR), and compliance were monitored. Left atrium pressure was set at 2-3 cmH2O. Hourly outflow perfusate samples (1 mL) were collected, immediately snap frozen in liquid nitrogen and stored at −80 °C.

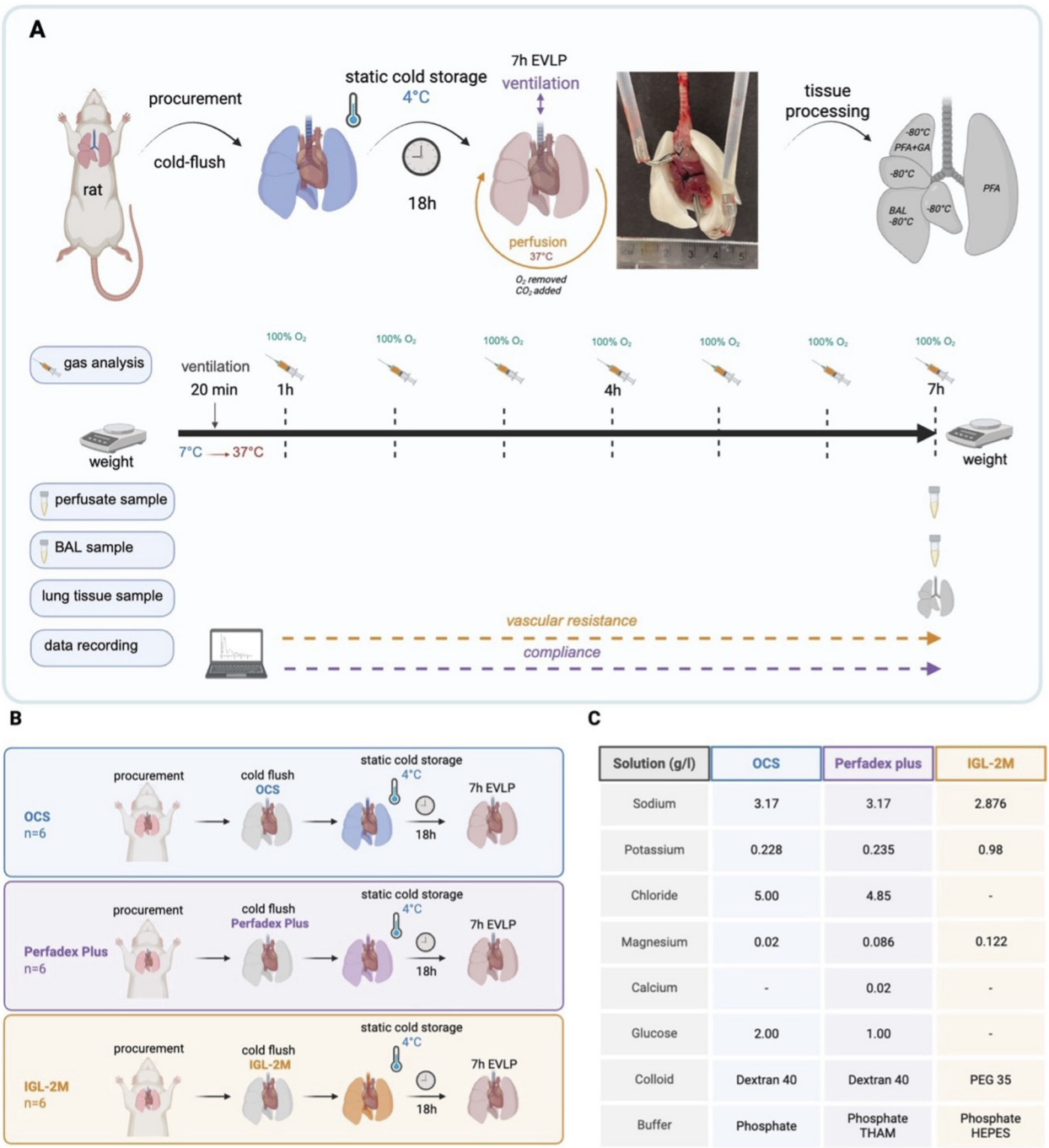
EVLP protocol was continued for a total of 7h, unless massive edema impeding further ventilation forced a premature stop to prevent fluid from reaching the trachea, afterwards heart-lung block was weighed. Prolonged EVLP (7 h) was chosen to allow clear detection of inflammatory differences between groups. An EVLP model to mimic reperfusion, rather than a transplant model, was used to reduce animals required. Bronchoalveolar lavage (BAL) samples of 1 mL 0.9% NaCl were collected from right lower lobe and snap-frozen in liquid nitrogen. Left lobe was instilled and fixed with 4% formaldehyde (Avantor, U.S). Remaining lobes were snap-frozen in liquid nitrogen and stored at −80 °C (**Figure 1A**).

Study Groups and Preservation Solutions

Cold pulmonary antegrade flush and static preservation were performed using one of three preservation solutions: *OCS* lung solution (TransMedics), *Perfadex Plus* (XVIVO), or *IGL-2M* (*Institut Georges Lopez 2 modified*) after unblinded randomization.

For each group (n = 6/group), the lungs were flushed with 20cc sterile preservation solution, pre-cooled to 4 °C, via pulmonary artery during procurement. Lungs were then preserved in the specific preservation solution at 4 °C for 18 h. After the preservation phase, EVLP was performed (**Figure 1B**). During EVLP, Steen solution (XVIVO, Sweden) was used as perfusate for all groups.

OCS lung solution is an FDA-cleared extracellular, low-potassium solution based on colloid Dextran 40 (50 g/L). It is



C

Solution (g/l)	OCS	Perfadex plus	IGL-2M
Sodium	3.17	3.17	2.876
Potassium	0.228	0.235	0.98
Chloride	5.00	4.85	-
Magnesium	0.02	0.086	0.122
Calcium	-	0.02	-
Glucose	2.00	1.00	-
Colloid	Dextran 40	Dextran 40	PEG 35
Buffer	Phosphate	Phosphate THAM	Phosphate HEPES

FIGURE 1 | (A) Experimental protocol and *ex vivo* lung perfusion sampling. **(B)** Study groups; **(C)** Composition of preservation solutions. Information about *OCS* solution's composition was found on the clinic bag that held the solution. Details about *Perfadex Plus* were found on XVIVOgroup website, and information about *IGL-2M* was provided by IGL-company. Created in https://BioRender.com.

used in clinics for normothermic perfusion and in our center for static cold preservation of donor lungs.

Perfadex Plus is a ready-to-use extracellular colloid-based (Dextran 40, 50 g/L) and is used by most lung transplant centers for cold flush and static preservation. Unlike *OCS*, *Perfadex Plus* is pre-buffered and pre-supplemented with calcium ions and THAM.

IGL-2M is a novel polyethylene glycol (PEG-35kDa) based extracellular preservation solution, representing a modified version of IGL-1 and IGL-2 with improved antioxidant and anti-

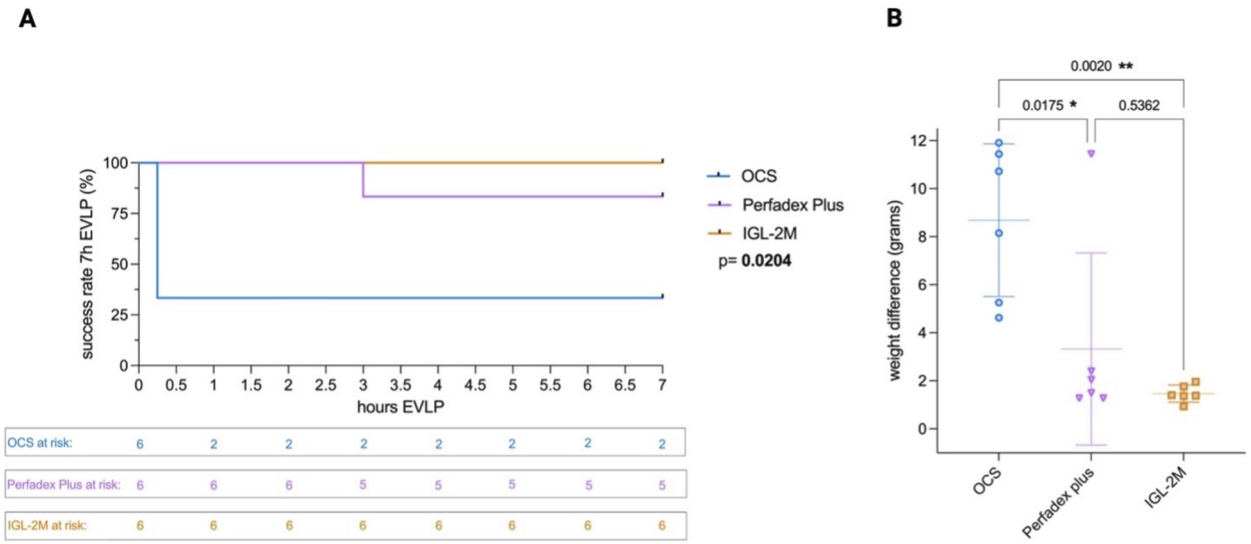


FIGURE 2 | Proportion of experiments developing edema. **(A)** 7 h *ex vivo* lung perfusion success rate. Log-rank test: $p < 0.05$. **(B)** Lung weight gain after *ex vivo* lung perfusion. Statistical analysis was performed using one-way ANOVA.

inflammatory properties. Originally designed to offer protection for steatotic livers [17]. It has not been used in clinic (**Figure 1C**)

Perfusate Samples, Bronchoalveolar Lavage Samples, and Lung Tissue Analysis

For analysis of cytokines, a 50 µL volume of either perfusates collected at 7 h EVLP and BAL was assayed using Rat Procarta 6-plex Luminex assay (Thermo Fisher Scientific).

RNA was isolated from frozen tissue biopsies. 25mg of frozen tissue RNA from the upper, middle, lower, and accessory lobes was combined with a 3 mm tungsten carbide bead (Qiagen, Ref#69997) and 300 µL of Aurum™ RNA lysis buffer (Bio-Rad, Ref#7326820), then homogenized using a TissueLyser II (Qiagen). RNA extraction was performed with TRIzol™ Reagent (Thermo Fisher Scientific, Ref#15596026) following the manufacturer's protocol, and the RNA phase was further purified using the Aurum™ Total RNA Mini Kit (Bio-Rad, Ref#7326820). The RNA concentration and purity were assessed using NanoDrop oneC (Thermo Fisher Scientific). c-DNA was synthesized from 200 ng RNA using Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV, Life Technologies, CA, USA). Next, the real-time qPCR reaction was performed on a LightCycler 96W system (Roche Diagnostics, Vilvoorde, Belgium) with Taqman Fast Universal PCR Master Mix and Taqman Gene Expression Assays IL-1β (Gene expression assay: Rn00580432_m1), IL-6 (Gene expression assay: Rn01410330_m1), TNF-α (Gene expression assay: Rn00562055_m1), NFKB (Gene expression assay: Rn01399572_m1), VCAM-1 (Gene expression assay: Rn00563627_m1), Bcl2 (Gene expression assay: Rn99999125_m1), Bcl2l1 (Gene expression assay: Rn00437783_m1), bax (Gene expression assay: Rn01480160_g1) and TaqMan® Fast Universal PCR Master Mix (Applied Biosystems®, Life Technologies, CA, USA). Thermocycling conditions consisted of an initial denaturation of 60s at 95 °C, followed by 45 cycles of 95 °C for 5s and 60 °C for 30s. Quantification of input target amount was analyzed by cycle threshold (Ct) value, the point at which the sample PCR amplification plot crosses the threshold. All data were normalized to GAPDH (Gene expression assay: Rn00562055_m1), and differences in gene expression were calculated as dCt values.

Lung Tissue Staining for Hyaluronan-Binding Protein

Paraffin-embedded sections were used for hyaluronic acid staining using a biotinylated hyaluronan binding protein (Sigma, 385911-50UG). Sections were baked at 65 °C and allowed to cool completely. Deparaffinization and dehydration were performed by sequentially immersing slides in xylene followed by a reducing ethanol gradient (100%, 95%, 70%, 50%). Slides were then washed once with distilled water before performing antigen retrieval (Target Retrieval Solution, Dako). Following incubation with protein blocking solution (TNB protein blocking solution) and endogenous biotin blocking (Biolegend) samples were incubated in solution containing hyaluronan binding protein (1/100) and αSMA-Cy3 antibody (1/400, Sigma, C6198) overnight with gentle rocking. Slides were washed three times with TNT Buffer (0.1M Tris.HCl (pH 7.5), 0.15M NaCl, 0.05% Tween®-20). To detect bound hyaluronan, slides were incubated in the dark with streptavidin conjugated to

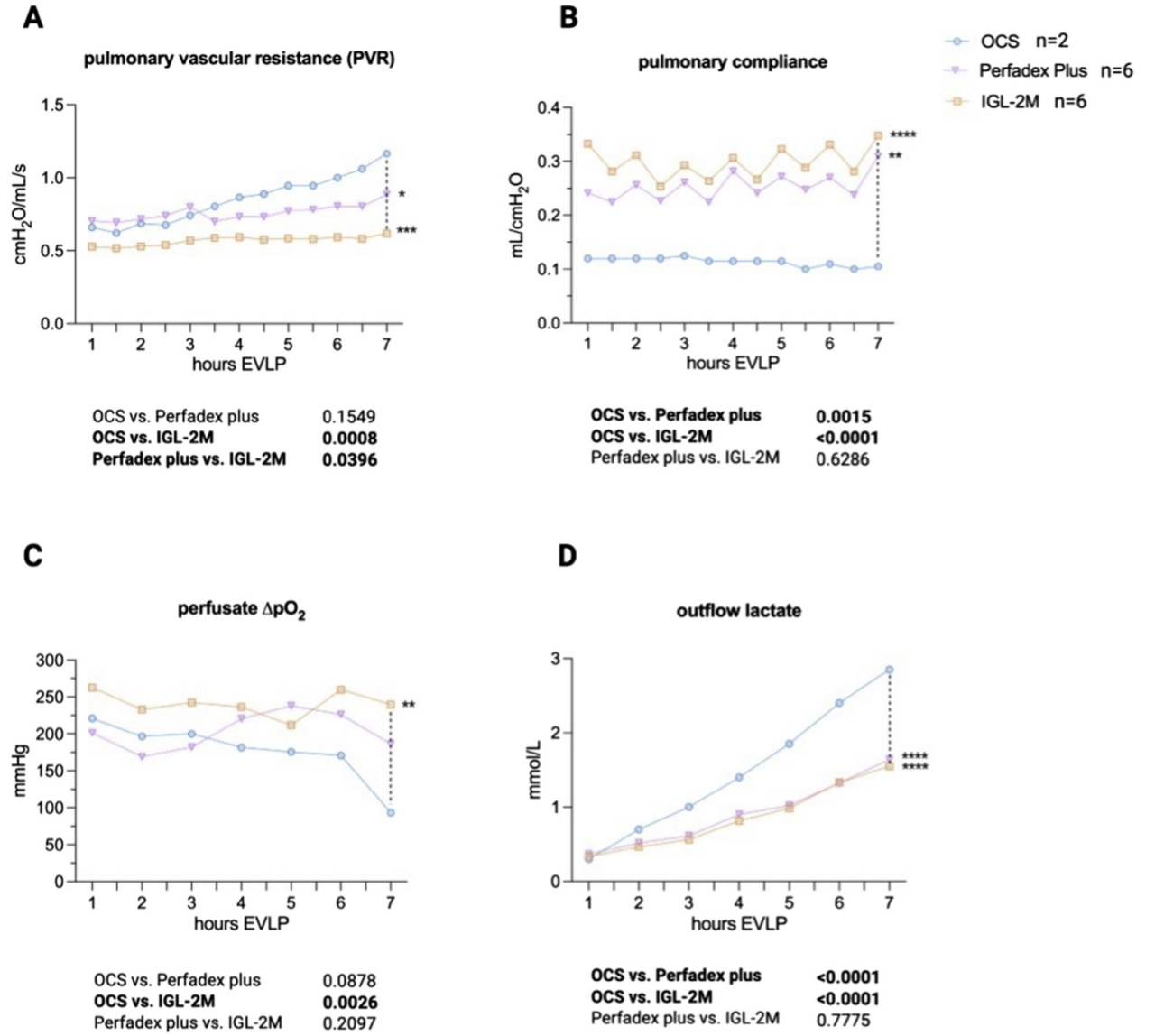


FIGURE 3 | Physiological data during 7 h *ex vivo* lung perfusion. **(A)** Pulmonary vascular resistance. **(B)** Pulmonary compliance. **(C)** Perfusate oxygenation (differential outflow minus inflow partial O2 pressure). **(D)** Outflow lactate levels. Statistical analysis was performed using two-way ANOVA.

Alexa488 (1/400, Thermo Fisher Scientific) for 1 h. Slides were washed three times in TNT, counterstained with DAPI and mounted with ProLong™ Gold Antifade Mountant (Thermo Fisher Scientific). All steps were performed at room temperature.

Fluorescent images were taken with a Zeiss 700 M confocal microscope (×20 magnification). Per animal, 3-4 tiled regions were captured. Images were analyzed using ImageJ software (Fiji). First each channel underwent background subtraction. Image segmentation was achieved using Cy3 channel to identify αSMA positive vessels. Images were then binarized using the triangle algorithm for thresholding. The binary image was converted to a mask and Cy3+ve areas were eroded to remove small particles, remaining positive areas were then dilated to capture the areas surrounding vessels. The segmented image was used to define measured areas on 488-channel image by redirecting measurements and using the "Analyze Particles" function in ImageJ. Results per image were written to csv files analyzed in R4.5.1.

Statistics

Continuous physiology and weight data were reported as mean with SD. Edema was quantified by measuring difference in heart-lung block weight after vs. before EVLP. Repeated measurements were compared using 2-way analysis of variance (ANOVA). Static data were compared using one-way ANOVA. Statistical significance was assigned to a $p < 0.05$. GraphPad Prism Version 10.3.1 was used for all statistical analyses.

RESULTS

IGL-2M Prevents Edema Development After Prolonged Static Cold Preservation

All six *IGL-2M* experiments reached 7 h EVLP. In *Perfadex Plus* group, 5/6 experiments reached 7 h EVLP while one experiment failed prematurely after 3 h due to massive edema. In *OCS* group 2/6 reached 7 h EVLP while 4 experiments failed prematurely after 0.25 h due to massive edema ($p < 0.05$) (**Figure 2A**).

Weight difference between heart-lung block after vs. before EVLP was taken as indicator of edema development. Weight gain in *IGL-2M* group was lower compared to *OCS* ($p < 0.01$) and no significant difference was found compared to *Perfadex Plus* ($p = 0.54$) (**Figure 2B**).

IGL-2M Results in Stable Pulmonary Vascular Resistance and Results in Higher Pulmonary Compliance Compared to OCS

After 7 h EVLP, pulmonary vascular resistance remained stable and was lower in *IGL-2M* compared to *OCS*, which showed a worsening trend over time ($p < 0.01$) and *Perfadex Plus* ($p < 0.05$) (**Figure 3A**). Compliance was higher in *IGL-2M* than in *OCS*, which started with a lower value from the beginning ($p < 0.01$), with no difference compared to *Perfadex Plus* ($p = 0.63$) (**Figure 3B**). Pulmonary gas exchange was evaluated using perfusate $\Delta pO2$, showing better oxygenation in *IGL-2M* than in *OCS* ($p < 0.01$) and no difference compared to *Perfadex Plus* ($p = 0.21$) (**Figure 3C**). Cellular damage was assessed by lactate levels in the effluent, with both *IGL-2M* and *Perfadex Plus* having lower lactate levels than *OCS* ($p < 0.01$) with a trend over time (**Figure 3D**).

IGL-2M Results in a Limited Release of Inflammatory Biomarkers in the Perfusate

Perfusate samples collected after 7 h EVLP in *IGL-2M* showed lower concentrations of pro-inflammatory cytokines IL-1β ($p < 0.05$) and IL-6 ($p < 0.05$) compared to *OCS*, and no difference compared to *Perfadex Plus* ($p = 0.96$; $p = 0.99$). *IGL-2M* showed the lowest concentration of TNF-α (vs. *OCS* $p = 0.08$; vs. *Perfadex plus* $p < 0.05$). Compared to *OCS*, *Perfadex Plus* showed a trend towards lower inflammatory cytokine release (**Figure 4**).

IGL-2M Results in a Limited Release of Inflammatory Biomarkers in the Bronchoalveolar Lavage

BAL samples collected after 7 h EVLP in *IGL-2M* showed reduced release of pro-inflammatory cytokines IL-1β ($p < 0.01$); IL-6 ($p < 0.01$); TNF-α ($p < 0.01$); CXCL1 ($p < 0.01$) and anti-inflammatory cytokine IL-10 ($p = 0.05$) compared to *OCS*; and no difference for the same biomarkers compared to *Perfadex Plus*. Compared to *OCS*, *Perfadex Plus* showed reduced release of IL-1β ($p < 0.05$); IL-6 ($p < 0.01$); TNF-α ($p < 0.01$); CXCL1 ($p < 0.01$) and anti-inflammatory cytokine IL-10 ($p < 0.01$) (**Figure 5**).

IGL-2M and Perfadex Plus Reduce Inflammation in Lung Tissue

Lung tissue analysis with RT-qPCR showed lower IL-6 gene expression ($p < 0.05$) in *IGL-2M* compared to *OCS*, with no difference compared to *Perfadex Plus* ($p = 0.82$). Compared to *OCS*, *Perfadex Plus* showed lower gene expression of IL-6 ($p < 0.01$); TNF-α ($p < 0.05$); VCAM-1 ($p < 0.05$) (**Figure 6**).

IGL-2M Induces the Expression of Anti-apoptotic Gene

The anti-apoptotic Bcl2 gene expression was higher in *IGL-2M* (vs. OCS, $p < 0.01$) with no difference compared to *Perfadex Plus* ($p = 0.31$) (**Figure 6**).

Hyaluronan Associated With the Lung Endothelium Is Preserved With IGL-2M

Hyaluronan detected by biotinylated hyaluronan-binding protein was present in the adventitia of αSMA positive vessels, as well as in the bronchial and bronchiolar epithelium, in the region of the basement membrane. Hyaluronan levels were decreased in αSMA positive vessels across the groups ($F(2, 14) = 4.384$, $p < 0.05$). Lungs preserved with *OCS* showed reduced vascular hyaluronan levels as compared to *IGL-2M* preserved lungs (**Figure 7**, $p < 0.05$).

IGL-2M and Perfadex Plus Preserve Endothelial Glycocalyx Integrity Compared to OCS

Perfusate samples collected after 7 h EVLP in *IGL-2M* and *Perfadex Plus* showed lower concentrations of Syndecan-1 compared to *OCS* ($p < 0.05$; $p < 0.01$) (**Figure 8**).

DISCUSSION

This rat model of prolonged lung preservation assessed over 7 h EVLP shows for the first time that *IGL-2M*, a PEG-based preservation solution, can be successfully used to flush and preserve rat lungs subjected to prolonged cold ischemia of 18 h. Compared to *OCS*, *IGL-2M* markedly reduced edema formation, preserved pulmonary vascular resistance and compliance, improved oxygenation, reduced lactate release, and attenuated the inflammatory cytokine response. *IGL-2M* was not inferior to *Perfadex Plus*, the current gold standard, and showed an additional effect of TNF-α suppression.

PGD remains a leading cause of early morbidity and mortality after lung transplantation, with an incidence of 30%, largely driven by IRI. While IRI encompasses the cascade of cellular

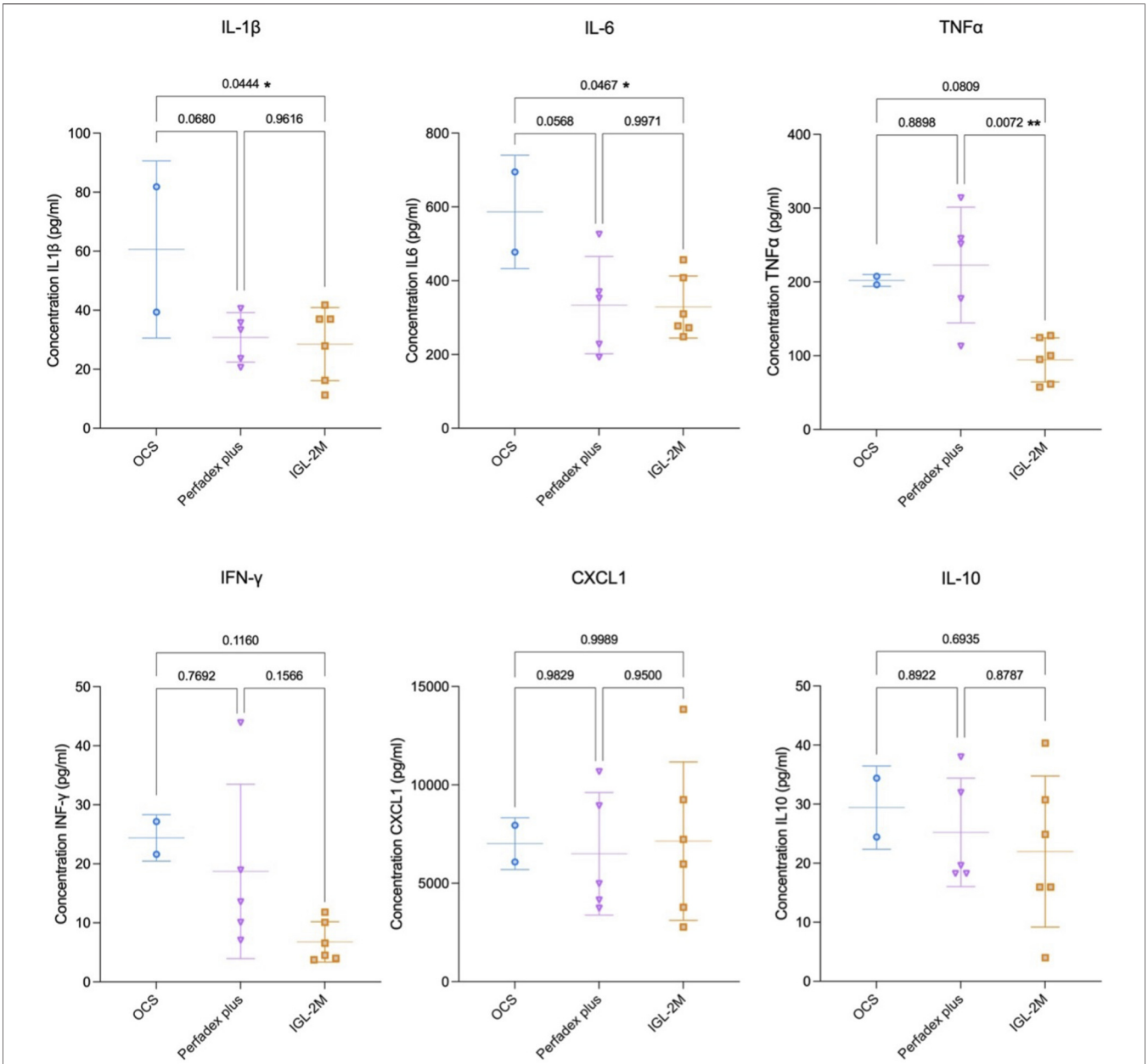


FIGURE 4 | Cytokine expression in the perfusate at 7 h of *ex vivo* lung perfusion. Data are plotted as effective concentrations (pg/mL). Statistical analysis was performed using one-way ANOVA.

metabolic, inflammatory, and endothelial responses initiated by ischemia and reperfusion, PGD is the clinical manifestation characterized by pulmonary edema, hypoxemia, and impaired compliance [1]. One key feature of IRI is microvascular leakage secondary to endothelial barrier disruption, resulting in uncontrolled transvascular fluid and leukocyte extravasation [4, 18].

Controlled hypothermic storage (CHS) has been introduced in clinical practice, reshaping static lung preservation and allowing prolonged ischemic time [12, 19, 20]. Therefore, minimizing ischemic damage is a key area of investigation. One of the strategies to reduce IRI is to explore new preservation solutions, where the components should aim to prevent the inflammatory processes that typically start during preservation. Minimizing the inflammatory response to ischemia and strengthening endothelial barrier integrity are key therapeutic strategies for reducing IRI and decreasing the incidence of PGD. Our data show that lungs preserved with *IGL-2M* had less edema, lower expression of pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α, and CXCL1, and reduced shedding of syndecan-1, supporting improved preservation of endothelial integrity compared to *OCS*.

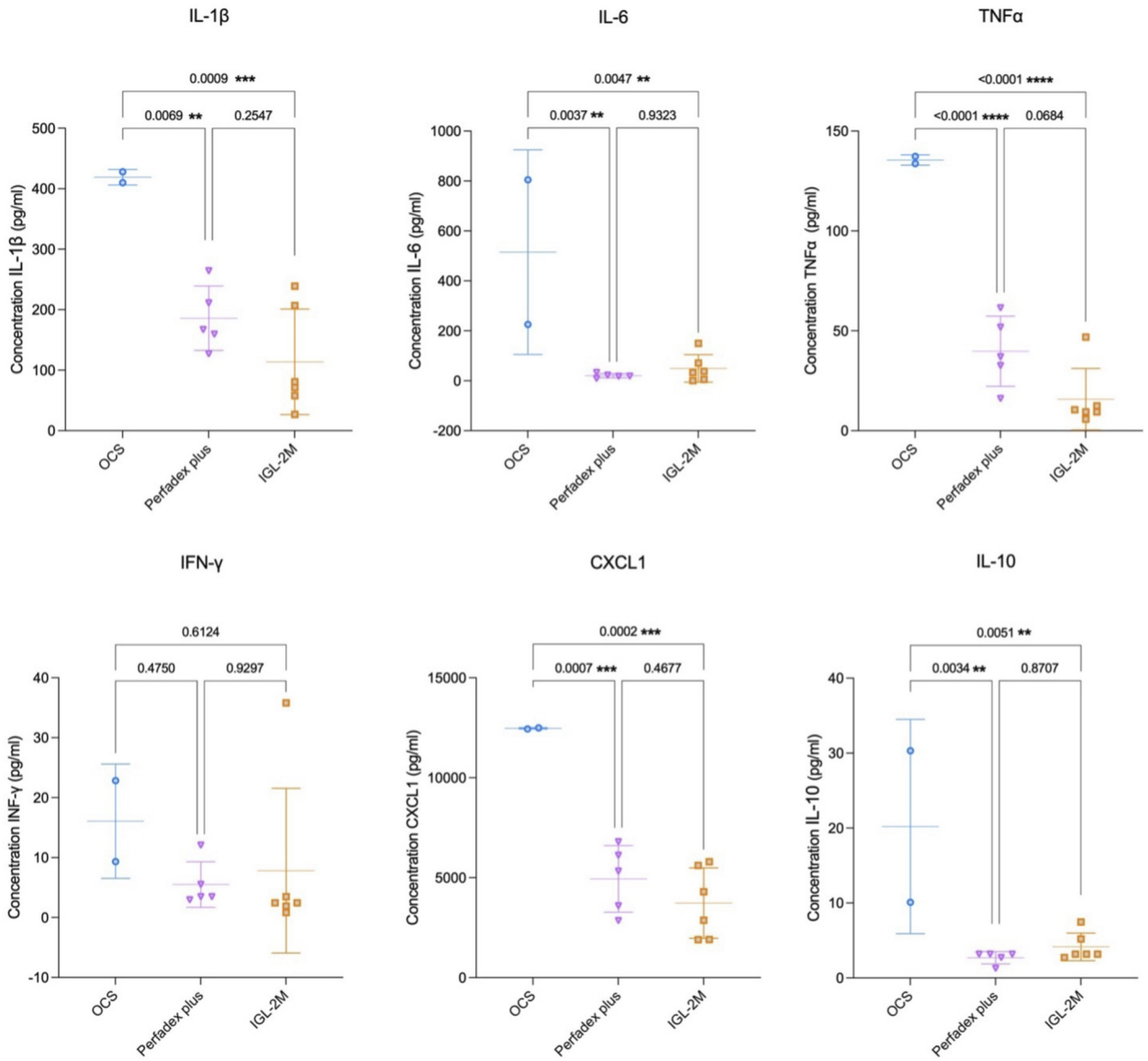


FIGURE 5 | Cytokine expression in the bronchoalveolar lavage samples at 7 h of *ex vivo* lung perfusion. Data are plotted as effective concentrations (pg/mL). Statistical analysis was performed using one-way ANOVA.

The Perfadex Plus is the most widely used lung preservation solution [21], with *OCS* lung solution used in the context of portable normothermic perfusion [22]. Both solutions are dextran-based. *Perfadex Plus* is pre-buffered with THAM and supplemented with calcium, which stabilizes endothelial junctions and maintains contractile tone [23]. This likely explains the superior protection compared to *OCS* observed in our model, where lungs preserved with *Perfadex Plus* developed less edema and released lower levels of inflammatory mediators in perfusate, BAL, and tissue. On the other hand, *IGL-2M* replaces dextran with PEG 35kDa, adding anti-inflammatory, antioxidant, and membrane-stabilizing effects [24, 25]. Our data indicate that *IGL-2M* achieves similar preservation compared to *Perfadex Plus*, while showing an additional anti-inflammatory effect through TNF-α suppression.

Physiologically, *IGL-2M*-preserved lungs showed stable and lower pulmonary vascular resistance from the onset of reperfusion, whereas *OCS*-preserved lungs had a progressive rise in resistance during EVLP. Compliance followed a similar pattern, with better compliance in the *IGL-2M* group already at the start of reperfusion. These early differences suggest that lungs preserved with *IGL-2M* experienced less structural and molecular damage during ischemia, allowing for more favorable baseline function when reperfusion began. The vascular staining of HA is likely around veins and venules, both based on structure and previous reports [26]. HA is essential in controlling permeability,

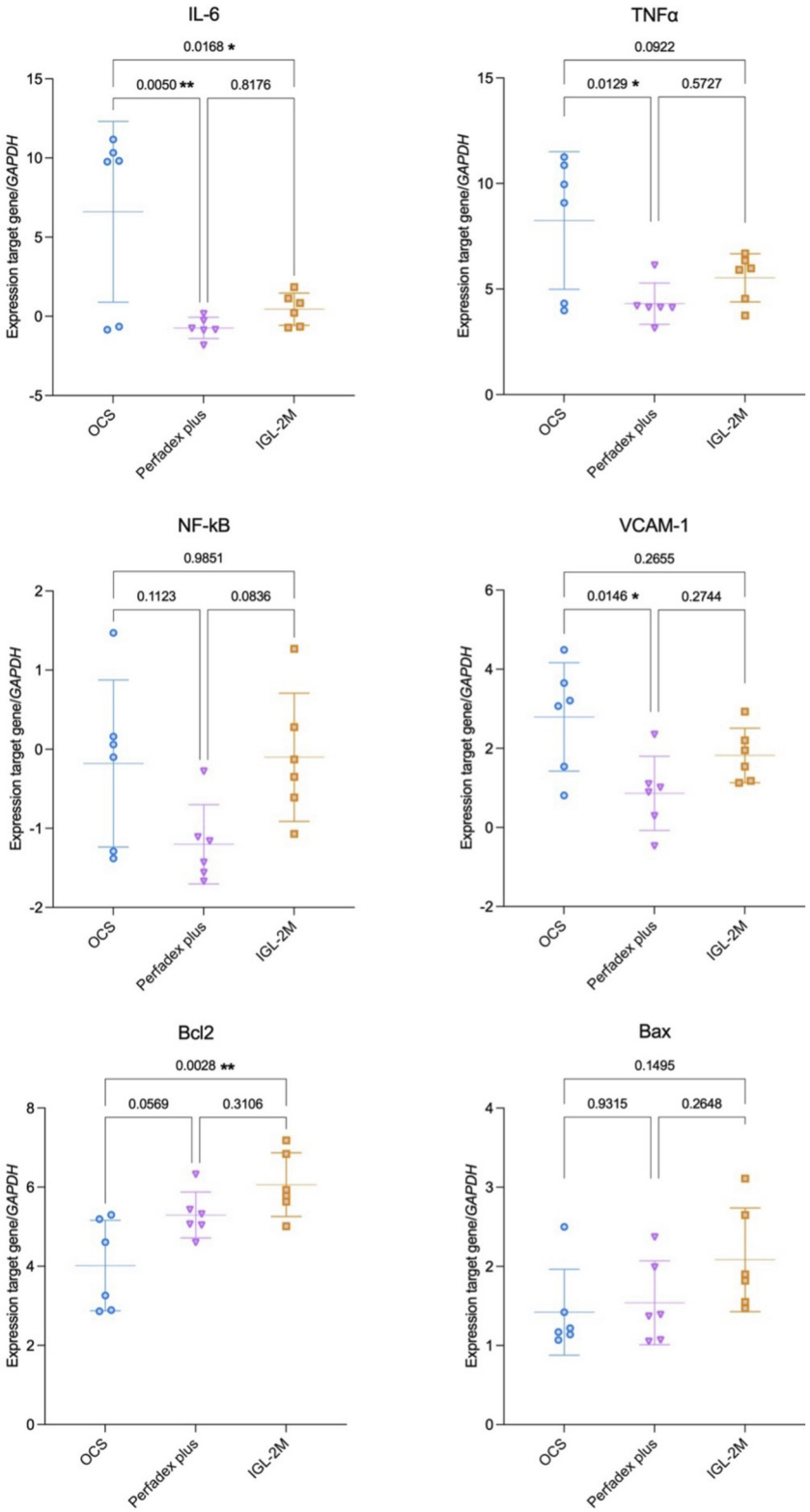


FIGURE 6 | The mRNA expression of cytokines, transcription factor (NF-kB), adhesion molecule (VCAM-1) and proteins involved in the apoptotic signaling in the lung tissue were quantified relative to the housekeeping gene GAPDH after *ex vivo* lung perfusion. Statistical analysis was performed using one-way ANOVA.

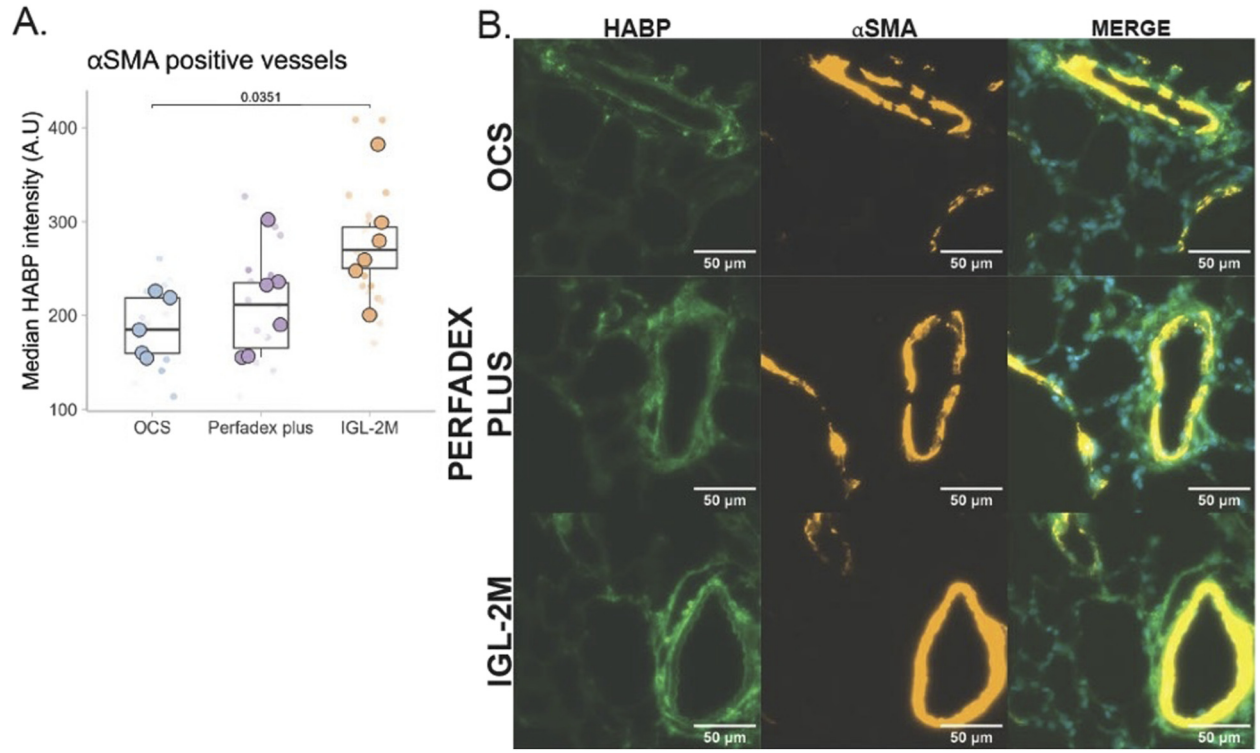


FIGURE 7 | Vascular hyaluronan was quantified using the Hyaluronan Binding Protein (HABP), co-stained with α smooth muscle actin (αSMA), in the rat lung after *ex vivo* lung perfusion. **(A)** Median intensity of HABP signal around αSMA positive vessels. Smaller points represent median intensity values from individual images. Large circles represent the average intensity derived from the individual images obtained per animal. **(B)** representative image of binding protein (green) positive co-localized with αSMA (orange). Statistical analysis was performed using one-way ANOVA.

but also plays a structural role and affects inflammation [27]. PEG has been shown to stabilize endothelial junctions by promoting VE-cadherin clustering and reducing paracellular gap formation [28]. The protective role of PEG 35 kDa in *IGL-2M* is further supported by *in vitro* studies demonstrating up to a 125% increase in transendothelial electrical resistance (TEER), sustained for over 40 h, compared to the transient effects of other agents. PEG reduces paracellular permeability to FITC-dextran by more than fourfold [28]. Together, these cellular mechanisms likely explain the lack of edema, the immediate physiological stability during EVLP, the decreased vascular leak, lower pulmonary vascular resistance, and reduced inflammatory cytokine release observed in *IGL-2M*-preserved lungs.

IGL-2M showed an anti-inflammatory effect at multiple levels. Perfusate analysis demonstrated reduced IL-1β and IL-6 compared to *OCS*, while BAL samples showed lower release of IL-1β, IL-6, TNF-α, and CXCL1. These findings were corroborated by tissue analysis, where IL-6 expression was reduced, and Bcl2 expression increased, indicating anti-apoptotic signaling. Moreover, *IGL-2M* suppressed TNF-α release compared to both *OCS* and *Perfadex Plus*. TNF-α plays a central role in IRI by amplifying endothelial activation, promoting leukocyte adhesion, and driving vascular leak [5]. Its reduction in the *IGL-2M* group points to PEG-mediated modulation of intracellular signaling pathways, beyond colloid effects alone.

Whether this effect will translate into reduced rates of PGD in clinical transplantation remains to be determined.

Perfadex Plus also demonstrated clear anti-inflammatory effects compared to *OCS*, lower levels of IL-1β, IL-6, TNF-α, CXCL-1 and IL-10, consistent with its calcium supplementation. Calcium is essential for preserving endothelial and epithelial integrity by stabilizing adherens junctions. *Perfadex Plus* contains a modified dextran formulation with a lower viscosity and carefully balanced electrolyte/osmolarity profile, reducing the risk of vascular leak. This likely accounts for the lower edema and reduced cytokine release observed in the *Perfadex Plus* group. Thus, while both solutions limit inflammation and preserve function, they probably act through different mechanisms: calcium-mediated stabilization for *Perfadex Plus* and PEG-mediated for *IGL-2M*.

Our findings are consistent with prior studies in liver transplantation, where PEG-containing solutions have been shown to preserve mitochondrial function, redox balance, and endothelial glycocalyx integrity more effectively than traditional solutions [25, 29–32]. PEG has also demonstrated anti-inflammatory, immunosuppressive, and cell-membrane-stabilization effects in the setting of intestinal IRI, increasing

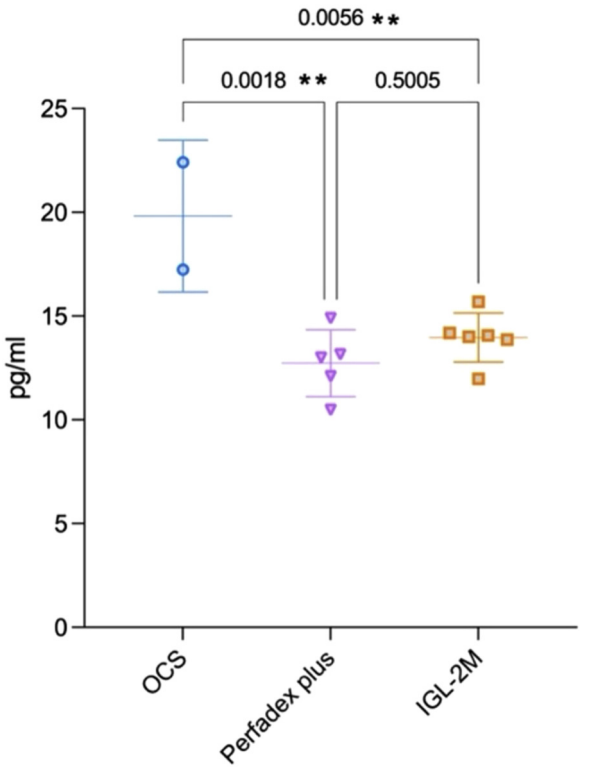


FIGURE 8 | Syndecan-1 expression in the perfusate at 7 h of *ex vivo* lung perfusion. Data are plotted as effective concentrations (pg/mL). Statistical analysis was performed using one-way ANOVA.

with higher dose [33–36]. The reproducibility of PEG's protective effects across organs suggests the feasibility of a universal preservation solution. A single PEG-based formulation for multiple organs could streamline procurement, simplify logistics, and expand the use of marginal donors.

This study has several limitations. Firstly, the high failure rate in the *OCS* group reduced the number of evaluable data points at the 7 h EVLP time point to only n = 2. This affects the statistical power, so the 7 h comparison should be interpreted with caution. However, this reflects the inability of *OCS*-preserved lungs to withstand prolonged cold ischemia followed by EVLP, resulting in edema and endothelial injury, which serve as important biological indicators of inferior preservation capacity under extended cold ischemia, rather than a technical failure. Secondly, 18 h of cold ischemia may exceed the tolerance of the *OCS*, potentially leading to early EVLP failure. However, this observation is indicative of solution-specific performance rather than a real limitation inherent to the study design. Third, direct visualization of endothelial glycocalyx integrity was not possible due to the experimental setup, as HA is only one component of the glycocalyx. Nonetheless, an ongoing transmission electron microscopy study will offer further evidence. Fourth, since this is a rodent model, caution is needed when translating findings to human lungs. Fifth, mechanistic studies were limited by the limitation of obtaining sequential tissue samples during ischemia and reperfusion. Nevertheless, the robust physiological, biochemical, and inflammatory data provide consistent evidence supporting the protective role of *IGL-2M*.

CONCLUSION

IGL-2M preserved lung function during prolonged cold ischemia at least as effectively as *Perfadex plus*, with additional anti-inflammatory benefits on TNF-α. Compared with *OCS*, *IGL-2M* limited edema formation and improved physiological stability. These findings justify further evaluation of *IGL-2M* in large animal models and provide a strong rationale for clinical translation, with the potential to establish a single PEG-based fluid as a universal organ preservation solution.

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 Local Ethical Committee for Animal Experimentation (Ethische Commissie Dierproeven) (P128/2023). The study was conducted in accordance with the local legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

AB: conceptualization; data curation; formal analysis; investigation; methodology; writing-original draft; writing-review and editing. JS: conceptualization; methodology; writing-review and editing. CT: methodology; writing-original draft; writing-review and editing. PK: methodology; writing-original draft; writing-review and editing. CV: writing-review and editing. AM: methodology; writing-review and editing. XJ: writing-review and editing. NJ: methodology; writing-review and editing. BO: writing-review and editing. SC: methodology; writing-review and editing. DS: writing-review and editing. TW: methodology; writing-original draft; writing-review and editing. KM: methodology; writing-review and editing. SS: writing-review and editing AR: writing-review and editing. II: writing-review and editing. PL: writing-review and editing. BV: writing-review and editing. JP: writing-review and editing. EJ: investigation; methodology; writing-original draft; writing-review and editing. LC: conceptualization; formal analysis; investigation; project administration; writing-original draft;

writing-review and editing. All authors contributed to the article and approved the submitted version.

FUNDING

The author(s) declared that financial support was received for this work and/or its publication. CT and EJ recognize the Human Frontiers Program (RGP015/2023) and Additional Ventures (1449617) for support of this research. LC is senior clinical investigator for FWO 18E2B24N.

CONFLICT OF INTEREST

JP and LC received an unrestricted University chair by IGL and IGL-2M preservation solution. LC received Perfadex plus and Steen solution from XVIVO.

The remaining author(s) declared that this work 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) declared that generative AI was used in the creation of this manuscript. During the preparation of this work the authors used ChatGPT (OpenAI) to improve readability and language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

ACKNOWLEDGEMENTS

The authors would like to thank all members of the Laboratory of respiratory diseases and thoracic Surgery (BREATHE) for their contribution.

