ESOT

Volume 35 | Issue 9
September 2022

Transplant International

Prehabilitation for liver transplantation

Transplant International

frontiers | Publishing Partnerships

Transplant International

Editorial Office
Nathan Masters
tieditorialoffice@frontierspartnerships.org

ESOT

Transplant International

Prehabilitation for liver transplantation

ISSN 1432-2277
ISBN 978-2-8325-5273-5
DOI 10.3389/978-2-8325-5273-5

Transplant International

Table of contents

Cover article

Original Research


ESOT
Grants
31 AUG - 31 OCT

ESOT TLJ3.0

TRANSPLANTATION LEARNING JOURNEY

PRAGUE, 13-15 NOV 2022

Consensus

#ESOTTLJ

ESOT Mentorship Programme

APPLY BY 31 OCT

TRANSPLANT TRIAL WATCH
published: 01 September 2022
doi: 10.3389/ti.2022.10769

Transplant Trial Watch

John M. O'Callaghan[1,2]*

[1]University Hospitals Coventry and Warwickshire, Coventry, United Kingdom, [2]Centre for Evidence in Transplantation, University of Oxford, Oxford, United Kingdom

Keywords: systematic review, kidney transplantation, living donor liver transplantation, randomised controlled trial, synbiotics

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

RANDOMISED CONTROLLED TRIAL 1

The Role of Renal Resistive Index as a Prognostic Tool in Kidney Transplantation: A Systematic Review.
by Azzouz, S., et al. Nephrology Dialysis Transplantation 2022 [record in progress].

Aims
This study aimed to summarise the available evidence investigating the prognostic role of renal resistive index (RRI) in kidney transplant recipients (KTRs).

Interventions
A literature search was performed on databases including MEDLINE, Cochrane CENTRAL, Embase and Scopus. Study screening and data extraction were conducted by two independent reviewers. The risk of bias was assessed using the Newcastle-Ottawa Scale for case-control studies and the Agency for Healthcare Research and Quality score for cross-sectional studies.

Participants
26 studies were included in the review.

Outcomes
Patient death, graft failure, measures of graft function and proteinuria.

Follow-Up
Not applicable.

OPEN ACCESS

***Correspondence:**
John M. O'Callaghan
john.ocallaghan@uhcw.nhs.uk

Received: *15 July 2022*
Accepted: *21 July 2022*
Published: *01 September 2022*

Citation:
O'Callaghan JM (2022) Transplant Trial Watch. Transpl Int 35:10769. doi: 10.3389/ti.2022.10769

CET Conclusion

This systematic review of Renal Resistive Index (RRI) in renal transplantation does have some good quality markers but also has some elements in the methodology and data quality that make strong conclusions difficult. The study was registered in advance with a review protocol on the PROSPERO system. A search was conducted on multiple database and 2 authors screened abstracts independently. Two authors extracted data and quality assessed studies independently. 26 studies were included, including 7049 renal transplant recipients, all studies were observational. 19 studies in languages other than English were excluded, as were an additional 19 studies for "erroneous data, unclear methods of analysis, or when data extraction could not be performed," which is a very significant proportion of the data that could have been available. Meta-analysis was not possible due to significant heterogeneity in study design and outcomes within the remaining papers; Some studies reported RRI as a continuous variable and others as categorical, in others it is reported as median for a whole population. There was also inconsistency in the timing of RRI assessment after transplantation. Overall risk of bias was concluded to be moderate to high. Most studies that reported on death showed an association between higher RRI and risk of patient death, but this was not clearly associated with graft-related outcomes across the breadth of other studies. It may be that RRI is one representation of the patients' overall health status rather than a graft-specific indicator. It is also possible that drawing firm conclusion from a disparate group of studies limits this review, particularly with the exclusion of a relatively large number of papers and data.

Trial Registration

PROSPERO—CRD42020170822.

Funding Source

No funding received.

RANDOMISED CONTROLLED TRIAL 2

A Randomized, Double-Blinded, Placebo-Controlled Trial Analyzing