REFERENCES

1. Van Slambrouck J, Van Raemdonck D, Vos R, Vanluyten C, Vanstapel A, Prisciandaro E, et al. A Focused Review on Primary Graft Dysfunction After Clinical Lung Transplantation: A Multilevel Syndrome. *Cells* (2022) 11(4):745. doi:10.3390/cells11040745
2. Van Slambrouck J, Loopmans S, Prisciandaro E, Barbarossa A, Kortleven P, Feys S, et al. The Effect of Rewarming Ischemia on Tissue Transcriptome and Metabolome Signatures: A Clinical Observational Study in Lung Transplantation. *J Heart Lung Transpl* (2024) 44:S1053249824019053. doi:10.1016/j.healun.2024.10.020
3. Pizanis N, Gillner S, Kamler M, De Groot H, Jakob H, Rauen U. Cold-Induced Injury to Lung Epithelial Cells Can Be Inhibited by Iron Chelators — Implications for Lung Preservation. *Eur J Cardiothorac Surg* (2011) 12: S1010794011001199–955. doi:10.1016/j.ejcts.2011.01.052
4. Ta HQ, Kuppusamy M, Sonkusare SK, Roeser ME, Laubach VE. The Endothelium: Gatekeeper to Lung Ischemia-Reperfusion Injury. *Respir Res* (2024) 25(1):172. doi:10.1186/s12931-024-02776-4
5. Krishnadasan B, Naidu BV, Byrne K, Fraga C, Verrier ED, Mulligan MS. The Role of Proinflammatory Cytokines in Lung Ischemia-Reperfusion Injury. *J Thorac Cardiovasc Surg* (2003) 125(2):261–72. doi:10.1067/mtc.2003.16
6. Ali MH, Schlidt SA, Chandel NS, Hynes KL, Schumacker PT, Gewertz BL. Endothelial Permeability and IL-6 Production During Hypoxia: Role of ROS in Signal Transduction. *Am J Physiol-Lung Cell Mol Physiol* (1999) 277(5): L1057–65. doi:10.1152/ajplung.1999.277.5.L1057
7. Eppinger MJ, Ward PA, Bolling SF, Deeb GM. Regulatory Effects of Interleukin-10 on Lung Ischemia-Reperfusion Injury. *Cardiovasc Surg* (1996) 112(5):1301–5. doi:10.1016/S0022-5223(96)70144-7
8. Sharma AK, Mulloy DP, Le LT, Laubach VE. NADPH Oxidase Mediates Synergistic Effects of IL-17 and TNF-α on CXCL1 Expression by Epithelial Cells After Lung Ischemia-Reperfusion. *Am J Physiol-Lung Cell Mol Physiol* (2014) 306(1):L69–79. doi:10.1152/ajplung.00205.2013
9. Den HWA, Gielis JF, Lin JY, Van Schil PE, De Windt LJ, Moens AL. Lung Ischemia-Reperfusion Injury: A Molecular and Clinical View on a Complex Pathophysiological Process. *Am J Physiol-Heart Circ Physiol* (2010) 299(5): H1283–99. doi:10.1152/ajpheart.00251.2010
10. Ran Q, Zhang J, Zhong J, Lin J, Zhang S, Li G, et al. Organ Preservation: Current Limitations and Optimization Approaches. *Front Med* (2025) 12: 1566080. doi:10.3389/fmed.2025.1566080
11. Latchana N, Peck JR, Whitson B, Black SM. Preservation Solutions for Cardiac and Pulmonary Donor Grafts: A Review of the Current Literature. *J Thorac Dis* (2014) 6(8):1143–9. doi:10.3978/j.issn.2072-1439.2014.05.14
12. Provoost AL, Novysedlak R, Van Raemdonck D, Van Slambrouck J, Prisciandaro E, Vandervelde CM, et al. Lung Transplantation Following Controlled Hypothermic Storage with a Portable Lung Preservation Device: First Multicenter European Experience. *Front Cardiovasc Med* (2024) 11: 1370543. doi:10.3389/fcvm.2024.1370543
13. Asong-Fontem N, Panisello-Rosello A, Sebagh M, Gonin M, Rosello-Catafau J, Adam R. The Role of IGL-2 Preservation Solution on Rat Livers During SCS and HOPE. *Int J Mol Sci* (2022) 23(20):12615. doi:10.3390/ijms232012615
14. Bardallo RG, Chullo G, Alva N, Rosello-Catafau J, Fundora-Suárez Y, Carbonell T, et al. Mitigating Cold Ischemic Injury: HTK, UW and IGL-2 Solution's Role in Enhancing Antioxidant Defence and Reducing Inflammation in Steatotic Livers. *Int J Mol Sci* (2024) 25(17):9318. doi:10.3390/ijms25179318
15. Wang X, Parapanov R, Debonneville A, Wang Y, Abdelnour-Berchtold E, Gonzalez M, et al. Treatment with 3-Aminobenzamide During Ex Vivo Lung Perfusion of Damaged Rat Lungs Reduces Graft Injury and Dysfunction After Transplantation. *Am J Transpl* (2020) 20(4):967–76. doi:10.1111/ajt.15695
16. Arni S, Maeyashiki T, Latshang T, Opitz I, Inci I. *Ex Vivo* Lung Perfusion with K(ATP) Channel Modulators Antagonize Ischemia Reperfusion Injury. *Cells* (2021) 10(9):2296. doi:10.3390/cells10092296
17. Bardallo RG, Da Silva RT, Carbonell T, Folch-Puy E, Palmeira C, Roselló-Catafau J, et al. Role of PEG35, Mitochondrial ALDH2, and Glutathione in Cold Fatty Liver Graft Preservation: An IGL-2 Approach. *Int J Mol Sci* (2021) 22(10):5332. doi:10.3390/ijms22105332
18. Carden DL, Granger DN. Pathophysiology of Ischaemia-Reperfusion Injury. *J Pathol* (2000) 190(3):255–66. doi:10.1002/(SICI)1096-9896(200002)190:3<255::AID-PATH526>3.0.CO;2-6
19. Novysedlak R, Provoost AL, Langer NB, Van Slambrouck J, Barbarossa A, Cenik I, et al. Extended Ischemic Time (>15 Hours) Using Controlled Hypothermic Storage in Lung Transplantation: A Multicenter Experience. *J Heart Lung Transpl* (2024) 43(6):999–1004. doi:10.1016/j.healun.2024.02.006
20. Cenik I, Van Slambrouck J, Provoost AL, Barbarossa A, Vanluyten C, Boelhouwer C, et al. Controlled Hypothermic Storage for Lung Preservation: Leaving the Ice Age Behind. *Transpl Int* (2024) 37:12601. doi:10.3389/ti.2024.12601
21. Sigvardsson. United States Patent Application Publication. In: *Organ Preservation and/or Perfusion Solutions*. US20180199566A1.
22. OCS™ Lung Solution, Transmedics (2019). Available online at: https://www.transmedics.com/lung-solution/ (Accessed September 12, 2025).
23. Dai C, Khalil RA. Calcium Signaling Dynamics in Vascular Cells and Their Dysregulation in Vascular Disease. *Biomolecules* (2025) 15(6):892. doi:10.3390/biom15060892

24. Xu X, Philip JL, Razzaque MA, Lloyd JW, Muller CM, Akhter SA. High-Molecular-Weight Polyethylene Glycol Inhibits Myocardial Ischemia–Reperfusion Injury *in vivo*. *J Thorac Cardiovasc Surg* (2015) 149(2):588–93. doi:10.1016/j.jtcvs.2014.10.074
25. Zaouali MA, Bejaoui M, Calvo M, Folch-Puy E, Pantazi E, Pasut G, et al. Polyethylene Glycol Rinse Solution: An Effective Way to Prevent Ischemia-Reperfusion Injury. *World J Gastroenterol* (2014) 20(43):16203–14. doi:10.3748/wjg.v20.i43.16203
26. Hellström M, Johansson B, Engström-Laurent A. Hyaluronan and Its Receptor CD44 in the Heart of Newborn and Adult Rats. *Anat Rec A Discov Mol Cell Evol Biol* (2006) 288A(6):587–92. doi:10.1002/ar.a.20332
27. Hoarau A, Polette M, Coraux C. Lung Hyaluronasome: Involvement of Low Molecular Weight Ha (Lmw-Ha) in Innate Immunity. *Biomolecules* (2022) 12(5):658. doi:10.3390/biom12050658
28. Chiang ET, Camp SM, Dudek SM, Brown ME, Usatyuk PV, Zaborina O, et al. Protective Effects of High-Molecular Weight Polyethylene Glycol (PEG) in Human Lung Endothelial Cell Barrier Regulation: Role of Actin Cytoskeletal Rearrangement. *Microvasc Res* (2009) 77(2):174–86. doi:10.1016/j.mvr.2008.11.007
29. Pasut G, Panisello A, Folch-Puy E, Lopez A, Castro-Benítez C, Calvo M, et al. Polyethylene Glycols: An Effective Strategy for Limiting Liver Ischemia Reperfusion Injury. *World J Gastroenterol* (2016) 22(28):6501–8. doi:10.3748/wjg.v22.i28.6501
30. Bejaoui M, Pantazi E, Calvo M, Folch-Puy E, Serafín A, Pasut G, et al. Polyethylene Glycol Preconditioning: An Effective Strategy to Prevent Liver Ischemia Reperfusion Injury. *Oxid Med Cell Longev* (2016) 2016(1):9096549. doi:10.1155/2016/9096549
31. Lopez A, Panisello-Rosello A, Castro-Benitez C, Adam R. Glycocalyx Preservation and NO Production in Fatty Livers—The Protective Role of High Molecular Polyethylene Glycol in Cold Ischemia Injury. *Int J Mol Sci* (2018) 19(8):2375. doi:10.3390/ijms19082375
32. Panisello RA, Teixeira DSR, Castro C, Bardallo R G, Calvo M, Folch-Puy E, et al. Polyethylene Glycol 35 as a Perfusate Additive for Mitochondrial and Glycocalyx Protection in HOPE Liver Preservation. *Int J Mol Sci* (2020) 21(16):5703. doi:10.3390/ijms21165703
33. Canovai E, Oltean M, Herlenius G, Akyürek LM, Trentadue G, Dijkstra G, et al. IGL-1 as a Preservation Solution in Intestinal Transplantation: A Multicenter Experience. *Transpl Int* (2020) 33(8):963–5. doi:10.1111/tri.13625
34. Oltean M, Joshi M, Björkman E, Oltean S, Casselbrant A, Herlenius G, et al. Intraluminal Polyethylene Glycol Stabilizes Tight Junctions and Improves Intestinal Preservation in the Rat. *Am J Transpl* (2012) 12(8):2044–51. doi:10.1111/j.1600-6143.2012.04067.x
35. Søfteland JM, Bagge J, Padma AM, Casselbrant A, Zhu C, Wang Y, et al. Luminal Polyethylene Glycol Solution Delays the Onset of Preservation Injury in the Human Intestine. *Am J Transpl* (2021) 21(6):2220–30. doi:10.1111/ajt.16418
36. Clarysse M, Accarie A, Panisello-Roselló A, Farré R, Canovai E, Monbaliu D, et al. Intravenous Polyethylene Glycol Alleviates Intestinal Ischemia-Reperfusion Injury in a Rodent Model. *Int J Mol Sci* (2023) 24(13):10775. doi:10.3390/ijms241310775

ESOT | Transplant International

ORIGINAL RESEARCH
published: 19 February 2026
doi: 10.3389/ti.2026.15769

Use of Maribavir in Adult Patients With Post-Transplant Refractory Cytomegalovirus Infection in the Real-Life Setting

Nassim Kamar[1], Fanny Vuotto[2], Catherine Cordonnier[3], Philippe Gatault[4], Faouzi Saliba[5,6], Lionel Couzi[7], Vincent Bunel[8,9], Cinira Lefevre[10], Michèle Maric[10], Abdelkrim Ziad[11] and Sophie Alain[12]*

[1]Department of Nephrology and Organ Transplantation, Axe TImE, Toulouse Rangueil University Hospital, INSERM UMR 1291, Toulouse Institute for Infectious and Inflammatory Diseases (Infinity), University Paul Sabatier, Toulouse, France, [2]Infectious Diseases Department, CHU Lille, Lille, France, [3]University Paris-Est-Créteil, Créteil, France, [4]Department of Nephrology-Hypertension, Dialysis and Renal Transplantation, Hôpital Bretonneau et Hôpital Clocheville, Tours, France, [5]Hepato-Biliary Centre, AP-HP Hôpital Paul Brousse, Villejuif, France, [6]University Paris Saclay, and INSERM No 1193, Gif-sur-Yvette, France, [7]Nephrology, Transplantation, Dialysis - Apheresis Department, Pellegrin Hospital, CHU Bordeaux, Bordeaux, France, [8]University Paris Cité, INSERM, Inflammation Research Center, Paris, France, [9]Pneumology, Allergology and Transplant Department, AP-HP, Bichat Hospital, Paris, France, [10]Takeda France SAS, Paris, France, [11]Clinsearch, Malakoff, France, [12]National Reference Center for Cytomegaloviruses, Microbiology Department, CHU Limoges, FHU SUPORT, UMR Inserm 1092, Omega Health Institute, Faculty of Medicine-Limoges University, Limoges, France

Maribavir is indicated for the treatment of refractory cytomegalovirus (CMV) infection/disease in patients who have undergone a solid organ transplant (SOT) or hematopoietic cell transplant (HCT). Only limited data on its use in real-world settings have been published from retrospective series. This retrospective study describes the real-world effectiveness of maribavir in 79 transplant patients with refractory CMV infection (67 SOT and 12 HCT) treated under a compassionate use program in France between October 2021 and April 2023. Maribavir was administered for <8 weeks, 8 weeks, and >8 weeks in 17, 32, and 30 patients, respectively. The response rate, defined as viremia clearance, was 53.2%, with a median time to first CMV clearance of 59 days. CMV clearance was observed in patients beyond 8 weeks of treatment. *De novo* maribavir resistance mutations were observed in 13.9% of patients, and CMV recurrence occurred in 45.2% of patients. Presence of CMV disease at baseline was associated with a lower likelihood of maribavir response. Compared to the pivotal SOLSTICE trial, real-world maribavir use demonstrated

OPEN ACCESS

***Correspondence**
Nassim Kamar,
kamar.n@chu-toulouse.fr

Received: 20 October 2025
Revised: 13 January 2026
Accepted: 11 February 2026
Published: 19 February 2026

Citation:
Kamar N, Vuotto F, Cordonnier C, Gatault P, Saliba F, Couzi L, Bunel V, Lefevre C, Maric M, Ziad A and Alain S (2026) Use of Maribavir in Adult Patients With Post-Transplant Refractory Cytomegalovirus Infection in the Real-Life Setting. Transpl. Int. 39:15769. doi: 10.3389/ti.2026.15769

Abbreviations: ALAT, alanine aminotransferase; ANSM, Agence Nationale de Sécurité du Médicament et des produits de santé; ASAT, aspartate aminotransferase; CI, confidence intervals; CMV, cytomegalovirus; CUP, compassionate use program; DNA, deoxyribonucleic acid; DNAemia, CMV DNA level in blood or plasma; ECIL, European Conference on Infections in Leukaemia; eGFR, estimated glomerular filtration rate; EU, European Union; GDPR, General Data Protection Regulation; HCT, hematopoietic cell transplant; HR, hazard ratio; IQR, interquartile range; IU, international unit; PCR, polymerase chain reaction; PUT-RD, protocole d'utilisation thérapeutique - recueil des données; SD, standard deviation; SOT, solid organ transplant; W, week.

comparable effectiveness and a lower emergence of maribavir resistance. Moreover, outcomes of patients with a longer treatment duration suggested potential benefits of extending maribavir therapy beyond the recommended 8 weeks.

Keywords: antiviral agents, cytomegalovirus, drug resistance, maribavir, transplantation

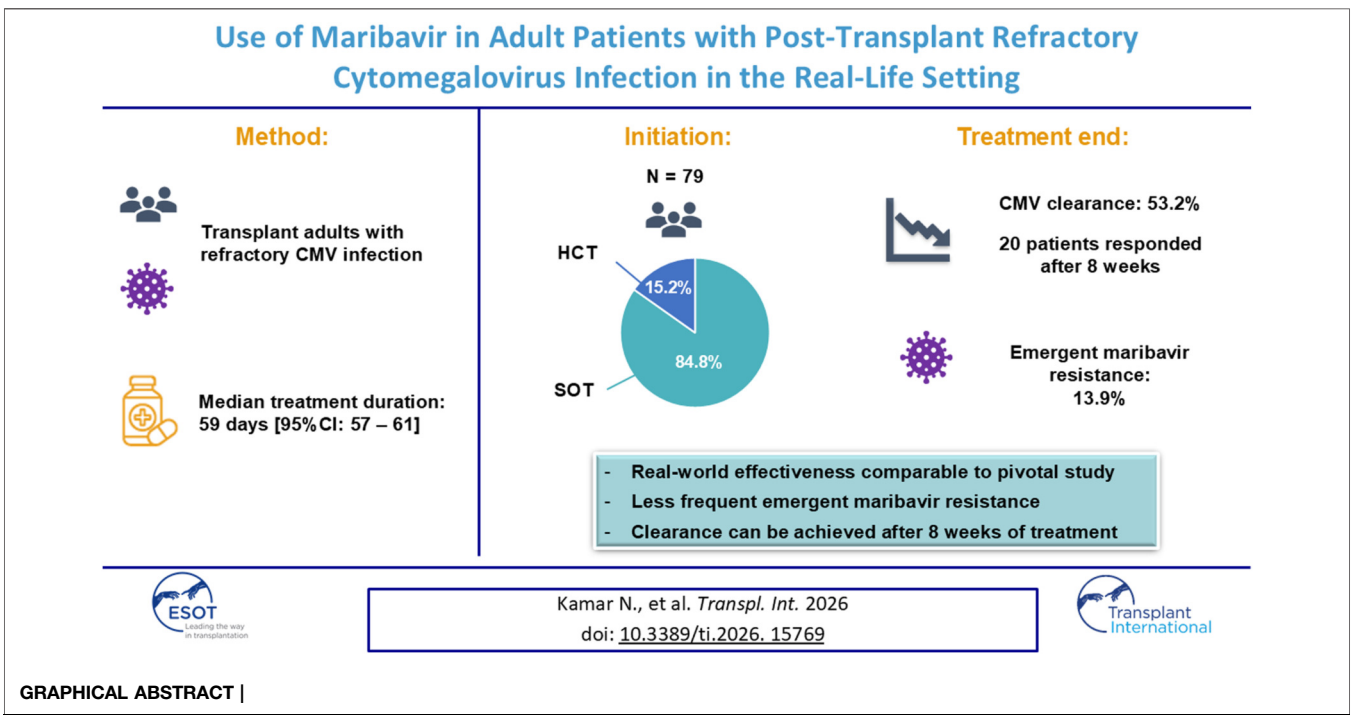
INTRODUCTION

Cytomegalovirus (CMV) is the leading cause of opportunistic infection in recipients of solid organ transplants (SOT) and allogeneic hematopoietic cell transplants (HCT) [1], with CMV infections occurring in 10%–40% of SOT recipients [2] and in approximately half of seropositive allogeneic HCT recipients in the absence of prophylaxis [3, 4].

In 3%–10% of SOT recipients and 11%–50% of HCT recipients, CMV infection is refractory to antivirals, defined as a decrease in blood or plasma viral load of less than one log10 IU/mL after 14 days of antiviral treatment [5, 6]. Refractory CMV can result from host, treatment, or viral factors, including mutations in viral genes that confer drug resistance and possible cross-resistance to antivirals. The most commonly identified drug resistance mutations are located in the genes encoding CMV DNA polymerase (UL54) and CMV protein kinase (UL97) [7]. Refractory CMV infections are associated with increased morbidity and mortality due to the direct and indirect effects of CMV infection, the immunosuppressed status of patients, and the side effects resulting from prior treatment with conventional antivirals [8, 9]. New therapies against CMV are therefore needed to provide alternative approaches to CMV management with the potential to improve patient outcomes [5, 10–12].

Maribavir is an orally bioavailable benzimidazole riboside with potent and selective multimodal anti-CMV activity through competitive inhibition of the CMV protein kinase [13]. The SOLSTICE trial (NCT02931539), a pivotal phase III clinical trial, was designed to evaluate the efficacy and safety of maribavir in adult patients with refractory or resistant CMV infection or disease following transplantation. Patients received maribavir at a dose of 400 mg twice daily for up to 8 weeks. The primary endpoint, CMV viremia clearance at Week 8, demonstrated superior efficacy and lower toxicity of maribavir compared with standard antiviral treatments (ganciclovir, valganciclovir, foscarnet, or cidofovir) for refractory CMV infection or disease with or without antiviral resistance [8, 14].

Maribavir received European marketing authorization on November 11, 2022, for the treatment of CMV infection and/or disease (with or without resistance) that is refractory to one or more prior therapies, including ganciclovir, valganciclovir, cidofovir, or foscarnet, in adult patients who have undergone HCT or SOT. The updated European Conference on Infections in Leukaemia (ECIL) guidelines for the management of CMV in patients after allogeneic HCT strongly recommend the use of maribavir for resistant or refractory CMV infection and disease, highlighting its lower risk of side effects compared to other available treatments [15]. Hence, maribavir can be considered for first-line preemptive therapy in patients with neutropenia or impaired renal function. Furthermore, the Fourth International



GRAPHICAL ABSTRACT |

Consensus Guidelines on the management of CMV in SOT strongly recommend the use of maribavir in patients who are intolerant to valganciclovir or ganciclovir during the treatment phase, as second-line therapy and as the principal alternative in cases of resistance to either ganciclovir or foscarnet [5]. Nevertheless, foscarnet may be preferred over maribavir in clinically severe patients with high viral load.

In France, the compassionate use program (CUP) allowed transplanted patients with refractory CMV infection with or without antiviral resistance to access maribavir (Agence Nationale de Sécurité du Médicament et des produits de santé, ANSM; 2021), prior to European marketing authorization. In accordance with French Health Authority requirements, data on the conditions of prescription and use of maribavir were collected and analyzed within the framework of the CUP. The purpose of the current study is to describe the use of maribavir treatment in a real-world setting.

PATIENTS AND METHODS

Study Design and Setting

This was a retrospective longitudinal study based on the maribavir CUP database. This database was compiled using prospective data collection (from 13 October 2021 to 31 August 2023), in accordance with the therapeutic use protocol (Protocole d'Utilisation Thérapeutique - Recueil des données; PUT-RD) validated by the French Health Authority (ANSM). All French centers had access to the CUP. Requests were submitted by physicians from transplant units and evaluated on a case-by-case basis by ANSM according to the PUT-RD. After inclusion in the CUP, no invasive or specific examinations were performed. All patients were managed by their transplant physicians in accordance with local procedures.

Ethics

According to French legislation, this study protocol, based on the retrospective analysis of CUP data, was declared to the French authorities under reference methodology MR-004. Patients provided informed consent regarding the use of their data as part of the CUP, and for the reuse of these data for the purposes of this research.

Processing of personal data was performed in accordance with Regulation (EU) No 2016/679 on the General Data Protection Regulation (GDPR) of natural persons.

Study Population

The patients included in this study were participating in the CUP and completed both treatment initiation and end-of-treatment forms. According to the PUT-RD, the main eligibility criteria to be included in the CUP were: age >18 years, history of a HCT or SOT, ongoing CMV refractory infection (i.e., failed to reach 1 log10 reduction in blood or plasma viral load after 14 days of standard antiviral treatment) with or without antiviral resistance, and estimated glomerular filtration rate (eGFR) >15 mL/min/1.73 m^2 (calculated using the Modification of Diet in Renal Disease Equation). The definition of refractory infection used in the present study is consistent with recent recommendations [5, 6, 15]. The complete list of inclusion criteria in CUP program is provided in the **Supplementary Material**.

Treatment Administration

Maribavir was administered orally at the recommended dose of 400 mg (two 200-mg tablets) twice daily (i.e., total daily dose of 800 mg). Although the recommended duration according to the maribavir label is 8 weeks, the treatment duration could be adjusted by the physician based on the clinical characteristics of each patient.

Data Collection Timepoints

Data on baseline variables were collected at the time of CUP request or at treatment initiation, then on follow-up visits: at week 4 (W4), W8, W12, and W20. Data on the use of maribavir were collected at each follow-up visit. Given the real-life setting of the study, there were no mandatory visits or interventions. In the event of inconsistencies regarding clinical values, quality controls were conducted by contacting the centers.

Variables of Interest

Baseline patient characteristics were collected on demographics (age, sex), biological parameters (hemoglobin, leukocyte, and platelet counts, eGFR), transplantation parameters (type and timing), and CMV-related variables (viral load, CMV drug resistance mutations, presence of CMV disease), when available. The collection of all CMV-related variables was not mandatory in routine clinical care.

Viral load levels were measured in local laboratories using a highly sensitive polymerase chain reaction (PCR) with a limit of detection of 200–500 IU/mL to quantify CMV deoxyribonucleic acid (DNA) in blood or plasma, i.e., DNAemia. DNAemia categories were defined as follows:

- High: ≥91,000 IU/mL (4.95 log10) for plasma, or ≥273,000 IU/mL (5.4 log10) for whole blood;
- Intermediate: <91,000 IU/mL (4.95 log10) and ≥9,100 IU/mL (3.95 log10) for plasma, <273,000 IU/mL (5.44 log10) and ≥27,300 IU/mL (4.44 log10) for whole blood;
- Low: <9,100 IU/mL (3.95 log10) and ≥910 IU/mL (2.95 log10) for plasma, <27,300 IU/mL (4.44 log10) and ≥2,730 IU/mL (3.44 log10) for whole blood.

These thresholds were those used in the pivotal SOLSTICE study [14].

No standardized definition of CMV disease was provided in the CUP protocol (PUT-RD); physicians were expected to rely on international recommendations and clinical judgment [16].

Co-prescriptions were recorded at treatment initiation and during follow-up. Treatment duration was measured from therapy start to discontinuation.

For effectiveness assessment, patients were considered treatment responders if they met either of the following criteria: they achieved viremia clearance, defined as a viral load concentration below the lower limit of quantification

of <137 IU/mL of plasma, or <411 IU/mL of whole blood); or 2) the viral load concentration was reported as below a threshold determined by the local laboratory or "not detectable" or "not quantifiable". The proportion of responders was estimated both across the full maribavir treatment period (i.e., between treatment initiation and treatment termination), regardless of treatment duration; and by treatment duration according to three categories: <8 weeks, 8 weeks ±6 days and ≥8 weeks.

Patients were followed for viral load up to 20 weeks after maribavir initiation, which allowed the identification of CMV recurrence after an initial response, defined as a PCR value above reference values for plasma or whole blood. Known CMV drug resistance mutations [17] were identified by Sanger sequencing genotyping before, during, and after maribavir treatment, at the physician's request.

Statistical Analysis

Descriptive statistics on patient characteristics, maribavir treatment use, and effectiveness were conducted within the overall study population. Kaplan Meier curves were provided for descriptive purposes. The median time to first clearance and time to treatment discontinuation were calculated, along with their 95% confidence intervals (CI).

Regression models were performed to identify potential predictive factors of treatment effectiveness. The construction of this model included the following steps: 1) selection of clinically relevant variables, 2) univariate analyses, 3) initial multivariate model and 4) final multivariate model. First, clinically relevant variables at treatment request or initiation were considered in univariate analyses [i.e., presence of CMV disease, CMV drug resistance mutations, biological measures (categorized according to population distribution, > or ≤ median), and viral load levels (categorized according to low, intermediate, and high levels)]. Then, univariate analyses were conducted using Chi2 tests or Fisher's exact test, with variables retained being those with $p < 0.05$. Statistically significant factors identified in the univariate analyses were then modeled using a Cox regression model to estimate the hazard ratio (HR) adjusted for significantly associated factors (after having verified that they were not highly correlated, i.e., $R < 0.7$; in case of highly correlated variables, the most significant variables in the univariate analysis were retained in the model). At the end, a final model was obtained considering model performance based on the Akaike information criterion, given the limited sample size.

RESULTS

Patient Characteristics

Between October 2021 and April 2023 (18 months), 79 patients initiated treatment with maribavir across 61 transplant departments and completed both initiation and end-of-treatment forms. The completeness rate of collected data exceeded 90% (CUP report ANSM, 2024). At baseline, patients had a mean age of 56.2 years and were predominantly male (69.6%). The majority had received SOT (67 patients; 84.8%), mainly kidney (52; 77.6%), followed by heart (7; 10.4%), lung (7; 10.4%), and pancreas (1; 1.5%). HCT recipients (12 patients) accounted for 15.2% of the study population. Additionally, 18.9% (15 patients) had cytopenia (neutrophil <1,000/mm^3, platelet <25,000/mm^3 or hemoglobin <8 g/dL), 16.5% (13 patients) had severe renal impairment (eGFR ≤30 mL/min/1.73 m^2), and 48.1% (38 patients) had viral loads (DNAemia) in the intermediate to high range (**Table 1**). Of the 79 infected patients, 57 (72.2%) were asymptomatic, and 22 (27.9%) had CMV disease. Co-prescription of maribavir with other anti-CMV agents was observed in 12 patients, including anti-CMV immunoglobulins Cytotect® (9 patients), foscarnet (2 patients), and cidofovir (1 patient).

Among the 79 patients with refractory CMV, 57 (72.2%) had at least one known CMV drug resistance mutation at baseline (resistant CMV), whereas 22 (27.9%) had no drug resistance mutations detected. One patient presented a mutation conferring resistance to maribavir, while the remaining mutations were associated with resistance to standard antiviral agents. The majority of detected CMV drug resistance mutations were in the UL97 gene, with additional mutations in the UL54 gene, conferring multidrug resistance (**Table 1**).

Maribavir Conditions of Use

The mean treatment duration of maribavir was 65.2 ± 4.4 days (median 59 days, [95% CI: 57–61] days). Most patients had a complete 8-week treatment (40.5%, 32 patients) or a treatment prolongation ≥8 weeks (38.0%, 30 patients), while 21.5% of patients (17 patients) received treatment for less than 8 weeks.

Treatment Effectiveness

Overall, 53.2% of patients (42/79) responded to maribavir at any time during treatment. The median time to first CMV clearance was 59 days [95% CI: 39–69] days (**Figure 1A**). Moreover, as shown in **Figure 1B** and **Supplementary Table S1**, the median time to first CMV clearance according to treatment duration (<8 weeks, 8 weeks ± 6 days, or >8 weeks) was 39, 56, and 63 days, respectively. These data indicate that patients may continue to achieve initial CMV clearance beyond the 8-week mark. Notably, 66.7% (20/30) of patients treated for >8 weeks experienced their first clearance after the 8th week.

Regarding potential predictive factors of treatment effectiveness, clinically relevant variables that could be related to viremia clearance under treatment were first considered in univariate analyses. These analyses showed that only three variables had p-values <0.05: the presence of CMV disease, leucocyte levels, and viral load levels at baseline (**Table 2**). These variables were then considered in multivariate models. In the final model, the presence of CMV disease at baseline was statistically significantly associated with viremia clearance (**Table 3**), indicating that patients with CMV disease at baseline were less likely to achieve CMV viremia clearance under maribavir treatment (adjusted HR = 0.44 [95% CI: 0.20–0.96]). However, baseline leucocyte levels (>4,180 mm^3 vs. ≤4,180 mm^3) and viral load levels (low vs. intermediate/high) were not statistically associated with response to

TABLE 1 | Patient characteristics at treatment access request.

Demographics	Patients (N = 79)
Age, years mean ± SD	56.2 ± 13.6
Male/Female sex, n (%)	55 (69.6)/24 (30.4)
Biological parameters	
Absolute neutrophil count,/mm^3	
<1,000, n/N (%)	8/76 (10.5)
≥1,000, n/N (%)	68/76 (89.5)
Missing data, n	3
Platelet count,/mm^3	
<25,000, n/N (%)	2/78 (2.6)
≥25,000, n/N (%)	76/78 (97.4)
Missing data, n	1
Hemoglobin level, g/L	
<80, n/N (%)	5/78 (6.4)
≥80, n/N (%)	73/78 (93.6)
Missing data, n	1
Glomerular filtration rate mL/min/1.73 m^2	
<15, n/N (%)	1/78 (1.3)
15–30, n/N (%)	12/78 (15.4)
>30, n/N (%)	65/78 (83.3)
Missing data, n	1
Solid organ transplant, n (%)	67 (84.8)
Organ transplanted, n/N (%)	
Kidney	52/67 (77.6)
Heart	7/67 (10.4)
Lung	7/67 (10.4)
Pancreas	1/67 (1.5)
Liver	0/67 (0.0)
History of graft rejection, n/N (%)	
No	59/67 (88.1)
Yes	8/67 (11.9)
Hematopoietic stem cell transplant, n (%)	12 (15.2)
Type of hematopoietic transplant, n/N (%)	
Autologous	2/12 (16.7)
Allogeneic	10/12 (83.3)
Graft-versus-host disease (GvHD), n (%)	13 (16.5)
Acute grade ≥ 2, n/N (%)	6/9 (66.7)
Chronic, n/N (%)	3/9 (33.3)
Missing data, n	4
Patients asymptomatic/with CMV disease, n (%)	57 (72.2)/22 (27.9)
Organ infected in patients with CMV disease, n/N (%)	
Gastrointestinal tract (GI) alone or in combination with other symptoms	18/21 (85.7)
Kidney	1/21 (4.8)
Lung	1/21 (4.8)
Eye	1/21 (4.8)
Bone marrow + kidney	1/21 (4.8)
Missing data, n	1
Refractory CMV infections, n	79
Refractory to ganciclovir/valganciclovir, n/N (%)	
Yes	75/78 (96.2)
No	3/78 (3.8)
Missing data, n	1
Refractory to foscarnet or cidofovir, n/N (%)	
Yes	24/74 (34.5)
No	50/74 (65.5)
Missing data, n	5
Analysis of baseline CMV drug resistance mutation, n	79
CMV gene with mutation, n/N (%)	57/79 (72.2)
UL97	51/57 (89.5)
UL54	14/57 (24.6)
Other	7/57 (12.3)
Patients with more than one drug mutation	15/57 (26.3)
Patient with maribavir resistance	1/57 (1.8)
Baseline DNAemia level categories[a], n	79
High, n/N (%)	18/79 (22.8)
Intermediate, n/N (%)	20/79 (25.3)
Low, n/N (%)	41/79 (51.9)

Abbreviations: CMV, cytomegalovirus; DNAemia; level of human CMV DNA in plasma or whole blood samples; SD, standard deviation.

[a]*The definition of CMV DNAemia level categories is provided in the methods of the article.*

maribavir: adjusted HR = 1.76 [95% CI: 0.92–3.37] and adjusted HR = 1.56 [95% CI: 0.82–2.97], respectively.

Post-Maribavir CMV Mutations and CMV Recurrence

Post-treatment, emergent maribavir resistance was detected in 13.9% (11/79) of patients. All CMV maribavir resistance mutations were located in the UL97 gene and included: T409M (5 patients), F342Y (3 patients), C480F (2 patients), C480R (1 patient), H411L (1 patient), and H411Y (1 patient). Of note, a single patient could present with multiple mutations. The median time to first maribavir resistance mutation occurrence was 45 days. Maribavir resistance mutations occurred during treatment in 8 patients and post-treatment in 3 patients. Among the 42 patients who achieved a treatment response, 19 (45.2%) experienced CMV recurrence within the 20-week follow-up period. The median time to recurrence was 91 days.

DISCUSSION

Since its marketing authorization in the European Union, maribavir has significantly changed the management of CMV infection and disease due to its efficacy, tolerability, and oral administration. These advantages are reflected in recent updates of international guidelines [5, 15]. Since the pivotal SOLSTICE trial, which demonstrated superior efficacy of maribavir over standard antiviral therapies [14], only a few publications have reported data on maribavir in transplant recipients in real-life settings [18–21].

The national-level retrospective study presented in this article is based on prospectively collected CUP data on the use of maribavir treatment in SOT and HCT recipients with refractory CMV infection or disease. Compared with the SOLSTICE study, the French CUP included patients with more severe hematological and renal impairment. At baseline, several patients in the CUP population presented cytopenia and/or renal impairment, criteria that led to exclusion in the SOLSTICE study. Specifically, 16.5% of patients had severe renal failure and 18.9% had cytopenia, compared with none in the SOLSTICE study. Additionally, more patients in the CUP had high CMV viral load levels (23.0% versus 6.0% in SOLSTICE), and presented with baseline CMV mutations conferring resistance to standard antivirals (72.2% versus 51.5% in SOLSTICE).

The response rate at any time during maribavir treatment in the CUP study was 53.2%, with a median time to first response of 59 days. These findings are consistent with other real-world studies, which reported response rates ranging from 40% to 69% [18–21]. However, the time to response observed in this study was longer than the 22 days reported in the SOLSTICE trial. This difference may be attributed to the absence of standardized PCR testing schedules in the CUP cohort—unlike in the clinical trial—as well as to higher baseline viral loads in CUP patients.

Regarding effectiveness and treatment duration, this real-life study showed that among the 30 patients who received treatment for more than 8 weeks, 67% experienced their first viremia

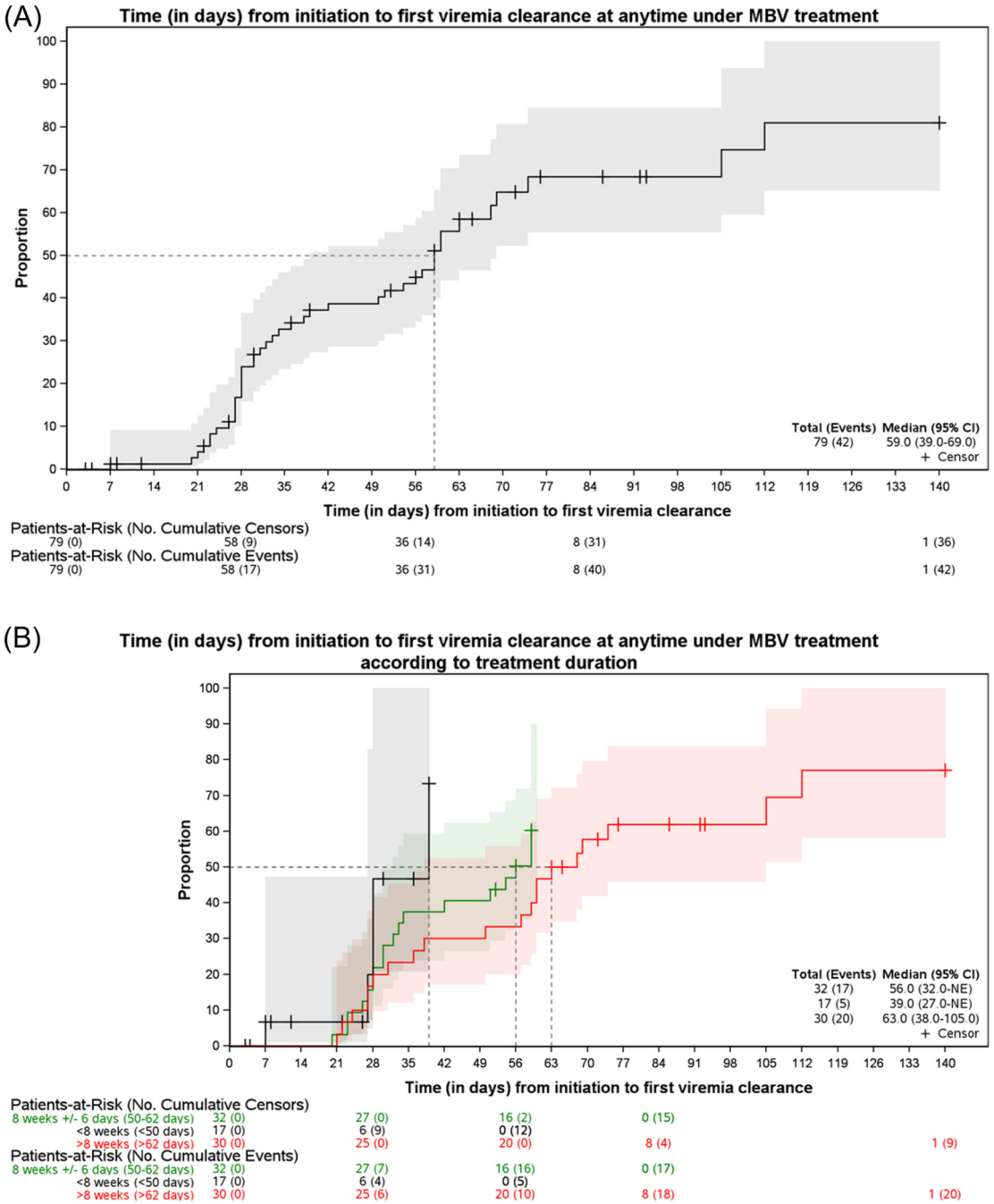


FIGURE 1 | Kaplan Meier estimates of time to first viremia clearance. **(A)** At any time under maribavir (MBV) treatment. **(B)** At any time under maribavir (MBV) treatment, stratified by treatment duration.

clearance after the 8th week, suggesting that late treatment response can occur beyond the 8-week mark. Furthermore, this analysis indicated that maribavir-emerging resistance during/after treatment occurred in around 14% of patients, which was lower than the 26% reported in the SOLSTICE trial. Other real-world studies have reported varying rates of treatment-emergent resistance, ranging from 8% to 47%, based on small series from single centers [18–20, 22]. Recently, a sub-analysis of the SOLSTICE trial showed that among SOT patients who developed maribavir-resistance, 58% achieved a viral clearance after switching to an alternative therapy [23].

In addition, this analysis describes CMV recurrences regardless of maribavir treatment duration. A potential hypothesis to explain the high percentage of recurrence

TABLE 2 | Univariate analysis of factors potentially associated with viremia clearance at any time under maribavir treatment.

Variables	Viremia clearance at any time		Total (N = 79)	P-value
	No (N = 37)	Yes (N = 42)		
Age (year)				0.3211[a]
N (missing)	37 (0)	42 (0)	79 (0)	
Mean (SD)	54.4 (14.67)	57.7 (12.53)	56.2 (13.59)	
Range	23.9–78.4	29.3–78.8	23.9–78.8	
Median (IQR)	57.0 (43.8–65.8)	61.2 (50.1–66.1)	58.6 (47.5–66.1)	
Gender, N (%)				0.0653[b]
Male	22 (59.5%)	33 (78.6%)	55 (69.6%)	
Female	15 (40.5%)	9 (21.4%)	24 (30.4%)	
Type of transplant, N (%)				0.8115[b]
SOT	31 (83.8%)	36 (85.7%)	67 (84.8%)	
HCT	6 (16.2%)	6 (14.3%)	12 (15.2%)	
Time between the most recent transplantation and treatment initiation (days)				0.9765[a]
N (missing)	37 (0)	42 (0)	79 (0)	
Mean (SD)	734.0 (1,303.29)	551.5 (1,007.45)	637.0 (1,151.46)	
Range	37.0–5,582.0	42.0–6,066.0	37.0–6,066.0	
Median (IQR)	288.0 (198.0–516.0)	270.0 (201.0–437.0)	270.0 (198.0–485.0)	
Resistance at initiation of maribavir (CMV drug mutation identified), N (%)				0.3936[b]
No	12 (32.4%)	10 (23.8%)	22 (27.8%)	
Yes	25 (67.6%)	32 (76.2%)	57 (72.2%)	
Platelet counts at baseline, N (%)				0.1408[b]
≤157000 mm^3	22 (59.5%)	18 (42.9%)	40 (50.6%)	
>157000 mm^3	15 (40.5%)	24 (57.1%)	39 (49.4%)	
Leucocyte counts at baseline, N (%)				0.0047[b]
≤4,180 mm^3	25 (67.6%)	15 (35.7%)	40 (50.6%)	
>4,180 mm^3	12 (32.4%)	27 (64.3%)	39 (49.4%)	
Neutrophil polynuclear at baseline, N (%)				0.2487[b]
≤2,500 mm^3	21 (58.3%)	19 (45.2%)	40 (51.3%)	
>2,500 mm^3	15 (41.7%)	23 (54.8%)	38 (48.7%)	
Missing	1	0	1	
ALAT at baseline, N (%)				0.6496[b]
≤25.5 IU/L	17 (47.2%)	22 (52.4%)	39 (50.0%)	
>25.5 IU/L	19 (52.8%)	20 (47.6%)	39 (50.0%)	
Missing	1	0	1	
ASAT at baseline, N (%)				0.9272[b]
≤29 IU/L	19 (51.4%)	22 (52.4%)	41 (51.9%)	
>29 IU/L	18 (48.6%)	20 (47.6%)	38 (48.1%)	
Total bilirubin at baseline, N (%)				0.3831[b]
≤7 µmol/L	22 (61.1%)	21 (51.2%)	43 (55.8%)	
>7 µmol/L	14 (38.9%)	20 (48.8%)	34 (44.2%)	
Missing	1	1	2	
Creatinine clearance at baseline, N(%)				0.2163[b]
>90 mL/min	4 (10.8%)	5 (11.9%)	9 (11.4%)	
60–90 mL/min	11 (29.7%)	10 (23.8%)	21 (26.6%)	
30–60 mL/min	11 (29.7%)	21 (50.0%)	32 (40.5%)	
≤30 mL/min	11 (29.7%)	6 (14.3%)	17 (21.5%)	
Concomitant treatment of CMV, N (%)				0.2646[b]
No	29 (78.4%)	36 (87.8%)	65 (83.3%)	
Yes	8 (21.6%)	5 (12.2%)	13 (16.7%)	
Missing	0	1	1	
Baseline viral load level (High)[c], N (%)				0.0087[b]
No	26 (70.3%)	39 (92.9%)	65 (82.3%)	
Yes	11 (29.7%)	3 (7.1%)	14 (17.7%)	
Baseline viral load level (Intermediate)[d], N (%)				0.7589[b]
No	25 (67.6%)	27 (64.3%)	52 (65.8%)	
Yes	12 (32.4%)	15 (35.7%)	27 (34.2%)	
Baseline viral load level (Low)[e], N (%)				0.0866[b]
No	23 (62.2%)	18 (42.9%)	41 (51.9%)	
Yes	14 (37.8%)	24 (57.1%)	38 (48.1%)	
Baseline viral load levels, N (%)				0.0267[b]
Low	14 (37.8%)	24 (57.1%)	38 (48.1%)	
Intermediate	12 (32.4%)	15 (35.7%)	27 (34.2%)	

(*Continued*)

TABLE 2 | Continued

Variables	Viremia clearance at any time		Total (N = 79)	P-value
	No (N = 37)	Yes (N = 42)		
High	11 (29.7%)	3 (7.1%)	14 (17.7%)	
Baseline CMV disease, N (%)				0.0230[b]
No	20 (57.1%)	34 (81.0%)	54 (70.1%)	
Yes	15 (42.9%)	8 (19.0%)	23 (29.9%)	
Missing	2	0	2	

Abbreviations: ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; CI, confidence interval; CMV, cytomegalovirus; HCT, hematopoietic cell transplant; IQR, interquartile range; IU, international unit; SD, standard deviation; SOT, solid organ transplant.

[a]*Kruskal-Wallis p-value.*

[b]*Chi-Square p-value.*

[c]*The variable "viral load level high" includes the following categories: "Yes" for patients with high viral load levels and "No" for patients with intermediate and low viral load levels.*

[d]*The variable "viral load level intermediate" includes the following categories: "Yes" for patients with intermediate viral load levels and "No" for patients with low and high viral load levels.*

[e]*The variable "viral load level low" includes the following categories: "Yes" for patients with low viral load levels and "No" for patients with intermediate and high viral load levels.*

TABLE 3 | Results of the multivariate model.

Baseline variables	Hazard ratio (95% CI)
Presence of CMV disease	
No	Reference
Yes	0.44 (0.20–0.96)
Leucocyte levels	
≤4,180 mm^3	Reference
>4,180 mm^3	1.76 (0.92–3.37)
Low viral load level	
No (intermediate/high)	Reference
Yes	1.56 (0.82–2.97)

Abbreviations: CI, confidence intervals; CMV, cytomegalovirus.

observed in this study (i.e., 45.2%) could be early treatment discontinuation, as 21.5% of patients stopped treatment before completing 8 weeks. However, these findings cannot be directly compared to those from the SOLSTICE trial since 1) recurrence in SOLSTICE was identified after a fixed treatment duration of 8 weeks; and 2) only clinically significant recurrences (those requiring treatment) were included.

Regarding potential predictive factors of treatment effectiveness, only the presence of CMV disease at baseline emerged as a significant predictor of a lower treatment response. Interestingly, leucocyte or CMV viral load levels at maribavir initiation were not significantly associated with CMV clearance in the final multivariate model. However, given the limited data available in this study to perform predictive modeling, we cannot rule out a potential clinical effect of these variables on CMV clearance following maribavir treatment. Indeed, Kasriel et al. have recently reported that high viral load was significantly associated with a higher rate of recurrence after maribavir treatment and a higher risk of mutation strain emergence [21].

The main study strengths were the study population, the quality of the prospectively collected clinical data, and the analytical approach. Patients were enrolled across the whole country, providing nationwide representativeness of maribavir use in both SOT and HCT populations. In addition, the data collected through the CUP were of high quality, with completeness rates exceeding 90%.

This study has several limitations. Unlike the SOLSTICE pivotal trial [14], in which CMV DNAemia levels were measured in plasma by a centralized laboratory, the CUP cohort data relied on measurements from plasma or whole blood samples tested at individual hospitals. This may have introduced measurement variability. Not all data were available at all timepoints as visits and interventions were not mandatory. Additionally, the follow-up period was limited to 20 weeks after treatment initiation, which precluded long-term assessment of CMV recurrence and maribavir-associated resistance. Finally, the study population included both SOT and HCT patients, with the SOT cohort primarily composed of kidney transplant recipients, which limits the extrapolation of findings to recipients of other organ types.

CONCLUSION

Overall, this study provides insight into the real-life use of maribavir in a population with a more severe health profile than in the pivotal SOLSTICE trial [14]. This included more patients with cytopenia, severe renal insufficiency, CMV disease, and elevated viral loads at baseline.

Our findings confirm that maribavir is an effective treatment for transplant recipients with refractory—eventually resistant—CMV infection or disease. The response rate observed was consistent with outcomes from the SOLSTICE trial. However, maribavir resistance occurred less frequently in this cohort, despite patients being more severely ill at baseline. Moreover, this study highlights the need for adaptable treatment duration, with potential benefits in extending maribavir therapy beyond the recommended 8 weeks to accommodate late treatment responders. Further studies are still needed to assess long-term outcomes in transplant recipients receiving anti-CMV therapy with maribavir.

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

Ethical approval was not required for the studies involving humans because According to French legislation, this study protocol, based on the retrospective analysis of CUP data, was declared to the French authorities under reference methodology MR-004. Patients provided informed consent regarding the use of their data as part of the CUP, and for the reuse of these data for the purposes of this research. Processing of personal data was performed in accordance with Regulation (EU) No 2016/679 on the General Data Protection Regulation (GDPR) of natural persons. The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from primarily isolated as part of your previous study for which ethical approval was obtained. 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

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

FUNDING

The author(s) declared that financial support was received for this work and/or its publication.

CONFLICT OF INTEREST

Authors CL and MM were employed by the company Takeda France SAS. AZ was employed by the company Clinsearch.

The remaining author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The authors declare that this study and medical writing support for this article were funded by Takeda France, 16 place de l'Iris, 92400 Courbevoie, France. The funder had the following involvement with the study: designed the study, analyzed and interpreted the data, and reviewed the manuscript.

GENERATIVE AI STATEMENT

The author(s) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

ACKNOWLEDGEMENTS

The authors thank the patients and healthcare providers from all centers who contributed to the data collection as part of the maribavir CUP program. Under the direction of the authors, medical writing support was provided by Drs. Françoise Nourrit-Poirette, Cécile Desjobert, Patrick Saunders, and Marielle Romet (Santé Active Edition, Paris, France), and was funded by Takeda France.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2026.15769/full#supplementary-material

REFERENCES

1. Haidar G, Boeckh M, Singh N. Cytomegalovirus Infection in Solid Organ and Hematopoietic Cell Transplantation: State of the Evidence. *The J Infect Dis* (2020) 221:S23–S31. doi:10.1093/infdis/jiz454
2. Tamzali Y, Pourcher V, Azoyan L, Ouali N, Barrou B, Conti F, et al. Factors Associated with Genotypic Resistance and Outcome Among Solid Organ Transplant Recipients with Refractory Cytomegalovirus Infection. *Transpl Int* (2023) 36:11295. doi:10.3389/ti.2023.11295
3. Hakimi Z, Aballéa S, Ferchichi S, Scharn M, Odeyemi IA, Toumi M, et al. Burden of Cytomegalovirus Disease in Solid Organ Transplant Recipients: A National Matched Cohort Study in an Inpatient Setting. *Transpl Infect Dis* (2017) 19. doi:10.1111/tid.12732
4. Peffault De Latour R, Chevallier P, Blaise D, Alami S, Lévy-Bachelot L, Allavoine T, et al. Clinical and Economic Impact of Treated CMV Infection in Adult CMV-Seropositive Patients After Allogeneic Hematopoietic Cell Transplantation. *J Med Virol* (2020) 92:3665–73. doi:10.1002/jmv.25895
5. Kotton CN, Kumar D, Manuel O, Chou S, Hayden RT, Danziger-Isakov L, et al. The Fourth International Consensus Guidelines on the Management of Cytomegalovirus in Solid Organ Transplantation. *Transplantation* (2025) 109:1066–110. doi:10.1097/TP.0000000000005374
6. Ljungman P, Chemaly RF, Khawaya F, Alain S, Avery R, Badshah C, et al. Consensus Definitions of Cytomegalovirus (CMV) Infection and Disease in Transplant Patients Including Resistant and Refractory CMV for Use in Clinical Trials: 2024 Update from the Transplant Associated Virus Infections Forum. *Clin Infect Dis* (2024) 79:787–94. doi:10.1093/cid/ciae321
7. Kleiboeker HL, Descourouez JL, Schulz LT, Mandelbrot DA, Odorico JS, Rice JP, et al. Maribavir for the Management of Cytomegalovirus in Adult Transplant Recipients: A Review of the Literature and Practical Considerations. *Ann Pharmacother* (2023) 57:597–608. doi:10.1177/10600280221118959
8. Kotton CN, Kamar N. New Insights on CMV Management in Solid Organ Transplant Patients: Prevention, Treatment, and Management of Resistant/Refractory Disease. *Infect Dis Ther* (2023) 12:333–42. doi:10.1007/s40121-022-00746-1
9. Walti CS, Khanna N, Avery RK, Helanterä I. New Treatment Options for Refractory/Resistant CMV Infection. *Transpl Int* (2023) 36:11785. doi:10.3389/ti.2023.11785
10. Khawaja F, Spallone A, Kotton CN, Chemaly RF. Cytomegalovirus Infection in Transplant Recipients: Newly Approved Additions to Our Armamentarium. *Clin Microbiol Infect* (2023) 29:44–50. doi:10.1016/j.cmi.2022.07.001
11. Piret J, Boivin G. Management of Cytomegalovirus Infections in the Era of the Novel Antiviral Players, Letermovir and Maribavir. *Infect Dis Rep* (2024) 16:65–82. doi:10.3390/idr16010005

12. Razonable RR. Oral Antiviral Drugs for Treatment of Cytomegalovirus in Transplant Recipients. *Clin Microbiol Infect* (2023) 29:1144–9. doi:10.1016/j.cmi.2023.03.020
13. Halpern-Cohen V, Blumberg EA. New Perspectives on Antimicrobial Agents: Maribavir. *Antimicrob Agents Chemother* (2022) 66:e0240521. doi:10.1128/aac.02405-21
14. Avery RK, Alain S, Alexander BD, Blumberg EA, Chemaly RF, Cordonnier C, et al. Maribavir for Refractory Cytomegalovirus Infections with or Without Resistance Post-Transplant: Results from a Phase 3 Randomized Clinical Trial. *Clin Infect Dis* (2022) 75:690–701. doi:10.1093/cid/ciab988
15. Ljungman P, Alain S, Chemaly RF, Einsele H, Galaverna F, Hirsch HH, et al. Recommendations from the 10th European Conference on Infections in Leukaemia for the Management of Cytomegalovirus in Patients After Allogeneic Haematopoietic Cell Transplantation and Other T-Cell-engaging Therapies. *The Lancet Infect Dis* (2025) 25:e451–e462. doi:10.1016/s1473-3099(25)00069-6
16. Ljungman P, Boeckh M, Hirsch HH, Josephson F, Lundgren J, Nichols G, et al. Definitions of Cytomegalovirus Infection and Disease in Transplant Patients for Use in Clinical Trials. *Clin Infect Dis* (2017) 64:87–91. doi:10.1093/cid/ciw668
17. Tilloy V, Díaz-González D, Laplace L, Bisserier E, Chou S, Rawlinson WD, et al. Comprehensive Herpesviruses Antiviral Drug Resistance Mutation Database (CHARMD). *Antiviral Res* (2024) 231:106016. doi:10.1016/j.antiviral.2024.106016
18. Beechar VB, Phadke VK, Pouch SM, Mehta AK, Karadkhele G, Larsen CP, et al. Evaluating Real-World Experience with Maribavir for Treatment of Refractory/Resistant Cytomegalovirus in Renal Transplant Recipients. *Clin Transpl* (2024) 38:e15480. doi:10.1111/ctr.15480
19. Daher M, Khawaja F, Spallone A, Shigle TL, Bhatti M, Vuong NN, et al. Real-World Experience with Maribavir for Treatment of Refractory or Resistant Cytomegalovirus Infection in Hematopoietic Cell Transplant Recipients and Hematologic Malignancy Patients. *Transpl Infect Dis* (2025) 27:e14444. doi:10.1111/tid.14444
20. Ni B, Wolfe CR, Arif S, Carugati M, Heldman MR, Messina JA, et al. Real-World Experience with Maribavir for Treatment of Cytomegalovirus Infection in High-Risk Solid Organ Transplant Recipients. *Open Forum Infect Dis* (2024) 11:ofae335. doi:10.1093/ofid/ofae335
21. Kasriel N, Alain S, Benotmane I, Kamar N, Danthu C, Tinel C, et al. Determinants of Virological Outcomes in Kidney Transplant Recipients Treated with Maribavir for Refractory Cytomegalovirus Infection. *Am J Transpl* (2025). doi:10.1016/j.ajt.2025.10.018
22. Lilleri D, Campanini G, Paolucci S, Lupia T, Tebaldi A, Gregorini M, et al. Emergence of Maribavir-Resistant Cytomegalovirus in Transplant Recipients Treated for Refractory/Resistant Cytomegalovirus Infection. *J Med Virol* (2025) 97:e70259. doi:10.1002/jmv.70259
23. Blumberg EA, Witzke O, Harber M, Ison MG, Saliba F, Kamar N, et al. Maribavir for Refractory Cytomegalovirus Infection (With or Without Resistance) in Solid Organ Transplant Recipients: Subgroup Analysis of the Phase 3 Randomized SOLSTICE Study. *The J Heart Lung Transplant* (2025) 44:986–94. doi:10.1016/j.healun.2024.11.026

ESOT | Transplant International

ORIGINAL RESEARCH
published: 10 February 2026
doi: 10.3389/ti.2026.15529

Combined Creatinine and Cystatin C Equations Improve Estimation of Glomerular Filtration Rate in Kidney Transplant Recipients

Etienne Mondésert[1], Anne-Sophie Bargnoux[1,2], Ilan Szwarc[3], Moglie Le Quintrec[3], Georges Mourad[3] and Jean-Paul Cristol[1,2,4]*

[1]Department of Biochemistry, University Hospital of Montpellier, Montpellier, France, [2]PhyMedExp, University of Montpellier, INSERM, CNRS, Montpellier, France, [3]Department of Nephrology, University Hospital of Montpellier, Montpellier, France, [4]AIDER Santé Fondation Charles Mion, Montpellier, France

Glomerular filtration rate (GFR) is a crucial parameter in post-transplant follow-up (PTF). CKD-EPI 2009 creatinine-based equation remains the most used estimated GFR (eGFR) and only few data are available on the other equations, based on creatinine, cystatin C or their combination. We evaluated 10 GFR estimation equations on 242 kidney-transplant recipient patients having measured GFR (mGFR) determination (urinary clearance of ^{99m}Tc-DTPA) with simultaneous plasma enzymatic creatinine and serum cystatin C (immunoturbidimetry or immunonephelemetry) assessments. Five creatinine (MDRD 2006, CKD-EPI 2009 and 2021, EKFC 2021, KRS 2023), two cystatin C (CKD-EPI 2012, EKFC 2023) and three combined eGFR (CKD-EPI 2012 and 2021, combined EKFC) were evaluated. All equations were significantly correlated with mGFR (R^2 = 0.672–0.745) with a low median bias (+4.2 to −1.1 mL/min/1.73 m^2). Chronic kidney disease staging agreements were all above 68% (maximum: 79.3% for CKD-EPI comb 2021). Percentages of eGFR comprised in between 30% of the mGFR ranged from 85.5% to 87.6% (combined equations), from 83.1% to 84.3% (cystatin C equations) and from 75.2% to 81.4% (creatinine equations). Combined creatinine/cystatin C eGFR equations with a P30 value greater to 85% of transplant recipients appeared closer to mGFR than cystatin C or creatinine eGFR.

Keywords: chronic kidney disease epidemiology collaboration CT, creatinine, cystatin C, glomerular filtration rate, kidney transplantation

OPEN ACCESS

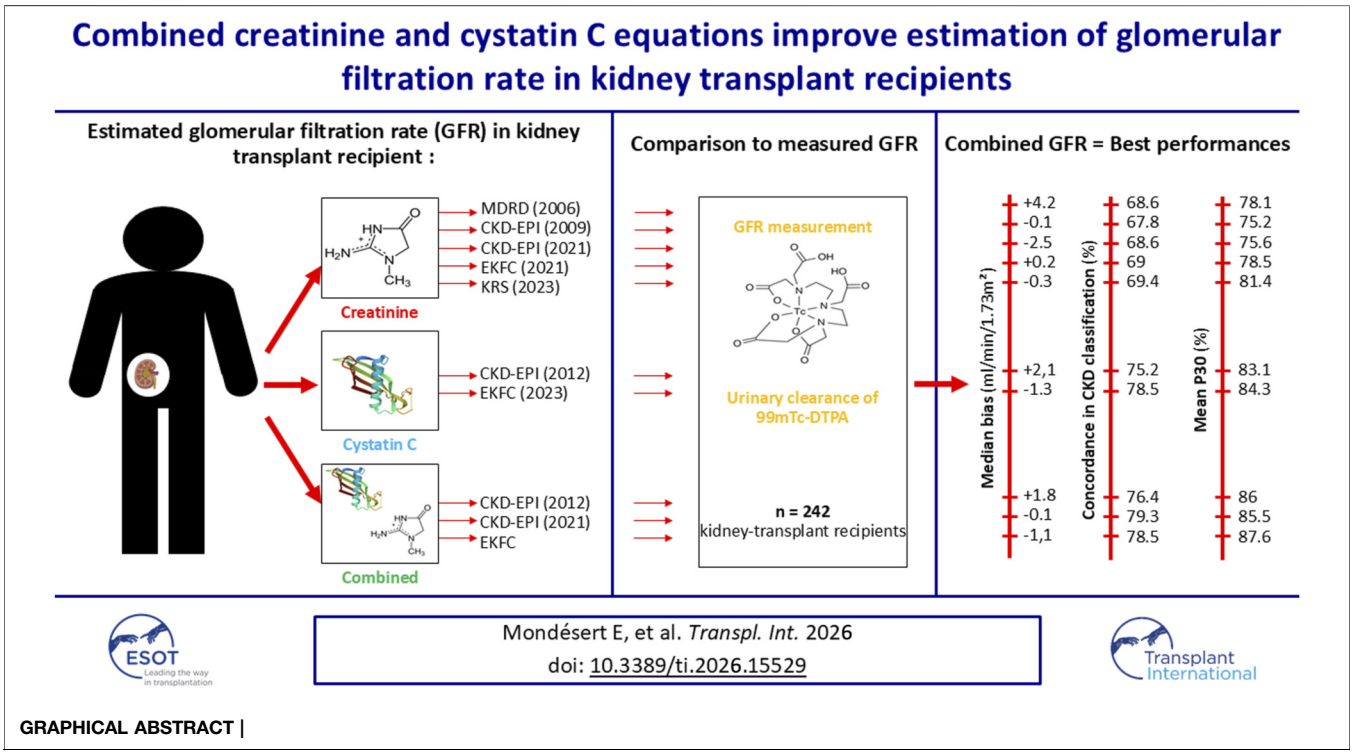
Correspondence
Etienne Mondésert,
e-mondesert@chu-montpellier.fr

Received: *02 September 2025*
Revised: *22 December 2025*
Accepted: *08 January 2026*
Published: *10 February 2026*

Citation:
Mondésert E, Bargnoux A-S, Szwarc I, Le Quintrec M, Mourad G and Cristol J-P (2026) Combined Creatinine and Cystatin C Equations Improve Estimation of Glomerular Filtration Rate in Kidney Transplant Recipients. Transpl. Int. 39:15529. doi: 10.3389/ti.2026.15529

INTRODUCTION

Post transplantation follow-up (PTF) of kidney transplant recipient (KTR) patient recommendations comprises a regular monitoring of estimated glomerular filtration rate (eGFR) [1]. Indeed, longitudinal follow-up of glomerular filtration rate (GFR) could determine transplant trajectories and predict graft failure or improvement of transplant function following adaptation of immunosuppressive treatment [2–5]. Although measured GFR (mGFR) with iohexol or radionuclides permits the most precise GFR assessment, its utilization requires outpatient hospitalization and is often limited to specialized medical structures and thus cannot be used to routinely monitor kidney function [6].



GRAPHICAL ABSTRACT |

Creatinine is the most used endogenous biomarker to estimate GFR. However, its levels can be influenced by several bias such as muscle mass variation [7] or tubular secretion [8]. KTR patients are particularly concerned by those biases since creatinine tubular secretion tends to increase with kidney function decline and could be impaired by anti-infectious drugs [8, 9]. Moreover, there is a high prevalence of sarcopenia in end stage renal disease patients undergoing hemodialysis [10, 11] and creatinine excretion rate (CER), a direct reflection of muscle mass could be decreased following transplantation [12].

Cystatin C (Cys C) is a complementary endogenous marker used to estimate GFR. Unlike creatinine, it is not secreted in renal tubules and is far less sensitive to muscle mass variations. Cys C have an important place in chronic kidney disease diagnosis and classification as it has been implemented since 2012 in kidney disease-improving global outcome (KDIGO) guidelines [13]. Moreover, the 2024 KDIGO recommendations clearly recommend Cys C use if creatinine estimation could be inaccurate, or if a more accurate assessment of GFR is needed for clinical decision-making or drug dosing [6]. Cys C has also been shown to be a strong predictor of cardiovascular mortality in the general population [14]. Several studies cite data in favor of the use of cystatin C in KTR. [15, 16], but Cys C has also its own bias in this population. For instance, corticosteroid medication that is a commonly prescribed immunosuppressive drug in kidney transplantation tends to increase Cys C concentration [17, 18].

Besides the specific variation factors for each marker, another factor of variability in eGFR is the multiplication of estimation equations (**Table 1**). Historically, Modification in Renal Diet Disease (MDRD 2006) equation was the first based on creatinine and estimating mGFR [19]. Chronic kidney disease–epidemiology equation based on creatinine (CKD-EPI creatinine 2009) equation had been developed afterwards in 2009 and is probably now the most used worldwide [20]. Nevertheless, the equation had been updated in 2021 (CKD-EPI creatinine 2021) to avoid the "race factor" that was complicated to implement in clinical settings [21]. In 2021, Pottel et al. developed in collaboration with the European kidney function consortium (EKFC) an eGFR equation (EKFC creatinine 2021) using a creatinine rescaling factor to control variation related to age and sex [22]. More recently, another "race-free" creatinine-based eGFR equation was specifically developed in KTR population datasets (KRS 2023), with superior performances compared to others cr-based eGFR equations [23]. Apart from the KRS 2023 equation, the development and validation dataset for eGFR typically includes a small number of KTR patients.

With Cys C use as the endogenous biomarker, the CKD-EPI Cys C 2012 equation was designed like CKD-EPI creatinine 2009 to be calculated with Cys C levels, age and sex [24]. EKFC Cys C 2023 was also designed like EKFC creatinine 2021 but with age as the only factor required to calculate eGFR [25].

Finally, combined equation based on both creatinine and Cys C levels had been developed: combined CKD-EPI 2012 (CKD-EPI comb 2012) which was updated in 2021 (CKD-EPI comb 2021) to overcome the limitation of incorporating a "race

TABLE 1 | eGFR equations formulas. CREA, creatinine; Cys C, cystatin C; comb, combination.

Equation/Sex [references]	Cr (μmol/L)	Cys (mg/L)	Equation
MDRD 2006 [19]			
Women & men	—	—	$175\times(CREA/88.4)^{-1.154}\times Age^{-0.203}[\times 0.742]^{a}$
CKD-Epi creatinine 2009 [20]			
Women	≤62	—	$144\times((CREA/88.4)/0.7)^{-0.329}\times 0.993^{Age}[\times 1.15]^{b}$
	>62	—	$144\times((CREA/88.4)/0.7)^{-1.209}\times 0.993^{Age}[\times 1.15]^{b}$
Men	≤80	—	$141\times((CREA/88.4)/0.9)^{-0.411}\times 0.993^{Age}[\times 1.14]^{b}$
	>80	—	$141\times((CREA/88.4)/0.9)^{-1.209}\times 0.993^{Age}[\times 1.14]^{b}$
CKD-Epi Cys C 2012 [24]			
Women & men	—	≤0.8	$133\times(Cys\ C/0.8)^{-0.499}\times 0.996^{Age}[\times 0.932]^{a}$
Women & men	—	>0.8	$133\times(Cys\ C/0.8)^{-1.328}\times 0.996^{Age}[\times 0.932]^{a}$
CKD-Epi comb 2012 [24]			
Women	≤62	≤0.8	$130\times((CREA/88.4)/0.7)^{-0.248}\times(Cys\ C/0.8)^{-0.375}\times 0.995^{Age}[\times 1.08]^{b}$
		>0.8	$130\times((CREA/88.4)/0.7)^{-0.248}\times(Cys\ C/0.8)^{-0.711}\times 0.995^{Age}[\times 1.08]^{b}$
Women	>62	≤0.8	$130\times((CREA/88.4)/0.7)^{-0.601}\times(Cys\ C/0.8)^{-0.375}\times 0.995^{Age}[\times 1.08]^{b}$
		>0.8	$130\times((CREA/88.4)/0.7)^{-0.601}\times(Cys\ C/0.8)^{-0.711}\times 0.995^{Age}[\times 1.08]^{b}$
Men	≤80	≤0.8	$135\times((CREA/88.4)/0.9)^{-0.207}\times(Cys\ C/0.8)^{-0.375}\times 0.995^{Age}[\times 1.08]^{b}$
		>0.8	$135\times((CREA/88.4)/0.9)^{-0.207}\times(Cys\ C/0.8)^{-0.711}\times 0.995^{Age}[\times 1.08]^{b}$
Men	>80	≤0.8	$135\times((CREA/88.4)/0.9)^{-0.601}\times(Cys\ C/0.8)^{-0.375}\times 0.995^{Age}[\times 1.08]^{b}$
		>0.8	$135\times((CREA/88.4)/0.9)^{-0.601}\times(Cys\ C/0.8)^{-0.711}\times 0.995^{Age}[\times 1.08]^{b}$
CKD-Epi creatinine 2021 [21]			
Women	≤61.6	—	$142\times((CREA/88.4)/0.7)^{-0.241}\times 0.9938^{Age}\times 1.012$
	>61.6	—	$142\times((CREA/88.4)/0.7)^{-1.200}\times 0.9938^{Age}\times 1.012$
Men	≤79.2	—	$142\times((CREA/88.4)/0.9)^{-0.302}\times 0.9938^{Age}\times 1.012$
	>79.2	—	$142\times((CREA/88.4)/0.9)^{-1.200}\times 0.9938^{Age}\times 1.012$
CKD-Epi comb 2021 [21]			
Women	≤62	≤0.8	$130\times((CREA/88.4)/0.7)^{-0.219}\times(Cys\ C/0.8)^{-0.323}\times 0.9961^{Age}$
		>0.8	$130\times((CREA/88.4)/0.7)^{-0.219}\times(Cys\ C/0.8)^{-0.778}\times 0.9961^{Age}$
Women	>62	≤0.8	$130\times((CREA/88.4)/0.7)^{-0.544}\times(Cys\ C/0.8)^{-0.323}\times 0.9961^{Age}$
		>0.8	$130\times((CREA/88.4)/0.7)^{-0.544}\times(Cys\ C/0.8)^{-0.778}\times 0.9961^{Age}$
Men	≤80	≤0.8	$135\times((CREA/88.4)/0.9)^{-0.144}\times(Cys\ C/0.8)^{-0.323}\times 0.9961^{Age}$
		>0.8	$135\times((CREA/88.4)/0.9)^{-0.144}\times(Cys\ C/0.8)^{-0.778}\times 0.9961^{Age}$
Men	>80	≤0.8	$135\times((CREA/88.4)/0.9)^{-0.544}\times(Cys\ C/0.8)^{-0.323}\times 0.9961^{Age}$
		>0.8	$135\times((CREA/88.4)/0.9)^{-0.544}\times(Cys\ C/0.8)^{-0.778}\times 0.9961^{Age}$
EKFC creatinine 2021 [22]			
Women & men	cr/Q ≤1	—	$107.3/(CREA/Q)^{0.322}\times[0.99^{Age-40}]^{c}$
	cr/Q >1	—	$107.3/(CREA/Q)^{1.132}\times[0.99^{Age-40}]^{c}$
EKFC Cys C 2023 [25]			
Women & men	—	cys/Q ≤1	$107.3/(CREA/Q)^{0.322}\times[0.99^{Age-40}]^{c}$
	—	cys/Q >1	$107.3/(CREA/Q)^{1.132}\times[0.99^{Age-40}]^{c}$
EKFC comb			
Women & men	—	—	(eGFR(EKFC CREA 2021)+eGFR(EKFC Cys C 2023))/2
KRS 2023 [23]			
Women	—	—	$e^{4.4275492-0.8230475*\ln(CREA/88.4)-0.0124264(CREA/88.4)2-0.055068*Age}$
Men	—	—	$e^{4.4275492-0.8230475*\ln(CREA/88.4)-0.0124264(CREA/88.4)2-0.055068*Age+0.1806494}$

[a]*Factor to apply if the patient is a woman.*
[b]*Factor to apply if the patient is African American.*
[c]*Factor to apply if age >40 years.*
Q = rescaling factor, calculated as follows:
Creatinine: For ages 2–25 years, Women: ln(Q) = 3.080 + 0.177 × Age − 0.223 × ln(Age) − 0.00596 × Age² + 0.0000686 × Age³.
Men: ln(Q) = 3.200 + 0.259 × Age − 0.543 × ln(Age) − 0.00763 × Age² + 0.0000790 × Age³. For ages >25 years, Women: Q = 62 μmol/L, Men Q = 80 μmol/L.
Cys C: For ages <50 years: Women & men: Q = 0.83 mg/L. For ages >50 years, Q = 0.83 + 0.005(Age-50) mg/L.*

factor" into the equation, which is a social construct that can influence medical decisions [21, 24]. The mean of EKFC creatinine 2021 and EKFC Cys C 2023 (EKFC comb) can be also used.

To date, few data comparing all these equations are available in KTR patients. We took advantage of simultaneous mGFR, creatinine and Cys C assessments in KTR patients to evaluate the performances of the ten eGFR equations mentioned above.

MATERIALS AND METHODS

Subjects

This single-center and retrospective study was carried out on patients seen in the Nephrology department of the Montpellier University Hospital between 2007 and 2009. KTR patients aged 18 or more recruited during their normal PTF protocol underwent GFR measurement by urinary clearance of technetium-labelled di-ethylene-triamino-penta acetic acid

TABLE 2 | Baseline characteristics of the 242 included patients. min, minimum; max, maximum; PTF, post-transplantation follow-up; IQR, interquartile range; SD, standard deviation.

Criteria	Value
Age (years, *median [min-max]*)	53.2 [18.3–76.6]
%Female/ %male (%)	35/ 65
PTF duration (years, *median ± IQR*)	2.1 ± 5
^{99m}Tc-DTPA mGFR (ml/min/1.73 m^2, *mean±SD*)	51.4 ± 19.8
CKD classification based on mGFR (%)	
G1 (>90 mL/min/1.73 m^2)	3.3
G2 (60–90 mL/min/1.73 m^2)	26
G3a (45–60 mL/min/1.73 m^2)	31
G3b (30–45 mL/min/1.73 m^2)	26
G4 (15–30 mL/min/1.73 m^2)	11.6
G5 (<15 mL/min/1.73 m^2)	2.1
Creatinine (µmol/L, *mean±SD*)	144.7 ± 62.3
Cystatin C (mg/L, *mean±SD*)	1.6 ± 0.6
Mean eGFR (ml/min/1.73 m^2, *mean±SD*)	
MDRD 2006	47.8 ± 19.3
CKD-Epi CREA 2009	51.6 ± 21.5
CKD-Epi CREA 2021	54.2 ± 22.3
EKFC CREA 2021	51.5 ± 20.3
KRS 2023	51.2 ± 16.2
CKD-Epi Cys C 2012	49.4 ± 21.5
EKFC Cys C 2023	52.8 ± 20
CKD-Epi comb 2012	49.4 ± 21
CKD-Epi comb 2021	51.8 ± 22
EKFC comb	52.2 ± 19.4

(^{99m}Tc-DTPA). Inclusion criteria comprised concomitant plasma creatinine and serum Cys C measurements and age superior to 18 years. Blood collection took place the same day before any injection and before the start of GFR measurement, whereas 24-hour urine sample was collected the day before GFR measurement. Basic demographical data (age, sex), graft date and medication at the time of mGFR measurement were extracted from medical records, in accordance with approval by our institution local ethics committee declared under the number DC-2008-417.

Methods

mGFR were measured by urinary clearance of ^{99m}Tc-DTPA using the constant infusion technique [26], results were expressed in mL/min/1.73 m^2, with body surface area calculated by Du Bois and Du Bois formula [27]. For mGFR measurement; four 20–30 min urine collections were obtained by spontaneous voiding after the induction of water diuresis and a 90-min equilibration period. At the end of each clearance period, patients drank a volume of water equal to the preceding urine volume. Radioactivity was determined on the 4 urine collection samples and on 4 plasma samples drawn at midpoint of each clearance period. The measured GFR values was the average of the four calculated clearance. Plasma creatinine assessment using an enzymatic isotope dilution mass spectrometry (IDMS) traceable assay on Olympus® analyzer (Olympus France, Rungis, France) using creatinine reagents from Olympus (Olympus France, Rungis, France) or on architect C8000® (Abott, Chicago, USA) using Abbott enzymatic creatinine method (Multigent® creatinine) was performed. Cys C serum levels were determined by particle-enhanced immunonephelometry (PENIA) using N Latex cys reagents from Siemens on the BNII® systems (Siemens, Marburg, Germany) or by particle-enhanced turbidimetry (PETIA) using Sentinel Diagnostics reagents (Sentinel CH. SpA, Milano, Italy) on Architect C8000® analyzer [15, 28]. BD vacutainer® (Franklin Lakes, New Jersey, United States of America) collection, with lithium heparinate anticoagulation for plasma samples and with clot activator additive (silica particles) for serum samples, were centrifuged at 2000g for 10 min. Creatinine measurements were performed shortly after centrifugation, while serum samples were frozen at −18 °C until analysis performed within 7 days from sampling. When available, CER expressed by urinary Creatinine measured by the same techniques than in the blood on a 24-h urine collection (mmol/24h) preceding mGFR assessment was also collected. eGFR was calculated with the formulas of the equations depicted in **Table 1**, comprising five CREA-based equations (MDRD 2006, CKD-EPI 2009, CKD-EPI 2021, EKFC creatinine 2021, KRS 2023), two Cys C-based equations (CKD-EPI Cys C 2012, EKFC Cys C 2023) and three combined equations (CKD-EPI comb 2012 and 2021, EKFC comb). For CKD-EPI creatinine 2009 and CKD-EPI comb 2012, the "African-American factor" was not taken into account in eGFR calculation.

Statistical Analysis

All quantitative values of GFR are expressed in mL/min/1.73 m^2 and as mean ± standard deviation (SD) in text. Normality was assessed with the Kolgomorov-Smirnov test. Passing-Bablok regression procedure was used to determine the slope (*a*) and the intercept (*b*) of the linear equation eGFR = *a**mGFR + *b*. Pearson determination coefficients (R^2) were determined to compare mGFR with eGFRs, eGFRs based on creatinine or combined eGFRs with 24-hours urinary CREA, eGFRs based on Cys C or combined eGFRs with corticosteroid dosage. Slope tests and t-tests for Pearson correlation were performed with alpha significance level set at 5% and a P-value under 0.05 considered as statistically significant. The scatter of differences was visualized by means of Bland-Altman plot. The concordance between CKD classification according to the 2012 KDIGO definition of patients using mGFR and eGFR was assessed using weighted Cohen's κ-test coefficient (wκ) with value in between 0.4 and 0.6 indicating moderate agreement, and values in between 0.6 and 0.8 indicating substantial agreement. Concordance was also evaluated by the determination of the proportion of patient with eGFR comprised within ±10% (P_{10}) ±30% (P_{30}) of mGFR. 95% confidence intervals were calculated for concordance measures. Net reclassification improvement (NRI) was calculated to assess the ability of the best performing eGFR equation (in terms of concordance with mGFR) to correctly classify individuals into CKD stages compared to other eGFR equations with mGFR as the reference standard. Category-based NRI was used with CKD stage cutoffs of 90, 60, 45, 30 and 15 mL/min.1.73 m^2.

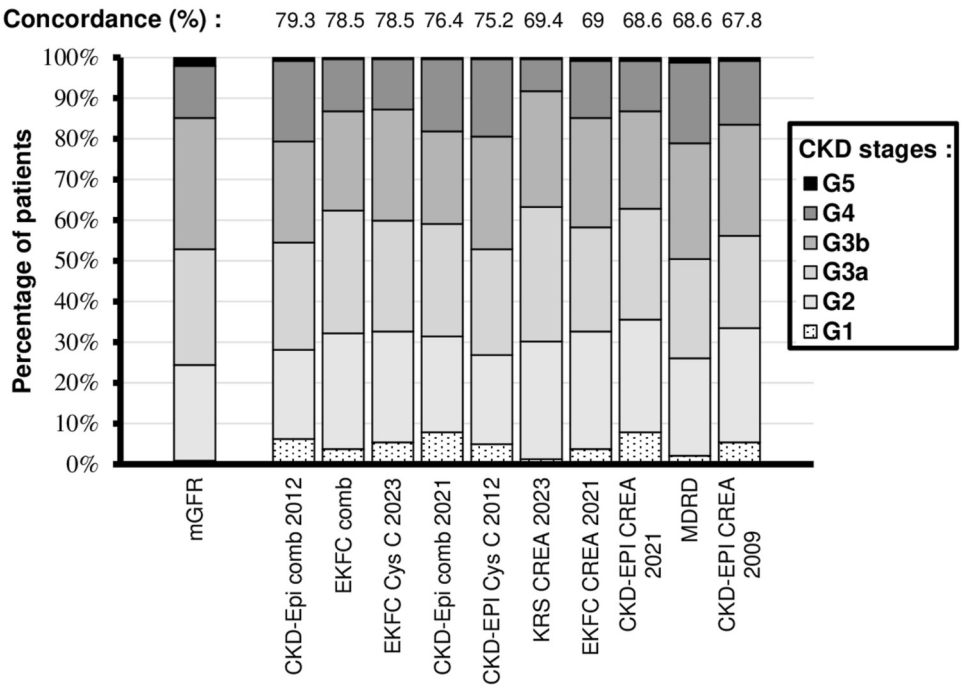


FIGURE 1 | Repartition of mGFR and eGFR values across the cohort. Percentage of concordance in CKD classification with mGFR is indicated for each eGFR equation.

RESULTS

Population, eGFR and mGFR

A total of 242 KTR patients (median [min-max] age: 53.2 [18.3–76.6] years, %female/male: 35/65, median ± Interquartile range PTF duration: 2.1 ± 5 years) with simultaneous mGFR, plasma creatinine and serum Cys C measurement were recruited (**Table 2**). Kolgomorov-Smirnov test showed that CREA, Cys C, mGFR and eGFRs values were normally distributed. Mean mGFR was at 51.4 ± 19.8 mL/min/1.73 m^2, CKD classifications based on it consisted mainly in G3a (N = 75, 31%) and G3b and G2 (N = 63, 26% for both) stages (**Figure 1**). Mean creatinine plasma level and Cys C serum level were 144.7 ± 62.3 μmol/L and 1.6 ± 0.6 mg/L respectively. Mean eGFRs ranged from 47.8 ± 19.3 (MDRD) to 54.2 ± 22.2 (CKD-EPI creatinine 2021) mL/min/1.73 m^2. A poor fit was observed between 24-h urinary creatinine (available for 97% of cases, 234/242 patients) and eGFRs based on creatinine or combined eGFRs (R^2 = 0.0005 to 0.015, P > 0.05 in every case). The relationship between immunosuppressive corticosteroid dosage (median ± Interquartile range: 5 ± 5 mg, 82% of patients treated) and eGFRs based on Cys C or combined eGFRs was limited in terms of explanatory power (R^2 = 0.004 to 0.01, P > 0.05 in every case).

Regression, Correlation, Bias and Concordance Between mGFR and eGFR

Regression and correlation between mGFR and the 10 tested equations are shown in **Figure 2** and values are summarized in **Table 3**. A statistically significant relationship with a slope test P-value <0.001 was found for all equations. Passing-Bablok regression yielded slopes values comprised between 0.8 and 0.96 for all equations except for KRS which was substantially lower (0.68). KRS equation presented also the highest intercept (16.2), while CKD-EPI Cys C and combined equations presented the lowest intercepts (2.2–2.5). Only three eGFRs (CKD-EPI Cys C, Comb 2012 and 2021) did not present a constant error compared to mGFR as 95% confidence intervals for the intercept included zero. On the other hand, only CKD-Epi creatinine 2021 and CKD-EPI Comb 2021 had a 95% confidence interval of the linear equation eGFR = a*mGFR + b slope (a) comprising one, indicating the absence of proportional error. R^2 coefficients were higher for combined equations (0.741–0.745) compared to Cys C (0.703–0.714) and creatinine ones (0.672–0.689). T-tests for Pearson correlation resulted in a statistically significant correlation (P < 0.001) between each eGFR and mGFR.

All equations presented a median bias (i.e., mGFR-eGFR) comprised in between ±2 mL/min/1.73 m^2 compared to mGFR except for MDRD median bias, underestimating mGFR by 4.2 mL/min/1.73 m^2 on average. Scatter of differences visualized on Bland-Altman plots (**Figure 3**) showed an equally positive and negative repartition, with a higher imprecision of eGFR when mGFR values are increasing.

Agreements measures are listed in **Table 3**. Overall, combined equations gave the best performances in terms of percentages of agreement in CKD classification, wκ index and P_{30} values followed by Cys C equations and then by creatinine equations.

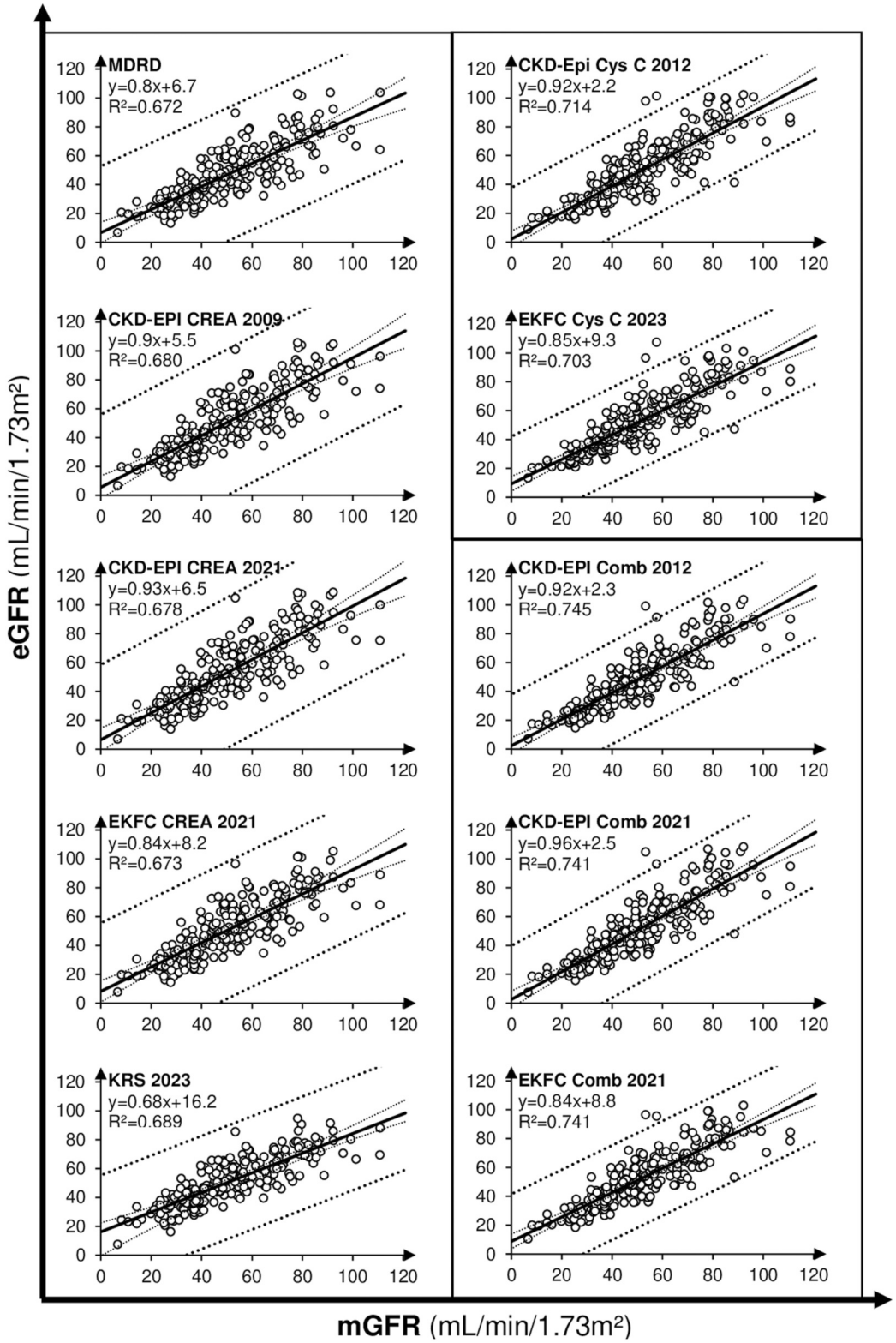


FIGURE 2 | Regression between mGFR (x axis, mL/min/1.73 m^2) and eGFRs (y axis, mL/min/1.73 m^2). Creatinine equation are presented in left, Cys C and Comb equations in right.

TABLE 3 | mGFR-eGFR equations correlation, regression and agreement measures with 95% confidence intervals. Passing-Bablok regression procedure fits the parameters *a* and *b* of the linear equation eGFR = *a**mGFR + *b*. CREA, creatinine; Cys C, cystatin C; comb, combination.

Equation	R^2	Slope (*a*)	Intercept (*b*)	Cohen wκ	Concordance in CKD classification (%)	Mean P30 (%)
MDRD 2006	0.672	0.80 [0.73, 0.87]	6.7 [2.8, 10.6]	0.566 [0.615, 0.517]	68.6 [62.7, 74.4]	78.1 [72.9, 83.3]
CKD-Epi CREA 2009	0.680	0.90 [0.82, 0.98]	5.5 [1.2, 9.8]	0.550 [0.600, 0.500]	67.8 [61.9, 73.7]	75.2 [69.8, 80.6]
CKD-Epi CREA 2021	0.678	0.93 [0.85, 1.01]	6.5 [2.0, 11.0]	0.546 [0.596, 0.496]	68.6 [62.7, 74.4]	75.6 [70.2, 81]
EKFC CREA 2021	0.673	0.84 [0.77, 0.92]	8.2 [4.1, 12.3]	0.552 [0.602, 0.502]	69 [63.2, 74.8]	78.5 [73.3, 83.7]
KRS 2023	0.698	0.68 [0.62, 0.74]	16.2 [13.0, 19.4]	0.515 [0.567, 0.463]	69.4 [63.6, 75.2]	81.4 [76.5, 86.3]
CKD-Epi Cys C 2012	0.714	0.92 [0.84, 0.99]	2.2 [−1.9, 6.2]	0.649 [0.696, 0.602]	75.2 [69.8, 80.6]	83.1 [78.3, 87.8]
EKFC Cys C 2023	0.703	0.85 [0.78, 0.92]	9.3 [5.4, 13.1]	0.594 [0.643, 0.545]	78.5 [73.3, 83.7]	84.3 [79.7, 88.9]
CKD-Epi comb 2012	0.745	0.92 [0.85, 0.98]	2.3 [−1.5, 6.1]	0.675 [0.720, 0.630]	76.4 [71.1, 81.8]	86 [81.6, 90.3]
CKD-Epi comb 2021	0.741	0.96 [0.89, 1.03]	2.5 [−1.4, 6.5]	0.712 [0.755, 0.669]	79.3 [74.2, 84.4]	85.5 [81.1, 90]
EKFC comb	0.741	0.84 [0.78, 0.91]	8.8 [5.3, 12.3]	0.686 [0.731, 0.641]	78.5 [73.3, 83.7]	87.6 [83.5, 91.8]

CKD-EPI comb 2021 had the highest percentage of agreement in CKD classification (79%) and highest wκ (0.712) indicating a substantial agreement. Substantial agreement was also noted for other combined equations and CKD-EPI cys 2012, whilst a moderate agreement was obtained for all other equations (wκ values from 0.515 to 0.594). P_{30} value was the highest for EKFC comb with 88%.

Using CKD-EPI comb 2021 as the reference eGFR equation, nine alternative eGFR equations were evaluated using CKD stages category-based NRI relative to mGFR (**Table 4**). A positive NRI was observed for 8 out of 9 equations (from 2.5 for EKFC Cys C to 12.4 for CKD-Epi CREA 2021), indicating a superior CKD classification ability of CKD-EPI comb 2021. A negative NRI was observed only for EKFC comb (−2.48).

P_{30} and Bias According to Age

Figure 4 shows the P_{30} and median bias according to age categories. Cys C and combined equations appeared to systematically underestimating mGFR for patients younger than 30 years, in contrast with patients aged 30–40 years where a systematic overestimation was observed. Creatinine equations presented more variable bias repartition, with CKD-EPI 2009 having the less important bias and MDRD the worst. In addition, a systematic underestimation of mGFR by all creatinine equations was noted in the patients aged over 70 years.

All equations presented a mean P_{30} greater than 75% for patient <70 years old which has been considered "sufficient for good clinical decision making" by the Kidney Disease Outcomes Quality Initiative [29]. However for older patients (>70 years-old) only EKFC Cys C and KRS creatinine remained around 80%, all other equations fell below 75%. Combined equations had the highest rates of P30 with some values greater than 90% which is the goal for optimal clinical decision making [29], except for patients aged above 70 years.

DISCUSSION

The present study was designed in order to determine the best biomarker/equation combination for eGFR determination in KTR settings. Our results clearly indicate that combined equations have better performances than Cys C and creatinine eGFR-based equations in terms of correlation and bias (**Figures 2–4**) with reference method and in terms of concordance performances for CKD classification (**Table 3**). CKD-Epi comb 2021 was notably the best performing equation when looking at Passing-Bablok regression (eGFR = *a**mGFR + *b* equation), being the only equation having slope (*a*) interval confidence including one (0.89–1.03) and intercept (*b*) confidence interval including zero (−1.4-6.5). Cys C is however not implemented in all laboratories yet and therefore the use of EKFC creatinine 2021 or CKD-EPI creatinine 2021 over CKD-EPI creatinine 2009 may be recommended because the "race factor" is not needed and the equations are simplest to calculate. Moreover, CKD-Epi comb 2021 showed a positive NRI of CKD classifications compared to most of the other equations with mGFR as a reference, only EKFC comb performing slightly better (**Table 4**).

Accurate GFR estimation is of critical importance for KTR patient, since eGFR trajectory changes can lead to important clinical decisions like immunosuppressive treatment management, dialysis reinstauration or reinscription in kidney transplantation list [1–5]. Gold standard measurement methods should be performed to have the most precise GFR determination, but cannot be routinely implemented in KTR patient follow-up [6]. Prompt GFR estimation with the help of endogenous markers and adequate equation is therefore advisable. KDIGO guidelines for KTR patient recommend a regular blood creatinine measurement for eGFR determination in PTF, at an increased rate the year after transplantation and every 2–3 months after the first year post-transplantation [1]. However, no precise eGFR formula is recommended in the guideline, and at the time of publication of the guideline (2009), only MDRD 2006 was available. Since then, multiplication of published equations has been observed (**Table 1**), and Cys C is being more widely used. eGFR based on blood Cys C assessment is notably included in the algorithm of eGFR initial assessment of the recent 2024 KDIGO guideline [6]. Numerous eGFR equations and cr/cys bias in the specific KTR population can lead to a substantial variability of eGFR results.

Interestingly, in our cohort CREA-based equations appeared to underestimate mGFR in older patients. A possible explanation could be the high prevalence of sarcopenia in this age category [10, 11]. However, no correlation was found between daily

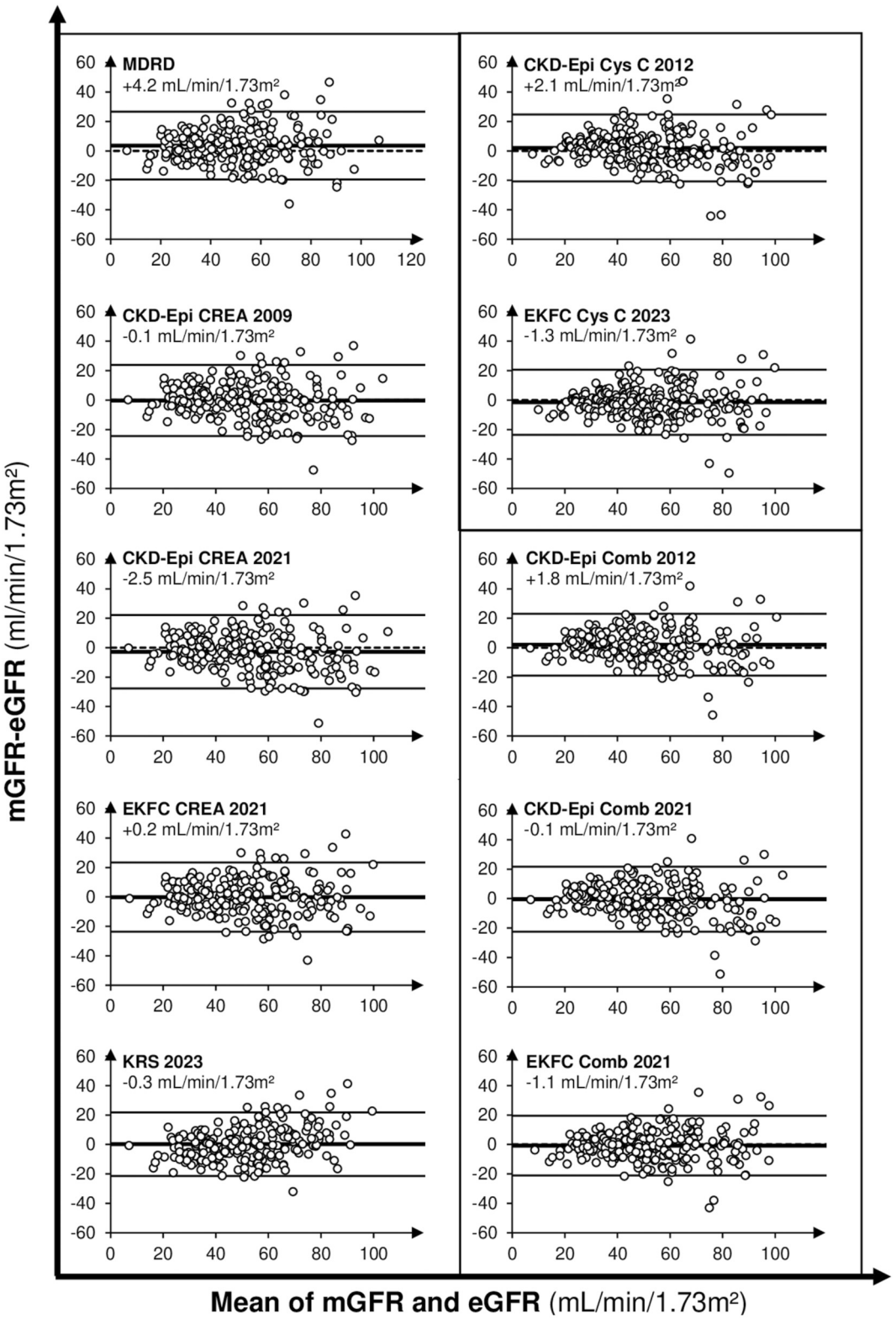


FIGURE 3 | Bland-Altman's plots between mGFR and eGFR. x axis represents mean of mGFR and eGFR (both in mL/min/1.73 m^2), y axis represents the difference between mGFR and eGFR. Creatinine equation are presented in left, Cys C and Comb equations in right. Median bias between mGFR and eGFR are indicated for each equation.

TABLE 4 | Net reclassification improvement (NRI) of eGFR CKD-Epi comb 2021 to correctly classify individuals into CKD stages compared to eGFR calculated with other equations with mGFR as the reference standard.

Equation	NRI
MDRD 2006	10.3
CKD-Epi CREA 2009	9.5
CKD-Epi CREA 2021	12.4
EKFC CREA 2021	10.7
KRS 2023	7.9
CKD-Epi Cys C 2012	6.6
EKFC Cys C 2023	2.5
CKD-Epi comb 2012	1.7
EKFC comb	–2.5

urinary excretion of creatinine and plasma creatinine or eGFR based on creatinine. On the counterpart, Cys C appeared to underestimate mGFR in younger patients aged under 30 and overestimate mGFR in patients aged between 30 and 40 years, without any clear explanation. Nonetheless, Cys C levels can be influenced notably by high corticosteroids dosages that can be encountered in transplantation for immunosuppression purposes [17, 18] but we did not find any correlation between Cys C or eGFR Cys C and corticosteroids dosages. Combined equations seem to limit those specific biomarker bias by taking into account another unbiased biomarker. Interestingly, a previous article demonstrated that a bias exists between eGFR based on Cys C and eGFR based on creatinine in KTR patient [30]. Moreover, it has been showed that GFR based on creatinine is more accurate than eGFR based on Cys C for determining associations with clinical outcomes due to low GFR when mGFR is not available [31]. This finding is consistent with the results published by Pottel and colleagues [22] where combined equations presented the best P_{30} values across ages in a general population of 12,832 patients. Also consistent with our results, Meeusun and colleagues showed as well that CKD-EPI comb 2012 performed better than CKD-EPI creatinine 2009 and CKD-EPI Cys C 2012 in a population of 568 KTR patients [32].

Regarding equation diversity, no equation seems to outperform another one in term of agreement when looking in each biomarker group. In term of absolute bias, no equation surpasses 3 mL/min/1.73 m^2 with the exception of MDRD 2006 equation with a median bias of +4.2 mL/min/1.73 m^2. This latter can lead to important errors and MDRD 2006 should therefore not be used in KTR patient.

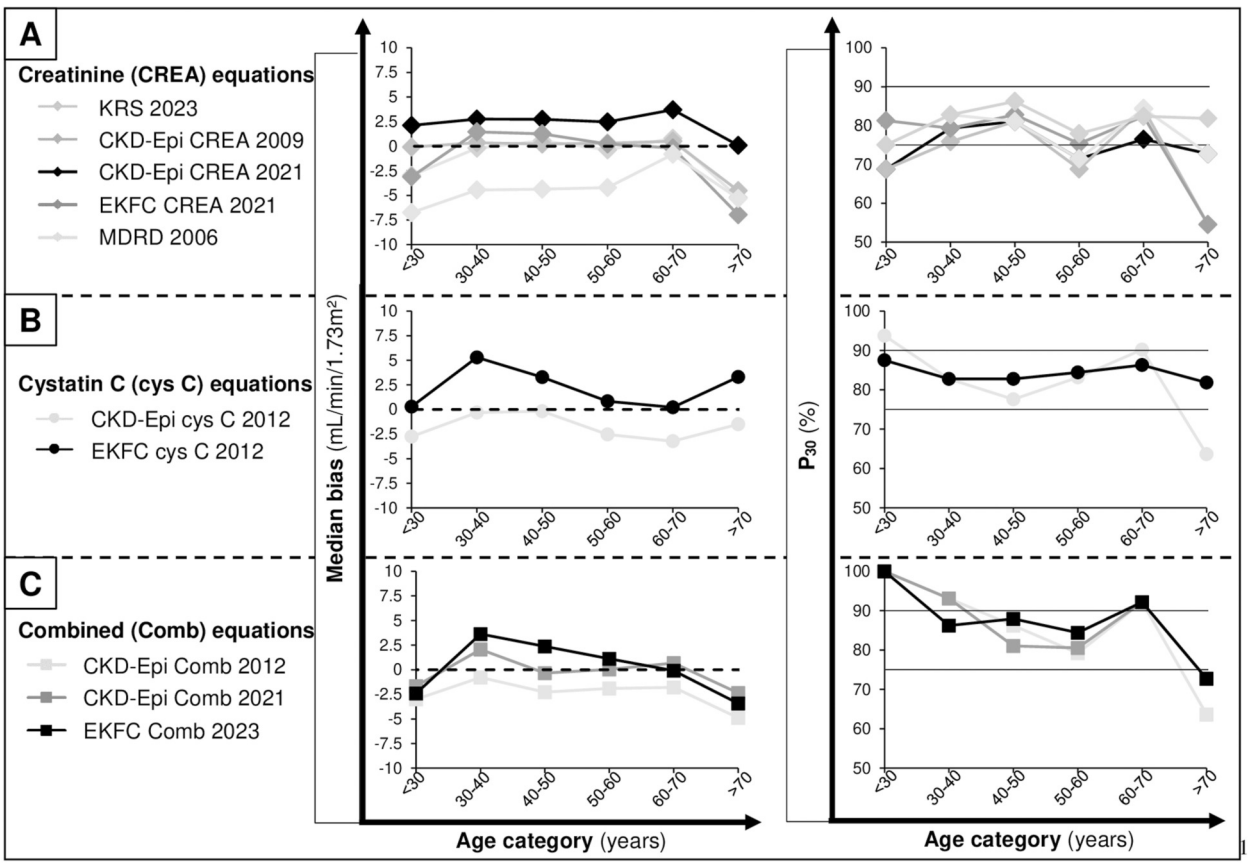


FIGURE 4 | Estimation equation performances in terms of median bias (left panel) and P_{30} (right panel) according to different ages categories. **(A)** Creatinine (CREA) equations performances. **(B)** Cystatin c (cys C) equations performances. **(C)** Combined (comb) equations.

In order to prevent some regional heterogeneity on creatinine level and eGFR estimation [25, 33], we tested the EKFC equations in our monocentric cohort. By contrast to previous reports [25], we did not observe a clear difference with the CKD-EPI equations. EKFC equation is being constructed with a Q-rescaling factor representing concentration of serum creatinine or Cys C in healthy males and females. Population-specific Q-rescaling factor have been proposed [34] providing remarkable performances in those populations. A specific Q-rescaling factor for KTR patients is maybe necessary to improve EKFC performances in this specific context.

Moreover, the KRS equation developed for KTR patient was not superior to other CREA-based equations. The overall P_{30} (81%) of KRS equation was lower of the overall P_{30} (90%) obtained in the development cohort [23]. Additionally, this equation had much worst Passing-Bablok regression results (eGFR = 0.68*mGFR+16.2) compared to all other equations, which could compromise his use.

We acknowledge that this study suffers from limitations: a limited patient number, a monocentric study setting and a variability of creatinine and Cys C employed assays. Nevertheless, all creatinine assays were based on a standardized enzymatic method as recommended by guidelines [35]. Regarding Cys C, measurements were realized before the establishment of ERM-DA471/IFCC reference material that have proven to improve performances of Cys c assays [36]. However, switch ability between the two PENIA and PETIA assays used in the present study were tested in a previous study [28]. Both assays yielded very close results on repeated measurements of five pooled serum samples with cystatin C values ranging from 0.6 to 1.4 mg/L. Furthermore, the Siemens PENIA assay was tested and showed good correlation with both IDMS reference method [37] and with many other PETIA assays [15, 38, 39]. The use of urinary clearance of ^{99m}Tc-DTPA method for mGFR determination is also a limitation because eGFR equations are generally build on other mGFR determination methods such as iotholamate for CKD-Epi and inulin, iohexol clearance and radionuclide clearances including 99mTc-DTPA for EKFC. Comparability between mGFR methods is often not observed, and steps towards standardization are needed [40, 41].

In conclusion, the conjoint use of creatinine and Cys C with combined equations in KTR patient follow-up offers the best performance in estimating GFR in our experience. Further studies on larger cohorts need to be conducted to confirm these results.

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 ethics committee of the Montpellier university hospital. 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

EM: Writing-original draft, Data curation. A-SB: Writing-review and editing, Data curation. IS: Writing-review and editing, Data curation, Investigation. ML: Writing-review and editing, Investigation. GM: Writing-review and editing, Investigation. J-PC: Writing-review and editing, conceptualization, supervision. All authors contributed to the article and approved the submitted version.

FUNDING

The author(s) declared that financial support was not received for this work and/or its publication.

CONFLICT OF INTEREST

The author(s) declared that this work 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) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

REFERENCES

1. Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group. KDIGO Clinical Practice Guideline for the Care of Kidney Transplant Recipients. *Am J Transpl* (2009) 9(Suppl. 3):S1–155. doi:10.1111/j.1600-6143.2009.02834.x
2. Raynaud M, Aubert O, Reese PP, Bouatou Y, Naesens M, Kamar N, et al. Trajectories of Glomerular Filtration Rate and Progression to End Stage Kidney Disease After Kidney Transplantation. *Kidney Int* (2021) 99(1):186–97. doi:10.1016/j.kint.2020.07.025
3. Magott-Procelewska M, Boratynska M, Janczak D, Chudoba P, Patrzałek D, Biecek P, et al. Estimated Glomerular Filtration Rate Evolution Between 6 and 24 Months Predicts Long-Term Kidney Transplant Survival Among Patients

with Inferior Graft Function. *Transpl Proc* (2009) 41(8):3028–32. doi:10.1016/j.transproceed.2009.07.105
4. Park JS, Oh IH, Lee CH, Kim GH, Kang CM. The Rate of Decline of Glomerular Filtration Rate Is a Predictor of Long-Term Graft Outcome After Kidney Transplantation. *Transpl Proc* (2013) 45(4):1438–41. doi:10.1016/j.transproceed.2012.10.052
5. Irish W, Nickerson P, Astor BC, Chong E, Wiebe C, Moreso F, et al. Change in Estimated GFR and Risk of Allograft Failure in Patients Diagnosed with Late Active Antibody-Mediated Rejection Following Kidney Transplantation. *Transplantation* (2021) 105(3):648–59. doi:10.1097/TP.0000000000003274
6. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2024 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. *Kidney Int* (2024) 105(4S):S117–S314. doi:10.1016/j.kint.2023.10.018
7. Mondesert E, Bargnoux AS, Portet F, Laoudj-Chenivesse D, Arbogast S, Badiou S, et al. Cystatin C for Kidney Function Assessment in Patients with Facioscapulohumeral Muscular Dystrophy. *Clin Chim Acta* (2023) 544:117328. doi:10.1016/j.cca.2023.117328
8. Mondesert E, Reynes J, Makinson A, Bargnoux AS, Plawecki M, Morquin D, et al. Cystatin C in Addition to Creatinine for Better Assessment of Glomerular Renal Function Decline in People with HIV Receiving Antiretroviral Therapy. *AIDS* (2023) 37(3):447–54. doi:10.1097/QAD.0000000000003434
9. Zhang X, Rule AD, McCulloch CE, Lieske JC, Ku E, Hsu CY. Tubular Secretion of Creatinine and Kidney Function: An Observational Study. *BMC Nephrol* (2020) 21(1):108. doi:10.1186/s12882-020-01736-6
10. Souweine JS, Pasquier G, Kuster N, Rodriguez A, Patrier L, Morena M, et al. Dynapaenia and Sarcopaenia in Chronic Haemodialysis Patients: Do Muscle Weakness and Atrophy Similarly Influence Poor Outcome? *Nephrol Dial Transpl* (2021) 36(10):1908–18. doi:10.1093/ndt/gfaa353
11. Sabatino A, Cuppari L, Stenvinkel P, Lindholm B, Avesani CM. Sarcopenia in Chronic Kidney Disease: What Have We Learned so Far? *J Nephrol* (2021) 34(4):1347–72. doi:10.1007/s40620-020-00840-y
12. Gaillard F, Ould Rabah M, Garcelon N, Touam M, Neuraz A, Legendre C, et al. Allograft Function and Muscle Mass Evolution After Kidney Transplantation. *J Cachexia Sarcopenia Muscle* (2022) 13(6):2875–87. doi:10.1002/jcsm.13066
13. Levey AS, de Jong PE, Coresh J, El Nahas M, Astor BC, Matsushita K, et al. The Definition, Classification, and Prognosis of Chronic Kidney Disease: A KDIGO Controversies Conference Report. *Kidney Int* (2011) 80(1):17–28. doi:10.1038/ki.2010.483
14. Jung E, Ro YS, Ryu HH, Kong SY, Shin SD, Hwang SO. Cystatin C and Mortality Risk in the General Population: Systematic Review and Dose Response Meta-Analysis. *Biomarkers* (2022) 27(3):222–9. doi:10.1080/1354750X.2021.1989489
15. Bargnoux AS, Cavalier E, Cristol JP, Simon N, Dupuy AM, Garrigue V, et al. Cystatin C Is a Reliable Marker for Estimation of Glomerular Filtration Rate in Renal Transplantation: Validation of a New Turbidimetric Assay Using Monospecific Sheep Antibodies. *Clin Chem Lab Med* (2011) 49(2):265–70. doi:10.1515/CCLM.2011.043
16. Foster MC, Weiner DE, Bostom AG, Carpenter MA, Inker LA, Jarolim P, et al. Filtration Markers, Cardiovascular Disease, Mortality, and Kidney Outcomes in Stable Kidney Transplant Recipients: The FAVORIT Trial. *Am J Transpl* (2017) 17(9):2390–9. doi:10.1111/ajt.14258
17. Risch L, Herklotz R, Blumberg A, Huber AR. Effects of Glucocorticoid Immunosuppression on Serum Cystatin C Concentrations in Renal Transplant Patients. *Clin Chem* (2001) 47(11):2055–9. doi:10.1093/clinchem/47.11.2055
18. Tsushita H, Tanaka R, Suzuki Y, Sato Y, Itoh H. Effects of Dose and Type of Corticosteroids on the Divergence Between Estimated Glomerular Filtration Rates Derived from Cystatin C and Creatinine. *J Clin Pharm Ther* (2020) 45(6):1390–7. doi:10.1111/jcpt.13235
19. Levey AS, Coresh J, Greene T, Stevens LA, Zhang YL, Hendriksen S, et al. Chronic Kidney Disease Epidemiology Collaboration. Using Standardized Serum Creatinine Values in the Modification of Diet in Renal Disease Study Equation for Estimating Glomerular Filtration Rate. *Ann Intern Med* (2006) 145(4):247–54. doi:10.7326/L21-0010
20. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, Feldman HI, et al. A New Equation to Estimate Glomerular Filtration Rate. *Ann Intern Med* (2009) 150(9):604–12. doi:10.7326/0003-4819-150-9-200905050-00006
21. Inker LA, Eneanya ND, Coresh J, Tighiouart H, Wang D, Sang Y, et al. New Creatinine- and Cystatin C-Based Equations to Estimate GFR Without Race. *N Engl J Med* (2021) 385(19):1737–49. doi:10.1056/NEJMoa2102953
22. Pottel H, Björk J, Courbebaisse M, Couzi L, Ebert N, Eriksen BO, et al. Development and Validation of a Modified Full Age Spectrum Creatinine-Based Equation to Estimate Glomerular Filtration Rate: A Cross-Sectional Analysis of Pooled Data. *Ann Intern Med* (2021) 174(2):183–91. doi:10.7326/M20-4366
23. Raynaud M, Al-Awadhi S, Juric I, Divard G, Lombardi Y, Basic-Jukic N, et al. Race-Free Estimated Glomerular Filtration Rate Equation in Kidney Transplant Recipients: Development and Validation Study. *BMJ* (2023) 381:e073654. doi:10.1136/bmj-2022-073654
24. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, et al. Estimating Glomerular Filtration Rate from Serum Creatinine and Cystatin C. *N Engl J Med* (2012) 367(1):20–9. doi:10.1056/NEJMoa1114248
25. Pottel H, Björk J, Rule AD, Ebert N, Eriksen BO, Dubourg L, et al. Cystatin C-Based Equation to Estimate GFR Without the Inclusion of Race and Sex. *N Engl J Med* (2023) 388(4):333–43. doi:10.1056/NEJMoa2203769
26. Vetromile F, Szwarc I, Garrigue V, Delmas S, Fesler P, Mimran A, et al. Early High Pulse Pressure Is Associated with Graft Dysfunction and Predicts Poor Kidney Allograft Survival. *Transplantation* (2009) 88(9):1088–94. doi:10.1097/TP.0b013e3181ba1585
27. Du Bois D, Du Bois EF. Clinical Calorimetry: Tenth Paper a Formula to Estimate the Approximate Surface Area if Height and Weight Be Known. *Arch Intern Med (Chic)* (1916) XVII(6_2):863–71. doi:10.1001/archinte.1916.00080130010002
28. Bargnoux AS, Servel AC, Piéroni L, Dupuy AM, Badiou S, Garrigue V, et al. SFBC Biology of Renal Function and Renal Failure Working Group. Accuracy of GFR Predictive Equations in Renal Transplantation: Validation of a New Turbidimetric Cystatin C Assay on Architect c8000. *Clin Biochem* (2012) 45(1-2):151–3. doi:10.1016/j.clinbiochem.2011.10.018
29. National Kidney Foundation. K/DOQI Clinical Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification, and Stratification. *Am J Kidney Dis* (2002) 39(2 Suppl. 1):S1–266.
30. Bohn MK, Asare-Werehene M, Leung F, Brinc D, Selvaratnam R. Discordance Between Creatinine and Cystatin C-Based Estimation of Glomerular Filtration Rate (eGFR) in Solid Organ Transplant Recipients. *Clin Biochem* (2025) 135:110853. doi:10.1016/j.clinbiochem.2024.110853
31. Keddis MT, Howard MR, Galapia L, Barreto EF, Zhang N, Butterfield RJ, et al. GFR Estimated with Creatinine Rather than Cystatin C Is More Reflective of the True Risk of Adverse Outcomes with Low GFR in Kidney Transplant Recipients. *Nephrol Dial Transpl* (2023) 38(8):1898–906. doi:10.1093/ndt/gfad007
32. Meeusen JW, Rule AD, Voskoboev N, Baumann NA, Lieske JC. Performance of Cystatin C- and Creatinine-Based Estimated Glomerular Filtration Rate Equations Depends on Patient Characteristics. *Clin Chem* (2015) 61(10):1265–72. doi:10.1373/clinchem.2015.243030
33. Delanaye P, Pottel H, Glassock RJ. Americentrism in Estimation of Glomerular Filtration Rate Equations. *Kidney Int* (2022) 101(5):856–8. doi:10.1016/j.kint.2022.02.022
34. Delanaye P, Vidal-Petiot E, Björk J, Ebert N, Eriksen BO, Dubourg L, et al. Performance of Creatinine-Based Equations to Estimate Glomerular Filtration Rate in White and Black Populations in Europe, Brazil and Africa. *Nephrol Dial Transpl* (2023) 38(1):106–18. doi:10.1093/ndt/gfac241
35. Pottel H, Cavalier E, Björk J, Nyman U, Grubb A, Ebert N, et al. Standardization of Serum Creatinine Is Essential for Accurate Use of Unbiased Estimated GFR Equations: Evidence from Three Cohorts Matched on Renal Function. *Clin Kidney J* (2022) 15(12):2258–65. doi:10.1093/ckj/sfac182
36. Karger AB, Long T, Inker LA, Eckfeldt JH, College of American Pathologists Accuracy Based Committee and Chemistry Resource Committee. Improved Performance in Measurement of Serum Cystatin C by Laboratories Participating in the College of American Pathologists 2019 CYS Survey. *Arch Pathol Lab Med* (2022) 146(10):1218–23. doi:10.5858/arpa.2021-0306-CP
37. Bargnoux AS, Piéroni L, Cristol JP, Kuster N, Delanaye P, Carlier MC, et al. Multicenter Evaluation of Cystatin C Measurement After Assay Standardization. *Clin Chem* (2017) 63(4):833–41. doi:10.1373/clinchem.2016.264325

38. Bargnoux AS, Perrin M, Garrigue V, Badiou S, Dupuy AM, Mourad G, et al. Analytical Performances of Cystatin C Turbidimetric Assay: Which Impact on Accuracy of Glomerular Filtration Rate Estimation in Renal Transplantation? *Clin Chem Lab Med* (2011) 50(1):133–8. doi:10.1515/CCLM.2011.725
39. Obiols J, Bargnoux AS, Kuster N, Fesler P, Piéroni L, Badiou S, et al. Validation of a New Standardized Cystatin C Turbidimetric Assay: Evaluation of the Three Novel CKD-EPI Equations in Hypertensive Patients. *Clin Biochem* (2013) 46(15):1542–7. doi:10.1016/j.clinbiochem.2013.05.056
40. White CA, Akbari A, Allen C, Day AG, Norman PA, Holland D, et al. Simultaneous Glomerular Filtration Rate Determination Using Inulin, Iohexol, and 99mTc-DTPA Demonstrates the Need for Customized Measurement Protocols. *Kidney Int* (2021) 99(4):957–66. doi:10.1016/j.kint.2020.06.044
41. Ebert N, Schaeffner E, Seegmiller JC, van Londen M, Bökenkamp A, Cavalier E, et al. Iohexol Plasma Clearance Measurement Protocol Standardization for Adults: A Consensus Paper of the European Kidney Function Consortium. *Kidney Int* (2024) 106(4):583–96. doi:10.1016/j.kint.2024.06.029

ESOT | Transplant International

ORIGINAL RESEARCH
published: 25 February 2026
doi: 10.3389/ti.2026.15472

The Multidisciplinary Support To Access living donor Kidney Transplant (MuST AKT) intervention: A Pilot Randomized Controlled Trial

Anne-Marie Selzler[1], Parastoo Molla Davoodi[2], Scott Klarenbach[1,3], Ngan N. Lam[4], Terry Smith[1], Abigail Ackroyd[3], Ben Vandermeer[3], Bonnie Corradetti[1], Aman Dhaliwal[1], Sharron Ferdinand[5], Dorothy Ikekekpolor[6], Gordon Smith[5], Aminu K. Bello[3], Kevin Wen[7] and Soroush Shojai[3] on behalf of the Multidisciplinary Support To Access living donor Kidney Transplant (MuST AKT) Research Group*

[1]Medicine Strategic Clinical Network, Alberta Health Services, AB, Canada, [2]Adult Cystic Fibrosis, Kaye Clinic, Alberta Health Services, Edmonton, AB, Canada, [3]Department of Medicine, University of Alberta, Edmonton, AB, Canada, [4]Department of Medicine, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada, [5]Alberta Kidney Care North, Alberta Health Services, Edmonton, AB, Canada, [6]Transplant Services, University of Alberta Hospital, Alberta Health Services, Edmonton, AB, Canada, [7]Department of Medicine, University of British Columbia, Vancouver, BC, Canada

We evaluated the feasibility and acceptability of the Multidisciplinary Support To Access living donor Kidney Transplant (MuST AKT) intervention, developed to increase living donor kidney transplantation (LDKT). In this pilot randomized controlled trial, we randomly assigned transplant candidates to receive standard care or the MuST AKT intervention, where transplant candidates and their social network addressed barriers to LDKT across four 60–90-minute sessions. Feasibility was assessed by consent/recruitment, retention, study protocol adherence, intervention adherence, and intervention engagement. Acceptability was assessed by questionnaire and post-intervention interviews. The recruitment rate was 61% (43/71), with 38 participants randomized 1:1. Among intervention participants, 1 was excluded for not meeting study criteria prior to start. Among those that started (18), 100% completed 1 session, 94% completed 2 sessions, 83% completed 3 sessions, and 56% completed all 4 sessions. The intervention was delivered in 71 days (mean), shorter than anticipated. The intervention participants reported increased confidence for communicating about LDKT from pre-to post-intervention, and all recommended MuST AKT to their peers. Intervention participants and invitees from their social network described a positive experience and provided recommendations for improvement. The MuST AKT intervention is feasible with minor modifications and acceptable to transplant candidates and their social network.

Keywords: kidney transplant, living kidney donation, pilot study, randomized controlled trial, transplant

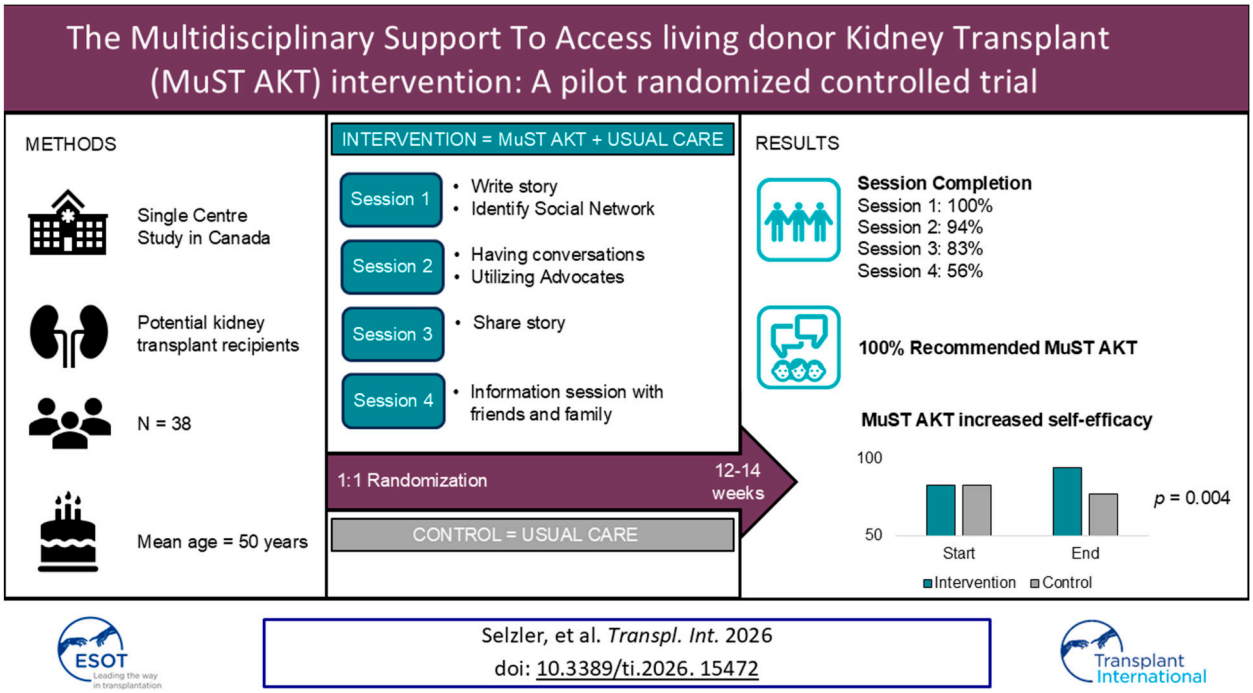
OPEN ACCESS

***Correspondence**
Soroush Shojai,
shojai@ualberta.ca

Received: 22 August 2025
Revised: 16 January 2026
Accepted: 19 January 2026
Published: 25 February 2026

Citation:
Selzler A-M, Davoodi PM, Klarenbach S, Lam NN, Smith T, Ackroyd A, Vandermeer B, Corradetti B, Dhaliwal A, Ferdinand S, Ikekekpolor D, Smith G, Bello AK, Wen K and Shojai S (2026) The Multidisciplinary Support To Access living donor Kidney Transplant (MuST AKT) intervention: A Pilot Randomized Controlled Trial. Transpl. Int. 39:15472. doi: 10.3389/ti.2026.15472

Abbreviations: COM-B, capability, opportunity, motivation, and behavior model; LDKT, living donor kidney transplantation; MuST AKT, Multidisciplinary Support To Access living donor Kidney Transplant; RCT, randomized controlled trial.



GRAPHICAL ABSTRACT |

INTRODUCTION

Compared with deceased donor kidney transplantation, living donor kidney transplant (LDKT) provides longer patient and graft survival [1] and shorter wait-time to transplantation [2]. Pre-emptive LDKT can prevent the need for dialysis and reduce the shortage of organs (kidney) for transplantation. However, LDKT is underutilized and many potential transplant recipients report being hesitant to approach potential living kidney donors due to fear, guilt, lack of knowledge, and lack of confidence to communicate effectively about living kidney donation [3–9].

Several interventions have been developed to increase LDKT. The most effective interventions are individually and culturally tailored, and designed to increase knowledge about LDKT and facilitate communication between potential recipients and their social network [10–13]. These interventions have led to increased potential donor evaluations [12, 14–17]. In a scoping review, similar interventions have been found to increase the number of potential donors contacting transplant programs by 40%–50%, the number of potential donors evaluated by 25%–47%, and the number of LDKT by 34% [18].

To address patient barriers to LDKT in our healthcare jurisdiction, we collaboratively developed the Multidisciplinary Support to Access living donor Kidney Transplant (MuST AKT) intervention [19] with patients, healthcare providers, and administrators, with the long-term goal of increasing LDKTs. Similar to previous successful interventions, MuST AKT targets transplant candidates and their social network to address knowledge and communication barriers to LDKT. In our design of MuST AKT, we applied the COM-B model [20] of behavior change to address barriers to LDKT and specifically enhance transplant candidates' motivation (M) and capability (C) to communicate about LDKT and support them with the creation of opportunities (O) for communication. Consistent with recommendations for developing, evaluating, and implementing complex interventions [21, 22], we first conducted a pilot study with the objective of assessing the feasibility and acceptability of the MuST AKT intervention.

MATERIALS AND METHODS

Study Design and Setting

We conducted a pilot parallel RCT with a nested qualitative study following qualitative description methodology [23, 24]. In this study we adopted a pragmatic worldview, acknowledging multiple realities (ontology) and valuing diverse approaches to knowledge (epistemology) to understand and evaluate a program to support people with their personal search for a living kidney donor. This study was conducted at a single regional academic transplant referral center with a catchment area population of >2 million. Participants were randomly assigned 1:1 to either the experimental (MuST AKT) or control (standard care) arm. Institutional review board approval was obtained

from the University of Alberta Health Research Ethics Board–Health Panel and Northern Alberta Clinical Trials and Research Centre (Pro00097902). The trial was registered with ClinicalTrials.gov (NCT04666545) and the CONSORT extension for pilot and feasibility trials [25] was used for reporting. Study enrollment occurred between May 2021 and February 2022. A comprehensive description of the MuST AKT intervention and study protocol is published elsewhere [19].

Participant Recruitment and Procedures

Patient Participants

Participants were recruited from the standard care 'Introduction to Kidney Transplant' education class or transplant waitlist at the University of Alberta Hospital. The inclusion criteria were referred or approved for kidney transplantation, aged between 18 and 75 years old, and English-speaking. The exclusion criteria were having a potential living kidney donor who contacted the living donor program, previously received a solid organ transplant, a candidate for a multi-organ transplant, scored <19 on the Rapid Estimate of Adult Literacy in Medicine (REALM-66) – indicating illiteracy in English [26] or scored >20 on the Stanford Integrated Psychosocial Assessment for Transplant (SIPAT) – indicating less than a good candidate for transplant [27]. The SIPAT is a standard assessment tool used at our transplant center and was conducted to support the inclusion of patients referred for transplant who were likely to be approved. No patients already approved for transplant were excluded by the SIPAT. A social worker conducted the REALM-66 and SIPAT using a virtual communications platform. The project manager obtained written informed consent electronically via the REDCap database [28]. Those who declined to participate were invited to take part in an interview to expand on their reasons for declining.

Subsequently, participants completed a baseline questionnaire (socio-demographics and self-efficacy) over the telephone with the project manager, who then randomized participants in the REDCap database [28] using predetermined randomly generated permuted blocks of 4 and 6 created by a statistician in Stata/MP 17.0 [29]. All participants completed a post-study questionnaire (acceptability and self-efficacy) after their intervention or time-control (12–14 weeks following enrollment), as appropriate. Participants in the experimental (MuST AKT) arm were invited to participate in a post-intervention interview.

Invitees of Patient Participants

All English-speaking individuals who attended the final MuST AKT intervention session at the invitation of the patient participant were invited to participate in a qualitative interview after the session. An independent qualitative researcher obtained written informed consent electronically via the REDCap database [28].

Interventions

Participants in the experimental arm received the MuST AKT intervention plus standard of care, whereas participants in the control arm received standard of care only.

Multidiscipinary Support To Access living donor Kidney Transplant (MuST AKT)

MuST AKT is a person-centered evidence-informed intervention designed to address barriers to LDKT, including lack of knowledge about LDKT and the process for potential donors, difficulty communicating about LDKT, and lack of social support, which were previously identified by transplant candidates in our healthcare jurisdiction [30]. The intervention was co-designed with patients and family advisors with lived experience of chronic kidney disease and kidney transplant, nephrologists and healthcare providers with expertise in kidney transplant, behavioural scientists, and social media experts from the Kidney Foundation of Canada–Alberta branches. Intervention strategies outlined in the COM-B model [20], including education (e.g., providing information, including how to use social media safely and appropriately), training (e.g., activities, including identifying donors and advocates), enablement (e.g., solution-focused interviewing to identify and overcome barriers to having conversations about LDKT), and modelling (e.g., role-playing on how to initiate and sustain conservations about LDKT) were applied to address these barriers. The sessions included structured activities and flexible time to support patients with identifying and overcoming their own personal barriers to LDKT. A complete description of the MuST AKT intervention has been published elsewhere [19]. In short, the MuST AKT intervention consists of an introductory session followed by 4 intervention sessions delivered over 12–14 weeks (approximately 1 session every 3 weeks), each ranging from 60–90 minutes in length, to identify and address barriers to LDKT. **Figure 1** outlines the main topics of each intervention session. All sessions were one-on-one with a behavioral scientist or a social worker, with the exception of the final session, in which the transplant candidate invited individuals from their social network to attend an information session on kidney disease and living kidney donation. This session also included an opportunity for participants to share their kidney disease and LDKT journey with invitees, and a question-and-answer period for which a transplant nephrologist was in attendance. The intervention was designed to be delivered in-person or virtually. Due to the COVID-19 pandemic, all sessions were conducted virtually.

Participants received reminder phone calls or emails 1–2 days prior to their scheduled sessions to promote session attendance. At their own request, participants could discontinue their participation in the intervention or withdraw from the study (i.e., no further data collection). Participants were withdrawn from the study if they did not respond to the study team after 3 contact attempts (as per HREB guidelines). Reasons for discontinuation and withdrawal were tracked.

Standard of Care

At our kidney transplant program, standard of care includes a standardized virtual group transplant education class (2 hours), arrangement of the required tests and consultations for transplant evaluation, and medical assessment for transplant suitability by a social worker, transplant nephrologist, and surgeon. Prior to the virtual education class, patients were provided with resources on

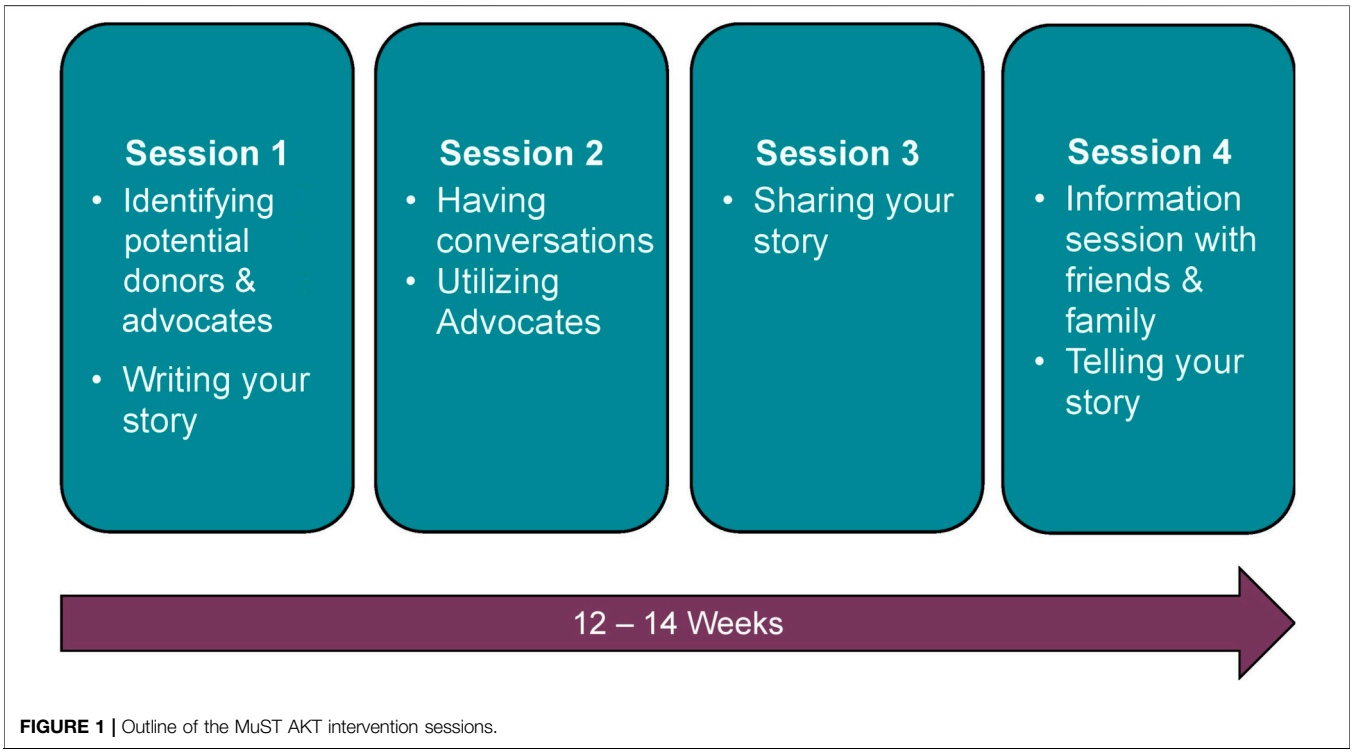


FIGURE 1 | Outline of the MuST AKT intervention sessions.

TABLE 1 | Feasibility and acceptability (questionnaire) outcomes.

Outcome	Details
Consent/recruitment	Consent rate ($n^{consented}/n^{approached}$) for patient participants and invitees of session 4 was tracked by consent logs, with reasons identified by structured survey
Retention	The number of patient participants that withdrew from the study and the number that were discontinued (session stoppage) were tracked by logs, with reasons identified by structured interview/survey
Adherence to study protocol (fidelity)	The duration of the intervention (from session 1–4) was tracked by logs of session attendance dates
Adherence to intervention	Attendance rate ($n^{attended}/n^{allocated}$ to MuST AKT) for each session, and the rate of sessions rescheduled combined across all 4 sessions ($n^{sessions\ rescheduled}/n^{total\ possible\ sessions}$) were tracked by attendance logs. Reasons for rescheduling sessions were identified by structured surveys
Engagement in intervention	Intervention engagement was measured by logs tracking completion of each step/component of the MuST AKT intervention (fidelity), the number of participants who wrote their stories at the end of MuST AKT session 1, the number of potential donors and advocates identified during session 1 of the MuST AKT intervention, the number of participants who found at least one potential advocate at the end of the MuST AKT intervention (12–14 weeks), and the number of participants who started conversation with at least one potential donor by the end of the last MuST AKT session
Acceptability (questionnaire)	A post-study questionnaire was conducted with patient participants 1–2 weeks after completing the MuST AKT intervention or time control. 'Recommendation of MuST AKT to others' and 'perception of intervention effectiveness' were measured using likert scales from 1 (completely disagree) to 7 (completely agree); and 'self-efficacy for finding a living kidney donor' and 'self-efficacy for communicating about LDKT' were measured on a scale from 0 (not at all confident) to 100 (completely confident). Self-efficacy was also assessed prior to randomization. Self-efficacy was assessed by items designed specifically for this study. These items were developed to assess the specific types of self-efficacy we were trying to impact with our intervention (i.e., confidence for communicating about LDKT, and confidence for finding a living kidney donor). Importantly, these items adhere to the theoretical and measurement guidelines outlined by Bandura [31].

how to use the virtual communication platform and one-on-one technical support to access the class. The transplant education is delivered by a nurse coordinator with topics including benefits and risks of kidney transplantation, living kidney donation, the kidney paired donation program, deceased kidney donation, evaluation and waitlist procedures, transplant surgery, post-transplant medicines and recovery. Additionally, patients were given a link to a kidney transplant educational website (https://myhealth.alberta.ca/KidneyTransplant) and access to the teaching materials. These resources were provided to all

patients prior to their participation in the study, with no additional internal resources provided as part of standard care during the study period. During their transplant work-up, patients are supported by a transplant nephrologist, nurse coordinator, and social worker.

Outcomes

Our joint primary outcomes were feasibility and acceptability, with feasibility assessed by consent/recruitment, retention, adherence to study protocol (fidelity), adherence to intervention, and engagement in intervention. Acceptability was assessed by questionnaire and interview (**Table 1**; **Supplementary Material** for guides and questions). A secured REDCap database [28] was used to collect and store survey and study process data. NVivo-12 software was used to organize and store qualitative data [32].

Acceptability (Interview)

The interview methodology was guided by qualitative description [24], where language is viewed as a vehicle of communication rather than an interpretive structure to be deciphered. In this approach the researcher seeks to present an accurate account of experiences using everyday language [24]. The interview guide was informed by the COM-B model [20] and written in everyday language to be consistent with qualitative description methodology. Semi-structured interviews were conducted over the telephone or virtual communication platform with (i) participants allocated to receive the MuST AKT intervention and (ii) their invitees who attended session 4 of the MuST AKT intervention. The purpose of the interviews was to explore their perspectives of the intervention sessions attended (including content), and to receive recommendations for improvement of the intervention. The interviewers were not healthcare professionals, had no pre-existing relationship with the participants, and were independent of the study team. The interviews were digitally recorded and transcribed clean verbatim. MuST AKT participant interviews ranged from 25–55 min and the invitee interviews ranged from 15–30 min.

Demographics, Social, and Clinical Characteristics

Participant age, gender, ethnicity, education, employment status, income, household status, dialysis (yes/no), duration of dialysis, and cause of kidney disease were collected from electronic medical databases or structured survey.

Changes to Trial Assessments

The following efficacy outcomes were collected in monthly reports and validated by comparing reports to individual electronic charts: proportion of participants with at least 1 potential donor who (i) contacts living donor services, (ii) starts evaluation, and (iii) completes evaluation; along with (iv) the proportion who receive a LDKT. After trial commencement but before completion, we decided to postpone the analysis of efficacy outcomes in order to pool the pilot data with the subsequent larger definitive RCT (funding secured during pilot for definitive RCT with power more favorable with pooling). The timeframe for collecting LDKTs was changed from 12 to 24 months in the pilot study to match the definitive RCT timeframe (clinicaltrials.gov: NCT04666545).

We planned to conduct interviews with people who declined to participate in the study to better understand their reasons for declining; however, no one consented to an interview.

Sample Size

Prior to the decision to postpone the analysis of the efficacy outcomes in this pilot study and pool the data with the definitive RCT data, we chose a sample size of 38 participants (19 per group), which is consistent with recommendations for pilot studies [33, 34]. Sample size calculations for the efficacy data are described elsewhere [19].

Blinding

It was not possible to blind the participants or the research team as to which intervention the participants received, although intervention allocation was not revealed until the participant was enrolled in the study and completed the baseline questionnaire. The quantitative and qualitative data analyses were conducted by a statistician and a qualitative research team, respectively, not involved in the conduct of the study.

Statistical Method

Quantitative Analysis

All analyses were conducted in Stata/MP 17.0 (www.stata.com) following an intention-to-treat approach. Descriptive statistics were conducted to summarize the data. T-tests were conducted to compare the difference in pre- and post-intervention self-efficacy (acceptability) scores across treatment arms, with $p < 0.05$ considered statistically significant.

Qualitative Design and Analysis

The interview data was analyzed using thematic analysis [35]. Two researchers read through the transcripts several times, while identifying meaningful information related to the research objectives. Next, key words or statements were selected from the data and then grouped into codes. A coding framework was established based on the COM-B informed interview guides and additional codes that emerged from the data. Including an inductive coding approach allowed us to stay close to the recounting of events as described by participants and interpret events beyond our theoretical disposition, consistent with qualitative description [24]. The coding framework was validated by two coders, with any disagreements resolved through discussion. The codes were revised and reviewed for each interview and then grouped into common themes. The themes were reviewed and compared across the interviews. Then, the themes were described and linked with quotes.

RESULTS

Recruitment, Enrollment, and Retention

Figure 2 displays the complete study flow with reasons for exclusion and discontinuation. In short, 43 of 71 transplant candidates consented (61%). The most common reason for

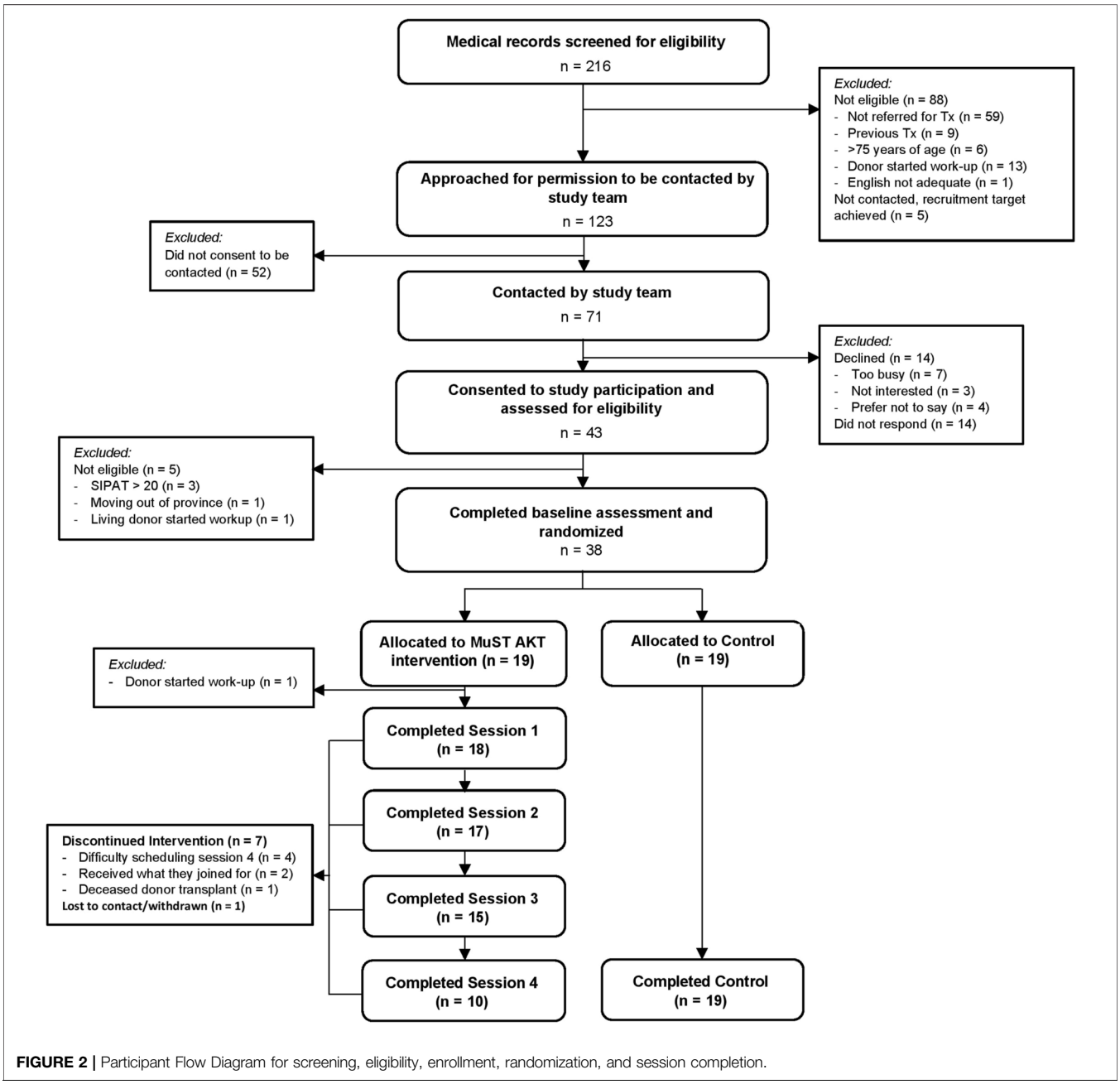


FIGURE 2 | Participant Flow Diagram for screening, eligibility, enrollment, randomization, and session completion.

non-consent was non-response after 3 contact attempts (n = 14). Of the 43 consenting participants, 5 were ineligible and excluded, and 38 were randomized 1:1 to the MuST AKT intervention or standard care arms. Of the 19 participants allocated to the MuST AKT arm, 1 did not receive the intervention as they were excluded after randomization (and from analysis) for having a living donor in evaluation. Of the 18 who started the intervention, 10 fully completed, and 8 partially completed (n = 7 discontinued, 1 lost-to contact/withdrew). Reasons for discontinuation include difficulty scheduling Session 4 (n = 4), received what they joined for–started communicating with social network about LDKT (n = 2), and received a deceased donor kidney transplant (n = 1). In the MuST AKT arm, 17 participants completed assessments (10 who completed the intervention, 7 who partially completed). In the standard of care arm, all 19 participants completed the assessments at the time control (12–14 weeks after enrollment).

Sociodemographic and Clinical Characteristics

Baseline characteristics of participants in the MuST AKT and control arms were similar, although, there was a greater representation of non-Caucasian and employed participants in

TABLE 2 | Participant sociodemographic and clinical characteristics at enrollment.

Characteristics	Experimental arm (n = 19)	Control arm (n = 19)
Age, years, mean (SD)	49.8 (16.2)	50.9 (15.8)
Gender, female, n (%)	6 (31.6)	8 (42.1)
Ethnicity[a], n (%)		
Caucasian	7 (36.8)	12 (63.2)
Indigenous	6 (31.6)	2 (10.5)
Non-Caucasian nor indigenous	7 (36.8)	5 (26.3)
Highest level of education, n (%)		
University Degree	7 (36.8)	2 (10.5)
College Diploma	5 (26.3)	4 (21.1)
Trade certification/some post- secondary	4 (21.1)	7 (36.8)
High school Diploma	3 (15.8)	4 (21.1)
Elementary/some high school	0 (0.0)	2 (10.5)
Employment status, n (%)		
Full-time	8 (42.1)	5 (26.3)
Part-time or casual	4 (21.1)	3 (15.8)
Not employed	1 (5.3)	3 (15.8)
Student	1 (5.3)	0 (0.0)
Disability	2 (10.5)	4 (21.1)
Retired	3 (15.8)	4 (21.1)
Household income, Canadian dollars, n (%)		
$0–49,999	5 (26.3)	2 (10.5)
$50,000-$99,999	4 (21.1)	9 (47.4)
$100,000-$149,999	1 (5.3)	2 (10.5)
≥$150,000	7 (36.8)	1 (5.3)
Prefer not to say	2 (10.5)	5 (26.3)
Household status, n (%)		
Alone	5 (26.3)	3 (15.8)
Spouse	10 (52.6)	12 (63.2)
Immediate family member	3 (15.8)	4 (21.1)
Friend(s) or roommate(s)	1 (5.3)	0 (0.0)
Dialysis at enrollment, n (%)	10 (52.6)	13 (68.4)
Days on dialysis at enrollment, median (IQR)	139 (67, 307)	151 (114, 456)
Cause of kidney disease		
Diabetic nephropathy	7	5
Glomerulonephritis	7	9
Hypertension	1	1
Congenital	1	0
Polycystic kidney disease	0	1
Thrombotic microangiopathy	1	0
Other	0	1
Unknown	2	2

[a]*One participant in "Treatment Group" identified as both Caucasian and Indigenous.*

the MuST AKT arm compared to the control arm (**Table 2**). Among the participants who chose to discontinue the intervention (n = 7), 86% (6/7) were male, 71% (5/7) were non-Caucasian, 86% (6/7) had at least some-post secondary or trade certification, 71% (5/7) were employed, 57% (4/7) had income > $90,000CAD, and 86% (6/7) lived with someone, 57% (4/7) lived with their spouse.

Intervention Adherence, Engagement, and Fidelity

Session attendance was 100% (18/18) for Session 1 but decreased throughout the sessions, with 94% (17/18) completing the first 2 sessions, 83% (15/18) completing the first 3 sessions, and 56% (10/18) completing all 4 sessions. A total of 25% of sessions were postponed and rescheduled (15/60) mainly as participants forgot/mixed up dates (n = 6) (**Figure 3**). The planned intervention duration, from session 1 to 4, was 84–98 days (12–14 weeks) and the average (SD) observed intervention duration was 71 (22) days.

Among those that attended, the completion rate across all in-session activities was 76% (142/187), with Session 2 and 4 having the lowest and the highest activity completion rate, respectively (40/68 = 59% and 10/10 = 100%) (**Table 3**). All participants who attended the first intervention session identified at least one potential advocate and one potential donor by the end of the MuST AKT sessions, with a median (IQR) of 17.5 (8, 25) advocates and 10.5 (7, 19) potential donors identified for each participant.

Acceptability

Questionnaire

There were statistically significant differences in self-efficacy for communicating about living kidney donation (p = 0.004) and

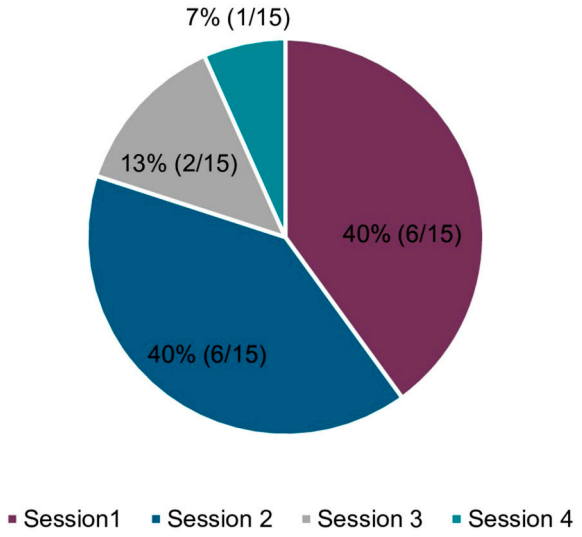


FIGURE 3 | The number of MuST AKT sessions rescheduled (15 total out of possible 60). Reasons for rescheduling included, forgot/mixed up dates (n = 6), unwell/emergent health visit (n = 3), work/school (n = 3), too busy (n = 1), caring for dependent (n = 1), wanted more time before next session (n = 1), facilitator scheduling conflict (n = 1), no friends/family members attended session 4 (n = 1). Multiple reasons for non-attendance were recorded, if applicable.

self-efficacy for finding a living kidney donor ($p < 0.001$) between the MuST AKT intervention and control arms from pre- to post-intervention. Participants in the intervention arm reported increased confidence for communicating about living kidney donation and finding a living kidney donor from pre- to post-study, whereas participants in the control arm reported decreased confidence (**Table 4**). All participants in the intervention arm recommended the program and found it effective.

Nested Qualitative Study

Interviews were conducted with participants in the experimental arm, referred to as "MuST AKT participants" and those who they invited to session 4, referred to as "invitees". Of the MuST AKT participants, 11 of 19 (58%) completed an interview (8 fully completed intervention, 3 partially completed). Of the invitees, 16 of 89 (18%) agreed to and completed an interview, with an average of 2 invitees per session, representing 7 of 10 (70%) session 4's. Most MuST AKT participants interviewed were male (6/11 = 55%), whereas most invitees were female (12/16 = 75%).

The report provided by the independent qualitative research team is summarized below [36]. Although the MuST AKT participants and invitees were interviewed with a different focus (the entire program versus one session) and using different interview guides, the data analysis revealed similar themes across both groups. As such, the results are presented together. We distinguish MuST AKT participant and invitee data when necessary. Additionally, similar themes were found across participants who fully and partially completed the intervention, and no distinction is made between these groups.

Participants' views were understood within 4 main themes and 9 sub-themes: (1) Intervention expectations and experience (exceeded expectations, positive & informative experience), (2) MuST AKT sessions (content, sharing my story, social media, logistics), (3) Intervention facilitators, and (4) Intervention Effectiveness (intervention impact, encouraged to be an advocate, encouraged to be a donor). Representative quotes for each theme and sub-theme are presented in **Table 5** and referred to in the text below (E.g., quote 1 [Q1], etc).

Intervention Expectations and Experience

Most MuST AKT participants did not have specific expectations of the intervention, whereas the invitees' expectations of session 4 varied according to what they were told by the MuST AKT participant (e.g., no specific expectation, a campaign to promote donation, learn about donation process). Regardless of expectations, MuST AKT participants and invitees reported a positive experience, which exceeded their expectations (Q1 and Q2). MuST AKT participants found the intervention informative, provided personalized support, and helped and guided them to share their story (Q1 and Q3). Invitees reported that session 4 was well facilitated and informative (Q4).

MuST AKT Sessions

MuST AKT participants and invitees reported that they valued learning clear and accurate information (Q5). MuST AKT participants emphasized the value of this approach over previous clinical interactions and felt more knowledgeable about their disease and circumstances (Q6). Invitees valued learning from healthcare professionals working with the MuST AKT participant compared to the internet (Q7). Both MuST AKT participants and invitees wanted a stronger focus on advocacy in the sessions (Q8).

MuST AKT participants reported that the intervention provided the support they needed to process their circumstances and emotions and share their story (Q9 and Q10). Most (10/11) reported sharing their story with others, with 50% (5/10) sharing their own story through social media, 20% (2/10) planning to share it on social media in the future, and 40% (4/10) asking others to share their story through social media. Sharing their story through social media and through advocates were considered important methods for communicating with their social network (Q11 and Q12). One-on-one conversations was another common way that participants shared their story.

MuST AKT participants were satisfied with the scheduling and logistics of the sessions (Q13). Invitees recommended having time without the MuST AKT participant present in session 4 to ask sensitive questions (e.g., drug and alcohol use) (Q14). See **Table 6** for a list of other logistical recommendations to improve MuST AKT sessions.

Intervention Facilitators

Both MuST AKT participants and invitees enjoyed the session facilitators and felt that the qualities of the facilitators (e.g., empathetic, emotionally intelligent, kind, personable) were critical to the success of the intervention (Q15 and Q16).

TABLE 3 | In-session activity completion rates and reasons for non-completion.

Activities across 4 sessions	Completion rate	Reasons for non-completion
Session 1 activity (n = 18)		
1. Write story	18/18 (100%)	
2. Family tree	18/18 (100%)	
3. Discussed social and financial challenges & solutions	3/18 (16.7%)	• Session delivered by non-social worker without this expertise (n = 12) • Not enough time (n = 2) • Covered in screening (n = 1)
Total session 1 activity completion	**39/54 (72.2%)**	
Session 2 activity (n = 17)		
1. Create plan for sharing story	3/17 (17.6%)	• Reasons not tracked
2. Short message prepared	9/17 (52.9%)	• Not enough time (n = 3) • Participant declined (n = 3) • Not relevant to participant (n = 2)
3. Discuss & identify best advocates	11/17 (64.7%)	• Not enough time (n = 5) • Participant declined (n = 1)
4. Discuss challenges & solutions to one-on-one conversations	17/17 (100%)	
Total session 2 activity completion	**40/68 (58.8%)**	
Session 3 activity (n = 15)		
1. Create story	15/15 (100%)	
2. Discuss challenges & solutions to group conversations	13/15 (86.7%)	• Not enough time (n = 2)
3. Session 3 slideshow reviewed in full (how to create effective messages)	15/15 (100%)	
Total session 3 activity completion	**43/45 (95.6%)**	
Session 4 activity (n = 10)		
1. Session 4 slideshow reviewed in full (information on kidney disease and LDKT)	10/10 (100%)	
2. Question and answer period for friends and family members	10/10 (100%)	
Total session 4 activity completion	**20/20 (100%)**	
Total activity completion across all sessions	**142/187 (76%)**	

Bold text indicates a cumulative completion rate across each individual session and all sessions combined.

Intervention Effectiveness

MuST AKT participants and invitees agreed that the program was valuable and important to continue (Q17). Invitees reported that session 4 encouraged and empowered them to be an advocate (Q18 and Q19). Some invitees reported that they were motivated to start the evaluation process amidst their uncertainty about donating, but (Q20) noted that it was helpful to have accurate information to help them make a decision (Q21).

DISCUSSION

The results of this pilot parallel RCT demonstrate feasibility of the MuST AKT intervention as assessed by outcomes of recruitment, intervention fidelity, adherence and engagement in the intervention, and acceptability of the intervention, with modifications needed to improve participant retention (completion of all 4 sessions) before further evaluation. The participant recruitment rate projects a recruitment timeline of 16–17 months for the definitive RCT (64 total participants/3.8 pilot participants per month), which is feasible for timely achievement of broader project commitments. This recruitment rate is satisfactory (61%) given the long-term and intensive commitment required for the intervention and is within the range of what has been reported in similar studies [14, 15]. Most intervention sessions were well attended (3 of 4 sessions with >80% attendance) and there were an acceptable number of intervention sessions rescheduled (15/60 = 25%), which was similar to what is observed for outpatient appointments at our center. We received positive feedback on the content, format, and delivery of the intervention, and had positive accounts of participant reported outcomes. The total intervention duration was shorter than anticipated, which bodes well for future implementation.

To the best of our knowledge, this is the first intervention developed to address barriers to LDKT that was delivered exclusively over a virtual communications platform. Previous similar interventions have included in-person visits between patients, healthcare providers, and the patient's social network [12, 14–17]. Although the virtual format was implemented out of necessity of the COVID-19 pandemic, a benefit of this format was that we were able to improve healthcare access by reaching patients

TABLE 4 | Participant reported acceptability by study arm.

Acceptability measure	Pre-study		Post study		*p*-value
	Experimental arm (n = 17)	Control arm (n = 19)	Experimental arm (n = 17)	Control arm (n = 19)	
Recommend to others, n (%)					
Completely agree			15 (88.2%)		
Moderately agree			1 (5.9%)		
Slightly agree			1 (5.9%)		
Neither agree nor disagree			0 (0.0%)		
Slightly disagree			0 (0.0%)		
Moderately disagree			0 (0.0%)		
Completely disagree			0 (0.0%)		
Program was effective, n (%)					
Completely agree			6 (31.6%)		
Moderately agree			5 (29.4%)		
Slightly agree			3 (17.7%)		
Neither agree nor disagree			2 (11.8%)		
Slightly disagree			1 (5.9%)		
Moderately disagree			0 (0.0%)		
Completely disagree			0 (0.0%)		
Self-efficacy[a], 0–100, mean (SD)					
Confidence for communicating about LDKT	83 (23)	83 (25)	94 (13)	77 (26)	0.004
Confidence for finding LKD	62 (27)	61 (25)	81 (22)	47 (28)	<0.001

LDKT, living donor kidney transplant; LKD, living kidney donor.
[a]*t-test*

across a large geographical area, many of whom would have been required to travel multiple hours to attend in-person visits posing a significant barrier to participation. Previous research has shown that virtual modalities are often preferred by patients for non-urgent care due to prompt appointments and convenience, although care should be taken to consider privacy, technology, and connectivity to maintain equity and quality of service [37]. We found that the virtual format was well accepted by both MuST AKT participants and invitees. While some challenges to using the virtual communication platform was noted by the facilitators (e.g., no audio), all challenges were able to be remedied before or during the session. We used the same secure virtual communication platform as the standard of care transplant education class at our health care center. Thus, prior patient experience with the virtual platform may have contributed to few participant challenges.

The finding that patients receiving the MuST AKT intervention reported stronger self-efficacy/confidence for communicating about LDKT after participating is an important benefit. While encouraging, this finding is preliminary, and will be re-evaluated in a definitive RCT. From the lens of the COM-B model, MuST AKT increased motivation to communicate about LDKT, making it more likely that such communication will take place, which is a critical and necessary step to receiving a LDKT. Previous interventions that were found to increase LDKTs and surrogate outcomes [10–12, 14–17] used communication with one's social network as a core component. Furthermore, self-efficacy has been shown to be a strong predictor of health behaviors across a variety of settings [38] and is associated with readiness to pursue LDKT [39].

Beyond increasing motivation for communicating about living kidney donation, qualitative reports indicate that MuST AKT increased key capabilities of transplant candidates related to finding a living donor, including story writing and sharing, and provided an opportunity (session 4) for communication about living kidney donation that was valued by both transplant candidates and their invitees. As a result of their participation in MuST AKT session 4, some invitees indicated they were encouraged to be advocates and/or living donors. While these are subjective reports, and not objective accounts of donor behaviour, the results provide support for our behaviour change approach guided by the COM-B model [20].

After a multi-session formal review of the results with the study team, intervention facilitators, and patient advisors, minor modifications to the intervention will be made to address participant retention and optimize the intervention before further evaluation (**Table 7**). We observed a moderate completion rate of all four MuST AKT sessions, which was similar to the TALK intervention [14], but lower than other interventions [15, 17]. The participants who chose to discontinue the MuST AKT intervention were predominantly non-Caucasian males currently employed and living with others. While we did not evaluate the role that these sociodemographic factors played in intervention completion, other studies have also found being male [40] and employed [41] led to lower-rates of health program completion. Based on participant report, almost half (4/9) of the participants who discontinued from this study did so for positive reasons (e.g., began communicating with their social network about LDKT or received deceased donor transplant). Of the remaining discontinuations, participants cited difficulty scheduling session 4 as the most common reason, suggesting this is a key facet to address. Scheduling session 4 with friends and family may present added logistical challenges for employed individuals. While difficulty scheduling session 4 may in part be logistical, the qualitative accounts highlight the emotional fortitude it takes to initiate communication about LDKT. In the

TABLE 5 | Themes, sub-themes, and representative quotes from participants.

Themes	Sub-themes	Representative sentence
Intervention expectation and experience	Expectations exceeded	"It was way beyond my expectations. [. . ..] it almost felt like it was personalized. [MuST AKT facilitator 1] was just fantastic. [. . .] I'm not saying I cannot do things like by myself, but it is really hard to do some of these things like [. . .] writing the story. Even though it sounds simple, it is not that simple." – MuST AKT participant (Q1) "Obviously it is a pretty serious issue, but even still, you do hear a lot of negative things more so than positive about the state of our healthcare system. To see two healthcare professionals come together for one person and do a 2-h info session on a zoom call in an evening, yeah it was really really impressive." – Friend and family (Q2)
	Positive & informative experience	"No, I just enjoyed it so much you would not believe. It was awesome. It was great. I learnt so much. I learnt to open up to friends and all that. I have good word for it." – MuST AKT participant (Q3) "I did not expect that it would be – I guess in the end it was so educational, such good information. I thought wow, we were not just looking at slides, we had professional people answering our questions and it went really well I thought." – Friend and family (Q4)
MuST AKT sessions	Content	"[. . .] I really appreciated the graphics and the slides and being able to see what [MA facilitator 1] was talking about and coming away with a clear understanding of where [MA participant] is going to be going as [they] continue on this journey." – Friend and family (Q5) "[. . .] with the specialist they had given me a binder. That was their extent of their education. When I compare my experience with the medical team I had and with what [MuST AKT facilitator 1] and that specialist provided, it was quite a difference. The session was much more informative and personal." – MuST AKT participant (Q6) " [. . .] I did have a lot of questions so I was curious to hear from professionals [. . .]. Google can be a bit daunting just because there is so much information, so it was nice to hear from people who are actively working with [MuST AKT participant] about the kidney transplant process." – Friends and family (Q7) "Just put more emphasis on what an advocate can do. [There is more emphasis] towards a living donor [. . .] which is great, but if you could also add to that advocacy part that would be beneficial." – MuST AKT participant (Q8)
	Sharing my story	"It helped me to talk about it because I was not ready to talk to anyone even my family, even my friends because I felt not guilty, it is hard to explain but I did not want to bother them with that. It was my burden. I'm taking care of it, but [they] helped me go through to open up to [my friends and family]." – MuST AKT participant (Q9) "It walked me through this is something do not think I'd even be able to get this far on my own because there was always that reluctance. I did not know how to go about it. It was there providing the support that I needed and helped me get through all these hurdles" – MuST AKT participant (Q10)
	Social media	"Yeah so if I post something and a couple of my friends will share it and let's say they have 500 friends on their friend list then maybe 10% will read it or curious and search it. That's 50 people that will find out about it. It is just ongoing right." – MuST AKT participant (Q11) "My [sibling] was a good help. When I sent [them] my story, [they] said can I share that? I said [. . .] share it to the family and friends first. Don't put it on Facebook [. . .]. [They] sent everything to everyone the nieces, the nephews. Everybody was calling me [. . .]. It was like social media." – MuST AKT participant (Q12)
	Logistics	"I think it was scheduled pretty good, [. . .] I was not overwhelmed. At times it was overwhelming because it seems I got a lot of appointments and busy, busy, busy." – MuST AKT participant (Q13) "If you take a [religious] household where maybe the kids are not religious, they drink, they smoke and they obviously cannot say that to their parents. [. . .] that was really important to ask that question, but I obviously could not go into detail." (Q14)
Intervention facilitators		"I think we lucked out with the people that were helping with the session. They were very personable and quite emotionally intelligent, responding to cues and humor [. . .] I think for this program to be successful, you really have to strike it with the people hosting because [they] are 80% of it. People will participate if dealing with someone more personable." – Friend and family (Q15) "I felt that [they were] with me and understood me very empathetic." – MuST AKT participant (Q16)
Intervention effectiveness	Intervention impact	"I think it is very important. I would hate for the program to not continue because in my opinion it improved our life and if it improves one or two other people, which I know it'll improve more, it's beneficial. It should happen." (Q17)
	Encouraged to be an advocates	"I think ultimately as a family member you do have to be an advocate regardless, but I think it made it easier. [. . .] it refines your knowledge a bit on the actual issues." – Friend and family (Q18) "It just really reassured us that this was something that we should be doing. We were supporting [them] personally, but not going out and letting other people know here we are, we are in this situation and we need that kidney." – Friend and family (Q19)
	Encouraged to be a donor	"I mean yes and no. It is very tough. The session definitely made me want to actually reach out to the next level of inquiry so I do plan on reaching out and calling that number and setting up a meeting to see what the first steps would be to run some tests. I do not know if it actually made me more likely to go through with it, but it has definitely made me more likely to go through the first steps of it." – Friend and family (Q20) "It gave me the information that would be needed to contemplate that decision with a little bit more clarity and a more informed decision." – Friend and family (Q21)

TABLE 6 | Logistical recommendations to improve MuST AKT sessions as provided by MuST AKT participants and friends and family.

MuST AKT participant recommendation (all sessions)	Friends and family recommendations (session 4 only)
• Incorporate more visuals in presentations	• Provide an agenda, session expectations, and session length before the meeting
• Record the sessions so they could share with others	• Clearly state the goal of the session at the beginning
• Have nephrologist attend session(s) to answer medical questions	• Provide another session for friends and family
• Provide more sessions to get more information and support	• Have the option to attend in person
• Provide additional friends and family session to provide more opportunity for them to ask questions	• Include more pauses and opportunities for discussion throughout the presentation
	• Provide assistance for using zoom
	• Include more resources after the session (e.g., how to share story and create effective messages, information discussed during the session)

definitive RCT, participants will be encouraged to invite a support person/advocate to the earlier intervention sessions to provide emotional and logistical support with arranging session 4, as previous research has found utilizing advocates or "donor champions" to be effective for initiating LDKT conversations and reducing communication barriers to LDKT [42]. We will also include resources for the intervention facilitators to better support patients who experience emotional challenges (e.g., fear, guilt) to communicating about LDKT, including how to utilize the narrative or story from session 1 to address patient challenges. This approach is consistent with techniques of Narrative Therapy [43], which has shown promise for addressing emotional challenges in medicine [44, 45].

Our approach to the MuST AKT initiative to increase LDKT in our healthcare jurisdiction aligns with continuous quality improvement processes, which are integral for ensuring quality in healthcare [46]. Delivery of a process of care/education, by its very nature, slowly changes over time. This adaptive approach ensures responsiveness to patient needs and jurisdictional circumstance.

Our findings should be interpreted within the following contexts and limitations. This pilot RCT was conducted virtually at a single transplant center in a Canadian city with English speaking participants, although there was representation from non-Caucasian racial groups and other groups disadvantaged for transplant including women, those with lower socioeconomic status, and non-urban dwellers. If the MuST AKT intervention is adopted as part of standard care, we will continue to work with our healthcare organization to ensure the intervention is accessible and comprehensible to all transplant candidates in our catchment area (e.g., provide real-time translation services, offer in-person sessions, ensure educational materials meet our organization's health literacy standards). Despite randomization, there appear to be differences between the study arms on sociodemographic characteristics. It is possible that sociodemographic factors including employment, gender, and ethnic background played a role in MuST AKT intervention completion. More research is needed to understand the interplay of these characteristics in program completion. We recruited participants without potential living donors who had not had a previous transplant and were either at the beginning of their transplant evaluation and/or were anticipated to have a long wait on the deceased donor waitlist determined by the transplant center. Those who consented to participate may have been particularly motivated to take the required steps to find a living kidney donor. Also, participants had pre-existing experience with virtual communication platforms prior to this study, which may have contributed to their willingness to participate in a virtual intervention. Satisfaction with the intervention was high, although a social desirability bias is possible for the acceptability questionnaire items, and only a small proportion of session 4 attendees completed interviews, which may not be representative of the patient's entire social network. Still, the qualitative interviews were conducted by researchers independent of the study team to mitigate social desirability bias, and the findings suggest that the MuST AKT intervention was acceptable. Although the results may not generalize to other healthcare jurisdictions and populations, this initiative outlines an approach for personalizing LDKT interventions that may be useful to others. The self-efficacy assessments were not validated tools, but they adhere to the theoretical and measurement guidelines [31]. The intervention was primarily delivered by a behavioral scientist out of necessity due to the organization's COVID-19 response, in which the social worker was unavailable to deliver the intervention. While these professions have different backgrounds and training, both individuals followed detailed intervention guides. In the definitive RCT, a social worker in kidney care will deliver the intervention, in which feasibility and acceptability will be re-evaluated. The sample size of the study was small but appropriate to evaluate feasibility. Results of statistical comparisons should be considered preliminary, and will be re-evaluated in a definitive RCT. While we did not set *a priori* cutoffs to evaluate feasibility, the comprehensiveness of the feasibility and acceptability metrics provide us with the required information to improve the intervention.

TABLE 7 | Forthcoming modifications to the Multidisciplinary Support To Access living donor Kidney Transplant (MuST AKT) intervention.

Identified area of improvement	Modification
Retention/Intervention adherence – difficulty getting participants to schedule and commit to session 4	• Provide more information in advance about what to expect in session 4 • During session 3, discuss barriers to scheduling session 4 and help identify solutions • Encourage participants to bring a support person/advocate to at least one intervention session (1–3) who could help arrange session 4
Intervention Fidelity/Engagement in intervention – unable to complete all session 1 and session 2 activities in the allotted time	
• Session 1: social and financial challenges	• Discussion of these challenges begins when screening potential participants for the study. Move in depth discussion of social and financial challenges from session 1 to session 2 and 3
• Session 2: create plan to share story	• Allow flexibility for facilitator to carry over discussion from session 2 to session 3, if required
• Session 2: prepare short message	• Remove – covered in session 3
• Session 2: discuss and identify best advocates	• Move description and discussion of advocates from session 2 to session 1
• Session 2&3: discuss challenges and solutions to one-on-one and group conversations	• Allow flexibility for facilitator to carry over discussion from session 2 to session 3, if required • Allow more participant generated discussion of LDKT barriers, including barriers that fall outside of 'having conversations' such as other social and financial barriers
Acceptability – participant recommended improvements to the sessions	
• Session 4: Invitation to friends and family	• Remind participants to send out standardized invitation to friends and family with session details. Describe why this is important
• Session 4: Introduction	• Clearly state the goals of the session at the beginning and provide assistance with using zoom, if required
• Session 4: Advocacy	• Include additional information and discussion about how to be an advocate
• Session 4: Question and answer period	• Provide an opportunity for friends and family to ask questions about LDKT without the participant present
• All sessions: Approach to presenting information	• Provide more opportunity for pauses and discussion throughout and reduce amount of time presenting • If presentation is required, incorporate more visuals • Develop narrated PowerPoint slides of key session materials so that session content can be shared with friends and family and reflected on afterwards
• Have nephrologist attend session(s)	• Not feasible to have nephrologist attend • Ensure training of facilitators so that they are able to answer medical questions • Develop a FAQ document that is vetted by nephrologists and clinicians
• More sessions	• Not feasible to include more sessions • Determine what specifically participants and friends and family members would like included if an extra session were to be available. Ask detailed follow-up questions during interviews for the definitive RCT.
Facilitator recommended improvement	
• Use narrative from session 1 to help address emotion-related barriers (e.g., fear)	• Utilize the narrative or 'write your story' activity from session 1 to help address emotional barriers to LDKT. • Develop materials for facilitators to better guide and incorporate the use of narrative in sessions 2 & 3

LDKT = living donor kidney transplantation; FAQ = frequently asked questions; RCT = randomized controlled trial.

Overall, this study demonstrated that the MuST AKT intervention designed to engage transplant candidates and their social network to address barriers to LDKT, is acceptable and is feasible with minor modifications. A larger definitive RCT will be conducted to evaluate the optimized MuST AKT intervention and efficacy to increase LDKTs.

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 Alberta Health Research Ethics Board–Health Panel (Pro00097902) and Northern Alberta Clinical Trials and Research Centre. 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

A-MS: Conceptualization, Methodology, Software, Writing – Original Draft, Visualization, interpretation of data, Supervision, Project Administration. PD: Conceptualization, Methodology, Resources, Writing – Review and Editing, interpretation of data, Funding. SK: Conceptualization, Methodology, Resources, Writing – Review and Editing, interpretation of data, Funding. NL: Conceptualization, Methodology, Resources, Writing – Review and Editing interpretation of data, Funding. TS: Conceptualization, Writing – Review and Editing, interpretation of data, Supervision, Project Administration. AA: Conceptualization, Writing – Original Draft, Visualization. BV: Methodology, Software, Resources, Writing – Review and Editing, Analysis. BC: Conceptualization, Writing – Review and Editing. AD: Writing – Review and Editing, Project Administration. SF: Conceptualization, Writing – Review and Editing. DI: Conceptualization, Writing – Review and Editing. GS: Conceptualization, Writing – Review and Editing. AB: Methodology, Writing – Review and Editing, interpretation of data. KW: Methodology, Writing – Review and Editing, interpretation of data. SS: Conceptualization, Methodology, Resources, Writing – Review and Editing, interpretation of data, Supervision, Project Administration, Funding Acquisition. All authors contributed to the article and approved the submitted version.

FUNDING

The author(s) declared that financial support was received for this work and/or its publication. University Hospital Foundation Kaye Competition provided funding for the conduct of the pilot study (grant number: RES0046891). Alberta Innovates Partnership for Research and Innovation in the Health System (PRIHS) Program provided funding for the full length of the project (grant number: G2020000182).

CONFLICT OF INTEREST

The author(s) declared that this work 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) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

ACKNOWLEDGEMENTS

The authors thank the members of the Multidisciplinary support To Access living donor Kidney Transplant (MuST AKT) Research Group that were active during the development and conduct of this research, who, in addition to the authors are: Branko Braam, Ross Bennett, Sandra Cockfield, Carol Easton, Manuel Escoto, Dyan Franco, Jill Goth, Michelle Hofer, Kailash Jindal, Shayroz Khosla, Lana Marchinko, Julia Moore, Louise Morrin, Dan Muruve, Nairne Scott-Douglas, Kyle Nash, Neesh Pannu, Deanna Paulson, Kara Penny, Flavia Robles, Mike Simoens, Lee Ann Tibbles, Marta Tsoukalas, Sandi Vanderzee, Joyce Van Deurzen, Nancy Verdin, and Kathy Yetzer. The authors thank the Kidney Health Section of the Medicine Strategic Clinical Network (now defunct) of Alberta Health Services for their support and contributions to help make this study possible. The authors also thank the Kidney Foundation of Canada–Northern and Southern Alberta Branches for their support and contributions to the study. Additionally, the authors would like to acknowledge and thank the participants of this research for inspiring this research and making it possible.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2026.15472/full#supplementary-material

REFERENCES

1. Hariharan S, Israni AK, Danovitch G. Long-Term Survival After Kidney Transplantation. *N Engl J Med* (2021) 385(8):729–43. doi:10.1056/NEJMra2014530
2. Information CIfH. *Summary Statistics on Organ Transplants, Wait-Lists and Donors*. Ottawa (2022). Available online at: https://www.cihi.ca/en/summary-statistics-on-organ-transplants-wait-lists-and-donors (Accessed May 26, 2023).
3. Bailey PK, Ben-Shlomo Y, Tomson CR, Owen-Smith A. Socioeconomic Deprivation and Barriers to Live-Donor Kidney Transplantation: A Qualitative Study of Deceased-Donor Kidney Transplant Recipients. *BMJ Open* (2016) 6(3):e010605. doi:10.1136/bmjopen-2015-010605
4. Bailey PK, Caskey FJ, MacNeill S, Tomson CRV, Dor F, Ben-Shlomo Y. Mediators of Socioeconomic Inequity in Living-Donor Kidney Transplantation: Results from a UK Multicenter Case-Control Study. *Transpl Direct* (2020) 6(4):e540. doi:10.1097/TXD.0000000000000986
5. Barnieh L, McLaughlin K, Manns BJ, Klarenbach S, Yilmaz S, Hemmelgarn BR, et al. Barriers to Living Kidney Donation Identified by Eligible Candidates with end-Stage Renal Disease. *Nephrol Dial Transpl* (2011) 26(2):732–8. doi:10.1093/ndt/gfq388
6. Getchell LE, McKenzie SQ, Sontrop JM, Hayward JS, McCallum MK, Garg AX. Increasing the Rate of Living Donor Kidney Transplantation in Ontario: Donor- and Recipient-Identified Barriers and Solutions. *Can J Kidney Health Dis* (2017) 4:2054358117698666. doi:10.1177/2054358117698666
7. Ismail SY, Luchtenburg AE, Kal VGJA, Zuidema WC, Weimar W, Busschbach JJ, et al. Modifiable Factors in Access to Living-Donor Kidney Transplantation Among Diverse Populations. *Transplantation* (2013) 96(6):586–90. doi:10.1097/TP.0b013e31829b754c
8. Taylor LA, Bahreman N, Hayat MJ, Hoey F, Rajasekaran G, Segev DL. Living Kidney Donors and Their Family Caregivers: Developing an Evidence-Based Educational and Social Support Website. *Prog Transpl* (2012) 22(2):119–28. doi:10.7182/pit2012611
9. Waterman AD, Peipert JD, Hyland SS, McCabe MS, Schenk EA, Liu J. Modifiable Patient Characteristics and Racial Disparities in Evaluation Completion and Living Donor Transplant. *Clin J Am Soc Nephrol* (2013) 8(6):995–1002. doi:10.2215/CJN.08880812
10. Boulware LE, Hill-Briggs F, Kraus ES, Melancon JK, McGuire R, Bonhage B, et al. Protocol of a Randomized Controlled Trial of Culturally Sensitive Interventions to Improve African Americans' and Non-African Americans' Early, Shared, and Informed Consideration of Live Kidney Transplantation: The Talking About Live Kidney Donation (TALK) Study. *BMC Nephrol* (2011) 12:34. doi:10.1186/1471-2369-12-34
11. Ismail SY, Luchtenburg AE, Zuidema WC, Boonstra C, Weimar W, Massey EK, et al. Multisystemic Engagement and Nephrology Based Educational Intervention: A Randomized Controlled Trial Protocol on the KidneyTteam at Home Study. *BMC Nephrol* (2012) 13:62. doi:10.1186/1471-2369-13-62
12. Rodrigue JR, Cornell DL, Lin JK, Kaplan B, Howard RJ. Increasing Live Donor Kidney Transplantation: A Randomized Controlled Trial of a Home-Based Educational Intervention. *Am J Transpl* (2007) 7(2):394–401. doi:10.1111/j.1600-6143.2006.01623.x
13. Rodrigue JR, Pavlakis M, Egbuna O, Paek M, Waterman AD, Mandelbrot DA. The "House Calls" Trial: A Randomized Controlled Trial to Reduce Racial Disparities in Live Donor Kidney Transplantation: Rationale and Design. *Contemp Clin Trials* (2012) 33(4):811–8. doi:10.1016/j.cct.2012.03.015
14. Boulware LE, Hill-Briggs F, Kraus ES, Melancon JK, Falcone B, Ephraim PL, et al. Effectiveness of Educational and Social Worker Interventions to Activate Patients' Discussion and Pursuit of Preemptive Living Donor Kidney Transplantation: A Randomized Controlled Trial. *Am J Kidney Dis* (2013) 61(3):476–86. doi:10.1053/j.ajkd.2012.08.039
15. Ismail SY, Luchtenburg AE, Timman R, Zuidema WC, Boonstra C, Weimar W, et al. Home-Based Family Intervention Increases Knowledge, Communication and Living Donation Rates: A Randomized Controlled Trial. *Am J Transpl* (2014) 14(8):1862–9. doi:10.1111/ajt.12751
16. Rodrigue JR, Cornell DL, Kaplan B, Howard RJ. A Randomized Trial of a Home-Based Educational Approach to Increase Live Donor Kidney Transplantation: Effects in Blacks and Whites. *Am J Kidney Dis* (2008) 51(4):663–70. doi:10.1053/j.ajkd.2007.11.027
17. Rodrigue JR, Paek MJ, Egbuna O, Waterman AD, Schold JD, Pavlakis M, et al. Making House Calls Increases Living Donor Inquiries and Evaluations for Blacks on the Kidney Transplant Waiting List. *Transplantation* (2014) 98(9):979–86. doi:10.1097/TP.0000000000000165
18. Barnieh L, Collister D, Manns B, Lam NN, Shojai S, Lorenzetti D, et al. A Scoping Review for Strategies to Increase Living Kidney Donation. *Clin J Am Soc Nephrol* (2017) 12(9):1518–27. doi:10.2215/CJN.01470217
19. Selzler AM, Davoodi PM, Klarenbach S, Lam NN, Smith T, Ackroyd A, et al. Multidisciplinary Support to Access Living Donor Kidney Transplant (Must AKT): A Clinical Research Protocol for a Pilot Randomized Controlled Trial to Increase Living Kidney Donation. *Can J Kidney Health Dis* (2023) 10:20543581231205340. doi:10.1177/20543581231205340
20. Michie S, van Stralen MM, West R. The Behaviour Change Wheel: A New Method for Characterising and Designing Behaviour Change Interventions. *Implement Sci* (2011) 6:42. doi:10.1186/1748-5908-6-42
21. Skivington K, Matthews L, Simpson SA, Craig P, Baird J, Blazeby JM, et al. A New Framework for Developing and Evaluating Complex Interventions: Update of Medical Research Council Guidance. *BMJ* (2021) 374:n2061. doi:10.1136/bmj.n2061
22. Thabane L, Ma J, Chu R, Cheng J, Ismaila A, Rios LP, et al. A Tutorial on Pilot Studies: The What, Why and How. *BMC Med Res Methodol* (2010) 10:1. doi:10.1186/1471-2288-10-1
23. Neergaard MA, Olesen F, Andersen RS, Sondergaard J. Qualitative Description - The Poor Cousin of Health Research? *BMC Med Res Methodol* (2009) 9:52. doi:10.1186/1471-2288-9-52
24. Sandelowski M. Whatever Happened to Qualitative Description? *Res Nurs Health* (2000) 23(4):334–40. doi:10.1002/1098-240x(200008)23:4<334::aid-nur9>3.0.co;2-g
25. Eldridge SM, Chan CL, Campbell MJ, Bond CM, Hopewell S, Thabane L, et al. CONSORT 2010 Statement: Extension to Randomised Pilot and Feasibility Trials. *Pilot Feasibility Stud* (2016) 2:64. doi:10.1186/s40814-016-0105-8
26. Davis TC, Long SW, Jackson RH, Mayeaux EJ, George RB, Murphy PW, et al. Rapid Estimate of Adult Literacy in Medicine: A Shortened Screening Instrument. *Fam Med* (1993) 25(6):391–5.
27. Maldonado JR, Dubois HC, David EE, Sher Y, Lolak S, Dyal J, et al. The Stanford Integrated Psychosocial Assessment for Transplantation (SIPAT): A New Tool for the Psychosocial Evaluation of Pre-Transplant Candidates. *Psychosomatics* (2012) 53(2):123–32. doi:10.1016/j.psym.2011.12.012
28. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research Electronic Data Capture (REDCap)--A Metadata-Driven Methodology and Workflow Process for Providing Translational Research Informatics Support. *J Biomed Inform* (2009) 42(2):377–81. doi:10.1016/j.jbi.2008.08.010
29. StataCorp. *Stata Statistical Software: Release 17*. College Station, TX: StataCorp LLC (2021).
30. Network KHSC. *Workshop Report: Identifying Best Practices and Ways to Increase the Use of Living Donor Kidney Transplantation in Alberta*. Alberta Health Services (2015).
31. Bandura A. Guide for Constructing Self-Efficacy Scales. In: Urdan TPF, editor. *Self-Efficacy Beliefs of Adolescence*. Greenwich: Information Age Publishing (2006). p. 307–37.
32. QSR International. Nvivo Qualitative Data Analysis. *Softw Version 12 [Software]* (1999).
33. Julious SA. Sample Size of 12 per Group Rule of Thumb for a Pilot Study. *Pharm Stat* (2005) 4(4):287–91. doi:10.1002/pst.185
34. Lancaster GA, Dodd S, Williamson PR. Design and Analysis of Pilot Studies: Recommendations for Good Practice. *J Eval Clin Pract* (2004) 10(2):307–12. doi:10.1111/j.2002.384.doc.x
35. Ritchie J, Lewis J, Nicholls CM, Ormston RE. *Qualitative Research Practice: A Guide for Social Science Students and Researchers*. 2nd ed. London, United Kingdom: Sage Publications Ltd. (2014).
36. Bourassa L, Stiphout M. *The Multidisciplinary Support to Access Living Donor Kidney Transplant (Must AKT) Pilot Program Evaluation Report: June 2022*. Edmonton, AB: Health Systems Knowledge and Evaluation, Alberta Health Services (2022).
37. Rogers JL, Kraft K, Johnson W, Forbes RC. Adoption of Telehealth as a Strategy for Pre-Transplant Evaluation and Post-Transplant Follow-Up. *Curr Transplant Rep* (2024) 11(3):140–52. doi:10.1007/s40472-024-00443-7

38. Luszczynska A, Schwarzer R. Self-Efficacy and Health Behaviours. In: Conner M, Norman P, editors. *Predicting Health Behaviour: Research and Practice with Social Cognition Models*. 2nd ed. Maidenhead, UK: Open University Press (2005). p. 127–69.
39. Waterman AD, Robbins ML, Paiva AL, Peipert JD, Davis LA, Hyland SS, et al. Measuring Kidney Patients' Motivation to Pursue Living Donor Kidney Transplant: Development of Stage of Change, Decisional Balance and Self-Efficacy Measures. *J Health Psychol* (2015) 20(2):210–21. doi:10.1177/1359105313501707
40. Karyotaki E, Kleiboer A, Smit F, Turner DT, Pastor AM, Andersson G, et al. Predictors of Treatment Dropout in Self-Guided Web-Based Interventions for Depression: An 'Individual Patient Data' Meta-Analysis. *Psychol Med* (2015) 45(13):2717–26. doi:10.1017/S0033291715000665
41. Reece J, Yu M, Bevens W, Simpson-Yap S, Davenport R, Jelinek G, et al. Sociodemographic, Health, and Lifestyle-Related Characteristics Associated with the Commencement and Completion of a Web-Based Lifestyle Educational Program for People with Multiple Sclerosis: Randomized Controlled Trial. *J Med Internet Res* (2024) 26:e58253. doi:10.2196/58253
42. Garonzik-Wang JM, Berger JC, Ros RL, Kucirka LM, Deshpande NA, Boyarsky BJ, et al. Live Donor Champion: Finding Live Kidney Donors by Separating the Advocate from the Patient. *Transplantation* (2012) 93(11):1147–50. doi:10.1097/TP.0b013e31824e75a5
43. White M. *Narrative Practice: Continuing the Conversations*. New York, NY: W. W. Norton (2011).
44. Petersen S, Bull C, Propst O, Dettinger S, Detwiler L. Narrative Therapy to Prevent Illness-Related Stress Disorder. *J Couns and Development* (2005) 83:41–7. doi:10.1002/j.1556-6678.2005.tb00578.x
45. Sun L, Liu X, Weng X, Deng H, Li Q, Liu J, et al. Narrative Therapy to Relieve Stigma in Oral Cancer Patients: A Randomized Controlled Trial. *Int J Nurs Pract* (2022) 28(4):e12926. doi:10.1111/ijn.12926
46. Endalamaw A, Khatri RB, Mengistu TS, Erku D, Wolka E, Zewdie A, et al. A Scoping Review of Continuous Quality Improvement in Healthcare System: Conceptualization, Models and Tools, Barriers and Facilitators, and Impact. *BMC Health Serv Res* (2024) 24(1):487. doi:10.1186/s12913-024-10828-0

ESOT | Transplant International

ORIGINAL RESEARCH
published: 20 February 2026
doi: 10.3389/ti.2026.15642

Immediate and Gradual Withdrawal of Immunosuppression After Kidney Graft Loss Lead to Similar Outcomes

Asmaa Nabil[1,2], Nicolas Congy-Jolivet[2,3,4†], Amandine Darres[1], Pierre Guy[1], Olivier Marion[1†], Jean Milhes[3], Thomas Prudhomme[2,5†], Nassim Kamar[1,2,6†] and Arnaud Del Bello[1,2,4†]*

[1]Department of Nephrology and Organ Transplantation, CHU de Toulouse, Toulouse, France, [2]Centre Hospitalier et Universitaire, Université Paul Sabatier Toulouse III, Toulouse, France, [3]Laboratory of Immunology, Biology Department, Centre Hospitalier et Universitaire (CHU) de Toulouse, Toulouse, France, [4]Institute of Metabolic and Cardiovascular Diseases (I2MC), Institut National de la Santé et de la Recherche Médicale (INSERM) U1297, University of Toulouse 3, Toulouse, France, [5]Department of Urology and Renal Transplantation, CHU de Toulouse, Toulouse, France, [6]Toulouse Institute for Infectious and Inflammatory Diseases (Infinity), INSERM UMR1043-CNRS 5282, Toulouse, France

The management of immunosuppression in dialysis patients with a failed kidney transplant remains a pending question, and different approaches to immunosuppression weaning have been proposed. We conducted a retrospective study of patients who experienced a graft failure, and compared the rates of immune and non-immune events, according to different modalities of immunosuppression withdrawal. Two hundred and eighteen patients were included. During the follow-up (45 (20–80) months post-graft failure), 53 patients (24.3%) experienced an intolerance syndrome. The time between graft failure and the occurrence of intolerance syndrome was 6 (3–13) months. Immunosuppression withdrawal was associated with the occurrence of intolerance syndrome. However, regarding the immunosuppression withdrawal modality, only a steroid cessation during the first 3 months post graft failure was independently associated with an earlier occurrence of intolerance syndrome [HR = 1.91, 95%CI (1.08–3.38), p = 0.025], while a longer time between transplantation to graft failure was independently associated with a delayed occurrence of intolerance syndrome [HR = 0.99, 95%CI (0.98–0.99), p = 0.009]. The immunosuppression withdrawal modality after graft failure didn't have an impact on infections and cardiovascular complications. Although discontinuation of immunosuppression strongly influences the occurrence of intolerance syndrome, immunosuppression withdrawal modality itself does not appear to.

Keywords: allograft nephrectomy, allo-sensitization, graft failure, immunosuppression, intolerance syndrome

OPEN ACCESS

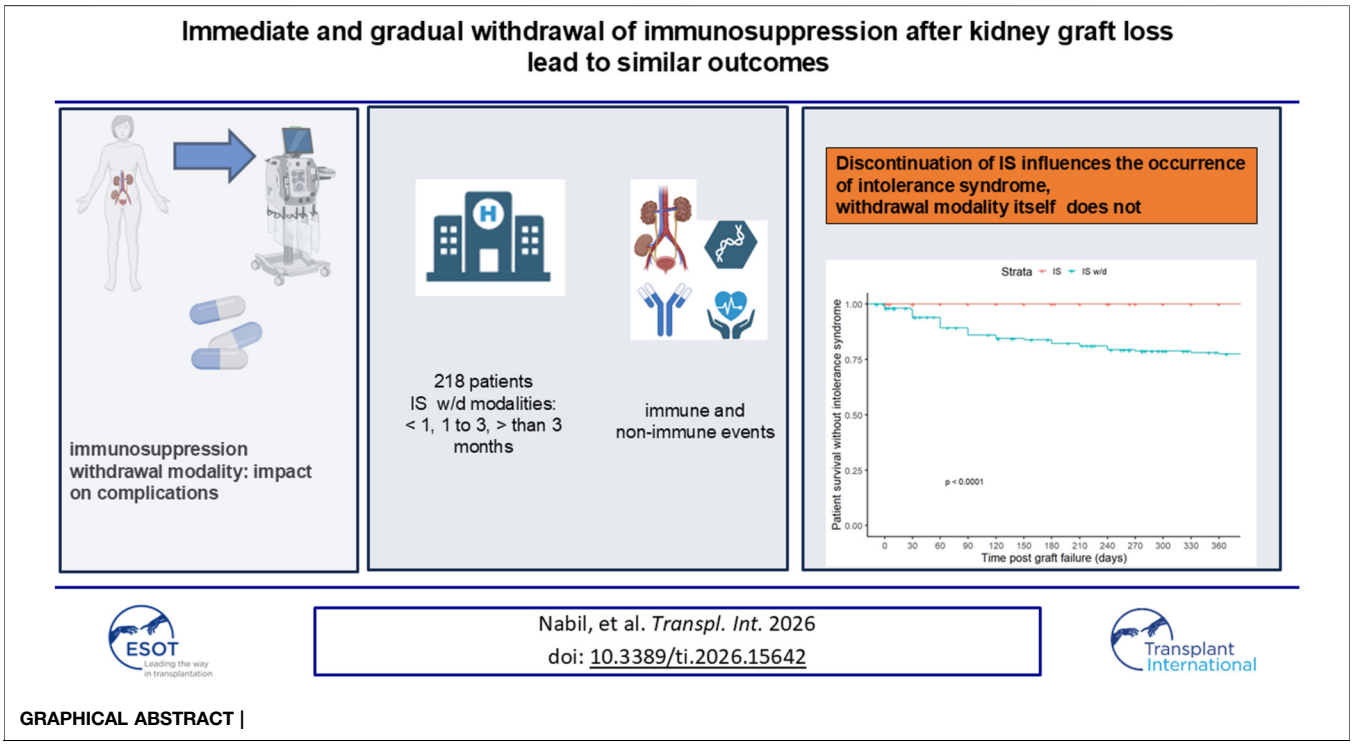
***Correspondence**
Arnaud Del Bello,
delbello.a@chu-toulouse.fr

†ORCID:
Nicolas Congy-Jolivet
orcid.org/0000-0003-4263-1700
Olivier Marion
orcid.org/0000-0001-9702-1833
Thomas Prudhomme
orcid.org/0000-0003-3601-9339
Nassim Kamar
orcid.org/0000-0003-1930-8964
Arnaud Del Bello
orcid.org/0000-0003-3115-868X

Received: 24 September 2025
Revised: 31 January 2026
Accepted: 03 February 2026
Published: 20 February 2026

Citation:
Nabil A, Congy-Jolivet N, Darres A, Guy P, Marion O, Milhes J, Prudhomme T, Kamar N and Del Bello A (2026) Immediate and Gradual Withdrawal of Immunosuppression After Kidney Graft Loss Lead to Similar Outcomes. Transpl. Int. 39:15642. doi: 10.3389/ti.2026.15642

Abbreviations: DSA, Donor Specific Antibodies; IF/TA, Interstitial Fibrosis / Tubular Atrophy; CNI, Calcineurin Inhibitors; MMF, mycophenolate mofetil; MPA, mycophenolic acid; mTOR inhibitors, mammalian Target of Rapamycin inhibitors; MACE, Major Adverse Cardiovascular Events; cPRA, calculated Panel Reactive Antibodies.



GRAPHICAL ABSTRACT |

INTRODUCTION

Anti-HLA sensitization remains a significant barrier in kidney transplantation because of the risk of antibody-mediated rejection in the setting of preformed DSA [1]. Despite the attention paid to pregnancy or blood transfusion, the rate of hypersensitized recipients on the waiting list didn't decrease over time [2–4], and a majority of these patients are candidates for a retransplantation [3, 4]. Although anti-HLA antibodies could develop after transplantation, the incidence of *de novo* DSA detection is less than 10% in recipients with a functioning graft [5, 6]. However, an intense allo-sensitization is observed after patients return to dialysis and stop immunosuppression while waiting for another transplant. Augustine and colleagues found an increase of highly sensitized recipients (defined as a cPRA≥80%) from 21% to 68% between the time of transplant failure and 2 years later [7]. Billen and colleagues found in a cohort of 56 patients that 16% presented detectable *de novo* DSA at graft failure, but the proportion increased to more than 80% after ceasing immunosuppression [8]. A majority of anti-HLA antibodies detected after graft loss are considered to be donor-specific at the epitope level [9].

After patients return to dialysis and immunosuppression is reduced or stopped, an allograft nephrectomy could be required in case of graft rejection occurring in a failed transplant (the so-called "intolerance syndrome"), graft malignancy, persistent C-reactive protein or to create space for another transplant [10]. The incidence of allograft nephrectomy in this setting can be as high as 30% [11], and appears to be more frequent during the first 6 months post-dialysis initiation [11–15]. Allograft nephrectomy is associated with non-immunological complications (mainly infections) in up to 30% [16] of case, including death. Furthermore, it is also responsible for an increase in anti-HLA antibodies occurrence, mainly in less sensitized patients [9, 17].

The management of immunosuppression in dialysis recipients with a failed transplant remains a pending question. Some but not all reports have suggested that maintaining immunosuppression could increase hospitalizations, and major adverse events such as infections, cardiovascular events, or cancers [12, 18–25]. Hence, currently, except for patients with a planned retransplantation from a living donor, most transplant societies propose ceasing immunosuppression during the first year after graft failure [10, 26, 27]. However, an immediate or progressive stop of immunosuppression over 1 year after graft failure was not assessed until now.

The present study first aimed to compare the incidence of intolerance syndrome after return to dialysis, according to the modality of immunosuppression withdrawal. The secondary aims were to compare the incidence of allo-sensitization, infection, neoplastic, and cardiovascular complications following graft failure according to the type of immunosuppressive withdrawal strategy.

PATIENTS AND METHODS

Patients

This retrospective study obtained approval from the Toulouse IRB (RC31/21/01/54).

The study was conducted using our Institution Electronic Medical Records. All adult kidney transplant recipients who

experienced graft failure between 01.01.2008 and 31.12.2022 were screened for inclusion (n = 418). The date of graft failure was defined as the date of starting hemodialysis or peritoneal dialysis.

Patients were excluded in case of preemptive transplantation (n = 3), combined transplantation that required the maintenance of immunosuppression (n = 23), need for graft nephrectomy of a functioning transplant (surgical complication, n = 31), immediate graft nephrectomy (and hence immunosuppression withdrawal) after transplantation (vascular complications during the first 8 days post transplantation, n = 125), and loss of follow-up immediately (<1 month after return to dialysis, n = 18). The last follow-up was considered as last medical appointment until July 01 2023. Finally, 218 patients were included in the study.

Immunosuppression

The time for immunosuppression discontinuation was defined as the time between the date of graft failure and the date of the last prescription recorded of any treatment, including calcineurin inihibitors (CNI), antimetabolites, mammalian target of rapamycin (mTOR) inhibitors, belatacept, and steroids. Since several recommendations were proposed, immunosuppressant discontinuation modalities were at the clinician's discretion:

a. Modality 1: brutal CNI and/or antimetabolites/mTOR inhibitors discontinuation at return to dialysis (no immunosuppression except steroids after 1 month post-graft failure).
b. Modality 2: CNI and antimetabolites/mTOR inhibitors were maintained at the same dose until they were discontinued between 1 and 3 months post-graft failure.
c. Modality 3: CNI and antimetabolites/mTOR inhibitors maintained at the same dose until they were discontinued more than 3 months post graft failure.

All patients had been given a low dose of steroids until graft failure (5 mg/day). After graft failure, steroids were converted to hydrocortisone or stopped (with or without ACTH stimulation test), at the clinician's discretion (in the absence of recommendations).

Outcomes

The primary outcome of this study was the occurrence of intolerance syndrome after return to dialysis. Intolerance syndrome was defined as the occurrence of graft pain with or without gross hematuria, fever, refractory anemia, or elevated C reactive protein (after exclusion of infection or cancer) [14]. Cases were identified and reviewed by 2 senior nephrologists from electronic medical records (AN, ADB). Secondary outcomes included the following: infection episodes requiring hospitalization, and cardiovascular complications (number, type, and time from return to dialysis to event were obtained from electronic medical records), allo-sensitization (as defined by the calculated panel reactive antibody cPRA with the vPRA online tool[1]. Opportunistic infections were defined as previously published [28, 29]. Major Adverse Cardiovascular Events (MACE) were defined as previously published [30]. The presence of anti-HLA antibodies was assessed every 6 months after return to dialysis in patients eligible for retransplantation, and detected using the Lifecodes™ single antigen technology (LMX deluxe Immucor, Gateway Drive, GA). The Lifecodes™ single antigen (LSA class I/II) determined the specificity of class I HLAs in A/B/Cw and class II in DR/DQ/DP IgG antibodies in the recipients' sera according to the manufacturer's instructions. The presence and specificity of antibodies were then detected, and the mean fluorescence intensity for each sample in each bead was evaluated. A mean fluorescence intensity value of >1,000 was considered positive.

Statistical Analyses

Reported values represent the means (±SD) or medians (IQR). Quantitative variables were compared using the student T-test or Mann-Whitney non-parametric test if appropriate. Categorical variables are expressed as percentages and compared between groups using the chi-squared tests or, if appropriate, Fisher's exact test. A p-value of <0.05 was considered statistically significant.

Analyses were performed with R, version 4.2.2 (R Development Core Team, Vienna, Austria).

Missing data represented less than 10% in each variable of the dataset (medical history [donor age, initial nephropathy, diabetes at dialysis initiation], initial immunosuppression [CNI type]) and were imputed (excluding outcomes) using the MICE package.

We used Kaplan-Meier curves (with log-rank tests) and univariate and multivariate Cox models (including all statistically significant variable in univariate analysis, and variables known to be clinically relevant such as donor and recipient age or class-I and II HLA mismatches) with backward elimination to estimate the association between the different immunosuppressant withdrawal modalities and the outcomes (intolerance syndrome, cardiovascular diseases, infections). The proportional hazards assumption was verified using Schenfeld residuals.

Survival without intolerance syndrome was analyzed using Cox proportional hazards models with time-dependant covariates. Time was measured from dialysis initiation until the occurrence of intolerance syndrome, or last-follow-up. The use of immunosuppression was modeled as a time-dependant covariate: patients contributed to the "immunosuppression" category until treatment discontinuation (except steroids), and to the "immunosuppression withdrawal" thereafter. This approach allowed patients to contribute risk time to both exposure groups according to their actual status during follow-up.

Survival analyses were performed with the survminer and survival packages.

RESULTS

Description of the Cohort

Baseline characteristics of the included patients are described in **Table 1**. The majority of patients were male, first kidney

[1]www.etrl.org/vPRA

TABLE 1 | Baseline characteristics of the cohort.

Variables	Modality- 1 <1 month	Modality-2 [1-3 months]	Modality – 3 >3 months	*P*-value
Number of patients	**84**	**80**	**54**	
Gender, male (%)	52 (61.9)	49 (61.3)	31 (57.4)	0.86
Recipient age at transplantation, mean (SD)	53.2 (15.1)	52.5 (13.9)	49.3 (17.2)	0.32
Initial nephropathy (%)	22 (26.2)	27 (33.8)	20 (37)	0.67
Interstitial - genetic	28 (33.3)	26 (32.5)	14 (25.9)	
Glomerular	8 (9.5)	10 (12.5)	5 (9.3)	
Diabetic	14 (16.7)	11 (13.8)	11 (20.4)	
Vascular	12 (14.3)	6 (7.5)	4 (7.4)	
Other-uknown				
Number of previous transplantations (%)	63 (75.0)	59 (73.8)	47 (87.0)	0.40
0	17 (20.2)	15 (18.9)	7 (13.0)	
1	2 (2.4)	5 (6.3)	0	
2	2 (2.4)	1 (1.3)	0	
3 or more				
Donor age at transplantation, mean (SD)	57.6 ± 14.8	57.5 ± 17.6	53.7 ± 19.1	0.22
T cell depleting agent at induction (%)	24 (28.6)	31 (38.8)	13 (24.1)	0.16
HLA A/B/DR/DQ mismatches, mean (SD)	4.88 (1.63)	5.01 (1.62)	4.83 (1.9)	0.81
cPRA at transplantation, median (IQR)	0 (0; 22)	0 (0; 31.5)	0 (0; 30)	0.96
Class I	0 (0; 50)	0 (0; 60)	0 (0; 32)	0.44
Class II				
Time between transplantation – return to dialysis, in months, mean (SD)	46.8 (35.9)	51.8 (38.2)	60.9 (38.6)	0.10
Diabetes at return to dialysis (%)	12 (14.3)	11 (13.8)	6 (11.1)	0.86
Cause of graft loss (%)	21 (25)	30 (37.5)	20 (37.0)	0.26
Chronic antibody mediated rejection	16 (19.0)	14 (17.5)	3 (5.6)	
Chronic T cell mediated rejection	7 (8.3)	2 (2.5)	3 (5.6)	
Reoccurrence of initial disease	9 (10.7)	5 (6.2)	5 (9.3)	
PVAN	3 (3.6)	1 (1.3)	1 (1.9)	
Chronic infection	28 (33.4)	28 (35.0)	22 (40.6)	
IFTA				
Immunosuppression at return to dialysis (%)	69 (82.1)	78 (97.5)	51 (94.4)	0.002
CNI	52 (61.9)	49 (61.3)	27 (50)	0.32
MPA mTOR inhibitors	20 (23.8)	12 (15)	13 (24.1)	0.29
Belatacept	13 (15.5)	3 (3.8)	10 (18.5)	0.016
Steroids	84 (100)	80 (100)	54 (100)	>0.99
Steroids withdrawal (%)	8 (9.5)	0	3 (5.6)	0.001
<1 month	22 (26.2)	22 (27.5)	3 (5.6)	
1-3 months	54 (64.3)	58 (72.5)	48 (88.9)	
>3 months				
Time between dialysis initiation – last follow-up, months, median (IQR)	22.5 (11.1; 51.7)	20.6 (9; 43.4)	30.5 (13.8; 42.4)	0.83
Time between IS withdrawal – last follow-up, months, median (IQR)	22.5 (11.1; 51.7)	20.0 (7.9; 42.8)	21.8 (2.8; 37.9)	0.05

Abbreviations: SD, standard deviation; cPRA, calculated panel reactive antibodies; IQR, interquartile range; PVAN, polyoma virus associated nephropathy; IFTA, interstitial fibrosis tubular atrophy; CNI, calcineurin inhibitors; MPA, mycophenolic acid; mTOR, mammalian target of rapamycin.

transplant recipients, non-HLA sensitized at transplantation, and did not receive a T-cell depleting agent at the time of transplantation. The median follow-up period between transplantation and graft failure was 45 (20-80) months. The primary causes of graft loss were chronic antibody-mediated rejection and interstitial fibrosis/tubular atrophy (IF/TA). Most patients began dialysis with a triple therapy regimen that included calcineurin inhibitors, mycophenolate, and steroids. The median follow-up period between graft failure and the last follow-up was 22.7 (10.9; 38.6) months. The median follow-up period between the cessation of immunosuppression and the last follow-up was 21.4 (8.8; 45.8) months. Except steroids, immunosuppression withdrawal was achieved in less than 1 month for 84 patients [median 0.0 (0.0; 0.0) months], between 1 and 3 months for 80 patients [median (1.1 (1.0; 2.9) months], and in more than 3 months for 54 patients [median 5.9 (4.9; 10.8) months]. With respect to steroids, they were stopped in 11, 47 and 160 patients, within the first month, between 1 and 3 months, and after 3 months after graft failure, respectively.

Intolerance Graft Syndrome and Allo-Sensitization

During the follow-up, fifty-three patients (24.3%) experienced an intolerance syndrome (**Table 2**). The time between graft failure and the occurrence of intolerance syndrome was 6 (3; 13) months. The time of intolerance syndrome and immunosuppression discontinuation was 1 (2; 9) months. All patients received a course of steroids for 1 week at 1 mg/kg. The treatment was effective and sufficient in 3 cases, without

TABLE 2 | Baseline characteristics of the cohort who presented or not an intolerance syndrome.

Variables	Intolerance syndrome	No intolerance syndrome	*P*-value
Number of patients	**53**	**165**	
Gender, male (%)	29 (54.7)	103 (62.4)	0.18
Recipient age at transplantation, mean (SD)	50.0 ± 15.0	52.3 ± 15.1	0.18
Initial nephropathy (%)	18 (34.0)	51 (30.9)	0.45
Interstitial - genetic	16 (30.2)	52 (31.5)	
Glomerular	6 (11.3)	17 (10.4)	
Diabetic	11 (20.7)	25 (15.1)	
Vascular	2 (3.8)	20 (12.1)	
Other-uknown			
Number of previous transplantations (%)	39 (73.6)	130 (78.8)	0.37
0	11 (20.7)	28 (17.0)	
1	2 (3.8)	5 (3.0)	
2	1 (1.9)	2 (1.2)	
3 or more			
Donor age at transplantation, mean (SD)	53.8 ± 18.0	57.4 ± 16.6	0.21
T cell depleting agent at induction (%)	18 (33.9)	50 (30.3)	0.74
HLA A/B/DR/DQ mismatches, mean (SD)	4.9 ± 1.6	4.9 ± 1.7	0.35
cPRA at transplantation, median (IQR)	0 (0; 22)	0 (0; 30)	0.41
Class I	0 (0; 8)	0 (0; 50)	0.10
Class II			
Time between transplantation – return to dialysis, in months, mean (SD)	38.6 ± 35.4	56.5 ± 37.5	0.38
Diabetes at return to dialysis (%)	8 (15.1)	21 (12.7)	0.83
Cause of graft loss (%)	26 (49.0)	78 (47.3)	0.95
Immune related, yes	18 (34.0)	53 (32.1)	
Chronic antibody mediated rejection	8 (15.0)	25 (15.1)	
Chronic T cell mediated rejection	27 (51)	87 (52.7)	
Other, yes	0	12 (7.3)	
Reoccurrence of initial disease	0	19 (11.6)	
PVAN	2 (3.8)	3 (1.8)	
Chronic infection	25 (47.2)	53 (32.1)	
IFTA			
Immunosuppression at return to dialysis (%)	49 (92.4)	149 (90.3)	0.97
CNI	32 (60.4)	96 (58.2)	
MPA mTOR inhibitors	9 (17.0)	36 (21.8)	
Belatacept	6 (11.3)	20 (12.1)	
Steroids	53 (100)	165 (100)	
Steroids withdrawal (%)	2 (3.8)	9 (5.4)	0.10
<1 month	17 (32.0)	30 (18.2)	
1-3 months	34 (64.2)	126 (76.4)	
>3 months			

Abbreviations: SD, standard deviation; cPRA, calculated panel reactive antibodies; IQR, interquartile range; PVAN, polyoma virus associated nephropathy; IFTA, interstitial fibrosis tubular atrophy; CNI, calcineurin inhibitors; MPA, mycophenolic acid; mTOR, mammalian target of rapamycin.

reoccurrence after a decrease in steroids over 1 month. The remaining 50 patients required a surgical graft nephrectomy (n = 43) or a renal artery embolization (n = 7). The impact of immunosuppression cessation on the development of intolerance syndrome was modelled using a Cox proportional hazards model with immunosuppression therapy as a time-dependant covariate. We observed that IS cessation was associated with the occurrence of intolerance syndrome [HR: 11.41, 95%CI (4.20–31.03), $p < 0.0001$, **Figure 1A**]. This remained true after adjustment for donor and recipient age, and time between transplantation and graft failure [aHR: 10.37, 95%CI (3.79–28.42), p < 0.0001]. When considering the modality of cessation of all immunosuppressants except steroids, we did not observe a difference for the rate or the time to intolerance syndrome after graft failure (**Figures 1B,C**) or after IS cessation (**Figures 1C,D**; **Table 1**). However, a steroid cessation during the first 3 months post graft failure was independently associated with a reduced time between graft failure and the occurence of intolerance syndrome [aHR = 2.31, 95% CI (2.13–2.50), $p < 0.001$], while a longer time between transplantation to graft failure was independently associated with a prolonged time between graft failure and the occurrence of intolerance syndrome [aHR = 0.99, 95% CI (0.98–0.99), $p < 0.001$], after adjustment for donor and recipient age, number of HLA mismatches, modality of cessation of immunosuppressants except steroids (**Figures 1C,D**; **Table 1**; **Supplementary Tables 1, 2**).

After graft failure, 99 patients (45.4%) were considered for a retransplantation and screened for anti-HLA sensitization. 56 of the 99 (58.6%) had developed *de novo* DSA at last follow-up [21 (9; 39) months post-graft failure]. We assessed the role of immunosuppression cessation modality on the occurrence of

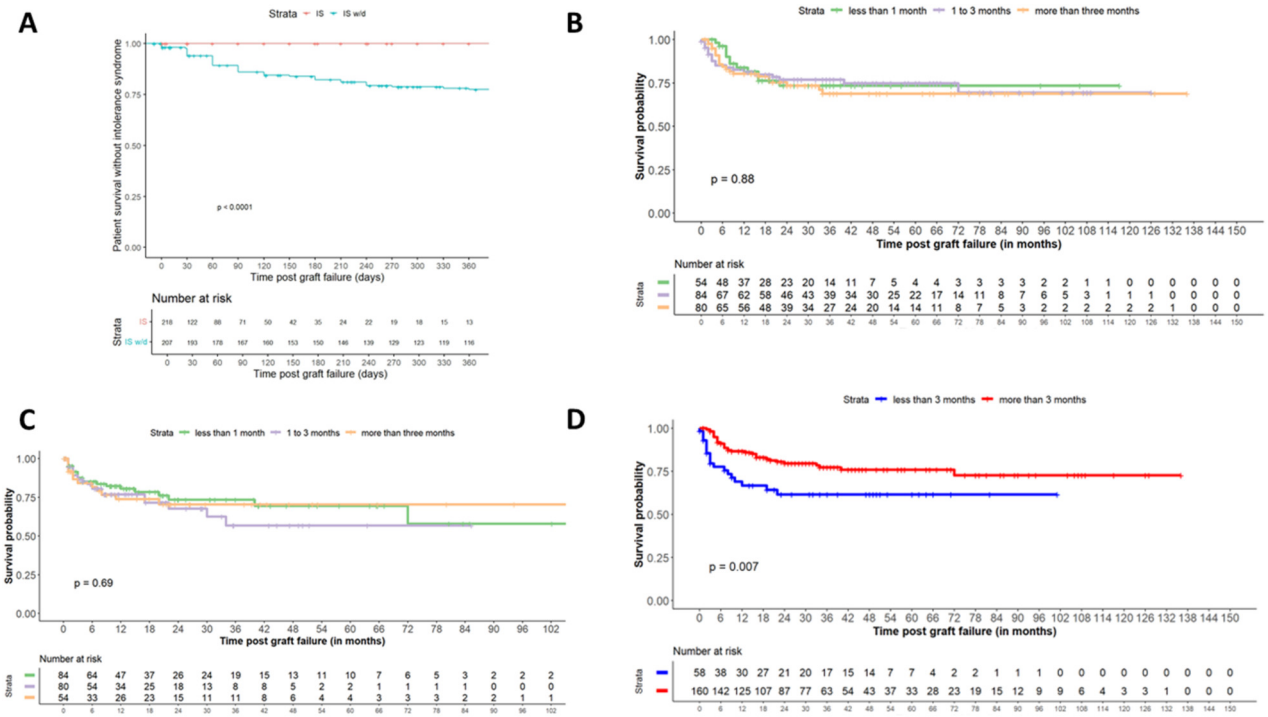
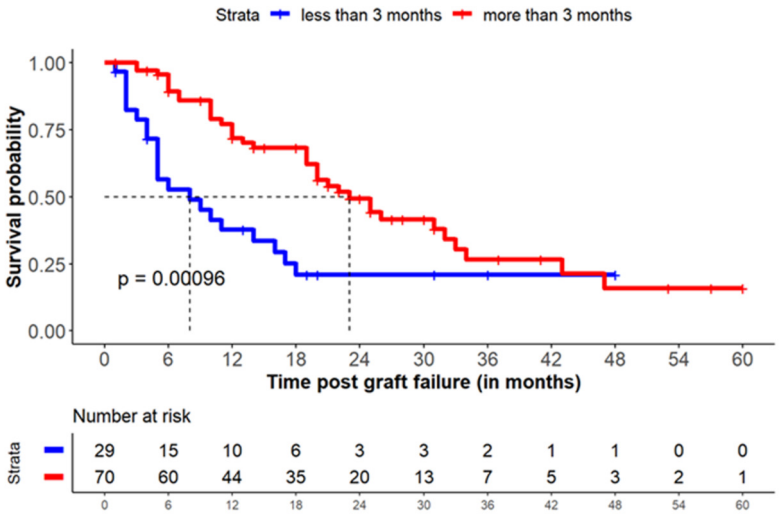


FIGURE 1 | Occurrence of intolerance syndrome according to immunosuppression withdrawal modality **(A)**. Patient survival free from intolerance syndrome according to immunosuppression exposition. Patients contributed to the "immunosuppression" category until treatment discontinuation (except steroids), and to the "immunosuppression withdrawal" thereafter. The *p*-value in the Kaplan-Meier curve was tested the log-rank test. **(B)**. Patient survival free from intolerance syndrome after graft failure. The *p*-value in the Kaplan-Meier curve was tested the log-rank test. **(C)** Patient survival free from intolerance syndrome after immunosuppression withdrawal (apart from steroids). The *p*-value was tested the log-rank test. **(D)** Patient survival free from intolerance syndrome after steroid withdrawal (less or more than 3 months). The p-value was tested the log-rank test.

post-graft failure allo-sensitization and found that a delayed withdrawal of immunosuppression other than steroids did not affect the occurrence of anti-HLA DSA (**Figure 2**; **Supplementary Table 2**). However, although a delayed steroids withdrawal was associated with a reduced occurrence of DSA (HR 0.41, 95% CI [0.24–0.71] p = 0.001, **Figure 2**; **Supplementary Table 2**), we did not observe any difference on cPRA values at last follow-up, according to immunosuppression discontinuation modality (**Figure 3**). 12 of the 99 patients received an allograft nephrectomy: 7 patients presented a DSA before the nephrectomy, 2 patients presented *de novo* DSA after the nephrectomy, and 3 patients did not presented DSA pre- or post nephrectomy. The cPRA increased before the nephrectomy (median cPRA: 0 (0; 93) at transplantation, 0 (0; 93) at graft failure, 52 (0; 100) before the nephrectomy [3.6 (0.9; 32.6) months post graft failure and 1.0 (0.0; 8.7) months before the nephrectomy], and after the nephrectomy (median cPRA: 96 (36; 100) at last follow-up, after 50.4 (5.7; 119.9) months post graft failure. Forty-nine (22.5%) patients received another transplantation during the follow-up. We did not observe any difference regarding the different modalities of immunosuppression withdrawal and the occurrence of retransplantation (retransplantation according to immunosuppression withdrawal modalities except steroids, withdrawal <1 month as the reference group, HR = 1.32, 95% CI [0.93; 1.87], p = 0.19; retransplantation according to steroid withdrawal modality [withdrawal <3 months as the reference group, HR = 0.82, 95% CI (0.47; 1.43), p = 0.99]).

Infection and Cardiovascular Complications

Sixty-three out of the 218 patients (28.9%) experienced at least one episode of infection requiring hospitalization during the follow-up, (19 of the 63 (30.2%) occurred in patients that were still under immunosuppression). Among them, 20 patients (31.7%) required an intensive care unit hospitalization at least once. Twelve patients (including 5 who were still under immunosuppression) developed at least one opportunistic infection [cytomegalovirus syndrome (n = 6), HSV-2 viremia (n = 1), invasive pulmonary aspergillosis (n = 1) and invasive non-aspergillosis mold (n = 1), candidemia (n = 2), cryptosporidiosis induced diarrhea (n = 1)]. We did not find any difference regarding the type and length of immunosuppression after graft failure and the occurrence of infectious complications (**Figures 4A,B**; **Supplementary Table 4**).



Steroids modality withdrawal	HR	95% CI [lower; upper]	p
more than 3 months vs less than 3 months	0.41	0.24 – 0.71	0.001

FIGURE 2 | Occurrence of *de novo*-Donor Specific Antibodies according to the timing of steroid withdrawal. Patient survival free from *de novo* DSA after graft failure. The p-value was tested the log-rank test. Association between the steroid withdrawal modality after graft failure and *de novo* DSA occurrence. The association was tested by using a univariate Cox proportional hazards model.

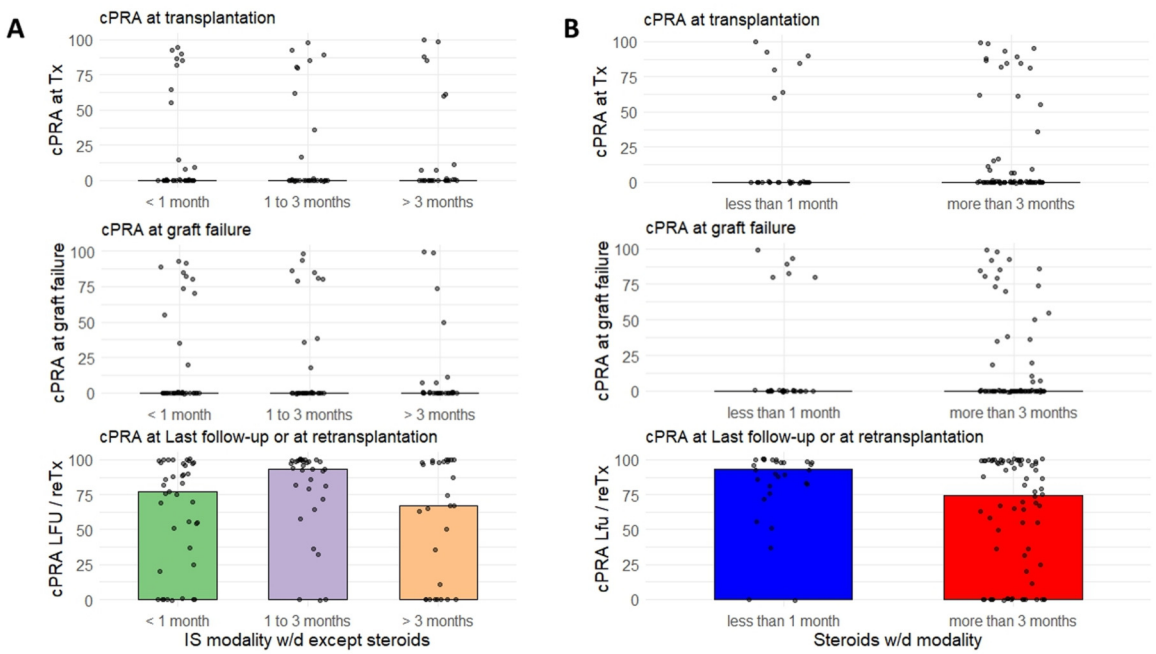


FIGURE 3 | Evolution of the cPRA at transplantation, graft failure and last-follow-up, according to **(A)** immunosuppression withdrawal modality except steroids, and **(B)** steroid withdrawal modality. Abbreviation: cPRA, calculated Panel Reactive Antibodies; Tx, Transplantation; LFU, Last Follow-up; reTx, re-Transplantation; w/d, withdrawal.

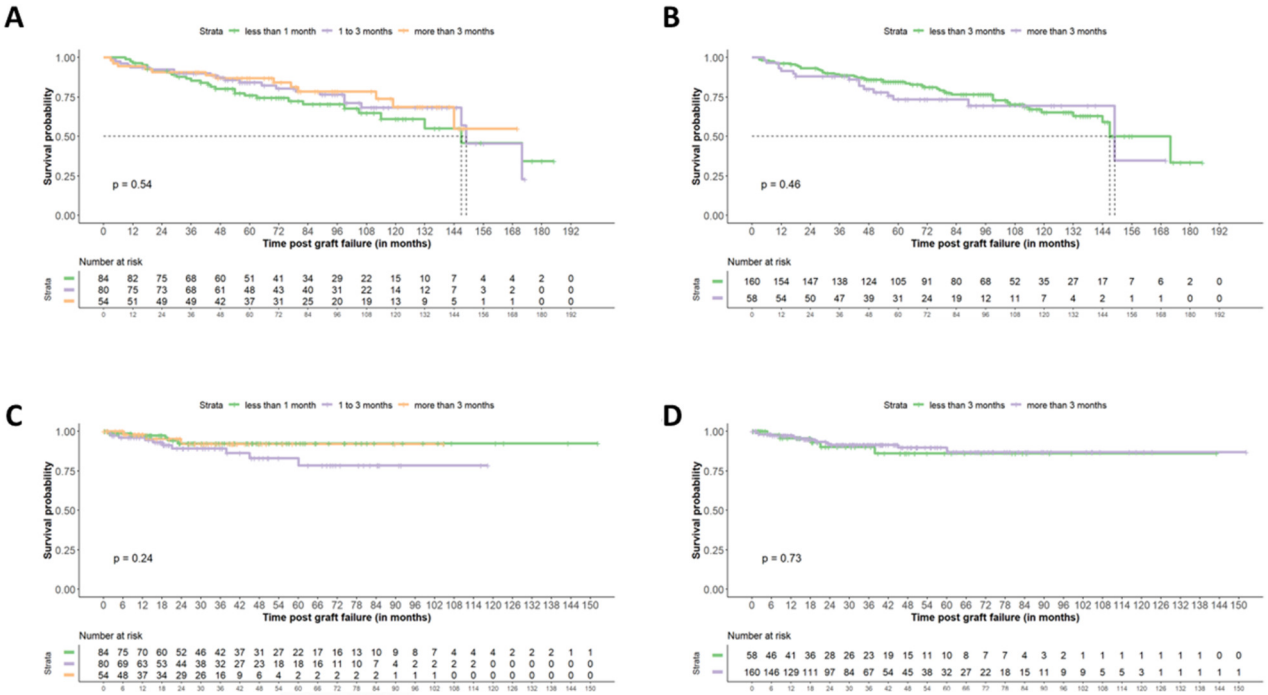


FIGURE 4 | Occurrence of infections and Major Adverse Cardiovascular Events (MACE) according to immunosuppression withdrawal **(A)**. Patient survival free from infection requiring a hospitalization after graft failure. The p-value was tested the log-rank test. **(B)** Patient survival free from infection requiring a hospitalization after graft failure according to the steroid withdrawal modality. **(C)** Patient survival free from MACE requiring an hospitalization after graft failure. The p-value was tested the log-rank test. **(D)** Patient survival free from MACE requiring an hospitalization after graft failure according to the steroid withdrawal modality.

Eighteen patients (8.3%) experienced at least one MACE during the follow-up (**Supplementary Table 5**). We did not find any difference regarding the occurrence of MACE and type and length of immunosuppression during dialysis (**Figures 4C,D**).

Fifty-nine patients (27.0%) died during the follow-up (**Supplementary Figure 1A,B**; **Supplementary Table 6**). We did not observe any difference regarding different modalities of immunosuppression withdrawal and death (death according to immunosuppression withdrawal modalities (except steroids): <1 month: 30/84, 1–3 months 16/80, more than 3 months 13/54, $p = 0.07$; death according to steroid withdrawal modality: <3 months, 15/58, >3 months 44/160, $p = 0.81$).

DISCUSSION

Although patients with a failing graft represent an increasing proportion among the dialysis population [27], return to dialysis remains a critical period. At that time, the management of immunosuppression is quite complex. Indeed, maintaining immunosuppression is considered to be risky because of the limited immediate expected benefit compared with potential complications. A higher risk for cardiovascular and infection disorders have been reported, leading to an increased risk of death compared to patients with poor allograft function, or incident dialysis patients without a history of transplantation [31–35]. However, clinicians may be prompted to pursue immunosuppressants in order to reduce the risk of allo-sensitization. Our study is in line with previous studies and shows that the risk of allo-sensitization is not very high during the graft functioning period [5, 6] and as long as the patient remains on immunosuppression [19], while returning to dialysis after immunosuppression withdrawal remains a high risk period when allo-sensitization occurs [7–9, 11, 17, 36–38], that represents a significant limitation for future transplantation. Moreover, intolerance syndrome, that is related to immunosuppressants' withdrawal, is a major complication that could lead to the need for allograft nephrectomy or arterial embolization that may induce other complications. Available guidelines to manage immunosuppression in those patients remain elusive [10, 26].

In the present retrospective study, we assessed the impact of different strategies of immunosuppression withdrawal on the occurrence of intolerance syndrome. The incidence of intolerance syndrome in our cohort was 24.3%, and occurred early after graft failure (median 6 (3; 13) months post-graft failure), which is concordant with previous studies [7, 11, 39]. We noted a strong association between immunosuppression withdrawal and intolerance syndrome occurrence. However, we did not observe any difference in the incidence or the timing of intolerance syndrome or anti-HLA sensitization after graft failure and the modality of immunosuppression withdrawal, except for steroid management

which was associated with a delay in the occurrence of this complication. This is in line with previous retrospective [23, 40, 41] and prospective studies [19, 42]. How to handle steroids after kidney graft failure is often not described and not considered in the strategy of management of immunosuppression in this critical period [10, 27]. However, our study suggest that the impact of low-dose steroids in this setting could be non-zero. Indeed, we found that patients who stopped their steroids during the first 3 months could present an intolerance syndrome earlier, and independently of the way of management of other immunosuppressants, i.e., CNIs, mTOR inhibitors and MPA. In a retrospective study of 89 patients who returned to dialysis, Garg and colleagues found that steroid continuation was associated with significantly lower odds of developing an absolute increase of allo-sensitization [43]. Low-dose steroids alter T-cell and antibody mediated responses [44–46]. Discontinuation of low-dose steroids was previously associated with immune reconstitution inflammatory syndrome after a prolonged course of treatment [47]. Taken together, these observations suggest that (i) reduced doses, without monitoring drug dose levels, for a limited period (i.e., not prolonged until retransplantation) is inefficient to prevent allo-sensitization or intolerance syndrome after graft failure, and (ii) in cases where early immunosuppression discontinuation is proposed, a brutal withdrawal of steroids could participate in the development of intolerance syndrome and allo-sensitization complications. Nonetheless, these findings should be interpreted as exploratory. Unmeasured factors not included in our analysis may have contributed to early steroid discontinuation and could therefore have introduced residual confounding. Validation of these results in future external cohorts will be important to confirm their generalizability. We didn't find an impact of a gradual maintenance immunosuppression withdrawal on the rate of infections requiring hospitalization, opportunistic infections, and cardiovascular complications in dialysis patients during follow-up. This could be explained by the low doses of CNIs/antimetabolites and early drug discontinuation. Some [23, 40], but not all [12], retrospective studies did not observe a higher risk for infections or MACE in the same seting. However, other adverse effects (e.g., hypertension, dyslipidemia, diabetes, etc...) that alter the long-term cardiovascular health should be kept in mind.

Our study presents several limitations. This is a retrospective, monocentric study. Immunosuppressants' doses and trough levels were not assessed. The number of intolerance syndromes included was small, limiting our ability to included multiple parameters in our multivariate analyses. Nonetheless, this helped us to obtain a granular assessment of immunosuppression management in these patients, particularly regarding the management of steroids. These results could guide the development of future large prospective clinical study.

In conclusion, although discontinuation of immunosuppression strongly influences the occurrence of intolerance syndrome, the modality of immunosuppression withdrawal according to available recommendations in patients with a failed graft do not prevent the occurrence of intolerance syndrome, or allo-sensitization. Only a brutal, early (<3 months) withdrawal of steroids seems to reduce the time between graft failure and the development of intolerance syndrome. Our data suggest further evaluation of the impact of current expert's opinion-based guidelines.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: Available upon request. Requests to access these datasets should be directed to AD, delbello.a@chu-toulouse.fr.

ETHICS STATEMENT

This retrospective study obtained approval from the Toulouse IRB (RC31/21/01/54). 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. Written informed consent was not obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article. According to the French law (Loi Jardé) this retrospective study obtained approval from the Toulouse IRB (RC31/ 21/01/54).

AUTHOR CONTRIBUTIONS

AD and NK designed the study, performed statistical analyses, and wrote the paper. AN collected the data. PG, NC-J, JM, OM, AD, and TP followed the patients, reviewed and completed the paper. All authors contributed to the article and approved the submitted version.

FUNDING

The author(s) declared that financial support was not received for this work and/or its publication.

CONFLICT OF INTEREST

The authors(s) declared that this work 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) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2026.15642/full#supplementary-material

REFERENCES

1. Loupy A, Lefaucheur C. Antibody-Mediated Rejection of Solid-Organ Allografts. *New England Journal of Medicine. Massachussetts Med Soc* (2018) 379(12):1150–60. doi:10.1056/NEJMra1802677
2. Lentine KL, Smith JM, Hart A, Miller J, Skeans MA, Larkin L, et al. OPTN/SRTR 2020 Annual Data Report: Kidney. *Am J Transplant* (2022) 22:21–136. doi:10.1111/ajt.16982
3. Lentine KL, Smith JM, Lyden GR, Miller JM, Dolan TG, Bradbrook K, et al. OPTN/SRTR 2022 Annual Data Report: Kidney. *Am J Transplant* (2024) 24: S19–S118. doi:10.1016/j.ajt.2024.01.012
4. Agence De La Biomédecine. (2025). Available online at: https://rams.agence-biomedecine.fr/ (Accessed April 3, 2025).
5. Wiebe C, Balshaw R, Gibson IW, Ho J, Shaw J, Karpinski M, et al. A Rational Approach to Guide Cost-Effective De Novo Donor-Specific Antibody Surveillance with Tacrolimus Immunosuppression. *Am J Transpl* (2023) 23(12):1882–92. doi:10.1016/J.AJT.2023.07.025
6. Salhi S, Congy-Jolivet N, Hebral AL, Esposito L, Vieu G, Milhès J, et al. Utility of Routine Post Kidney Transplant Anti-HLA Antibody Screening. *Kidney Int Rep* (2024) 9(5):1343–53. doi:10.1016/J.EKIR.2024.02.1394
7. Augustine JJ, Woodside KJ, Padiyar A, Sanchez EQ, Hricik DE, Schulak JA. Independent of Nephrectomy, Weaning Immunosuppression Leads to Late Sensitization After Kidney Transplant Failure. *Transplantation* (2012) 94(7): 738–43. doi:10.1097/TP.0B013E3182612921
8. Billen EVA, Christiaans MHL, Lee J, Van Den Berg-Loonen EM. Donor-Directed HLA Antibodies Before and After Transplantectomy Detected by the Luminex Single Antigen Assay. *Transplantation* (2009) 87(4):563–9. doi:10.1097/TP.0B013E3181949E37
9. Del Bello A, Congy-Jolivet N, Sallusto F, Guilbeau-Frugier C, Cardeau-Desangles I, Fort M, et al. Donor-Specific Antibodies After Ceasing Immunosuppressive Therapy, with or Without an Allograft Nephrectomy. *Clin J Am Soc Nephrol* (2012) 7(8):1310–9. doi:10.2215/CJN.00260112
10. Andrews PA, Standards Committee of the British Transplantation Society. Summary of the British Transplantation Society Guidelines for Management of the Failing Kidney Transplant. *Transplantation* (2014) 98(11):1130–3. doi:10.1097/TP.0000000000000426
11. Johnston O, Rose C, Landsberg D, Gourlay WA, Gill JS. Nephrectomy After Transplant Failure: Current Practice and Outcomes. *Am J Transplant* (2007) 7(8):1961–7. doi:10.1111/j.1600-6143.2007.01884.x
12. Woodside KJ, Schirm ZW, Noon KA, Huml AM, Padiyar A, Sanchez EQ, et al. Fever, Infection, and Rejection After Kidney Transplant Failure. *Transplantation* (2014) 97(6):648–53. doi:10.1097/01.TP.0000437558.75574.9C
13. Pham PT, Pham PC. Immunosuppressive Management of Dialysis Patients with Recently Failed Transplants. *Semin Dial* (2011) 24(3):307–13. doi:10.1111/j.1525-139X.2011.00864.x
14. Bunthof KLW, Verhoeks CM, van den Brand JAJG, Hilbrands LB. Graft Intolerance Syndrome Requiring Graft Nephrectomy After Late Kidney Graft Failure: Can It Be Predicted? *A Retrospective Cohort Study. Transpl Int* (2018) 31(2):220–9. doi:10.1111/tri.13088
15. Budhiraja P, Nguyen M, Heilman R, Kaplan B. The Role of Allograft Nephrectomy in the Failing Kidney Transplant. *Transplantation* (2023) 107(12):2486–96. doi:10.1097/TP.0000000000004625
16. Alberts VP, Minnee RC, Bemelman FJ, van Donselaar-van der Pant KAMI, Idu MM. Transplant Nephrectomy: What Are the Surgical Risks? *Ann Transpl* (2013) 18(1):174–81. doi:10.12659/AOT.883887
17. Lucisano G, Brookes P, Santos-Nunez E, Firmin N, Gunby N, Hassan S, et al. Allosensitization After Transplant Failure: The Role of Graft Nephrectomy and Immunosuppression – A Retrospective Study. *Transpl Int* (2019) 32(9):949–59. doi:10.1111/tri.13442
18. Yanik EL, Clarke CA, Snyder JJ, Pfeiffer RM, Engels EA. Variation in Cancer Incidence Among Patients With Esrd During Kidney Function and Nonfunction Intervals. *J Am Soc Nephrol* (2016) 27(5):1495–504. doi:10.1681/ASN.2015040373
19. Knoll G, Campbell P, Chasse M, Fergusson D, Ramsay T, Karnabi P, et al. Immunosuppressant Medication Use in Patients With Kidney Allograft Failure: A Prospective Multi-Center Canadian Cohort Study. *J Am Soc Nephrol* (2022) 33(6):1182–92. doi:10.1681/ASN.2021121642
20. Smak Gregoor PJH, Zietse R, Van Saase JLCM, op de Hoek CT, Ijzermans JN, Lavrijssen AT, et al. Immunosuppression Should Be Stopped in Patients with Renal Allograft Failure. *Clin Transpl* (2001) 15(6):397–401. doi:10.1034/J.1399-0012.2001.150606.X
21. Van Leeuwen MT, Webster AC, McCredie MRE, Stewart JH, McDonald SP, Amin J, et al. Effect of Reduced Immunosuppression After Kidney Transplant Failure on Risk of Cancer: Population Based Retrospective Cohort Study. *BMJ (Online)* (2010) 340(7744):463. doi:10.1136/BMJ.C570
22. Sapir-Pichhadze R, Tinckam KJ, Laupacis A, Logan AG, Beyene J, Kim SJ. Immune Sensitization and Mortality in Wait-Listed Kidney Transplant Candidates. *J Am Soc Nephrol* (2016) 27(2):570–8. doi:10.1681/ASN.2014090894
23. Martin K, Cantwell L, Barraclough KA, Lian M, Masterson R, Hughes PD, et al. Prolonged Immunosuppression Does Not Improve Risk of Sensitization or Likelihood of Retransplantation After Kidney Transplant Graft Failure. *Transpl Int* (2021) 34(11):2353–62. doi:10.1111/TRI.13998
24. Nimmo AMSA, McIntyre S, Turner DM, Henderson LK, Battle RK. The Impact of Withdrawal of Maintenance Immunosuppression and Graft Nephrectomy on HLA Sensitization and Calculated Chance of Future Transplant. *Transpl Direct* (2018) 4(12):e409. doi:10.1097/TXD.0000000000000848
25. del Moral Cuesta CL, Foz SG, Pereda DG, et al. Immunosuppression with Calcineurin Inhibitor After Renal Transplant Failure Inhibits Allosensitization. *Biomedicines* (2020) 8(4). doi:10.3390/BIOMEDICINES8040072
26. Lubetzky M, Tantisattamo E, Molnar MZ, Lentine KL, Basu A, Parsons RF, et al. The Failing Kidney Allograft: A Review and Recommendations for the Care and Management of a Complex Group of Patients. *Am J Transplant* (2021) 21(9):2937–49. doi:10.1111/AJT.16717
27. Davis S, Mohan S. Managing Patients with Failing Kidney Allograft Many Questions Remain. *Clin J Am Soc Nephrol* (2022) 17(3):444–51. doi:10.2215/CJN.14620920
28. Fishman JA. Infection in solid-organ Transplant Recipients. *N Engl J Med* (2007) 357(25):2601–14. doi:10.1056/NEJMRA064928
29. Roberts MB, Fishman JA. Immunosuppressive Agents and Infectious Risk in Transplantation: Managing the "Net State of Immunosuppression.". *Clin Infect Dis* (2021) 73(7):E1302–E1317. doi:10.1093/CID/CIAA1189
30. Botto F, Alonso-Coello P, Chan MTV, Villar JC, Xavier D, Srinathan S, et al. Myocardial Injury After Noncardiac Surgery: A Large, International, Prospective Cohort Study Establishing Diagnostic Criteria, Characteristics, Predictors, and 30-day Outcomes. *Anesthesiology* (2014) 120(3):564–78. doi:10.1097/ALN.0000000000000113
31. Fuquay R, Teitelbaum I. Care of the Patient After Renal Allograft Failure: Managing the Present and Planning for the Future. *Am J Nephrol* (2012) 36(4): 348–54. doi:10.1159/000342626
32. Weiner DE, Carpenter MA, Levey AS, Ivanova A, Cole EH, Hunsicker L, et al. Kidney Function and Risk of Cardiovascular Disease and Mortality in Kidney Transplant Recipients: The FAVORIT Trial. *Am J Transplant* (2012) 12(9): 2437–45. doi:10.1111/j.1600-6143.2012.04101.x
33. Rao PS, Schaubel DE, Jia X, Li S, Port FK, Saran R. Survival on Dialysis Post-kidney Transplant Failure: Results from the Scientific Registry of Transplant

Recipients. *Am J Kidney Dis* (2007) 49(2):294–300. doi:10.1053/j.ajkd.2006.11.022
34. Perl J, Zhang J, Gillespie B, Wikström B, Fort J, Hasegawa T, et al. Reduced Survival and Quality of Life Following Return to Dialysis After Transplant Failure: The Dialysis Outcomes and Practice Patterns Study. *Nephrol Dial Transplant* (2012) 27(12):4464–72. doi:10.1093/NDT/GFS386
35. Kabani R, Quinn RR, Palmer S, Lewin AM, Yilmaz S, Tibbles LA, et al. Risk of Death Following Kidney Allograft Failure: A Systematic Review and meta-analysis of Cohort Studies. *Nephrol Dial Transplant* (2014) 29(9):1778–86. doi:10.1093/ndt/gfu205
36. Knight MG, Tiong HY, Li J, Pidwell D, Goldfarb D. Transplant Nephrectomy After Allograft Failure Is Associated with Allosensitization. *Urology* (2011) 78(2):314–8. doi:10.1016/j.urology.2011.02.068
37. Kosmoliaptsis V, Gjorgjimajkoska O, Sharples LD, Chaudhry AN, Chatzizacharias N, Peacock S, et al. Impact of Donor Mismatches at Individual HLA-A, -B, -C, -DR, and -DQ Loci on the Development of HLA-specific Antibodies in Patients Listed for Repeat Renal Transplantation. *Kidney Int* (2014) 86(5):1039–48. doi:10.1038/ki.2014.106
38. Marrari M, Duquesnoy RJ. Detection of Donor-Specific HLA Antibodies Before and After Removal of a Rejected Kidney Transplant. *Transpl Immunol* (2010) 22(3-4):105–9. doi:10.1016/J.TRIM.2009.12.005
39. Delgado P, Diaz F, Gonzalez A, Sanchez E, Gutierrez P, Hernandez D, et al. Intolerance Syndrome in Failed Renal Allografts: Incidence and Efficacy of Percutaneous Embolization. *Am J Kidney Dis* (2005) 46(2):339–44. doi:10.1053/j.ajkd.2005.04.024
40. Ferrari K, Aarnink A, Ayav C, Frimat L, Couchoud C, Audry B, et al. Evolution of HLA-Sensitization According to Immunosuppressive Therapy Management Among Kidney Transplant Patients Returning to Dialysis Between 2008 and 2019: A French Retrospective Study. *Clin Transpl* (2024) 38(1):e15160. doi:10.1111/ctr.15160
41. Elgenidy A, Shemies RS, Atef M, Awad AK, El-Leithy HH, Helmy M, et al. Revisiting Maintenance Immunosuppression in Patients with Renal Transplant Failure: Early Weaning of Immunosuppression Versus Prolonged Maintenance—Systematic Review and Meta-Analysis. *J Nephrol* (2023) 36(2):537–50. doi:10.1007/s40620-022-01458-y
42. Leal R, Fragoso P, Venda J, Gomes J, Inácio M, Guedes Marques M, et al. Prolonging Calcineurin Inhibitor Therapy Post Kidney Allograft Failure: A Prospective Study. *Ren Fail* (2025) 47(1):2483386. doi:10.1080/0886022X.2025.2483386
43. Garg N, Viney K, Burger J, Hidalgo L, Parajuli S, Aziz F, et al. Factors Affecting Sensitization Following Kidney Allograft Failure. *Clin Transpl* (2022) 36(3):e14558. doi:10.1111/CTR.14558
44. Fedor ME, Rubinstein A. Effects of Long-Term Low-Dose Corticosteroid Therapy on Humoral Immunity. *Ann Allergy Asthma & Immunol* (2006) 97(1):113–6. doi:10.1016/S1081-1206(10)61380-4
45. Ashwell JD, Lu FWM, Vacchio MS. Glucocorticoids in T Cell Development and Function. *Annu Rev Immunol* (2000) 18:309–45. doi:10.1146/ANNUREV.IMMUNOL.18.1.309
46. Tokunaga A, Sugiyama D, Maeda Y, Warner AB, Panageas KS, Ito S, et al. Selective Inhibition of Low-affinity Memory CD8+ T Cells by Corticosteroids. *J Exp Med* (2019) 216(12):2701–13. doi:10.1084/JEM.20190738
47. Batool N, Song D, Almas T, Alsubai AK, Thakur T, Ismail H, et al. Too Much of a Good Thing: Immune Reconstitution Inflammatory Syndrome in a Patient with Still's Disease. *Ann Med Surg* (2022) 82:104590. doi:10.1016/J.AMSU.2022.104590

ESOT | Transplant International

ORIGINAL RESEARCH
published: 18 February 2026
doi: 10.3389/ti.2026.16021

Long-Term Renal Outcomes Following Left Renal Vein Ligation Versus Direct Splenorenal Shunt Ligation in Living Donor Liver Transplantation: A 10-Year Single-Center Study

Woo-Hyoung Kang, Deok-Bog Moon, Shin Hwang, Ki-Hun Kim, Chul-Soo Ahn, Tae-Yong Ha, Gi-Won Song, Dong-Hwan Jung, Gil-Chun Park, Young-In Yoon, Byeong-Gon Na, Sang-Hoon Kim, Sung-Min Kim and Sung-Gyu Lee*

Division of Liver Transplantation and Hepatobiliary Surgery, Department of Surgery, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea

In living donor liver transplantation (LDLT), large splenorenal shunts (SRS) can divert portal inflow and negatively affect graft function due to portal steal syndrome. Direct SRS ligation (SRSL) and left renal vein ligation (LRVL) are used to prevent this complication; however, the long-term renal impact of LRVL remains unclear, particularly in recipients requiring nephrotoxic immunosuppression. We retrospectively analyzed adult LDLT recipients with large SRS (>1 cm) and normal baseline renal function who underwent SRSL (n = 120) or LRVL (n = 74). Patient and graft survival, serial renal function profiles, and tacrolimus trough levels were evaluated. Survival outcomes were comparable between the two groups. LRVL was more frequently performed in patients with higher preoperative Model for End-Stage Liver Disease (MELD) scores or increased transfusion requirements. During long-term follow-up, the LRVL group showed a more evident decline in renal function, with persistently higher serum creatinine levels, despite similar tacrolimus exposure. Four recipients in the LRVL group progressed to end-stage renal disease requiring dialysis within 10 years, whereas no dialysis cases occurred following SRSL. Although both strategies are clinically feasible, LRVL demonstrated a stronger association with progressive renal deterioration. These findings suggest that SRSL may be preferred in recipients with renal vulnerability to minimize cumulative renal burden.

Keywords: end-stage liver disease, left renal vein ligation, living donor liver transplantation, nephrotoxicity, splenorenal shunt ligation

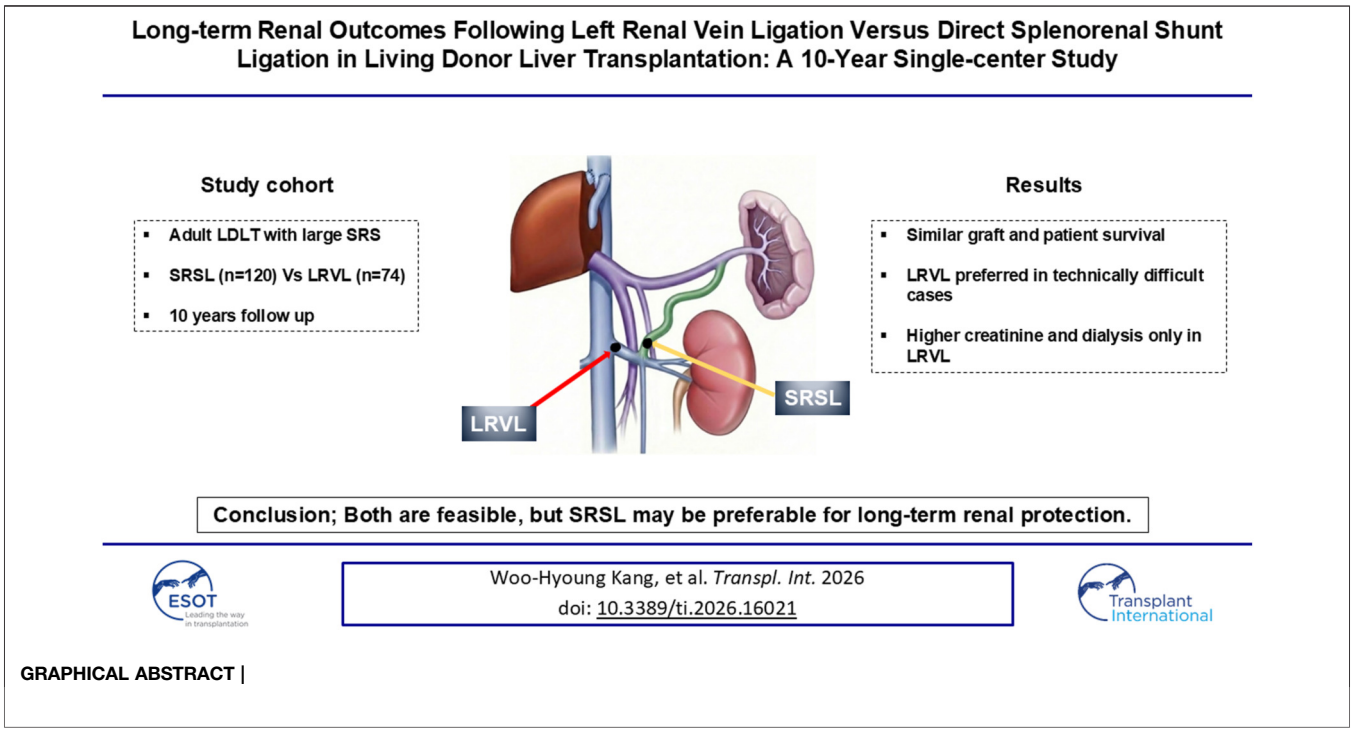
OPEN ACCESS

***Correspondence**
Deok-Bog Moon,
mdb1@amc.seoul.kr

Received: *09 December 2025*
Revised: *31 January 2026*
Accepted: *02 February 2026*
Published: *18 February 2026*

Citation:
Kang W-H, Moon D-B, Hwang S, Kim K-H, Ahn C-S, Ha T-Y, Song G-W, Jung D-H, Park G-C, Yoon Y-I, Na B-G, Kim S-H, Kim S-M and Lee S-G (2026) Long-Term Renal Outcomes Following Left Renal Vein Ligation Versus Direct Splenorenal Shunt Ligation in Living Donor Liver Transplantation: A 10-Year Single-Center Study. Transpl. Int. 39:16021. doi: 10.3389/ti.2026.16021

Abbreviations: MELD, Model for End-Stage Liver Disease; LDLT, living donor liver transplantation; LRVL, left renal vein ligation; LRV, left renal vein; SRS, splenorenal shunt; SRSL, splenorenal shunt ligation.



GRAPHICAL ABSTRACT |

INTRODUCTION

In patients with end-stage liver disease, portal hypertension frequently leads to the formation of spontaneous portosystemic shunts, including coronary, periumbilical, and splenorenal shunts (SRS). Among these, large SRSs can divert portal blood flow away from the liver graft after living donor liver transplantation (LDLT), resulting in portal steal syndrome. This condition may compromise graft regeneration and function, potentially leading to graft failure. Therefore, intraoperative interruption of large shunts is essential to ensure adequate portal inflow and graft survival [1–3].

The following two major surgical approaches are used to interrupt SRSs: Direct splenorenal shunt ligation (SRSL) and left renal vein ligation (LRVL) [4, 5]. SRSL involves anatomically precise identification and ligation of the shunt vessel itself, but often present technical challenges during the procedure (particularly in patients with severe adhesions or complex vascular anatomy) and carries a risk of bleeding from surrounding tissues or from the shunt itself. In contrast, LRVL achieves functional interruption of the SRS by ligating the left renal vein (LRV), and is often preferred due to its simplicity and ease of access.

Our institution previously reported the safety and feasibility of LRVL in LDLT, and several subsequent studies have confirmed its effectiveness in preventing portal steal syndrome and maintaining stable portal perfusion [1, 4]. However, concerns persist regarding the potential impact of LRVL on renal function. Because the LRV is the major venous drainage pathway for the left kidney, its ligation may lead to venous congestion and impaired renal perfusion. Although previous nontransplant studies have suggested that LRV ligation has minimal long-term effects on renal function—because collateral pathways such as the left gonadal vein can provide sufficient outflow—the applicability of these findings to transplant recipients remains to be evaluated [6–9].

To date, most studies on LRVL in LDLT have focused on surgical feasibility and short-term safety, with few addressing long-term renal outcomes. Moreover, there is a lack of direct comparisons between SRSL and LRVL in the transplant setting, especially using methodologies that adjust for baseline differences in renal function and comorbidities.

We aimed to compare the long-term outcomes of patients undergoing either LRVL or SRSL during LDLT, focusing on graft-related endpoints such as shunt recanalization, graft failure, and patient survival, as well as changes in renal function. To account for baseline differences, propensity score matching was applied, and renal function was assessed over a 10-year follow-up period in a single-center setting using a consistent immunosuppressive protocol.

MATERIALS AND METHODS

Study Design and Patient Selection

This retrospective single-center cohort study was conducted at Asan Medical Center and included adult patients who underwent LDLT between January 2009 and December 2015. Among 267 patients with spontaneous SRSs >1 cm in diameter and preserved renal function (serum creatinine ≤1.4 mg/dL), 194 were selected for analysis

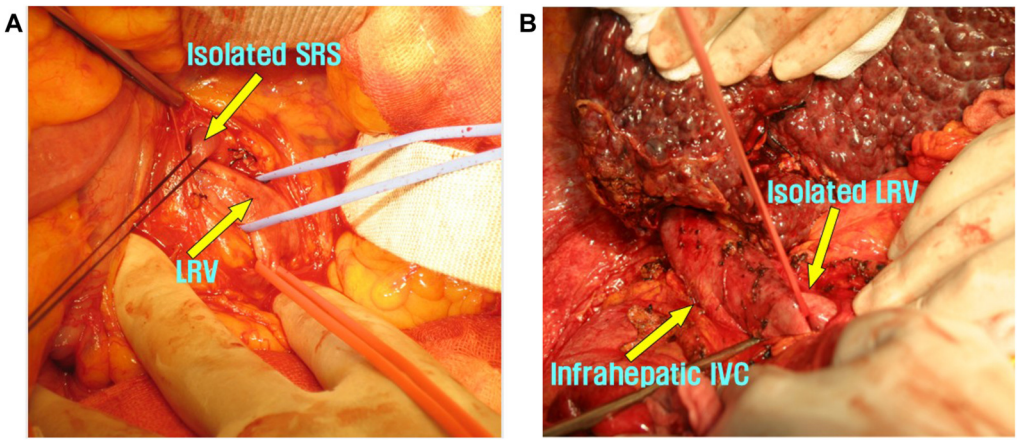


FIGURE 1 | Representative illustrations of the available techniques to prevent splenorenal shunt (SRS). **(A)** Isolation of the splenorenal shunt for direct ligation (SRSL). **(B)** Isolation of the left renal vein after Kocherization for left renal vein ligation (LRVL).

based on having undergone one of the two intraoperative shunt interruption techniques: LRVL (n = 74) or SRSL (n = 120). The remaining 73 patients were excluded because they had received alternative treatments for SRS—such as selective embolization, plug-assisted retrograde transvenous obliteration, or proximal splenic vein embolization [5]—or were managed with immunosuppressive regimens other than tacrolimus.

Surgical Techniques

The method for SRS interruption was determined intraoperatively based on a comprehensive assessment of anatomical feasibility, severity of adhesions, complexity of collateral circulation, and hemodynamic stability. Portal hemodynamics were primarily evaluated using real-time cine-portography. Portal pressure was not routinely measured. The presence of portal flow diversion through collateral vessels, as well as the velocity and direction of portal flow, was directly visualized on cine-portography. Restoration of hepatopetal flow after ligation was confirmed jointly by the transplant surgeon and the interventional radiologist. In all cases, intraoperative portography was performed to verify adequate portal inflow and confirm the absence of residual portal steal [10].

Dissection of the SRS or the LRV was carried out either during total hepatectomy or after graft implantation. However, ligation was typically performed after portal reperfusion to avoid portal hypertension and ensure adequate graft inflow.

In the SRSL group, patients with a single collateral draining into the LRV underwent direct ligation of the shunt. The mesocolon was dissected to expose the LRV near the ligament of Treitz or the inferior mesenteric vein. The shunt was then isolated circumferentially and ligated using thick silk ties or vascular nylon tape (**Figure 1A**). In the LRVL group, Kocherization was performed to mobilize the duodenum and mesocolon from the inferior vena cava, thereby exposing the LRV along its left side. After careful dissection, the LRV was completely encircled and fully ligated (**Figure 1B**). Compared with SRSL, this approach was technically simpler and provided a safer access route with lower risk of injury to adjacent vessels.

Study Objectives and Endpoints

This study had two predefined objectives. The primary objective was to compare the long-term clinical outcomes of the two surgical approaches, including patient mortality, graft failure or need for retransplantation, and shunt recannulation. The secondary objective was to evaluate the differential effects of SRSL and LRVL on long-term renal function following a consistent immunosuppressive regimen.

Renal Function and Immunosuppression Assessment

All patients had normal preoperative renal function and no history of kidney disease. Renal function was assessed using serum creatinine levels at postoperative intervals of 1 week, 1 month, 6 months, and 1, 3, 5, and 10 years. We also documented whether any patients required initiation of dialysis during the follow-up period. Patients in both groups received tacrolimus-based immunosuppression, and tacrolimus trough levels were analyzed as a supportive indicator to ensure comparable immunosuppressive exposure between the two groups. When tacrolimus dose reduction was required because of nephrotoxicity, mycophenolate mofetil or other non-nephrotoxic agents were added according to standard institutional protocols to maintain adequate immunosuppression.

Statistical Analysis

To minimize baseline imbalances between the two groups and control for potential confounders affecting renal function, propensity score matching was performed. Matched variables included age, sex, graft-to-recipient weight ratio, Model for End-Stage Liver Disease (MELD) score, presence of diabetes mellitus and hypertension, preoperative serum creatinine, and

TABLE 1 | Baseline cohort characteristics.

Characteristic	Category	SRS ligation	LRV ligation	p-value	SMD
		120	74		
Sex (%)	Male	81 (67.5)	50 (67.6)	1.000	0.001
	Female	39 (32.5)	24 (32.4)		
Age (mean [SD])		52.33 (6.96)	53.00 (6.19)	0.500	0.101
Original disease (%)	HBV	83 (69.2)	51 (68.9)	0.661	0.287
	HCV	6 (5.0)	2 (2.7)		
	ALD	17 (14.2)	11 (14.9)		
	PSC	1 (0.8)	0 (0.0)		
	AI	2 (1.7)	0 (0.0)		
	Other	11 (9.2)	10 (13.5)		
GRWR (mean [SD])		1.17 (0.28)	1.14 (0.25)	0.437	0.117
MELD (mean [SD])		14.21 (6.27)	17.73 (5.98)	<0.001	0.575
DM (%)	No	93 (77.5)	52 (70.3)	0.339	0.165
	Yes	27 (22.5)	22 (29.7)		
HTN (%)	No	104 (86.7)	64 (86.5)	1.000	0.005
	Yes	16 (13.3)	10 (13.5)		
Preoperative serum creatinine (mean [SD])		0.72 (0.23)	0.72 (0.20)	0.882	0.022
Intraoperative RBC transfusion, unit (mean [SD])		7.85 (7.06)	15.36 (10.66)	<0.001	0.831

HBV, hepatitis B virus; HCV, hepatitis C virus; ALD, alcoholic liver disease; PSC, primary sclerosing cholangitis; AI, autoimmune hepatitis; GRWR, graft-to-recipient weight ratio; MELD, model for end-stage liver disease; DM, diabetes mellitus; HTN, hypertension; Cr, creatinine; RBC, red blood cell; SMD, standardized mean difference; SRS, splenorenal shunt; LRV, left renal vein.

intraoperative red blood cell transfusion volume [11]. One-to-one nearest-neighbor matching with a caliper of 0.2 was applied. After matching, balance between the groups was assessed using standardized mean differences. Because procedural selection was influenced by intraoperative findings and surgeon judgment, we included MELD score and intraoperative RBC transfusion volume as pragmatic surrogate indicators of overall surgical complexity and physiological instability. Although prior abdominal surgery and previous episodes of spontaneous bacterial peritonitis may partially reflect surgical difficulty, their impact is variable and difficult to quantify objectively. Surgeon-related factors could not be quantified and therefore could not be directly adjusted for.

For the longitudinal assessment of renal function, both fixed-effects models and linear mixed-effects models were used to account for repeated measurements over time. The postoperative follow-up period was divided into an early (0–1 years), an intermediate (1–5 years), and a late (5–10 years) phase, and temporal changes in creatinine levels as well as group-by-time interactions were evaluated accordingly. Period-specific changes in creatinine levels (ΔCr) were also analyzed to compare long-term renal trajectories between the LRVL and SRSL groups. Tacrolimus trough levels were analyzed using similar phase-specific fixed-effects models to evaluate differences in immunosuppression tapering patterns between the groups. Patient and graft survival were analyzed using the Kaplan–Meier method, and between-group differences were assessed with the log-rank test. Continuous variables were compared using Student's t-test or the Mann–Whitney U test, while categorical variables were analyzed using the chi-square test or Fisher's exact test, as appropriate. All statistical analyses were performed under the guidance of a medical statistician from our institution to ensure appropriate model selection and methodological validity.

This study was conducted with approval from the Institutional Review Board of Asan Medical Center (IRB No. AMC 2022-0763).

RESULTS

Baseline Characteristics of the Original Cohort

A total of 194 adult LDLT recipients with large spontaneous SRSs were included in the analysis, comprising 120 patients in the SRSL group and 74 in the LRVL group. Most baseline characteristics were comparable between the two groups. The mean age was similar (52.3 vs. 53.0 years, $p = 0.500$), and the sex distribution was nearly identical (male participants: 67.5% vs. 67.6%). The presence of diabetes mellitus and hypertension, the primary liver disease patterns, the graft-to-recipient weight ratio, and the preoperative serum creatinine levels were also well balanced (**Table 1**).

Two variables demonstrated significant differences: The LRVL group exhibited a higher mean MELD score (17.7 ± 6.0 vs. 14.2 ± 6.3, $p < 0.001$), suggesting increased disease severity at transplantation. Additionally, intraoperative red blood cell transfusion volumes were noticeably higher in the LRVL group (15.4 ± 10.7 vs. 7.9 ± 7.1 units, $p < 0.001$). These findings suggest that LRVL was more frequently selected in surgically complex or hemodynamically challenging situations, rather than being applied uniformly across the cohort.

Baseline Characteristics After Propensity Score Matching

To reduce potential confounding from preoperative or intraoperative variables that may influence long-term renal outcomes, 1:1 propensity score matching was performed using age, sex, MELD score, graft-to-recipient weight ratio, presence of

TABLE 2 | Baseline characteristics in matched cohort.

Characteristic	Category	SRS ligation	LRV ligation	p-value	SMD
		58	58		
Sex (%)	Male	41 (70.7)	41 (70.7)	1	<0.001
	Female	17 (29.3)	17 (29.3)		
Age (mean [SD])		53.48 (7.68)	53.47 (5.32)	0.989	0.003
Original disease (%)	HBV	32 (55.2)	45 (77.6)	0.129	0.512
	HCV	4 (6.9)	2 (3.4)		
	ALD	13 (22.4)	6 (10.3)		
	PSC	-	-		
	AI	1 (1.7)	0 (0.0)		
	Other	8 (13.8)	5 (8.6)		
GRWR (mean [SD])		1.15 (0.29)	1.14 (0.26)	0.843	0.037
MELD (mean [SD])		16.93 (7.42)	16.33 (5.13)	0.612	0.095
DM (%)	No	40 (69.0)	39 (67.2)	1	0.037
	Yes	18 (31.0)	19 (32.8)		
HTN (%)	No	51 (87.9)	51 (87.9)	1	<0.001
	Yes	7 (12.1)	7 (12.1)		
Preoperative serum creatinine (mean [SD])		0.74 (0.25)	0.71 (0.19)	0.558	0.109
Intraoperative RBC transfusion, unit (mean [SD])		11.45 (8.07)	11.59 (8.22)	0.928	0.017

HBV, hepatitis B virus; HCV, hepatitis C virus; ALD, alcoholic liver disease; PSC, primary sclerosing cholangitis; AI, autoimmune hepatitis; GRWR, graft-to-recipient weight ratio; MELD, Model For End-Stage Liver Disease; DM, diabetes mellitus; HTN, hypertension; Cr, creatinine; RBC, red blood cell; SMD, standardized mean difference; SRS, splenorenal shunt; LRV, left renal vein.

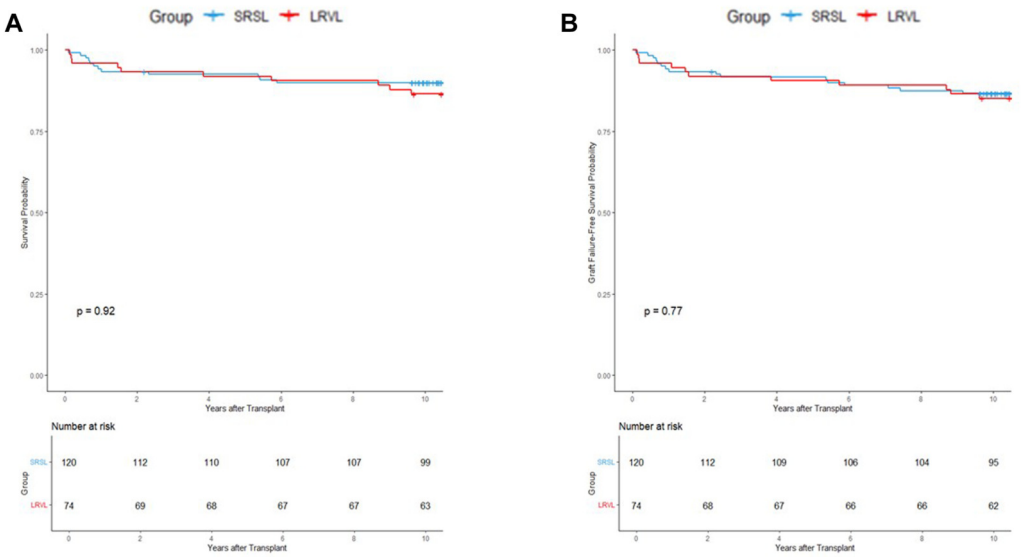


FIGURE 2 | Kaplan–Meier survival curves for the overall cohort showing **(A)** patient survival and **(B)** graft survival. No significant differences were observed between the splenorenal shunt ligation (SRSL) and left renal vein ligation (LRVL) groups (p = 0.92 and 0.77, respectively).

diabetes and hypertension, preoperative serum creatinine levels, and intraoperative RBC transfusion volume. Matching with a caliper of 0.2 yielded 58 well-balanced pairs (n = 116).

After matching, most covariates achieved acceptable balance. Although minor residual imbalance remained in baseline creatinine, this difference was limited and clinically marginal. Overall, the matching process substantially improved comparability between the two groups (**Table 2**).

Clinical Outcomes in the Original and the Matched Cohorts

Patient and Graft Survival

In the original cohort (n = 194), Kaplan–Meier analysis demonstrated no significant difference in patient survival between the SRSL and LRVL groups (p = 0.92; **Figure 2A**). Similarly, graft failure–free survival did not differ significantly (p = 0.77; **Figure 2B**).

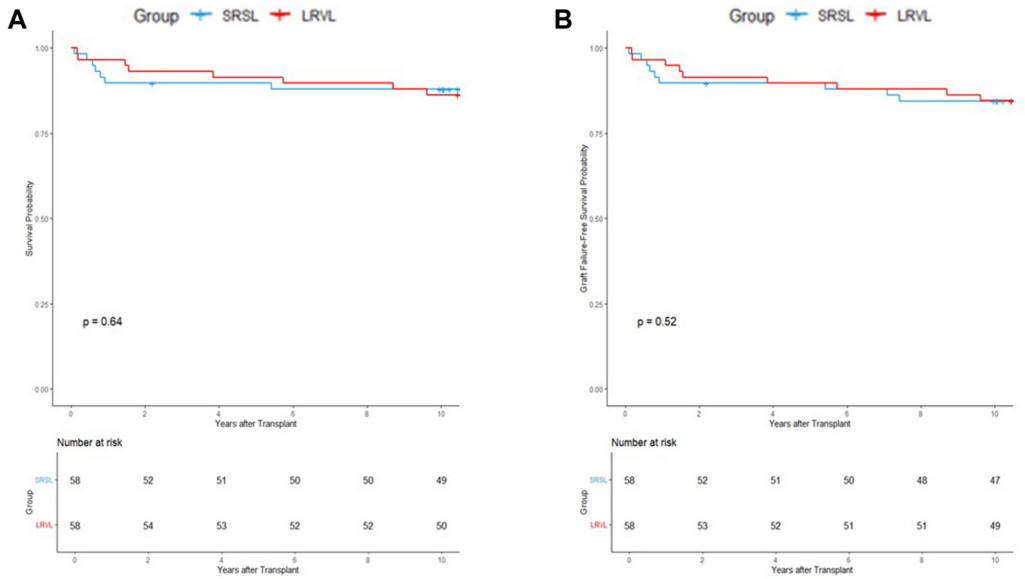


FIGURE 3 | Kaplan–Meier survival curves for the propensity score–matched cohort showing **(A)** patient survival and **(B)** graft survival. No significant differences were observed between the splenorenal shunt ligation (SRSL) and left renal vein ligation (LRVL) groups (p = 0.64 and 0.52, respectively).

The findings were consistent with those from the propensity-matched cohort (n = 116). Neither patient survival (p = 0.64) nor graft survival (p = 0.52) showed intergroup differences (**Figures 3A,B**). These results indicate that both SRS interruption techniques offer comparable long-term survival outcomes.

Post-Transplant Dialysis Requirement

In the original cohort, four patients required chronic dialysis within the 10-year follow-up period, with all cases occurring in the LRVL group (4/74, 5.4%). Notably, each of these patients developed end-stage renal failure during the late postoperative period, between 5 and 10 years after transplantation. This pattern was consistent with that observed in the propensity score–matched cohort, in which all three cases requiring dialysis were likewise confined to the LRVL group, with none observed in the SRSL group.

Beyond the initial 10-year follow-up period, two additional patients in the LRVL group subsequently initiated dialysis at 11 and 14 years after transplantation, respectively (see **Table 3**). This suggests that the cumulative number of patients requiring dialysis is likely to increase further with a longer follow-up period. Taken together, these findings indicate that LRVL may be associated with a late-onset decline in renal function, underscoring the need for long-term renal surveillance in this population.

Shunt Recanalization

Clinically significant shunt recanalization occurred only in the SRSL group (2/120, 1.7%). One case was detected at 1 month and the other at 10 years post-transplantation. Both were managed successfully using plug-assisted retrograde transvenous obliteration, with preserved graft function and stable renal parameters during subsequent follow-up. No recanalization events were identified in the LRVL group.

Long-Term Renal Function Analysis

Temporal Trends in Serum Creatinine

Serum creatinine was analyzed at seven time points: After 1 week, 1 month, 6 months, 1 year, 3 years, 5 years, and 10 years. Both groups maintained generally stable renal function, with mean creatinine values near 1.2 mg/dL across the 10-year follow-up period (**Figures 4A,B**). However, the LRVL group demonstrated consistently higher mean creatinine levels at most time points. At year 10, mean creatinine was 1.11 ± 0.26 mg/dL in the SRSL group and 1.33 ± 1.57 mg/dL in the LRVL group. The large standard deviation in the LRVL group resulted from occasional extreme elevations, including one outlier with creatinine levels as high as 12.0 mg/dL.

Overall, renal function remained stable in most patients, but the LRVL group exhibited a broader range of creatinine variability that included occasional extreme elevations along with a gradual upward trend during long-term follow-up.

Fixed-Effects Modeling

To characterize temporal patterns, the follow-up period was divided into an early (0–6 months), an intermediate (1–5 years), and a late (5–10 years) phase. Overall, serum creatinine increased significantly with time (estimate +0.25, p = 0.018).

Phase-specific changes revealed that baseline creatinine at the start of the intermediate phase was significantly higher than in the early phase (estimate +0.153, p = 0.032), although the subsequent rate of increase slowed down (Time × Phase 1–5 years: −0.283, p = 0.010). A similar pattern was observed in the late phase (−0.216, p = 0.043).

LRVL group assignment was not independently associated with a statistically significant elevation in creatinine (estimate +0.092, p = 0.185). Nonetheless, the LRVL group showed a consistent longitudinal trend toward

TABLE 3 | Clinical characteristics of patients who progressed to dialysis after modulation of preoperative splenorenal shunt.

Patient No.	SRS Modulation	Sex	Etiology	Age (yr)	GRWR	MELD	Intraoperative RBC Transfusion (Units)	Pre-op Cr (mg/dL)	DM	HTN	Time to Dialysis (months)	Survival Status	Remarks
1	LRVL	M	HBV	44	1.05	14	16	0.8	Yes	No	83	Alive	—
2	LRVL	M	HBV	47	0.93	9	1	0.6	Yes	No	115	Alive	—
3	LRVL	M	HBV	47	1.08	24	80	0.7	No	No	95	Dead	Death due to pneumonia
4	LRVL	M	HBV	52	1.50	22	33	0.8	Yes	Yes	167	Alive	Dialysis initiated beyond 10-year follow-up
5	LRVL	M	HCV	41	0.94	21	59	0.6	No	No	95	Dead	Death due to post-retransplant bleeding
6	LRVL	M	HBV	47	1.38	31	15	0.6	Yes	Yes	137	Alive	Dialysis initiated beyond 10-year follow-up

LRVL, left renal vein ligation; HBV, hepatitis B virus; HCV, hepatitis C virus; GRWR, graft-to-recipient weight ratio; MELD, Model for End-Stage Liver Disease; RBC, red blood cell; Cr, creatinine; DM, diabetes mellitus; HTN, hypertension; SRS, splenorenal shunt.

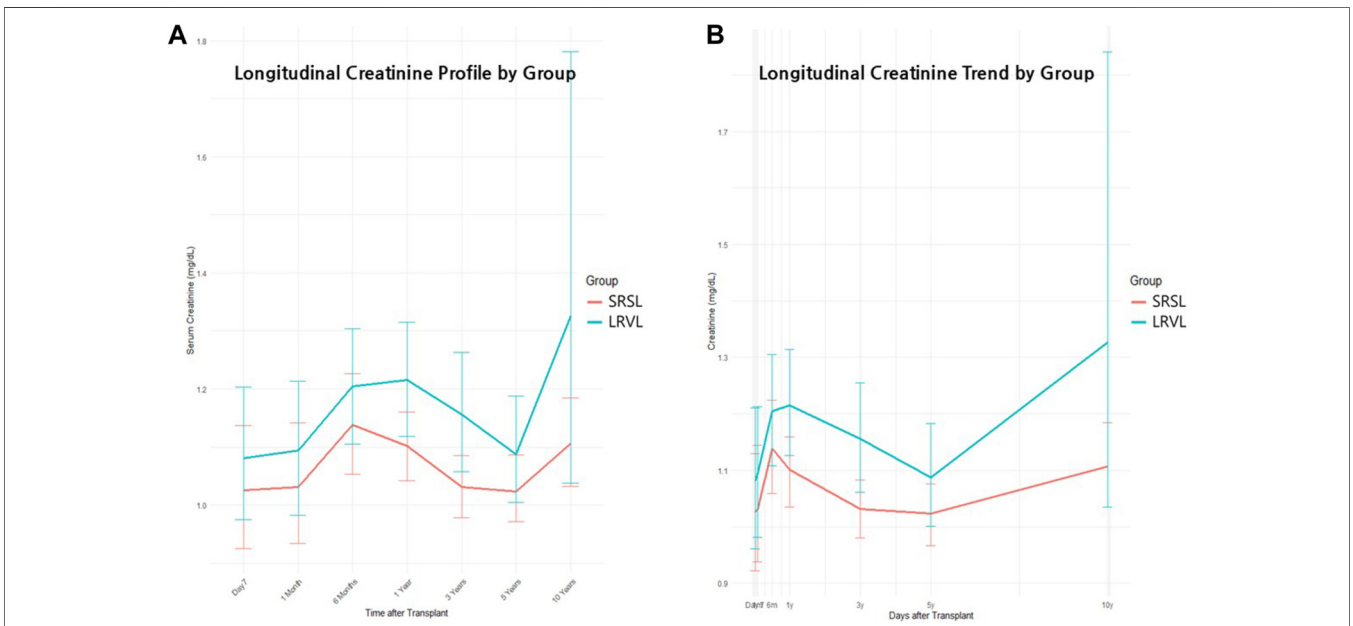


FIGURE 4 | Longitudinal changes in serum creatinine after living donor liver transplantation. **(A)** Longitudinal creatinine profile by group. Mean serum creatinine levels with standard deviations after 1 week, 1 month, 6 months, 1 year, 3 years, 5 years, and 10 years are shown. The left renal vein ligation (LRVL) group (blue) demonstrated consistently higher creatinine values compared to the splenorenal shunt ligation (SRSL) group (red) across most time points. **(B)** Longitudinal trends for creatinine levels in each group. Creatinine trajectories during the same follow-up interval illustrate overall trends in both groups. The LRVL group exhibited a wider range of variability and relatively higher creatinine levels during long-term follow-up, with greater divergence observed at later time points.

higher creatinine values, although this did not reach statistical significance (**Table 4**).

Linear Mixed-Effects Model for Changes in Creatinine

Changes in creatinine (ΔCr) were compared across the three defined phases. Although intergroup differences were not statistically significant in any of the phases, the pattern for each of the phases differed (**Table 5**): During the early phase, the value for the SRSL group was +0.098 mg/dL and that for the LRVL group was +0.147 mg/dL. The increase in the LRVL group approached significance (95% CI: −0.011 to +0.306). During the intermediate phase, both groups showed mild declines (SRSL: −0.080 mg/dL; LRVL: −0.125 mg/dL). Finally, only the LRVL group demonstrated a significant increase in the late phase

TABLE 4 | Fixed-effects analysis of serum creatinine changes over time and by subgroup (SRSL vs. LRVL).

Term	Estimate	Std. Error	df	t value	p-value	Interpretation
Intercept	1.000	0.056	182.88	17.97	<0.001	Baseline Cr at time 0 in the reference phase
Time (per year)	0.250	0.106	644.87	2.37	0.018	Cr increases over time in reference phase
Phase: Year 1–5	0.153	0.071	644.17	2.16	0.032	Higher baseline Cr between intermediate phase vs. early phase
Phase: Year ≥5	–0.151	0.098	643.20	–1.54	0.123	Lower baseline Cr in 5+ years phase (not significant)
Group (LRVL vs. SRSL)	0.092	0.069	110.37	1.33	0.185	Slightly higher Cr in LRVL group (not significant)
Time × phase (Year 1–5)	–0.283	0.110	644.61	–2.58	0.010	Cr rises slower over time between 1 and 5 years
Time × phase (Year ≥5)	–0.216	0.106	644.82	–2.03	0.043	Slower Cr increase in late phase

Cr, serum creatinine; SRSL, splenorenal shunt ligation; LRVL, left renal vein ligation; y, year; df, degrees of freedom; Std., standard.

TABLE 5 | Estimated change in serum creatinine level (ΔCr) across follow-up intervals according to group (SRSL vs. LRVL).

Phase	Group	Estimated ΔCr (mg/dL)	Std. Error	95% CI	Interpretation
Early	SRSL	0.0983	0.0827	[–0.0645, +0.2611]	Slight increase (not significant)
0 → 1 year	LRVL	0.1473	0.0805	[–0.0111, +0.3057]	Slight increase, marginally close to significance
Intermediate	SRSL	–0.0800	0.0844	[–0.2460, +0.0860]	Mild decrease (not significant)
1 → 5 years	LRVL	–0.1250	0.0828	[–0.2878, +0.0379]	Slightly larger decrease, not significant
Late	SRSL	0.0865	0.0870	[–0.0847, +0.2576]	Small increase (not significant)
5 → 10 years	LRVL	0.2463	0.0836	[+0.0819, +0.4108]	Significant increase

Cr, serum creatinine; SRSL, splenorenal shunt ligation; LRVL, left renal vein ligation; CI, confidence interval; Std., standard; ΔCr, change in serum creatinine.

(+0.246 mg/dL; 95% CI: +0.082 to +0.411, $p < 0.05$), whereas the SRSL group did not (+0.087 mg/dL, NS).

Despite the absence of statistically significant intergroup interactions, the pronounced late-phase increase in the LRVL group suggests a trend toward accumulating renal burden during extended follow-up.

Longitudinal Trends in Tacrolimus Trough Levels

Tacrolimus trough levels decreased steadily over time in both groups, reflecting routine clinical tapering practices.

Overall Trends in Tacrolimus Trough Levels Over Time

The LRVL group generally exhibited slightly lower tacrolimus levels than the SRSL group at all time points, with the most noticeable difference occurring during the early postoperative period (**Figure 5**).

Phase-specific Modeling

The fixed-effects model showed that tacrolimus levels declined rapidly in the early phase ($p < 0.001$), with a significantly faster rate of decrease in the LRVL group (interaction estimate: −4.820, $p = 0.004$). Levels continued to decrease during the intermediate phase ($p < 0.001$), but group differences were no longer significant. A mild further decline was observed in both groups during the late phase ($p < 0.001$), with no intergroup differences. These findings suggest that early tacrolimus tapering may have been more aggressive in the LRVL group, possibly reflecting concerns for renal function by the attending clinicians, while tapering strategies later converged for both groups. Overall, despite a tendency toward earlier tacrolimus tapering in the LRVL group, these patients demonstrated a more pronounced long-term decline in renal function, reflected by progressively higher creatinine levels and the exclusive occurrence of renal failure requiring dialysis treatment within this group during the late phase of the follow-up period (**Table 6**).

DISCUSSION

Effective management of large spontaneous SRSs during LDLT is essential to ensure adequate portal inflow and prevent portal steal syndrome. Two intraoperative techniques are currently employed for this purpose: SRSL, which anatomically targets the shunt itself, and LRVL, which functionally interrupts shunt outflow by occluding the LRV. SRSL provides anatomically precise control but can be technically demanding in patients with dense adhesions or distorted venous anatomy. In contrast, LRVL is less technically challenging and is often preferred in hemodynamically unstable or surgically complex cases [1–3].

In this study, both techniques were effective in preventing portal steal and maintaining adequate graft perfusion. Patient and graft survival, re-transplantation rates, and shunt recanalization were comparable between groups, supporting LRVL as a feasible and effective alternative when direct shunt ligation is difficult or deemed unsafe.

Nevertheless, concerns remain regarding the potential long-term renal consequences of LRVL. Because the LRV is the primary venous drainage route of the left kidney, its ligation may predispose patients to impaired renal perfusion and progressive renal dysfunction, particularly in LDLT recipients who require lifelong exposure to nephrotoxic immunosuppressants such as tacrolimus. Although previous nontransplant studies have suggested minimal long-term renal impact after LRV ligation,

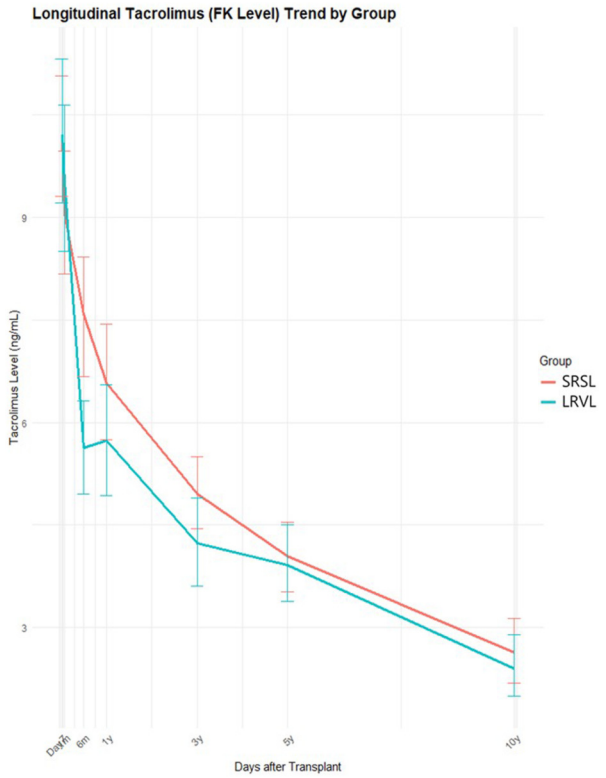


FIGURE 5 | Longitudinal changes in tacrolimus levels in blood after transplantation. Tacrolimus levels were analyzed at seven fixed time points: at 1 week, 1 month, 6 months and 1, 3, 5, and 10 years. The left renal vein ligation (LRVL) group showed a more rapid decline in tacrolimus levels during the early phase compared to the splenorenal shunt ligation (SRSL) group, followed by a similar tapering pattern of immunosuppression in both groups. The early decrease in tacrolimus levels is presumed to be due to a rise in serum creatinine during the immediate postoperative period.

these findings may not be fully applicable to liver transplant recipients under chronic immunosuppression. Beyond simple mechanical obstruction of venous outflow, progressive renal dysfunction after LRVL may involve multiple pathophysiological mechanisms, including microvascular injury, altered renal autoregulation, and synergistic nephrotoxicity associated with prolonged calcineurin inhibitor exposure. These mechanisms may partly explain the greater variability in creatinine levels and the exclusive occurrence of dialysis in the LRVL group during long-term follow-up.

To evaluate renal outcomes more rigorously, we performed 1: 1 propensity score matching using the baseline values for relevant variables. After matching, long-term tacrolimus exposure was comparable between the groups, although the LRVL group demonstrated a tendency toward earlier tacrolimus tapering during the early postoperative period, likely reflecting concerns by the clinicians about renal function. Despite this early tapering, the LRVL group exhibited a sustained trend toward higher serum creatinine throughout follow-up, and all cases of renal failure requiring dialysis occurred exclusively in the LRVL groups between 5 and 10 years post-transplant. Although not definitive, these findings raise the possibility that LRVL may be associated with a subtle but progressive long-term renal burden.

These observations align with prior experience at our institution. In an early report, Lee et al. found no short-term deterioration in renal function, proteinuria, or hematuria following LRVL [4]. However, a 20-year follow-up study by Hwang et al. in the same patient cohort revealed that 9.1% of them eventually required dialysis, and more than half experienced a decline in the estimated glomerular filtration rate [12]. Although underlying comorbidities such as diabetes or glomerulonephritis likely contributed to this outcome, chronic venous congestion from LRV ligation may also have played a role in the progressive renal decline. These longer-term findings underscore the importance of careful patient selection and appropriate renal monitoring following LRVL.

Our study provides follow-up data up to 10 years after transplantation. While no statistically significant differences in renal failure were observed during this interval, the trends in creatinine elevation and the exclusive occurrence of cases requiring dialysis in the LRVL group suggest that more pronounced differences may emerge with a more extended follow-up, as suggested by the long-term data reported by a Hwang et al. [12] This highlights the need for continued

TABLE 6 | Changes in tacrolimus trough levels over time by phase and group.

Phase	Variable	Estimate	Std. Error	p-value	Interpretation
Early	Intercept	9.875	0.395	<0.001	FK level at time = SRSL group is higher
0 → 1 year	Time_year	−4.709	1.196	0.000	FK level significantly declines over time
	Group (LRVL vs. SRSL)	0.481	0.558	0.390	LRVL group shows slightly higher FK level (not significant)
	Time × group	−4.820	1.691	0.004	FK level in LRVL group declines faster than in SRSL group
Intermediate	Intercept	7.411	0.512	<0.001	FK level starts lower than in early phase
1 → 5 years	Time_year	−0.832	0.204	0.000	FK level continues to decrease over time
	Group (LRVL vs. SRSL)	−0.915	0.714	0.202	LRVL group shows slightly lower FK baseline level (not significant)
	Time × group	0.069	0.284	0.807	No significant group difference in slope
Late	Intercept	5.501	0.468	<0.001	FK level further reduced
5 → 10 years	Time_year	−0.291	0.055	<0.001	Decline continues at a slower rate
	Group (LRVL vs. SRSL)	−0.055	0.654	0.933	No baseline difference between groups
	Time × group	−0.016	0.077	0.839	No group difference in slope (not significant)

FK, level, Tacrolimus trough level (FK506); SRSL, splenorenal shunt ligation; LRVL, left renal vein ligation; NS, not significant.

surveillance and emphasizes the importance of choosing surgical strategies that minimize long-term renal risk.

In this context, the potential advantages of SRSL warrant renewed attention. SRSL preserves physiological renal venous drainage and avoids direct compromise of kidney perfusion. Although it may be technically challenging, particularly in the presence of dense adhesions, it provides targeted shunt interruption while minimizing collateral vessel injury. By avoiding alterations in systemic venous return, SRSL may offer the advantage of sparing the kidney, which is particularly important in patients with pre-existing renal risk factors.

At our institution, SRSL has consistently provided reliable portal decompression and stable long-term renal outcomes. Given the rising prevalence of chronic kidney disease after liver transplantation and the widespread use of tacrolimus, SRSL may represent the preferred SRS management strategy when anatomically feasible. Surgical planning should incorporate both anatomical considerations and long-term organ protection, particularly in recipients predisposed to renal dysfunction.

This study has several strengths, including a relatively large cohort of LDLT recipients with large SRS, long-term follow-up of up to 10 years, and rigorous propensity score matching to minimize selection bias. In addition, standardized immunosuppressive protocols and objective renal biomarkers further strengthened the validity of the comparative analyses. However, several limitations should be acknowledged. This was a retrospective single-center study, which limits generalizability, and residual confounding cannot be completely excluded. Procedural selection was influenced by intraoperative assessment, surgeon preference, and individual judgment, introducing potential selection bias. Although we attempted to mitigate this by using MELD score and RBC transfusion as surrogate markers in the propensity score model, unmeasured surgeon-related factors may still have contributed to residual confounding. Furthermore, technical difficulty in determining the ligation strategy was difficult to quantify objectively, and renal function was primarily assessed using only serum creatinine. and the limited number of patients requiring dialysis reduced the statistical power for detecting differences in rare outcomes. Another limitation is that detailed graft function parameters, including rejection and longitudinal liver function tests, were not systematically analyzed. Therefore, subtle differences in graft function between groups may not have been fully captured. These strengths and limitations should be considered when interpreting our findings, and further prospective multicenter studies are warranted to validate our results.

In conclusion, both SRSL and LRVL are effective strategies for managing SRS during LDLT. Although survival outcomes were comparable, LRVL may be associated with potential long-term renal risks, even when early tacrolimus tapering is implemented. When anatomically feasible, SRSL may be considered, whereas LRVL remains an important alternative in technically challenging or hemodynamically unstable cases. In patients requiring LRVL, strategies aimed at renal protection, including careful titration of nephrotoxic immunosuppressants, should be implemented, and long-term renal function should be closely monitored. Further prospective multicenter studies with extended follow-up are warranted to validate these findings and guide optimal surgical decision-making in the management of SRS during liver transplantation.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: The dataset analyzed during this study contains sensitive patient medical information obtained from a single tertiary transplant center. Due to institutional and ethical restrictions, the raw data cannot be made publicly available. Access to the dataset may be granted upon reasonable request to the corresponding author and subject to approval by the Institutional Review Board and data-sharing agreements in compliance with patient privacy protection regulations. Requests to access these datasets should be directed to romikwh@gmail.com.

ETHICS STATEMENT

The studies involving humans were approved by Institutional Review Board of Asan Medical Center (IRB No. AMC 2022-0763). 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

WHK contributed to study design, transplantation surgeries, data collection, statistical analysis, and drafting of the manuscript as the first author. DBM contributed to study design, performed transplantation surgeries, supervised clinical management, critically revised the manuscript, and served as the corresponding author with final responsibility for the research. SH, CSA, KHK, DHJ, TYH, GWS, GCP, SGL, YIY, BGN, SHK, and SMK performed or assisted in transplantation surgeries, provided operative expertise, contributed to perioperative clinical decision-making, and participated in data acquisition. All authors contributed to the article and approved the submitted version.

FUNDING

The author(s) declared that financial support was not received for this work and/or its publication.

CONFLICT OF INTEREST

The author(s) declared that this work 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) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

ACKNOWLEDGEMENTS

Assistance with the study: The authors gratefully acknowledge Professor Seunghee Baek, PhD from the Department of Medical Statistics, Asan Medical Center, University of Ulsan College of Medicine, for her expert advice in the conduction of statistical analyses, which greatly contributed to the completion of this study.

REFERENCES

1. Moon DB, Lee SG, Kim KH, Ahn CS, Hwang S, Park KM, et al. The Significance of Complete Interruption of Large Spontaneous Portosystemic Collaterals in Adult Living Donor Liver Transplantation as a Graft Salvage Procedure. *Transpl Int* (2008) 21(7):698–700. doi:10.1111/j.1432-2277.2008.00639.x
2. Ikegami T, Shirabe K, Nakagawara H, Yoshizumi T, Toshima T, Soejima Y, et al. Obstructing Spontaneous Major Shunt Vessels Is Mandatory to Keep Adequate Portal Inflow in Living-Donor Liver Transplantation. *Transplantation* (2013) 95(10):1270–7. doi:10.1097/TP.0b013e318288cadc
3. Sadamori H, Yagi T, Matsukawa H, Matsuda H, Shinoura S, Umeda Y, et al. The Outcome of Living Donor Liver Transplantation with Prior Spontaneous Large Portasystemic Shunts. *Transpl Int* (2008) 21(2):156–62. doi:10.1111/j.1432-2277.2007.00593.x
4. Lee SG, Moon DB, Ahn CS, Kim KH, Hwang S, Park KM, et al. Ligation of Left Renal Vein for Large Spontaneous Splenorenal Shunt to Prevent Portal Flow Steal in Adult Living Donor Liver Transplantation. *Transpl Int* (2007) 20(1):45–50. doi:10.1111/j.1432-2277.2006.00392.x
5. Kang WH, Moon DB, Ko GY, Gwon DI, Yoon YI, Cho HD, et al. Application of Proximal Splenic Vein Embolization to Interrupt Complicated Large Splenorenal Shunts in Adult Living Donor Liver Transplantation. *Ann Surg* (2022) 276(6):e834–e41. doi:10.1097/SLA.0000000000004868
6. Loveday BPT, Dib MJ, Sequeira S, Alotaiby N, Visser R, Barbas AS, et al. Renal Outcomes Following Left Renal Vein Harvest for Venous Reconstruction During Pancreas and Liver Surgery. *HPB (Oxford)* (2019) 21(1):114–20. doi:10.1016/j.hpb.2018.07.015
7. Ojo AO, Held PJ, Port FK, Wolfe RA, Leichtman AB, Young EW, et al. Chronic Renal Failure After Transplantation of a Nonrenal Organ. *N Engl J Med* (2003) 349(10):931–40. doi:10.1056/NEJMoa021744
8. Samson RH, Lepore MR, Jr., Showalter DP, Nair DG, Lanoue JB. Long-Term Safety of Left Renal Vein Division and Ligation to Expedite Complex Abdominal Aortic Surgery. *J Vasc Surg* (2009) 50(3):500–4. ; discussion 4. doi:10.1016/j.jvs.2009.04.041
9. Pandirajan K, Katsogridakis E, Sidloff D, Sayers RD, Bown MJ, Saratzis A. Effects of Left Renal Vein Ligation During Open Abdominal Aortic Aneurysm Repair on Renal Function. *Eur J Vasc Endovasc Surg* (2020) 60(6):829–35. doi:10.1016/j.ejvs.2020.08.003
10. Moon DB, Lee SG, Ahn C, Hwang S, Kim KH, Ha T, et al. Application of Intraoperative Cine-Portogram to Detect Spontaneous Portosystemic Collaterals Missed by Intraoperative Doppler Exam in Adult Living Donor Liver Transplantation. *Liver Transpl* (2007) 13(9):1279–84. doi:10.1002/lt.21252
11. Lim SY, Wang R, Tan DJH, Ng CH, Lim WH, Quek J, et al. A Meta-Analysis of the Cumulative Incidence, Risk Factors, and Clinical Outcomes Associated with Chronic Kidney Disease After Liver Transplantation. *Transpl Int* (2021) 34(12):2524–33. doi:10.1111/tri.14149
12. Ko HJ, Hwang S, Kang J, Moon DB, Ahn CS, Ha TY, et al. Long-Term Outcomes of Left Renal Vein Ligation in Living Donor Liver Transplantation: A 20-Year Study. *Ann Transpl* (2025) 30:e947492. doi:10.12659/AOT.947492

ESOT | Transplant International

BRIEF RESEARCH REPORT
published: 12 February 2026
doi: 10.3389/ti.2026.15432

The Clinical Impact of Early Steroid Withdrawal on Diabetes Mellitus After Liver Transplantation: A Population-Based Cohort Study

Haeseon Lee[1,2†], *YoungRok Choi*[3†] *and Hae Sun Suh*[2,4*]

[1]Department of Pharmacotherapy, College of Pharmacy, University of Utah, Salt Lake City, UT, United States, [2]Institute of Regulatory Innovation Through Science (IRIS), Kyung Hee University, Seoul, Republic of Korea, [3]Seoul National University Hospital, College of Medicine, Seoul National University, Seoul, Republic of Korea, [4]College of Pharmacy, Kyung Hee University, Seoul, Republic of Korea

Despite the metabolic risks associated with corticosteroids after liver transplantation (LT), the optimal timing for their withdrawal remains uncertain due to limited and inconsistent evidence. To evaluate the impact of corticosteroid withdrawal timing on the development of *de-novo* post-transplant diabetes mellitus (PTDM), we performed a retrospective cohort study of 6,295 adult recipients who underwent LT between 2009 and 2021 in South Korea, utilizing a national health insurance claims database. A landmark analysis with time-varying propensity score matching was conducted at one-, three-, and six-month post-transplantation to compare the incidence of PTDM between steroid withdrawal and maintenance groups. Early steroid withdrawal within 3 months significantly reduced PTDM risk (HR = 0.586; 95% CI = 0.407–0.846 at 1 month, HR = 0.766; 95% CI = 0.611–0.960 at 3 month), whereas withdrawal after 3 months showed no significant benefit (HR = 0.844; 95% CI = 0.619–1.152 at 6 month). Rejection events were rare, suggesting no substantial compromise in graft function. These findings indicate that corticosteroid withdrawal within the first three months post-LT can lower the risk of PTDM without increasing rejection risk, supporting timely steroid tapering as part of post-transplant immunosuppressive strategies to reduce long-term metabolic complications.

Keywords: corticosteroid withdrawal, immunosuppression, landmark analysis, liver transplantation, post-transplant diabetes mellitus

OPEN ACCESS

Correspondence
Hae Sun Suh,
haesun.suh@khu.ac.kr

[†]These authors have contributed equally to this work

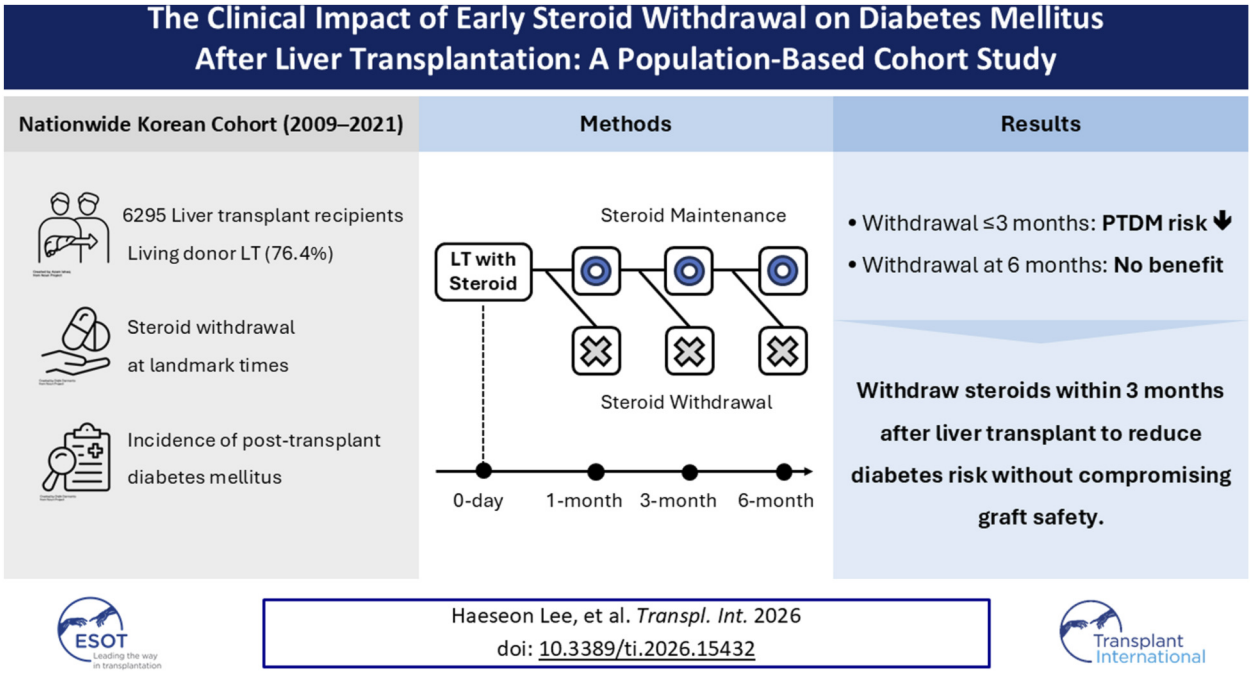
Received: *14 August 2025*
Revised: *10 January 2026*
Accepted: *27 January 2026*
Published: *12 February 2026*

Citation:
Lee H, Choi Y and Suh HS (2026) The Clinical Impact of Early Steroid Withdrawal on Diabetes Mellitus After Liver Transplantation: A Population-Based Cohort Study. Transpl. Int. 39:15432. doi: 10.3389/ti.2026.15432

INTRODUCTION

Liver transplantation (LT) has become the most effective treatment for patients with liver cancer or end-stage liver diseases [1], with 5-year graft survival rate exceeding 75% [2]. Long-term care after LT encompasses the management of hypertension, diabetes, bone health, and cancer surveillance [1, 3], underscoring the importance of reducing preventable complications such as post-transplant diabetes mellitus (PTDM) [4]. PTDM affects 30%–40% of recipients and increases the risks of infection, mortality, and cardiovascular disease, the leading cause of non-graft-related death after transplantation [5–7].

Steroids are the primary modifiable risk factor for PTDM due to their multiple interactions within glucose metabolism [8, 9]. While traditionally essential for induction of immunosuppression and



GRAPHICAL ABSTRACT |

rejection control [10], their prolonged use has come under critical review. The 2018 International Liver Transplantation Society Consensus Statement recommended minimizing steroids use to prevent metabolic complications [11]. Many centers empirically adopt steroid-tapering protocols [12], often around 3 months [13], but robust evidence and formal guidelines on optimal timing remain limited.

Given the complex nature of diabetes and the heterogeneity of transplant care, population-based studies reflecting real-world practice are crucial [14]. South Korea, with one of the highest global rates of living donor LT [15], provides a valuable setting to investigate LT outcomes across diverse clinical scenarios. Using nationwide healthcare insurance data, we aimed to evaluate the association between the timing of steroid withdrawal after LT and PTDM without compromising the liver graft function in liver recipients.

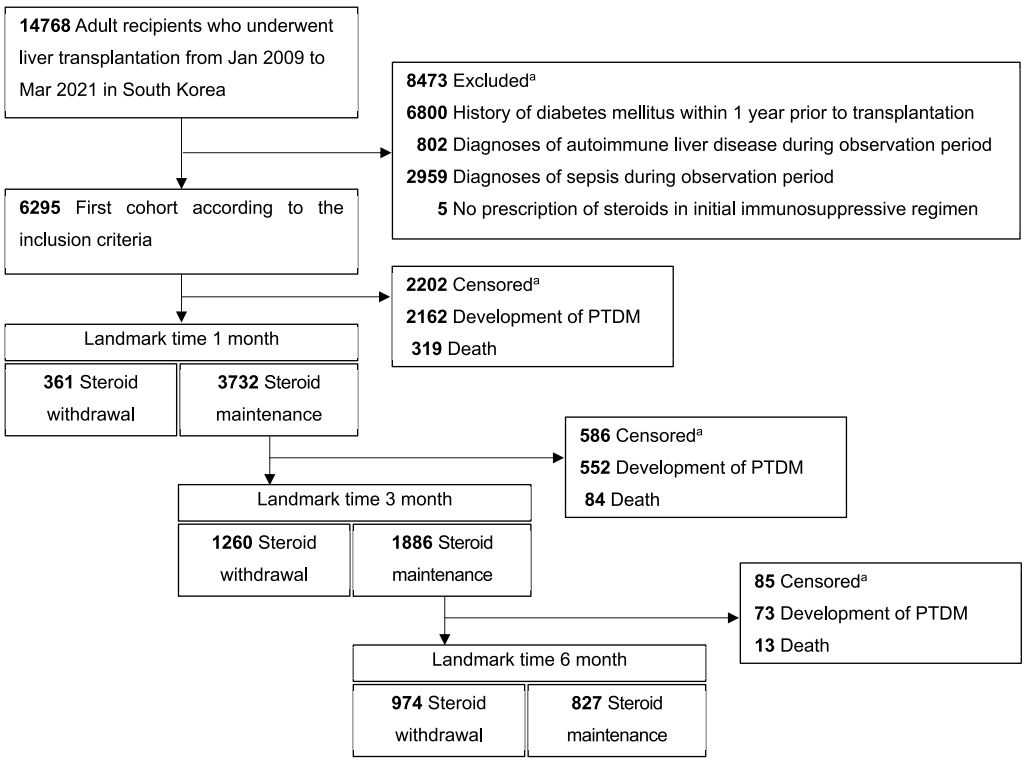
METHODS

Study Design and Population

This retrospective cohort study utilized the Health Insurance Review and Assessment Service database from South Korea, which includes comprehensive information on patient characteristics, diagnoses, treatments, prescription, and medical expenses for the entire population of approximately 50 million people [16]. Patients aged >18 years who received LT between 2009 and 2021, with initial steroid-containing immunosuppressive regimen, were included and followed up until December 2021. Liver recipients were identified as those with an electronic data interchange (EDI) code related to LT for health insurance reimbursement (EDI code of Q80 for cadaver donor LT and Q81 for living donor LT). Patients were excluded if they had a history of diabetes mellitus within 1 year prior to LT. Furthermore, patients requiring continuous steroid use for specific conditions (e.g., autoimmune liver disease) were excluded, irrespective of the timing of diagnosis. This exclusion was implemented to delineate steroid exposure attributable to the immunosuppressive regimen from steroid use for the management of other medical conditions [17, 18]. All diagnoses were identified using ICD-10-CM codes (**Supplementary Table S1**). This study was approved by the Institutional Review Board of Kyung Hee University (No. KHSIRB-22-176(EA)) and was presented according to Strengthening the Reporting of Observational Studies in Epidemiology reporting guideline [19].

Landmark Analyses Based on Steroid Use

To assess the potential impact of steroid withdrawal at various time points after LT, we employed a landmark approach to facilitate the identification of causal effects, adhering to the foundational assumptions of propensity score analyses [20]. We set 1-, 3-, and 6-month post-transplantation as landmark times. At each time point, the patients were categorized into 'steroid withdrawal' or 'steroid maintenance' groups according to their steroid use status from the previous landmark time to the current landmark time. We calculated propensity score using logistic regression models at each landmark time point based on the latest covariates, including comorbidities [21] and hepatitis C virus infections [22], alongside demographics and



[a] Patients may have been excluded for more than one reason; therefore, the sum of distinct exclusions exceeds the total number of excluded individuals.

FIGURE 1 | Flow diagram of cohort construction.

immunosuppressive regimen. Subsequently, we performed 1:1 matching of patients from the steroid withdrawal group with those from the steroid maintenance group, creating two cohorts differentiated only by their steroid use at a specified landmark time. Patients were followed up from the day of LT surgery (index date) until the earliest day of occurrence of PTDM, cessation due to death, or the end of the dataset as censored observations. Additional information and the study scheme are shown in **Supplementary Methods 1.1**, **Supplementary Figure S1**.

Operational Definitions

The exposure of interest was steroid withdrawal, which is a dichotomous time-dependent variable. We defined steroids as the three corticosteroid medications commonly used after LT in South Korea; prednisolone/prednisone, methylprednisolone, and deflazacort [23].

In this study, PTDM was defined as a type 2 diabetes diagnosis along with a prescription for antidiabetic medication including insulin, with the date of the first prescription considered the occurrence of the outcome [24]. Steroid use was defined with a 30-day grace period, with maintenance as continuous use with a prescription refill within 30 days of the last fill date and withdrawal as no refill within 30 days. To prevent the inclusion of steroid use for other conditions, we considered only prescriptions where the diagnosis of "liver transplant status" (ICD-10-CM code of Z94.4) was confirmed on the same date as the prescription. Deceased individuals were identified by the absence of medical claims for over a year from their last visit. Lastly, we investigated whether steroid withdrawal compromised the goal of preventing allograft rejection, focusing on incidents occurring within 2 months of withdrawal [25]. Rejection events were defined as prescriptions of 500 mg or more of intravenous methylprednisolone, based on medical advice. A detailed explanation is provided in **Supplementary Methods 1.2**.

Statistical Analysis

Data are summarized as frequencies with percentages or means with standard deviations. The chi-square test or Fisher's exact test was used for categorical variables, while continuous variables were analyzed using Student's t-test. Kaplan-Meier analysis was used to estimate the probability of outcomes at daily intervals with censoring. If the day of the outcome of interest in a patient's record was within the analysis timeframe, the patient was censored on that day. For these matched cohorts, we measured the incidence of PTDM. The incidence rate was calculated by dividing the number of events by the total number of person years of follow-up, then multiplying by 1,000. We performed Cox proportional hazards regression analysis to describe the relative hazard of the outcomes, comparing the time-to-event rates with HRs with 95% Confidence Interval (CI). Statistical significance was set as less than 0.05. The proportional hazards assumption was verified using a log minus log plot. All analyses were conducted using the SAS software (version 9.4.2 (SAS Institute, Inc., Cary, NC, USA)).

TABLE 1 | Baseline characteristics of study participants with liver transplantation.

Characteristics	No. (%)	*P* value
Total no. of patients	6,295 (100)	
Age at LT, mean (SD), y	53.0 (15.2)	-
Male	4,451 (70.7)	<0.0001
Insurance type		<0.0001
National health insurance	5,945 (94.4)	
Medical aid	350 (5.6)	
Types of transplantation		<0.0001
Living donor LT	4,812 (76.4)	
Deceased donor LT	1,483 (23.6)	
Immunosuppression		<0.0001
Tacrolimus-based regimen	5,975 (94.9)	
Cyclosporin A-based regimen	115 (1.8)	
Other regimen	205 (3.3)	
CCI score, mean (SD)	4.3 (2.2)	-
Comorbidities		<0.0001
Hypertension	841 (13.4)	
Dyslipidemia	102 (1.6)	
Osteoporosis	152 (2.4)	
Congestive heart failure	304 (4.8)	
Peripheral vascular disease	241 (3.8)	
Chronic pulmonary disease	1,379 (21.9)	
Rheumatologic disease	122 (1.9)	
Renal disease	157 (2.5)	

Abbreviations: CCI, charlson comorbidity index; LT, liver transplantation; n, number; SD, standard deviation; Y, year.

RESULTS

Patient Characteristics at Transplantation

A total of 14,768 adult patients who underwent LT in South Korea between January 2009 and March 2021 were identified. For the analysis of incident PTDM, the study cohort included 6,295 patients with no history of diabetes (**Figure 1**). The mean age at LT was 53.0 years, with approximately 400–600 recipients annually. The majority were male (70.7%), and most underwent living donor LT (76.4%) (**Table 1**). The tacrolimus-based regimen for initial post-transplantation immunosuppression was dominant (94.9%), with the use of a cyclosporin-based regimen becoming infrequent in 2016.

Among the patients followed up with, 41.5% withdrew from steroid use within 6 months after LT, and 44.5% were diagnosed with PTDM within the same period. The mean follow-up duration was 5.3 years (median 4.8 years; interquartile range 2.0–8.3 years). After the patients were classified based on steroid use and matched by propensity scores, the two groups were balanced at each landmark time. The patient characteristics before and after matching are presented in **Supplementary Table S2**.

PTDM in LT Recipients at 1-Month Landmark Time

Among the initial cohort of 6,295 patients, 34.3% were diagnosed with PTDM within 1-month post-LT and 5.1% died. These patients were excluded from analysis at the 1-month landmark. The steroid maintenance group (hereafter SMG) included 3,732 patients, while the steroid withdrawal group (hereafter SWG) included 361 patients. After propensity score matching, each cohort group consisted of 351 patients. The incidence rate was 54.9 cases per 1,000 person-years in the SMG and 30.0 cases per 1,000 person-years in the SWG, indicating that the rate of new PTDM cases in the SMG was 1.8 times higher than that in the SWG ($P < 0.00001$). The Kaplan-Meier cumulative survival curves showed a higher risk of PTDM at every time point in the SMG than in the SWG. The log-rank test demonstrated that the observed difference in risk between the two groups was statistically significant ($P = 0.0018$). Cox proportional hazards model analysis revealed that at the 1-month landmark time, the SWG had a significantly lower risk of PTDM compared to the matched SMG (5.8% vs. 14.0%; HR, 0.59; 95% CI, 0.41–0.85) (**Figure 2**).

PTDM in LT Recipients at 3-Month Landmark Time

Among the 3,732 uncensored patients at the 1-month landmark, 14.8% were diagnosed with PTDM within 3-month post-LT and 2.3% died. These patients were excluded from the 3-month landmark analysis, which included 1,886 patients in the SMG and 1,260 in the SWG. After propensity score matching, each cohort group consisted of 1,257 patients. The incidence rate was 30.2 cases per 1,000 person-years in the SMG and 22.1 cases per 1,000 person-years in the SWG, indicating that the rate of new PTDM cases in the SMG was 1.4 times higher than that in the SWG ($P = 0.0006$). The Kaplan-Meier cumulative survival curves showed a slightly higher risk of PTDM at every time point in the SMG than in the SWG. The log-rank test demonstrated that the observed difference in risk between the two groups was also statistically significant ($P = 0.0073$). Cox proportional hazards model analysis revealed that at the 3-month landmark time, the SWG had a significantly lower risk of PTDM compared to the matched SMG (5.1% vs. 7.4%; HR, 0.77; 95% CI, 0.61–0.96) (**Figure 2**).

PTDM in LT Recipients at 6-Month Landmark Time

Among the 1,886 uncensored patients at the 3-month landmark, 3.9% were diagnosed with PTDM within 6-month post-LT and 0.7% died. These patients were excluded from the 6-month landmark analysis, which included 827 patients in the SMG and 974 in the SWG. After propensity score matching, each cohort comprised of 782 patients. The incidence rate were 23.5 cases per 1,000 person-years in the SMG and 18.7 cases per 1,000 person-years in the SWG; however, the difference was not statistically significant ($P = 0.1159$). The Kaplan-Meier cumulative survival curves did not show a clear difference in the risk of PTDM occurrence between the two groups at any time point. The log-rank test confirmed that the observed risk difference between the groups was not statistically significant ($P = 0.1393$). Cox proportional hazards model analysis showed that at the 6-month landmark time, the SWG had a lower risk of PTDM compared to the matched SMG, but this difference was

Landmark Time	Incidence rate		Hazard Ratio (95% CI)	P value
	Withdrawal	Maintenance		
1 month	41 (30.00)	98 (54.85)	0.586 (0.407-0.846)	0.0043
3 month	128 (22.13)	185 (30.19)	0.766 (0.611-0.960)	0.0205
6 month	72 (18.70)	91 (23.51)	0.844 (0.619-1.152)	0.2860

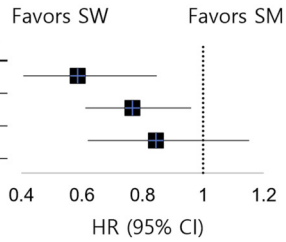


FIGURE 2 | Incidence rate (per 1000 person-years) and adjusted hazard ratio for post-transplant diabetes mellitus according to steroid withdrawal timing at each landmark time after liver transplantation. Abbreviations: CI, confidence intervals; HR, hazard ratio; SM, steroid maintenance; SW, steroid withdrawal.

not statistically significant (4.6% vs. 5.8%; HR, 0.84; 95% CI, 0.62–1.15) (**Figure 2**).

Allograft Risk After Steroid Withdrawal

Although rejection is becoming less frequent in LT recipients, we monitored for allograft rejection to ensure the safety of steroid withdrawal. We examined the requirement for high-dose steroid pulse (≥500 mg/dose of prednisolone) as a treatment for acute cellular or humoral rejection within 2 months post-withdrawal. Two patients received such therapy, both of whom belonged to the 3-month landmark withdrawal group.

DISCUSSION

Minimizing exposure to immunosuppressive agents, especially steroids, in solid organ transplant recipients has been discussed for decades. However, no international consensus has been reached on its optimal timing or the appropriate withdrawal protocol. This was attributable to substantial heterogeneity in regimens and withdrawal schedules across LT studies. A systematic review of 16 randomized controlled trials (RCT) reported persistent ambiguity regarding the benefits and harms of steroid avoidance or withdrawal after LT, finding no significant differences in mortality, graft loss, or infection between steroid-free and steroid-containing regimens [26]. Regarding diabetes outcomes, a subgroup analysis yielded inconsistent results depending on the analytical approach: the fixed-effects model showed a significant reduction in risk (Relative Risk (RR), 0.81; 95% CI, 0.66–0.99), whereas the random-effects model did not (RR, 0.82; 95% CI, 0.64–1.07) [26]. Furthermore, substantial risk of bias and wide variability in withdrawal timing (64–180 days) among the included trials limited the reliability of comparisons across specific time points.

In a recent RCT comparing early withdrawal (2 weeks ±3 days) with later withdrawal (3 months ±2 weeks), the incidence of new-onset diabetes was significantly higher in the earlier-withdrawal group (23.3% vs. 5.5%; $P = 0.008$), likely attributable to intensified tacrolimus exposure [27]. However, that study was limited by a relatively short follow-up period of 1 year, restricting evaluation of long-term outcomes.

Despite these uncertainties, efforts to reduce steroid use have continued, supported by the liver's unique immune privilege, which may allow for safer minimization of immunosuppression [28]. This paradigm reflects a shift in focus from short-term graft survival toward long-term patient-centered outcomes.

In this context, our study provided robust evidence using a large cohort with up to 13 years of follow-up. We demonstrated that early steroid withdrawal reduced the risk of PTDM by 23%–41%. In the era of predominantly tacrolimus-based regimens, withdrawal within 3 months appeared to be a safe and effective strategy. Notably, continuing steroids more than 3 months offered no additional benefit even when withdrawal occurred before 6 months, underscoring the importance of discontinuing steroids as early as feasible within the first 3 months after transplantation. By applying a landmark design, we minimized immortal time bias and achieved an accurate assessment of time-dependent drug effects. In addition, the strict delineation of protocol-driven steroid exposure from therapeutic administration for comorbidities allowed us to mitigate confounding by indication. Combined with the external validity of a nationwide dataset reflecting real-world clinical practice, this study provides actionable evidence to guide immunosuppressive management and prognosis after LT.

Several limitations should be acknowledged. As steroid withdrawal may have reflected centre-led clinical decision-making, residual confounding from unmeasured factors cannot be excluded, despite landmark-specific propensity score matching to balance measured covariates [29]. Reliance on claims data also introduced the possibility of coding errors, and clinical parameters such as glycemic profiles and body mass index were unavailable. Our design also limited our ability to examine pre-transplant risk factors for developing PTDM or to evaluate the clinical rationale underlying decisions regarding steroid withdrawal, which may vary across institutions or among individual clinicians. This study addressed only the duration of steroid use without considering cumulative dosage or relative potency. Steroid withdrawal may have been accompanied by intensification or substitution with other immunosuppressants (e.g., calcineurin inhibitors, antimetabolites). Nevertheless, we prioritized steroid withdrawal as the primary exposure of interest, consistent with the conclusion of Van Hooff et al (2004) that avoidance or early withdrawal represents the best preventive strategy against diabetes [30]. Finally, as our cohort was derived exclusively from the South Korean population, caution is warranted when

extrapolating these results to other geographic regions with different baseline patient characteristics or genetic backgrounds.

A substantial proportion of recipients developed hyperglycemia or diabetes within the first month after LT. These early cases were excluded from the landmark analyses for methodological reasons. Yet, accumulating evidence suggests that this early period represents a clinically important metabolic phase. Studies in kidney and other solid-organ transplant populations indicated that early post-transplant hyperglycemia was associated with adverse outcomes, including infections, rehospitalizations, and graft dysfunction [31, 32]. Together, these findings underscore the need for dedicated investigations focused on this early post-transplant period.

In conclusion, this nationwide cohort study showed that early steroid withdrawal after LT was associated with a lower risk of PTDM, with the greatest benefit observed when steroids were discontinued within 3 months. Using a landmark-based approach in a large, real-world population, our findings provide time-specific evidence relevant to contemporary tacrolimus-based practice. These results support earlier consideration of steroid minimization and may help inform clinical decision-making aimed at improving long-term metabolic outcomes in LT recipients.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: Data were obtained from the Health Insurance Review and Assessment Service (HIRA) under license and are not publicly available. Access requires administrative approval, and all patient information was anonymized in accordance with the Personal Information Protection Act. Requests to access these datasets should be directed to Health Insurance Review and Assessment Service (HIRA).

ETHICS STATEMENT

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

AUTHOR CONTRIBUTIONS

HL, YC, and HS conceived and designed the study. HL and YC acquired, analyzed, and interpreted the data. HL drafted the manuscript, and YC and HS critically revised it. HS secured funding, provided administrative and technical support, and supervised the study with YC. All authors contributed to the article and approved the submitted version.

FUNDING

The author(s) declared that financial support was received for this work and/or its publication. This research was supported by a grant (21153MFDS601) from the Ministry of Food and Drug Safety in 2023.

CONFLICT OF INTEREST

The author(s) declared that this work 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) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2026.15432/full#supplementary-material

REFERENCES

1. Lucey MR, Furuya KN, Foley DP. Liver Transplantation. *New Engl J Med* (2023) 389(20):1888–900. doi:10.1056/NEJMra2200923
2. Kwong A, Ebel N, Kim W, Lake J, Smith J, Schladt D, et al. OPTN/SRTR 2020 Annual Data Report: Liver. *Am J Transplant* (2022) 22:204–309. doi:10.1111/ajt.16978
3. Lucey MR, Terrault N, Ojo L, Hay JE, Neuberger J, Blumberg E, et al. Long-Term Management of the Successful Adult Liver Transplant: 2012 Practice Guideline by the American Association for the Study of Liver Diseases and the American Society of Transplantation. *Liver Transpl* (2013) 19(1):3–26. doi:10.1002/lt.23566
4. Serper M, Asrani S, VanWagner L, Reese PP, Kim M, Wolf MS. Redefining Success After Liver Transplantation: From Mortality Toward Function and Fulfillment. *Liver Transplant* (2022) 28(2):304–13. doi:10.1002/lt.26325
5. Neuberger JM, Bechstein WO, Kuypers DR, Burra P, Citterio F, De Geest S, et al. Practical Recommendations for Long-Term Management of Modifiable Risks in Kidney and Liver Transplant Recipients: A Guidance Report and Clinical Checklist by the Consensus on Managing Modifiable Risk in Transplantation (COMMIT) Group. *Transplantation* (2017) 101(4S): S1–S56. doi:10.1097/TP.0000000000001651

6. Gupta S, Pollack T, Fulkerson C, Schmidt K, Oakes DJ, Molitch ME, et al. Hyperglycemia in the Posttransplant Period: NODAT vs Posttransplant Diabetes Mellitus. *J Endocr Soc* (2018) 2(11):1314–9. doi:10.1210/js.2018-00227
7. Schmidt K, Spann A, Khan MQ, Izzy M, Watt KD. Minimizing Metabolic and Cardiac Risk Factors to Maximize Outcomes After Liver Transplantation. *Transplantation* (2024) 108(8):1689–99. doi:10.1097/tp.0000000000004875
8. Singh S, Watt KD, editors. Long-Term Medical Management of the Liver Transplant Recipient: What the Primary Care Physician Needs to Know. *Mayo Clinic Proceedings*. Elsevier (2012).
9. Chauhan K, Khan A, Chowdhury S, Ross HM, Parra NS, Halegoua-DeMarzio D. A Comprehensive Review on the Risk of Metabolic Syndrome and Cardiovascular Disease After Liver Transplantation. *Livers* (2022) 2(2): 85–96. doi:10.3390/livers2020006
10. Di Maira T, Little EC, Berenguer M. Immunosuppression in Liver Transplant. *Best Pract & Res Clin Gastroenterol* (2020) 46(47):101681. doi:10.1016/j.bpg.2020.101681
11. Charlton M, Levitsky J, Aqel B, O'Grady J, Hemibach J, Rinella M, et al. International Liver Transplantation Society Consensus Statement on Immunosuppression in Liver Transplant Recipients. *Transplantation* (2018) 102(5):727–43. doi:10.1097/TP.0000000000002147
12. Fairfield CJ, Harrison EM, Wigmore SJ, VanWagner LB. Steroid-Free Versus Steroid-Containing Immunosuppression for Liver Transplant Recipients. *Clin Liver Dis* (2020) 16(5):191–5. doi:10.1002/cld.992
13. Reding R. Steroid Withdrawal in Liver Transplantation: Benefits, Risks, and Unanswered Questions. *Transplantation* (2000) 70(3):405–10. doi:10.1097/00007890-200008150-00001
14. Murea M, Ma L, Freedman BI. Genetic and Environmental Factors Associated with Type 2 Diabetes and Diabetic Vascular Complications. *The Review Diabetic Studies: RDS* (2012) 9(1):6–22. doi:10.1900/RDS.2012.9.6
15. IRiODaT. *International Registry in Organ Donation and Transplantation* (2023).
16. Kim L, Kim J-A, Kim S. A Guide for the Utilization of Health Insurance Review and Assessment Service National Patient Samples. *Epidemiol Health* (2014) 36: e2014008. doi:10.4178/epih/e2014008
17. Jeong S-H. Current Epidemiology and Clinical Characteristics of Autoimmune Liver Diseases in South Korea. *Clin Mol Hepatol* (2018) 24(1):10–9. doi:10.3350/cmh.2017.0066
18. Annane D, Bellissant E, Bollaert PE, Briegel J, Keh D, Kupfer Y, et al. Corticosteroids for Treating Sepsis in Children and Adults. *Cochrane Database Syst Rev* (2019) 12(12):CD002243. doi:10.1002/14651858.CD002243.pub4
19. Vandenbroucke JP, von Elm E, Altman DG, Gøtzsche PC, Mulrow CD, Pocock SJ, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): Explanation and Elaboration. *Plos Med* (2007) 4(10):e297. doi:10.1371/journal.pmed.0040297
20. Heinze G, Jüni P. An Overview of the Objectives of and the Approaches to Propensity Score Analyses. *Eur Heart Journal* (2011) 32(14):1704–8. doi:10.1093/eurheartj/ehr031
21. Li D-W, Lu T-F, Hua X-W, Dai H-J, Cui X-L, Zhang J-J, et al. Risk Factors for New Onset Diabetes Mellitus After Liver Transplantation: A Meta-Analysis. *World J Gastroenterol WJG* (2015) 21(20):6329–40. doi:10.3748/wjg.v21.i20.6329
22. Chowdhury TA, Wahba M, Mallik R, Peracha J, Patel D, De P, et al. Association of British Clinical Diabetologists and Renal Association Guidelines on the Detection and Management of Diabetes Post Solid Organ Transplantation. *Diabetic Med* (2021) 38(6):e14523. doi:10.1111/dme.14523
23. Park J, Joo D, Kim J. Liver Transplantation and New Life: The Korean Society for Transplantation. Available online at: https://mykst.org/wp-content/uploads/2024/06/%EA%B0%84%EC%9D%B4%EC%8B%9D%EA%B3%BC-%EC%83%88%EB%A1%9C%EC%9A%B4-%EC%82%B6-%EB%82%B4%EC%A7%80.pdf. (Accessed June 30, 2023)
24. Lawendy B, Srinathan S, Kotha S, Gomes C, Misra S, Yu J, et al. Systematic Review and Meta-Analysis of Post-Transplant Diabetes Mellitus in Liver Transplant Recipients. *Clin Transpl* (2021) 35(7):e14340. doi:10.1111/ctr.14340
25. Park S, Kim M, Kim JE, Kim K, Park M, Kim YC, et al. Characteristics of Kidney Transplantation Recipients over Time in South Korea. *The Korean J Intern Med* (2020) 35(6):1457–67. doi:10.3904/kjim.2019.292
26. Fairfield C, Penninga L, Powell J, Harrison EM, Wigmore SJ. Glucocorticosteroid-Free Versus Glucocorticosteroid-Containing Immunosuppression for Liver Transplanted Patients. *Cochrane Database Syst Rev* (2018) 4(4):CD007606. doi:10.1002/14651858.CD007606.pub4
27. Kim J, Joh J-W, Lee K-W, Choi DL, Wang H-J. Safety and Efficacy of Early Corticosteroid Withdrawal in Liver Transplant Recipients: A Randomized Controlled Trial. *Ann Hepatobiliary Pancreat Surg* (2024) 28(2):238–47. doi:10.14701/ahbps.23-129
28. Forrester JV, Xu H, Lambe T, Cornall R. Immune Privilege or Privileged Immunity? *Mucosal Immunol* (2008) 1(5):372–81. doi:10.1038/mi.2008.27
29. Nørgaard M, Ehrenstein V, Vandenbroucke JP. Confounding in Observational Studies Based on Large Health Care Databases: Problems and Potential Solutions - A Primer for the Clinician. *Clin Epidemiol* (2017) 9:185–93. doi:10.2147/clep.S129879
30. Van Hooff JP, Christiaans MH, van Duijnhoven EM. Evaluating Mechanisms of Post-Transplant Diabetes Mellitus. *Nephrol Dial Transplant* (2004) 19(Suppl. l_6):vi8–vi12. doi:10.1093/ndt/gfh1063
31. Muir CA, Kuang W, Muthiah K, Greenfield JR, Raven LM. Association of Early Post-Transplant Hyperglycaemia and Diabetes Mellitus on Outcomes Following Heart Transplantation. *Diabet Med* (2025) 42(1):e15441. doi:10.1111/dme.15441
32. Iqbal A, Zhou K, Kashyap SR, Lansang MC. Early Post-Renal Transplant Hyperglycemia. *The J Clin Endocrinol & Metab* (2021) 107(2):549–62. doi:10.1210/clinem/dgab697

ESOT | Transplant International

BRIEF RESEARCH REPORT
published: 24 February 2026
doi: 10.3389/ti.2026.16099

Donor-Derived Cell-Free DNA as a Non-Invasive Readout of Activity Across the Rejection Continuum

*Louise Benning[1], Aylin Akifova[2], Bilgin Osmanodja[2], Christian Morath[1,3], Julia Beck[4], Ekkehard Schütz[4,5] and Klemens Budde[2]**

[1]Department of Nephrology, Heidelberg University Hospital, Heidelberg, Germany, [2]Department of Nephrology and Intensive Care, Charité Universitätsmedizin Berlin, Berlin, Germany, [3]Department of Nephrology and Hypertension, Klinikum Nuremberg, Paracelsus Medical University, Nuremberg, Germany, [4]Chronix Biomedical GmbH, Göttingen, Germany, [5]Insight Molecular Diagnostics Inc., Nashville, TN, United States

Recent work demonstrated that kidney allograft rejection unfolds along a biological continuum that can be quantified using histopathology-derived continuous indices. To investigate whether donor-derived cell-free DNA (dd-cfDNA) reflects this rejection continuum and complements these histopathology-derived indices, we analyzed the association between dd-cfDNA measures and the newly developed rejection indices in 249 indication biopsies from two independent cohorts. dd-cfDNA was analyzed as percentage, absolute copies/mL, and as a previously developed combined continuous model (CM) score integrating both measures to mitigate limitations of relative measurements. dd-cfDNA increased with histopathological activity and was highest in biopsies with microvascular inflammation (MVI), including antibody-mediated (AMR) and mixed rejection, paralleling high AMR/MVI and activity indices. T-cell-mediated rejection (TCMR) showed elevated TCMR/tubulointerstitial inflammation (TI) indices but lower and more variable dd-cfDNA, accompanied by increased total cfDNA, providing a plausible explanation for the reduced detectability of low-grade TCMR when dd-cfDNA is expressed as a percentage alone. Interclass correlation analyses revealed the strongest associations between dd-cfDNA and the AMR/MVI and activity indices. The combined CM score achieved the highest overall associations (sum R^2 = 3.4), outperforming absolute and relative dd-cfDNA measures. Thus, dd-cfDNA may serve as a non-invasive readout of graft inflammation and extends the rejection-continuum concept into the non-invasive space.

Keywords: dd-cfDNA, donor-derived cell-free DNA, kidney transplantation, rejection, rejection continuum

OPEN ACCESS

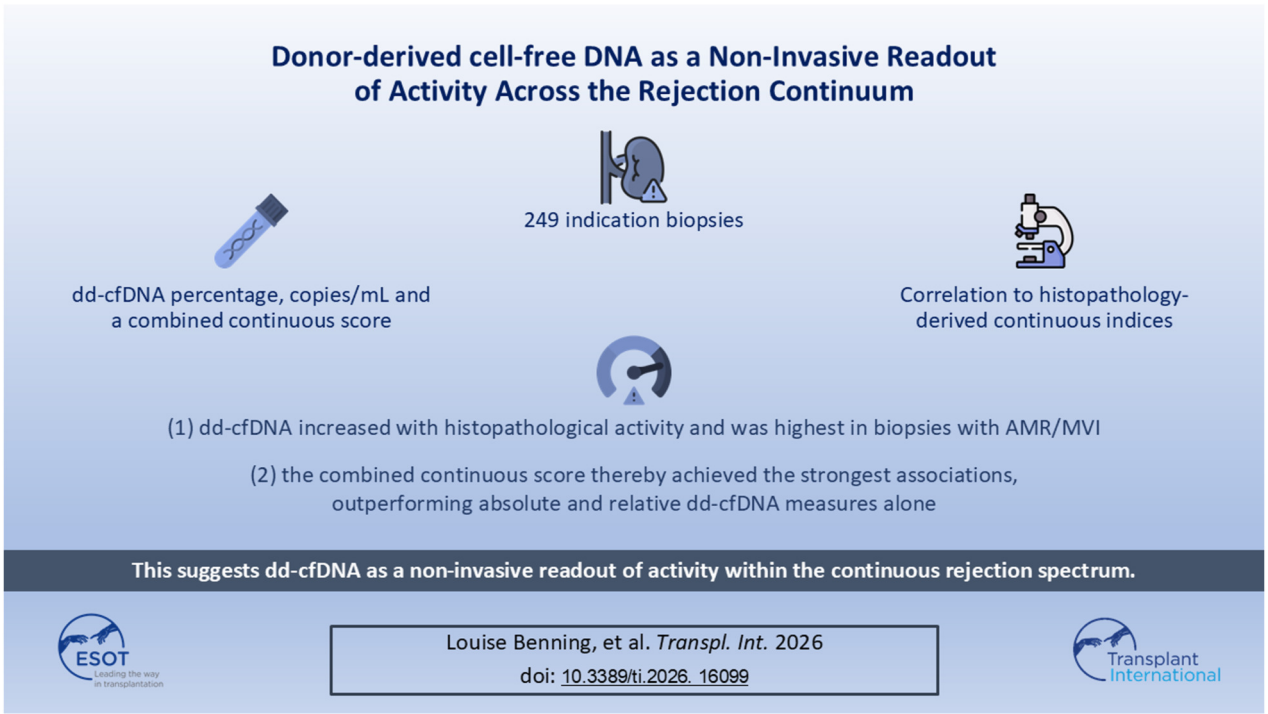
***Correspondence**
Klemens Budde,
klemens.budde@charite.de

Received: 19 December 2025
Revised: 05 February 2026
Accepted: 12 February 2026
Published: 24 February 2026

Citation:
Benning L, Akifova A, Osmanodja B, Morath C, Beck J, Schütz E and Budde K (2026) Donor-Derived Cell-Free DNA as a Non-Invasive Readout of Activity Across the Rejection Continuum. Transpl. Int. 39:16099. doi: 10.3389/ti.2026.16099

INTRODUCTION

The recently published study by Vaulet et al. compellingly demonstrates that kidney allograft rejection unfolds along a biological continuum rather than within rigid diagnostic subcategories [1]. By deriving continuous activity and chronicity indices from assessed histological lesion scores in a cohort study of 19,500 biopsies from 8,873 patients across 10 centers worldwide, the authors elegantly quantified the spectrum of rejection and distinguished microvascular inflammation from tubulointerstitial inflammation patterns. This is a huge step forward in the interpretation of Banff lesions in a more quantitative manner and shall provide a better and standardized patient care if implemented as histopathological standard.



GRAPHICAL ABSTRACT |

An important next step is determining whether non-invasive biomarkers map onto this continuum. Donor-derived cell-free DNA (dd-cfDNA) as an indicator of graft damage is now widely used in clinical kidney transplant care, in both for-cause and surveillance settings [2–4]. There is ongoing discussion about whether such a "liquid biopsy" may complement the histopathological and clinical assessment [3–7]. Usually, dd-cfDNA is measured as percentage of total cfDNA, which, however, may cause misinterpretation in patients with high or low total cfDNA [8]. Absolute dd-cfDNA copy numbers may address some of these limitations but require standardized measurement approaches [8, 9]. To address this, we recently developed a combined continuous model (CM) score that integrates relative and absolute dd-cfDNA measurements into a single composite metric, with the aim of reducing false classifications and improving the robustness of dd-cfDNA interpretation [10].

The aim of this study was therefore to determine whether, and how, dd-cfDNA measures including the combined CM score correlate with the newly developed histopathology-based continuous rejection indices [1] and to identify which Banff lesion patterns are the principal drivers of dd-cfDNA release.

METHODS

To this end, we analyzed a cohort of 249 indication biopsies from two previously published independent cohorts [11, 12]. Detailed descriptions of the patient populations have been reported previously. For each biopsy, histopathological diagnoses were assigned according to Banff criteria [13], and continuous rejection indices for antibody-mediated rejection/microvascular inflammation (AMR/MVI), T-cell-mediated rejection/tubulointerstitial inflammation (TCMR/TI), global activity, and chronicity were calculated as described by Vaulet et al. [1].

dd-cfDNA was analyzed as relative percentage of total cfDNA, as absolute copies per milliliter (cp/mL), and using the previously developed combined CM score integrating relative and absolute dd-cfDNA measurements [10]. Descriptive analyses were performed by histopathological diagnostic category, with indices and dd-cfDNA measures summarized as means ± standard deviations.

To assess associations between individual Banff lesion scores and cfDNA measures, Spearman rank correlation coefficients (ρ) were calculated between each lesion score and dd-cfDNA metrics (percentage, cp/mL, CM score) as well as total cfDNA. Lesions were grouped according to AMR-related, TCMR-related, and chronic injury categories [13].

To evaluate how well dd-cfDNA quantitatively tracks the continuous rejection indices across their dynamic range, interclass correlation analyses were performed. For each dd-cfDNA metric, biopsies were stratified into five equal-sized quintiles. Mean values and standard errors of dd-cfDNA measures and histopathology-derived indices were calculated within each quintile, and linear regression between quintile means was used to derive coefficients of determination (R^2) and corresponding *P*-values. Overall explanatory association was compared across dd-cfDNA metrics by summing R^2 values across indices.

Because the endpoints were continuous indices and ordinal lesion grades, Spearman's ρ and R^2 were used as descriptive

TABLE 1 | Indices and dd-cfDNA by histopathology.

Histopathological Diagnosis	N	AMR/MVI [index]	TCMR/TI [index]	Activity [index]	Chronicity [index]	dd-cfDNA [%]	dd-cfDNA [cp/mL]	dd-cfDNA [CM-Score]
AMR	46	5.04 ± 2.28	0.95 ± 0.85	4.85 ± 2	7.07 ± 3.52	2.01 ± 1.48	96.4 ± 99.8	0.46 ± 0.63
TCMR	16	0.43 ± 0.79	3.57 ± 1.25	4.56 ± 2.1	3.25 ± 2.65	0.56 ± 0.39	53.4 ± 84.4	–0.14 ± 0.52
Mixed Rej	8	5.29 ± 0.96	4.47 ± 1.27	9.13 ± 1.64	6.13 ± 3	1.82 ± 1.11	153.6 ± 111.6	0.73 ± 0.47
DSA- MVI	9	5.34 ± 2.17	1.31 ± 1.04	5.78 ± 2.22	6.22 ± 3.9	1.81 ± 1.41	113.2 ± 101.9	0.5 ± 0.63
Borderline	20	0.6 ± 0.86	1.87 ± 0.23	2.7 ± 0.73	2.85 ± 2.54	0.65 ± 0.54	46.1 ± 66.1	–0.16 ± 0.51
IFTA	46	0.34 ± 0.59	0.64 ± 0.63	0.8 ± 1.09	3.61 ± 1.95	0.41 ± 0.52	21.4 ± 28.4	–0.4 ± 0.33
GN	20	0.57 ± 1.18	1.23 ± 1.2	1.5 ± 1.57	5.1 ± 2.83	0.33 ± 0.2	14.5 ± 9.6	–0.46 ± 0.15
CNI Tox	20	0.47 ± 0.9	0.33 ± 0.37	0.35 ± 0.67	3.85 ± 2.46	0.58 ± 0.59	14.8 ± 11.2	–0.38 ± 0.27
UTI	6	1.16 ± 1.34	2.66 ± 1.85	3.67 ± 3.27	5.83 ± 3.54	0.51 ± 0.32	14.2 ± 6.3	–0.38 ± 0.16
BKVAN	19	0.89 ± 1.2	2.72 ± 1.6	4.11 ± 2.54	2.84 ± 2.06	0.56 ± 1.04	56.4 ± 88.7	–0.2 ± 0.68
Normal	14	0.16 ± 0.58	0.26 ± 0.47	0.21 ± 0.43	1.29 ± 2.37	0.6 ± 1.02	29.8 ± 47.7	–0.31 ± 0.5
ATI	8	0.1 ± 0.27	0.36 ± 0.38	0.38 ± 0.52	1.25 ± 1.04	0.87 ± 1.04	92.3 ± 101.7	0.12 ± 0.77
Other	17	0.45 ± 1.0	1.07 ± 1.61	1.35 ± 1.84	2.71 ± 2.47	0.32 ± 0.11	37.1 ± 49.5	–0.29 ± 0.31

Averages and standard deviations are given. AMR, antibody-mediated rejection; ATI, acute tubular injury; BKVAN, BK polyomavirus nephropathy; CM-score, combined model score; CNI Tox, calcineurin inhibitor toxicity; dd-cfDNA, donor-derived cell-free DNA; DSA, donor-specific antibodies; GN, glomerulonephritis; IFTA, interstitial fibrosis and tubular atrophy; MVI, microvascular inflammation; Rej, rejection; TCMR, T-cell-mediated rejection; TI, tubulointerstitial inflammation; UTI, urinary tract infection.

measures of association. Our analyses were not intended to assess diagnostic discrimination or calibration for binary outcomes.

RESULTS

Across all dd-cfDNA measures, the highest dd-cfDNA levels were observed in biopsies with high histopathological activity indices (**Table 1**). Biopsies characterized by MVI, including AMR, mixed rejection, and DSA-negative/C4d-negative MVI, showed the highest AMR/MVI and global activity index values and correspondingly elevated dd-cfDNA levels. Biopsies with TCMR displayed elevated TCMR/TI and activity indices but lower and more variable dd-cfDNA levels. Borderline changes were associated with moderately increased TCMR/TI and activity indices, falling between overt TCMR and non-rejection. Categories characterized by chronic or non-immune-mediated injury, such as interstitial fibrosis/tubular atrophy (IFTA), glomerulonephritis (GN), or calcineurin inhibitor (CNI) toxicity, exhibited low dd-cfDNA and low activity despite variable chronicity indices.

To further assess the contribution of individual Banff lesions to cfDNA release, Spearman correlation coefficients were calculated between each lesion score and dd-cfDNA measured as percentage, copies/mL, and combined CM score, as well as total cfDNA (**Figure 1**). Lesions associated with AMR showed the highest correlation coefficients with dd-cfDNA measures, with glomerulitis (g) and peritubular capillaritis (ptc) demonstrating the largest coefficients. For TCMR lesions, correlations with dd-cfDNA percentage were lower, whereas higher coefficients were observed with absolute dd-cfDNA copies/mL and total cfDNA. Chronic lesions showed lower correlation coefficients overall with dd-cfDNA: among chronic Banff lesions, only transplant glomerulopathy (cg) and, to a lesser extent, vascular fibrous intimal thickening (cv) were associated with increased dd-cfDNA levels, whereas interstitial fibrosis (ci) and tubular atrophy (ct) showed no positive association or an inverse relationship.

Building on this descriptive alignment, we next assessed how well dd-cfDNA quantitatively tracks the newly developed indices across their dynamic range. We therefore performed interclass (IC) correlations between the histopathology indices and the dd-cfDNA measures, with classes defined by quintiles (20% intervals) of the dd-cfDNA levels in the cohort. Across all dd-cfDNA measurements, the highest R^2 in the IC correlations were observed for the AMR/MVI and activity indices (**Figure 2**). In contrast, R^2 values for the TCMR/TI and chronicity indices were lower (**Figure 2**). Among the dd-cfDNA metrics, the combination score, which simultaneously considers percent and absolute dd-cfDNA, showed the highest overall association with the indices, with a sum R^2 of 3.4 across all indices, followed by absolute dd-cfDNA levels in cp/mL (2.9) and relative dd-cfDNA percentages (2.6) (**Figure 2**). **Supplementary Figure S1** shows the individual-biopsy relationships between each cfDNA measure and the histopathology-derived continuous rejection indices.

DISCUSSION

In this study, we evaluate the newly developed histopathology-derived continuous indices [1] in an independent cohort of clinically indicated biopsies and demonstrate how dd-cfDNA aligns with the inflammatory component of the rejection continuum. Together, our findings suggest that dd-cfDNA may serve as a non-invasive readout of the acuity of graft injury within the continuous rejection spectrum.

As expected, biopsies with MVI as seen in AMR, mixed rejection, and DSAnegC4dneg MVI showed the highest AMR/MVI and activity index values, along with the highest dd-cfDNA levels, highlighting once again the utility of dd-cfDNA, particularly in AMR contexts [3–5, 10, 12, 14–16]. Consistently, correlation analyses demonstrated strong associations between AMR lesions, notably g and ptc, and dd-cfDNA, as demonstrated previously [2, 12].

In contrast, dd-cfDNA levels were lower and more heterogenous in TCMR biopsies, a pattern that has been reported previously across

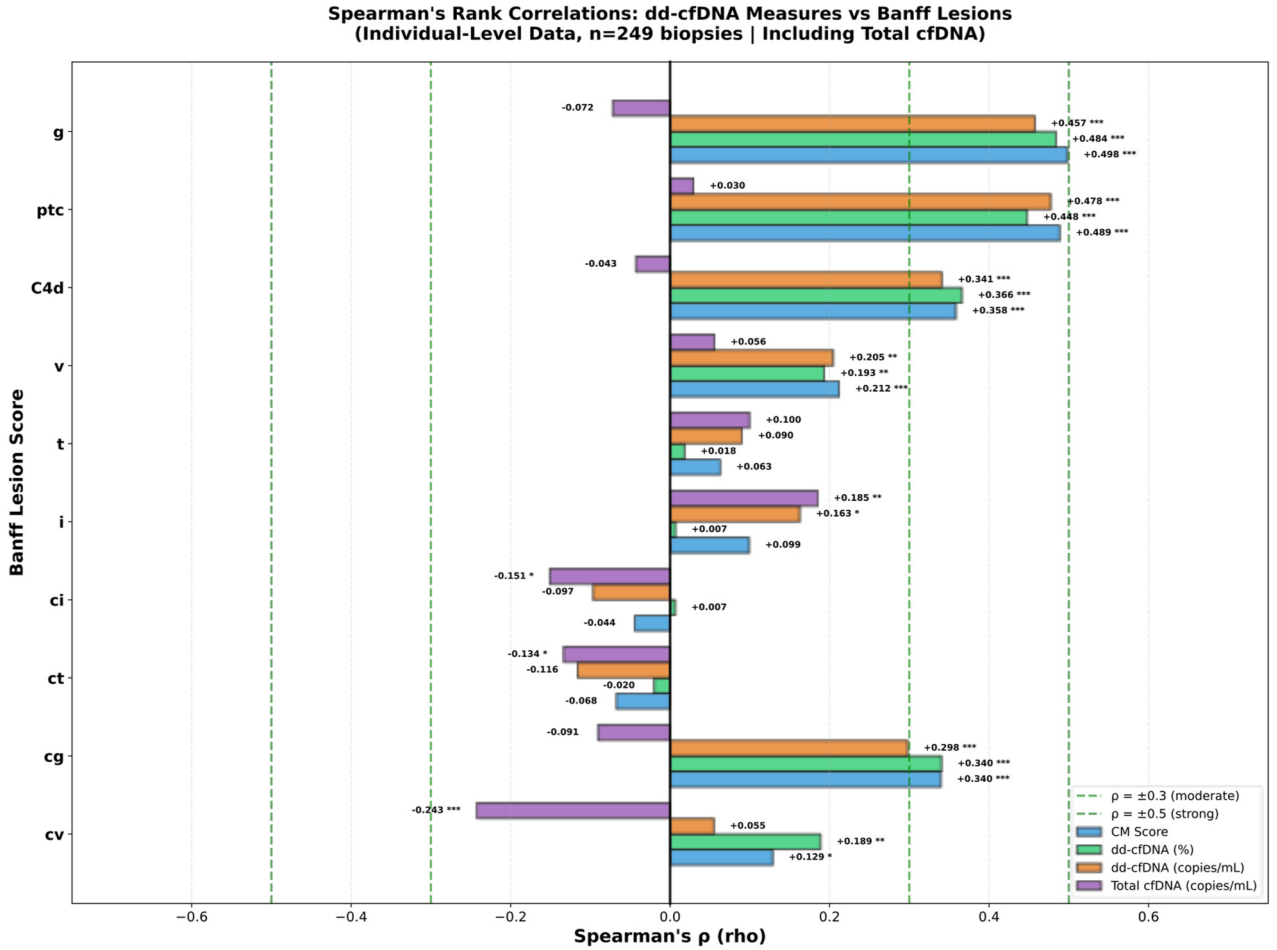


FIGURE 1 | Spearman rank correlations between individual Banff lesion scores and circulating cfDNA measures in 249 kidney transplant biopsies. Correlations are shown for donor-derived cell-free DNA (dd-cfDNA) expressed as combined continuous model (CM) score, percentage, and copies/mL, as well as total cfDNA. dd-cfDNA shows strongest associations with microvascular inflammation lesions (g, ptc), whereas correlations with tubulointerstitial and chronic lesions are weaker. Dashed lines indicate thresholds for moderate and strong correlations; statistical significance is indicated as $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***).

multiple studies [2, 3, 5, 10–12, 14]. For TCMR-associated lesions, total cfDNA showed a strong influence on dd-cfDNA measurements (**Figure 1**). As total cfDNA constitutes the denominator of the percentage-based metric, this provides a plausible explanation for the limited increase in relative dd-cfDNA often observed in TCMR. In contrast, absolute dd-cfDNA expressed as copies per milliliter is not affected by recipient-derived cfDNA and therefore showed a stronger association with TCMR-related injury (**Figure 2**). These observations are strikingly consistent with the limited association between the TCMR/TI index and dd-cfDNA when expressed as a percentage (**Figure 2**). A potential biological explanation is that infiltrating recipient immune cells undergo cell death within the graft during TCMR, contributing substantially to circulating total cfDNA levels and thereby diluting the relative dd-cfDNA fraction when expressed as a percentage. Independently of this effect on total cfDNA, variability in dd-cfDNA measures among TCMR biopsies may also reflect biological differences in injury mechanisms, whereby some cases exhibit interstitial inflammation accompanied by subtle microvascular injury, leading to increased dd-cfDNA, whereas others are dominated by tubular injury that may preferentially drain into the urine and therefore not be reflected in circulating cfDNA. Notably, in our cohort, seven TCMR cases were grade Ia, six were grade Ib, and none were grade III, resulting in a 25% lower mean of the TCMR/TI index compared to the cohort described by Vaulet et al. [1]. This limited dynamic range likely contributes to the weaker alignment of dd-cfDNA with the TCMR/TI index and to mean CM scores near (and slightly below) zero, rather than clearly positive values. Nonetheless, our findings for the first time provide a plausible mechanistic explanation for why low-grade TCMR is difficult to detect when dd-cfDNA is expressed as a percentage alone.

As expected, patients with Borderline changes had only moderately elevated TCMR/TI and activity indices, which were lower than those with overt TCMR. This is consistent with previous findings indicating that approximately 2/3 of Borderline cases have a molecular phenotype compatible with no rejection [17]. In such ambiguous cases, histological assessment and biomarkers may complement one another, with dd-cfDNA providing additional

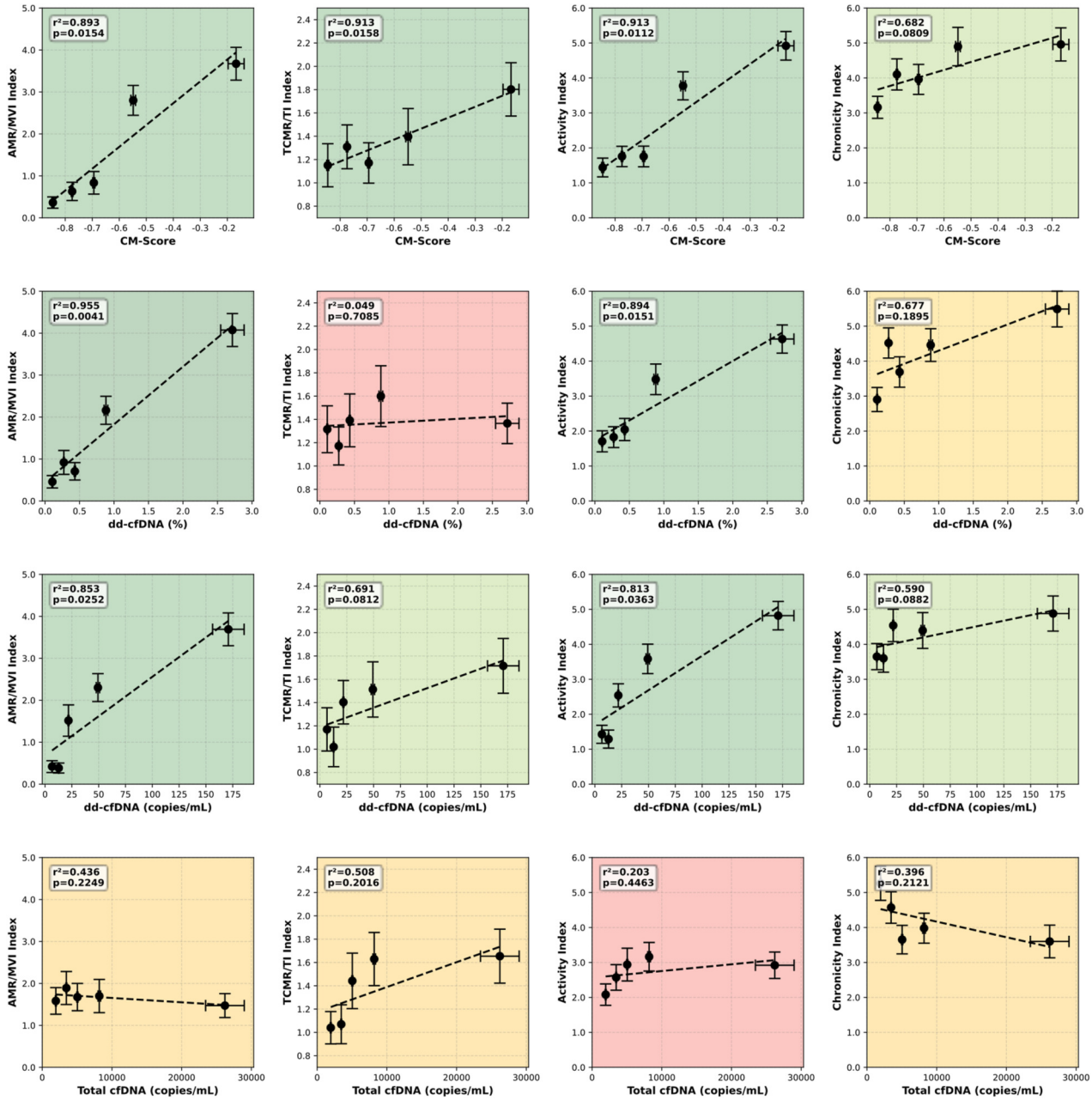


FIGURE 2 | Interclass correlations between continuous indices of kidney transplant rejection and circulating cfDNA measures in 249 kidney transplant biopsies. Biopsies were stratified into quintiles based on the four cell-free DNA (cfDNA) measures, including the combined continuous model (CM) score, donor-derived cell-free DNA (dd-cfDNA) percentage, dd-cfDNA copies/mL, and total cfDNA (rows, top to bottom). Mean values of (dd-)cfDNA and histopathology-derived indices (AMR/MVI, TCMR/TI, activity, chronicity) are shown across quintiles, with linear regression used to derive R^2 and P-values. The CM score showed the highest overall explanatory power compared with absolute and relative (dd-)cfDNA measures. Background colors indicate statistical significance.

support in distinguishing true rejection with ongoing graft injury from innocuous infiltrates, indicating which patients might profit from intensified surveillance [11]. Whether dd-cfDNA can distinguish Borderline lesions that require treatment from those that are clinically benign, however, remains speculative and will require confirmation in larger cohorts of borderline cases together with molecular phenotyping.

The consistently low dd-cfDNA levels observed in chronic and non-immune-mediated injury support the concept that dd-cfDNA primarily reflects acute inflammatory injury rather than chronic structural damage. Among chronic Banff lesions, only transplant glomerulopathy (cg) and, to a lesser extent, vascular fibrous intimal thickening (cv), both reflecting chronic vascular and endothelial injury, were associated with increased

dd-cfDNA levels, whereas interstitial fibrosis (ci) and tubular atrophy (ct), representing largely scarring, showed no positive association or an inverse relationship. Taken together, chronic graft injury represents a relatively weak driver of dd-cfDNA release, consistent with the findings observed for the chronicity index (**Figure 2**).

Thus, our dd-cfDNA data, and in particular the novel combined CM score (integrating the percentage and absolute dd-cfDNA) in relation to the rejection-continuum indices help integrate tissue-based and blood-based measures: whereas the continuous histology-based indices quantify the tissue phenotype of inflammation and injury, dd-cfDNA captures the real-time acuity of graft damage in the blood and may further support longitudinal monitoring of clinical response, as indicated previously [11, 18]. Despite the limited dataset, the overall patterns seem consistent with the biological behavior of dd-cfDNA, which reflects acute inflammation that causes cellular injury and death with subsequent release of dd-cfDNA but remains low in chronic structural damage. This supports the concept of a continuum of rejection activity, with microvascular inflammation representing the high-acuity end of the spectrum.

The results presented herein add to the scientific understanding of dd-cfDNA in relation to the specific lesions described by light microscopy and complement the biopsy-derived framework introduced by the publication of Vaulet et al. [1]. Particularly the increase of total cfDNA in TCMR is a novel observation, which offers an explanation, why low grade TCMR is notoriously hard to detect with dd-cfDNA when expressed as percentage only.

Our study has several limitations: (i) the study population is restricted to indication biopsies and does not include protocol biopsies, which may introduce selection bias and limits generalizability to low-pretest-probability surveillance settings and subclinical phenotypes; (ii) both cohorts are European, which constrains external validity across different populations and clinical practices; (iii) TCMR cases were predominantly low grade, which limits inference regarding cfDNA-index relationships in advanced/high-grade ("full-blown") TCMR; (iv) the combined CM score, while applied here as a pre-specified composite integrating dd-cfDNA percentage and copies/mL, still lacks independent external validation in prospective clinical cohorts; and (v) because our endpoints were the newly proposed continuous rejection indices and Banff ordinal lesion grades, the reported Spearman and R^2 metrics should be interpreted as descriptive measures of association and not as stand-alone diagnostic performance assessments.

Taken together, these invasive and non-invasive measures may contribute to a unified framework in which histology and circulating biomarkers (dd-cfDNA) converge to quantify active injury, track its trajectory, and potentially aid clinicians to more effectively treat graft inflammation with active injury patterns.

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 Medical Faculty of Heidelberg and by the Ethics Committee of Charité – Universitätsmedizin Berlin, respectively. 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, ES, and KB designed the study. LB, JB, ES, and KB analyzed and interpreted the data and drafted the manuscript. Patients were recruited and results generated by LB, AA, BO, CM, and KB. JB and ES established and performed the quantification of dd-cfDNA in their laboratory. CM and KB supervised the project and revised the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

The author(s) declared that financial support was received for this work and/or its publication. The authors declare that this study received in-kind support from Insight Molecular Diagnostics Inc. (iMDx; provision of dd-cfDNA kits/reagents and access to testing infrastructure).

ACKNOWLEDGEMENTS

The graphical abstract has been designed using resources from Flaticon.com.

CONFLICT OF INTEREST

Insight Molecular Diagnostics Inc. (iMDx; GraftAssure) provided kits and reagents for dd-cfDNA assessment and supplied the testing infrastructure used to generate study results. AA received travel support from Insight Molecular Diagnostics Inc. KB acts as a consultant to Insight Molecular Diagnostics Inc. and has received research funds and/or honoraria from: Aicuris, Alexion, Astellas, AstraZeneca, Biogen, Biohope, Carealytics, CareDx, Chiesi, CSL Behring, DTB GmbH, Eledon, Hansa, HiBio, iMDx, MSD, Natera, Neovii, Oncocyte, Oska, Otsuka, Paladin, Pfizer, Pirche, Sanofi, smart care solutions, Stada, Takeda, Veloxis, Vifor and Xenothera. JB was employed by Chronix Biomedical GmbH (an Insight Molecular Diagnostics Inc. company). ES was employed by Insight Molecular Diagnostics Inc., which holds intellectual property rights (EP 3004388B, EP3201361B1, US11155872B2, US10570443B2). The Department of Nephrology, University Hospital Heidelberg (LB and CM) has received kits and supplies for dd-cfDNA testing from CareDx and from Insight Molecular Diagnostics Inc. CM is co-founder and CSO of

TolerogenixX GmbH (Heidelberg, Germany), a biotechnology company that holds licenses and patents for MIC treatment, and has received honoraria from AstraZeneca, Boehringer, Hansa and Novartis.

The remaining author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The authors declare that this study received in-kind support from Insight Molecular Diagnostics Inc. (iMDx; provision of dd-cfDNA kits/reagents and access to testing infrastructure). The study was investigator-initiated and investigator-led. iMDx/Chronix personnel contributed to aspects of data analysis, interpretation, and manuscript drafting. However, the academic investigators retained responsibility for the study design, overall data interpretation, and the decision to submit the manuscript for publication.

GENERATIVE AI STATEMENT

The author(s) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2026.16099/full#supplementary-material

REFERENCES

1. Vaulet T, Koshy P, Wellekens K, Aubert O, Bottomley C, Callemeyn J, et al. Continuous Indices to Assess the Phenotypic Spectrum of Kidney Transplant Rejection. *Nat Commun* (2025) 16(1):10417. doi:10.1038/s41467-025-65153-9
2. Aubert O, Ursule-Dufait C, Brousse R, Gueguen J, Racapé M, Raynaud M, et al. Cell-Free DNA for the Detection of Kidney Allograft Rejection. *Nat Med* (2024) 30(8):2320–7. doi:10.1038/s41591-024-03087-3
3. Budde K, Osmanodja B, Schrezenmeier E, Halleck F, Mayer KA, Böhmig GA, et al. Clinical Utility of Donor-Derived Cell-Free DNA Testing for Kidney Transplant Monitoring in Selected Patients. *Kidney Int* (2025) 107(5):792–6. doi:10.1016/j.kint.2024.09.023
4. Naesens M, Wong G. Simply Measuring what Matters—Toward Clinical Utility of donor-derived Cell-Free DNA Testing? *Kidney Int* (2025) 107(5): 799–805. doi:10.1016/j.kint.2025.01.039
5. Seif N, Bloom RD. Using donor-Derived Cell-Free DNA in Kidney Transplant Patients: This Jury Needs More Evidence. *Kidney Int* (2025) 107(5):796–9. doi:10.1016/j.kint.2024.10.029
6. Pagliazzi A, Bestard O, Naesens M. Donor-Derived Cell-Free DNA: Attractive Biomarker Seeks a Context of Use. *Transpl Int* (2023) 36:12406. doi:10.3389/ti.2023.12406
7. Oellerich M, Sherwood K, Keown P, Schütz E, Beck J, Stegbauer J, et al. Liquid Biopsies: Donor-Derived Cell-Free DNA for the Detection of Kidney Allograft Injury. *Nat Rev Nephrol* (2021) 17(9):591–603. doi:10.1038/s41581-021-00428-0
8. Osmanodja B, Akifova A, Budde K, Choi M, Oellerich M, Schütz E, et al. Absolute or Relative Quantification of Donor-Derived Cell-Free DNA in Kidney Transplant Recipients: Case Series. *Transpl Direct* (2021) 7(11): e778. doi:10.1097/txd.0000000000001237
9. Oellerich M, Budde K, Osmanodja B, Bornemann-Kolatzki K, Beck J, Schütz E, et al. Donor-Derived Cell-Free DNA as a Diagnostic Tool in Transplantation. *Front Genet* (2022) 13:1031894. doi:10.3389/fgene.2022.1031894
10. Benning L, Akifova A, Oellerich M, Osmanodja B, Morath C, Beck J, et al. Improving diagnostic performance of kidney allograft rejection with a model combining relative fraction and absolute copies of donor-derived cell-free DNA: results from five independent cohorts [Preprint]. *medRxiv*. (2025). doi:10.64898/2025.12.01.25341374
11. Benning L, Morath C, Fink A, Rudek M, Speer C, Kälble F, et al. Donor-Derived Cell-Free DNA (dd-cfDNA) in Kidney Transplant Recipients with Indication Biopsy—Results of a Prospective Single-Center Trial. *Transpl Int* (2023) 36:11899. doi:10.3389/ti.2023.11899
12. Akifova A, Budde K, Choi M, Amann K, Buettner-Herold M, Oellerich M, et al. Association of Blood Donor-Derived Cell-Free DNA Levels with Banff Scores and Histopathological Lesions in Kidney Allograft Biopsies: Results from an Observational Study. *Transpl Direct* (2025) 11(5):e1794. doi:10.1097/txd.0000000000001794
13. Naesens M, Roufosse C, Haas M, Lefaucheur C, Mannon RB, Adam BA, et al. The Banff 2022 Kidney Meeting Report: Reappraisal of Microvascular Inflammation and the Role of Biopsy-Based Transcript Diagnostics. *Am J Transpl* (2024) 24(3):338–49. doi:10.1016/j.ajt.2023.10.016
14. Akifova A, Budde K, Oellerich M, Beck J, Bornemann-Kolatzki K, Schütz E, et al. Perspective for Donor-Derived Cell-Free DNA in Antibody-Mediated Rejection After Kidney Transplantation: Defining Context of Use and Clinical Implications. *Transpl Int* (2024) 37:13239. doi:10.3389/ti.2024.13239
15. Benning L, Bestard O. Shedding Light on Microvascular Inflammation: Understanding Outcomes, but what Sparks the Flame? *Transpl Int* (2024) 37:14032. doi:10.3389/ti.2024.14032
16. Akifova A, Budde K, Amann K, Buettner-Herold M, Choi M, Oellerich M, et al. Donor-Derived Cell-Free DNA Monitoring for Early Diagnosis of Antibody-Mediated Rejection After Kidney Transplantation: A Randomized Trial. *Nephrol Dial Transpl* (2024) 40(7):1384–95. doi:10.1093/ndt/gfae282
17. Freitas DGde, Sellarés J, Mengel M, Chang J, Hidalgo LG, Famulski KS, et al. The Nature of Biopsies with "Borderline Rejection" and Prospects for Eliminating This Category. *Am J Transpl* (2012) 12(1):191–201. doi:10.1111/j.1600-6143.2011.03784.x
18. Mayer KA, Schrezenmeier E, Diebold M, Halloran PF, Schatzl M, Schranz S, et al. A Randomized Phase 2 Trial of Felzartamab in Antibody-Mediated Rejection. *N Engl J Med* (2024) 391(2):122–32. doi:10.1056/nejmoa2400763

ESOT | Transplant International

LETTER TO THE EDITOR
published: 24 February 2026
doi: 10.3389/ti.2026.15766

Diagnostical Performances of the FilmArray Gastro-Intestinal Panel in Kidney Transplant Recipients

Elise Ferrie[1], Zoulkarnayni Nikiema[2], Arnaud Serret-Larmande[2,3], Noémie Simon-Tillaux[4], Nadia Arzouk-Snanoudj[1], Marta Cancella de Abreu[5,6], Brigitte Rached[7], Jérôme Robert[6,7], Pierre Galichon[1,8] and Inna Mohamadou[1,8]**

[1]Kidney Transplant Service, Pitié-Salpêtrière Hospital, APHP.Sorbonne Université, Paris, France, [2]Public Health Department, PSL-CFX Clinical Research Unit, Pitié-Salpêtrière Hospital, APHP.Sorbonne Université, Paris, France, [3]Sorbonne University, INSERM, Pierre Louis Epidemiology and Public Health Institute, Paris, France, [4]Office of Biostatistics and Epidemiology, Gustave Roussy, Oncostat U1018, Inserm, University Paris-Saclay, labeled Ligue Contre le Cancer, Villejuif, France, [5]Emergency Department, GHU APHP-Sorbonne Université, Hôpital Pitié-Salpêtrière, APHP.Sorbonne Université, Paris, France, [6]Centre d'Immunologie et des Maladies Infectieuses - CIMI-Paris, Sorbonne Université, and Infection control Unit, Paris, France, [7]Bacteriology Department, Pitié-Salpêtrière Hospital, APHP.Sorbonne Université, Paris, France, [8]INSERM, Sorbonne Université, Common and Rare Kidney Diseases: from Molecular Events to Precision Medicine, CoRaKid, Paris, France

Keywords: diagnosis, diarrhea, kidney transplantation, PCR, performances

Dear Editors,

Diarrhea is a major burden for kidney transplant recipients (KTR), associated with dehydration, sepsis, acute kidney injury, immunosuppressor overdose or discontinuation, and rejection [1, 2]. Infections and drug toxicity are the main causes of diarrhea in KTR, with infection being present in 23%–51% of all cases [3–7]. Because of the variety of potential pathogens involved, it requires multiple and repeated stool samples for identification and appropriate treatment.

Diagnosis relies on microscopy, antigen detection, culture and specific polymerase chain reaction (PCR) [8]. They are time-consuming and can lack sensitivity. Multiplex PCR enables the diagnosis of a large range of microbiological agents in one unique sample, in a shorter timespan (around 1 h), and with a limited cost. FilmArray GastroIntestinal Panel (FAGIP) is a multiplex FDA-approved PCR, enabling the detection in stools of most pathogens involved in diarrhea. FAGIP has shown promising results in children [9], liver transplant recipients [10], and hematologic patients [11]. In KTR, data are limited and based on retrospective studies [12].

In this study, we compare the sensibility and specificity of FAGIP vs. standard tests in a population of KTR with diarrhea.

We performed a double-blind, observational, prospective cohort study in KTR from a single university hospital. The study was approved by the Institutional Review Board (IRB) CERC-MIT.

KTR hospitalized for acute diarrhea (<7 days) or who developed diarrhea during their hospital stay from April 2022 to February 2024 (with an interruption from May 2023 to October 2023 for the replacement of expired FAGIP kits and the relocation of the PCR device) were included in the study. The inclusion criteria consisted of adult KTR with a functional graft who did not express opposition to the study. Exclusion criteria were patients with a non-functional kidney transplant, resolution of diarrhea before samples could be achieved, those already included in the study for a diarrhea episode, and patients with a known positive CMV-PCR in blood tests during the 7 days preceding the study, as the diagnosis of CMV-related diarrhea does not rely on stool tests. Patients were included prospectively at their hospital admission and followed throughout their entire hospital stay.

Standard tests were prescribed by the attending physician following the procedures of each local laboratory: (i) bacteriological cultures for classical digestive pathogens (*Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Yersinia* spp.) and enzyme immunoassay for shiga-toxin in case of bloody stools; (ii) tests for toxinogenic *C. difficile*; (iii) tests for enteropathogenic parasites (cryptosporidium

OPEN ACCESS

***Correspondence**
Pierre Galichon,
pierre.galichon@aphp.fr
Inna Mohamadou,
inna.mohamadou@aphp.fr

Received: 20 October 2025
Revised: 03 February 2026
Accepted: 09 February 2026
Published: 24 February 2026

Citation:
Ferrie E, Nikiema Z, Serret-Larmande A, Simon-Tillaux N, Arzouk-Snanoudj N, Cancella de Abreu M, Rached B, Robert J, Galichon P and Mohamadou I (2026) Diagnostical Performances of the FilmArray Gastro-Intestinal Panel in Kidney Transplant Recipients. Transpl. Int. 39:15766. doi: 10.3389/ti.2026.15766

TABLE 1 | Population's characteristics and FAGIP results.

Population's characteristics		Median [IQT] or number (percentage)
Age (median, IQT)		51.5 [43.3; 60.7]
Renal graft alone (n,%)		34 (100%)
Immunosuppressive treatment (n, %)		
Corticoids		34 (100%)
Mycophenolate mofetil		33 (97.1%)
Tacrolimus		33 (97.1%)
Ciclosporine		1 (2.9%)
mTORi		1 (2.9%)
Delay since graft (months) (median, IQT)		6.1 [1.17; 8.4]
Hospitalization (n,%)		26 (76%)
Length of hospitalization (days, median, IQT)		7 [2.0; 16.7]
Fever (n, %)		23 [67.6%]
IV hydration (n,%)		15 (44.1%)
Length of hydration (days, median, IQR)		4.5 [3.25; 6.75]
Acute kidney injury (n, %)		23 (34.6%)
Initial creatininemia (mg/L) (median, IQR)		2.79 [1.8; 4.08]
CRP (mg/L) (median, IQR)		4.02 [1.09; 20.74]
Stool samples (n, %)		
Standard stool culture		30 (88%)
Search for *C. difficile* toxin		34 (100%)
Parasitologic		31 (91%)
Virologic		9 (26%)
Delay before diagnostic (days, median, IQT)		6 [3; 9,7]
Delay before specific treatment (days, median, IQT)		7 [6; 9,5]
FAGIP and gold standard results		
Positive FAGIP		13 (38.2%)
- Positive FAGIP and positive gold standard		4
- Positive FAGIP and negative gold standard		9
Negative FAGIP		21 (61.8%)
- Negative FAGIP and negative gold standard		19
- Negative FAGIP and positive gold standard		2
Comparison between pathogens identified with FAGIP and gold standard		
FilmArray GI panel		
Pathogen 1	Pathogen 2	Gold standard
Enteropathogenic E.coli	*Campylobacter*	
Enteropathogenic E.coli	*C. difficile*	-
Shiga-like toxin-producing E.coli	Cryptosporidium	Enteropathogenic E.coli
Cryptosporidium	-	Cryptosporidium
Sapovirus	-	Cryptosporidium
Enteroaggregative E.coli	-	-
Enteropathogenic E.coli	-	-
Enteropathogenic E.coli	-	-
Enteropathogenic E.coli	Enterotoxigenic E.coli	-
Enteropathogenic E.coli	Cryptosporidium	-
Enteroinvasive E.coli	*Shigella*	Cryptosporidium
Rotavirus	-	-
Sapovirus	-	-

or microsporidium) in the parasitology department (three stool samples within 5 days); and (iv) tests for enteric viruses (adenovirus, enterovirus, sapovirus, or norovirus) in the virology department.

FAGIP was performed on a stool sample by an independent clinical study technician, blinded from the patient, the circumstances of diarrhea, and the results of standard analyses. Investigators and clinicians were blinded from the FAGIP results in order not to interfere with the management of the patients.

Clinical and biological data were prospectively collected from electronic medical files.

Thirty-four patients were included in the study. Clinical and microbiological characteristics are summarized in **Table 1**. The median age was 51 years. Immunosuppression included corticosteroid (100%), mycophenolate mofetil (97.1%), or tacrolimus (97.1%). The median time between transplantation and diarrhea was 6.1 months. Twenty-three (67.6%) patients had Acute Kidney Injury and 55.9% had intravenous hydration. The median serum creatinine was 2.79 mg/dL.

Amongst 34 patients, 30 (88.2%) had a stool culture and 31 (91.1%) a parasitological stool test, but only 9 (26.5%) had a virological test. FAGIP was performed in all patients.

Antimicrobial treatment for diarrhea was initiated in 4/34 (11.8%) patients, with a delay of 5 days.

Six (17.6%) patients had a diagnosis of infectious diarrhea (**Table 1**). In two of them, the diagnosis was made in spite of negative stool tests: one patient had a blood culture positive for *Klebsiella pneumoniae* and *E.coli* with a negative stool culture, and the second patient was diagnosed with CMV disease. FAGIP was negative in these two patients.

In the four patients with a positive standard test, FAGIP was also positive and identified the same pathogen. In three cases, FAGIP detected a second pathogen (**Table 1**). In the 30 patients with negative standard tests, FAGIP was positive in nine patients. Altogether, FAGIP was positive in 13 out of 34 patients (38.2%).

To investigate the significance of a positive FAGIP with negative standard tests, we studied clinical and demographic features: systolic arterial blood pressure (BP) was lower in patients with a positive FAGIP (median 121 vs. 138 mmHg, $p = 0.049$). Other features including temperature, kidney function, and length of stay were not significantly different.

These results highlight several key findings:

1. In our cohort of 34 patients, standard methods had limited yield since they could identify pathogens in only 4/34 (11.7%) patients (three parasites and one bacteria). The virological test could be performed in only 26.5% of patients, underscoring the difficulty of performing repeated stool sampling in real-life settings.
2. FAGIP was concordant with standard methods in the four patients with a positive standard test.
3. FAGIP was positive in 9/34 patients with negative standard tests (26.5%).

In these nine patients, FAGIP positivity with negative standard tests could have warranted a specific treatment or modification of immunosuppressive regimen. However, it is not possible at this stage to distinguish asymptomatic carriage from authentic active infection (as suggested by the slightly lower BP). Eventually, all these patients evolved well without a specific treatment. Therefore, it is also possible that FAGIP results were falsely positive, by detecting DNA or RNA of pathogens that can persist in stool after healing an infection or colonizing non-pathogenic agents.

An interventional study is thus needed to assess the effect of treating patients based on FAGIP results. Resolution of diarrhea, but also health costs and antibiotic resistance, are relevant outcomes, since the integration of highly sensitive multiplex PCR tests generally leads toward more antibiotics and anti-viral and anti-parasitic drug use.

In conclusion, FAGIP is an easy-to-implement and sensitive method for the identification of diarrhea-associated pathogens in KTR. Its increasing use in place of the standard microbiology tests raises concerns about overdiagnosis and overtreatment that will need further prospective evaluation.

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 studies involving humans were approved by CER-MIT (Comité d'éthique en infectiologie). 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

EF: inclusion, interpretation, drafting. ZN, ASL and NST: methodology and statistical analysis. MCA: design of the study and inclusion. BR and JR: bacteriological analyses and interpretation. PG and IM: design, interpretation and writing. All authors made critical revisions to the manuscript and approved the final version.

FUNDING

The author(s) declared that financial support was received for this work and/or its publication. The authors declare that this study received funding from Mécénat APHP. The funder was not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication.

CONFLICT OF INTEREST

The authors(s) declared that this work 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) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

REFERENCES

1. Gil-Vernet S, Amado A, Ortega F, Alarcón A, Bernal G, Capdevila L, et al. Gastrointestinal Complications in Renal Transplant Recipients: MITOS Study. *Transpl Proc. Sept* (2007) 39(7):2190–3. doi:10.1016/j.transproceed.2007.07.015
2. Patil AD, Saxena NG, Thakare SB, Pajai AE, Bajpai D, Jamale TE. Diarrhea After Kidney Transplantation: A Study of Risk Factors and Outcomes. *J Postgrad Med* (2023) 69(4):205–14. doi:10.4103/jpgm.jpgm_601_22
3. Hardinger KL, Brennan DC, Lowell J, Schnitzler MA. Long-Term Outcome of Gastrointestinal Complications in Renal Transplant Patients Treated With Mycophenolate Mofetil. *Transpl Int Off J Eur Soc Organ Transpl* (2004) 17(10):609–16. doi:10.1007/s00147-004-0768-6
4. Bunnapradist S, Neri L, Wong W, Lentine KL, Burroughs TE, Pinsky BW, et al. Incidence and Risk Factors for Diarrhea Following Kidney Transplantation and Association with Graft Loss and Mortality. *Am J Kidney Dis Off J Natl Kidney Found* (2008) 51(3):478–86. doi:10.1053/j.ajkd.2007.11.013
5. Maes B, Hadaya K, de Moor B, Cambier P, Peeters P, de Meester J, et al. Severe Diarrhea in Renal Transplant Patients: Results of the DIDACT Study. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2006) 6(6):1466–72. doi:10.1111/j.1600-6143.2006.01320.x
6. Coste JF, Vuiblet V, Moustapha B, Bouin A, Lavaud S, Toupance O, et al. Microbiological Diagnosis of Severe Diarrhea in Kidney Transplant Recipients by Use of Multiplex PCR Assays. *J Clin Microbiol* (2013) 51(6):1841–9. doi:10.1128/JCM.03366-12
7. Altiparmak MR, Trablus S, Pamuk ON, Apaydin S, Sariyar M, Oztürk R, et al. Diarrhoea Following Renal Transplantation. *Clin Transpl* (2002) 16(3):212–6. doi:10.1034/j.1399-0012.2002.01129.x
8. Angarone M, Snydman DR, AST ID Community of Practice. Diagnosis and Management of Diarrhea in Solid-Organ Transplant Recipients: Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. *Clin Transpl Sept* (2019) 33(9):e13550. doi:10.1111/ctr.13550
9. Spina A, Kerr KG, Cormican M, Barbut F, Eigentler A, Zerva L, et al. Spectrum of Enteropathogens Detected by the Filmarray GI Panel in a Multicentre Study of Community-Acquired Gastroenteritis. *Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis* (2015) 21(8):719–28. doi:10.1016/j.cmi.2015.04.007
10. Ching CK, Nobel YR, Pereira MR, Verna EC. The Role of Gastrointestinal Pathogen Polymerase Chain Reaction Testing in Liver Transplant Recipients Hospitalized With Diarrhea. *Transpl Infect Dis Off J Transpl Soc* (2022) 24(4):e13873. doi:10.1111/tid.13873
11. Alejo-Cancho I, Fernández Avilés F, Capón A, Rodríguez C, Barrachina J, Salvador P, et al. Evaluation of a Multiplex Panel for the Diagnosis of Acute Infectious Diarrhea in Immunocompromised Hematologic Patients. *PLoS One* (2017) 12(11):e0187458. doi:10.1371/journal.pone.0187458
12. Atamna A, Rahamimov R, Levit A, Saleh L, Ben Zvi H, Bishara J, et al. Enteric Pathogen Detection Using Multiplex PCR Assay in Kidney Transplant Recipients With Diarrhea—Retrospective Before–After Study. *Infect Dis Ther* (2024) 13(11):2415–22. doi:10.1007/s40121-024-01056-4

ESOT | Transplant International

LETTER TO THE EDITOR
published: 27 February 2026
doi: 10.3389/ti.2026.15400

Intraoperative Phrenic Nerve Monitoring in Lung Transplants: Results From a Single Center Prospective Cohort

Alberto Pérez de Vargas[1], Lucas Rafael Pérez-Cuesta[1], Andrés Varela de Ugarte[2], Edwin Ebrat Mancilla[1], Alejandra Sánchez Aparicio[1], Marta Vaquero Martínez[1], Luis López Pájaro[1], Paula Péndola Calero[1], Virginia Russu[1] and David Gómez de Antonio[2]*

[1]Clinical Neurophysiology Department Hospital Universitario Puerta de Hierro de Majadahonda, Madrid, Spain, [2]Thoracic Surgeon from Thoracic Surgery Department Hospital Universitario Puerta de Hierro de Majadahonda, Madrid, Spain

Keywords: lung transplant, mechanical ventilation weaning, phrenic intraoperative monitoring, postoperative nerve injury, postoperative phrenic damage

Dear editors,

Lung transplantation is a complex surgical procedure associated with significant perioperative morbidity. Adequate respiratory function after transplantation depends largely on proper diaphragmatic movement, which is exclusively innervated by the phrenic nerve. Injury to this nerve during surgical dissection, mediastinal manipulation, or thermal exposure may result in diaphragmatic paralysis or dysfunction, thereby compromising postoperative respiratory mechanics.

Phrenic nerve injury following lung transplantation has been reported with variable incidence and may significantly delay weaning from mechanical ventilation. Prolonged ventilatory support increases the risk of infection, ICU-related complications, and overall hospital costs. Early detection of phrenic nerve compromise during surgery may allow immediate corrective maneuvers and reduce the severity or permanence of nerve injury.

Intraoperative neurophysiological monitoring provides continuous functional assessment of neural pathways and allows surgeons and anesthesiologists to detect early warning signs of nerve injury [1, 2]. While its benefit has been well established in other surgical fields, experience with phrenic nerve monitoring in lung transplantation remains scarce [3]. The aim of this study was therefore to describe our institutional experience with intraoperative phrenic nerve monitoring and to evaluate its impact on postoperative outcomes and healthcare costs.

This longitudinal, prospective, parallel-group study was conducted at Hospital Universitario Puerta de Hierro Majadahonda in accordance with international ethical guidelines and the ethics statement of the International Society for Heart and Lung Transplantation (ISHLT). All patients undergoing lung transplantation between 11 January 2018, and 31 December 2019, were screened for inclusion.

Patients were excluded if they died within 4 weeks following transplantation, underwent redo lung transplantation, required preoperative non-invasive mechanical ventilation or extracorporeal membrane oxygenation (ECMO), or underwent combined heart–lung transplantation. Redo transplantations were excluded to maintain sample homogeneity, as these cases are typically more complex and associated with a higher risk of phrenic nerve injury.

A total of 58 patients met the inclusion criteria. Thirty-one patients underwent lung transplantation with intraoperative phrenic nerve monitoring (IOM group), while 27 patients formed the control group. Monitoring was applied consecutively depending on the availability of the neurophysiology team. Randomization and blinding were not performed due to ethical concerns regarding the potential withholding of a protective monitoring technique.

OPEN ACCESS

***Correspondence**
Alberto Pérez de Vargas,
albertoperezdv@hotmail.com

Received: *08 August 2025*
Revised: *27 January 2026*
Accepted: *17 February 2026*
Published: *27 February 2026*

Citation:
Pérez de Vargas A, Rafael Pérez-Cuesta L, Varela de Ugarte A, Ebrat Mancilla E, Sánchez Aparicio A, Vaquero Martínez M, López Pájaro L, Péndola Calero P, Russu V and Gómez de Antonio D (2026) Intraoperative Phrenic Nerve Monitoring in Lung Transplants: Results From a Single Center Prospective Cohort. Transpl. Int. 39:15400. doi: 10.3389/ti.2026.15400

All patients underwent a standardized preoperative neurophysiological assessment to document baseline phrenic nerve function. Patients in the monitored group also underwent postoperative neurophysiological evaluations at 1, 6, and 12 months after transplantation.

Intraoperative phrenic nerve monitoring was performed using a combination of compound muscle action potential (CMAP) recordings and free-running electromyography (EMG). CMAPs were obtained by electrical stimulation of the phrenic nerve in the cervical region, with recordings obtained from the diaphragm using both external and internal electrodes [4].

Baseline recordings were established at the beginning of the surgical procedure and served as a reference throughout the operation. Alarm criteria were defined as a permanent reduction in CMAP amplitude greater than 50% or an increase in distal latency greater than 10% relative to baseline values [5]. Lesser changes, such as amplitude reductions between 30% and 50%, were considered warning signs and prompted immediate communication with the surgical team [6].

Free-running EMG was used to detect mechanical or thermal irritation of the nerve in real time. In addition, train-of-four (TOF) stimulation was employed to monitor the degree of neuromuscular blockade and exclude anesthetic interference with CMAP amplitude. Throughout the procedures, neuromuscular blockade was carefully adjusted to maintain reliable and reproducible recordings.

No adverse events related to intraoperative monitoring were observed. Baseline demographic and clinical characteristics were comparable between the two groups, with the exception of a higher prevalence of diabetes mellitus in the monitored group. Importantly, none of the patients with diabetes demonstrated evidence of preoperative peripheral neuropathy on baseline testing.

Postoperative outcomes demonstrated a consistent trend toward improved recovery in the monitored group. Patients who underwent intraoperative monitoring experienced shorter durations of mechanical ventilation, reduced ICU stay, and shorter overall hospital stay compared with controls. Although these differences did not reach statistical significance, they were clinically meaningful (**Table 1**).

Seven cases of new-onset phrenic nerve dysfunction were detected postoperatively, most of which were transient and associated with reversible intraoperative changes. One patient exhibited a sustained reduction in CMAP amplitude greater than 50%, which correlated with persistent phrenic nerve dysfunction at 1-year follow-up.

Cost analysis revealed an average saving of €13,952.84 per patient in the monitored group, primarily attributable to reduced ICU and hospital length of stay.

The present study demonstrates that intraoperative phrenic nerve monitoring during lung transplantation is feasible, safe, and capable of providing clinically relevant information. Early detection of intraoperative changes allowed the surgical team to implement corrective maneuvers aimed at preventing permanent nerve injury [7].

TABLE 1 | Post-surgery variables comparative.

Variables	Group 1 (IOM)	Group 0 (control)	p
N	31	27	
ICU_st	11.35 ± 12.05	14.40 ± 15.1	0.39
MVT	6.77 ± 13.68	11.92 ± 20.01	0.13
Early extubation	14 (45.16%)	8 (29.62%)	0.11
Hospital_st	46.74 ± 19.69	69.48 ± 60.70	0.05
Total cost (euros)	37.226,91	51.179,76	0,09

The quantitative variables are expressed as mean standard ± deviation. The qualitative variables are expressed as absolute variables and percentages. IOM: intraoperative monitorization, ICU_st: stay in ICU, SD: standard deviation, MVT: mechanical ventilation time, n: number of cases, %: percentage, Hospital_st: hospital stay. Total cost per patient.

When warning criteria were met, recovery strategies summarized by the acronym TIPP (Time, Irrigation, Papaverine, Pressure) were applied [8]. The most frequently used interventions were temporary interruption of the surgical maneuver and irrigation with warm saline, both of which were effective in restoring baseline CMAP values in the majority of cases.

Our findings support previous observations suggesting that CMAP amplitude reductions greater than 50% are associated with severe and potentially permanent nerve injury, whereas reductions between 30% and 50% may result in mild to moderate dysfunction. Even mild phrenic nerve dysfunction was associated with prolonged hospital stay, underscoring the clinical relevance of early detection [9].

Although the study was not powered to demonstrate statistically significant differences in all clinical endpoints, the consistent trend toward improved outcomes and the substantial cost savings observed highlight the potential value of routine intraoperative monitoring.

Intraoperative phrenic nerve monitoring during lung transplantation represents a valuable adjunct to surgical care. The technique is safe, provides real-time functional information, and may reduce postoperative morbidity, length of hospital stay, and overall healthcare costs. Based on these findings, routine implementation of phrenic nerve monitoring should be considered in lung transplantation programs [10].

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 Ethics comittee of Hospital Puerta de Hierro Majadahonda. 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

All the authors have participated in the design of the study, the surgical procedure or the nerve monitoring, interpretation of results and writing or reviewing the article. However, depending on the knowledge of each writer they have participated more heavily in certain aspects of the process. AP, LRP-C, EE, AS, MV, LL, PP, and VR have been involved in the nerve monitoring during surgery, whereas DG and AV have participated in the surgical process. All the authors, have participated in the writing and reviewing of the article, with a more notable contribution being made by AP and DG. All authors contributed to the article and approved the submitted version.

FUNDING

The author(s) declared that financial support was received for this work and/or its publication. Funding for publication fees has been received by Fundación Investigación Biomédica H. Puerta de Hierro Majadahonda.

CONFLICT OF INTEREST

The authors(s) declared that this work 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) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

REFERENCES

1. Danks RA, Aglio LS, Gugino LD, Black PM. Craniotomy Under Local Anesthesia and Monitored Conscious Sedation for the Resection of Tumors Involving Eloquent Cortex. *J Neurooncol* (2000) 49(2):131–9. doi:10.1023/a:1026577518902
2. Monitoring Somatosensory Evoked Potentials. In: Deletis V, Shils Jay L, Sala FSK, editors. *Neurophysiology in Neurosurgery A Modern Approach*. 2nd ed. San Diego, California: Nikki Levy. (2021). p. 45–7. doi:10.1016/C2017-0-01299-5
3. Hernández-Hernández MA, Sánchez-Moreno L, Orizaola P, Iturbe D, Álvaréz C, Fernández-Rozas S, et al. A Prospective Evaluation of Phrenic Nerve Injury After Lung Transplantation: Incidence, Risk Factors, and Analysis of the Surgical Procedure. *J Heart Lung Transpl* (2022) 41(1):50–60. doi:10.1016/j.healun.2021.09.013
4. Morélot-Panzini C, Fournier E, Donzel-Raynaud C, Dubourg O, Willer J-C, Similowski T. Conduction Velocity of the Human Phrenic Nerve in the Neck. *J Electromyogr Kinesiol* (2009) 19(1):122–30. doi:10.1016/j.jelekin.2007.06.017
5. Grande-Martín A, Martínez-Moreno A, Sánchez-Honrubia RM, Pardal-Fernández JM. Intraoperative Neurophysiological Monitoring of the Phrenic Nerve: Utility and Descriptions of the Technique. *Cir Esp* (2019) 97(2):103–7. doi:10.1016/j.ciresp.2018.11.002
6. Lucente G, Martinez-Barenys C, Ramos-Fransi A, Almendrote-Muñoz M, López de Castro P, Deletis V, et al. A New Methodology for Intraoperative Monitoring of the Functional Integrity of the Phrenic Nerve During Cardiothoracic Surgery. *J Clin Neurophysiol* (2021) 38(3):226–30. doi:10.1097/WNP.0000000000000677
7. Crothers E, Kennedy DS, Emmanuel S, Molan N, Scott S, Rogers K, et al. Incidence of Early Diaphragmatic Dysfunction After Lung Transplantation: Results of a Prospective Observational Study. *Clin Transpl* (2021) 35(9):e14409. doi:10.1111/ctr.14409
8. Sala F, Coppola A, Tramontano V. Intraoperative Neurophysiology in Posterior Fossa tumor Surgery in Children. *Childs Nerv Syst* (2015) 31(10):1791–806. doi:10.1007/s00381-015-2893-1
9. Anwar O, Gunawardene MA, Dickow J, Scherschel K, Jungen C, Münkler P, et al. Contemporary Analysis of Phrenic Nerve Injuries Following Cryoballoon-Based Pulmonary Vein Isolation: A Single-Centre Experience with the Systematic Use of Compound Motor Action Potential Monitoring. *PLoS One* (2020) 15, e0235132. doi:10.1371/journal.pone.0235132
10. Sala F, Dvorak J, Faccioli F. Cost Effectiveness of Multimodal Intraoperative Monitoring During Spine Surgery. *Eur Spine J*. (2007) 16 S229–S231. doi:10.1007/s00586-007-0420-0

Transplant International

Official journal of the European Society for Organ Transplantation

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

tieditorialoffice@frontierspartnerships.org
frontierspartnerships.org/journals/transplant-international

frontiers | Publishing Partnerships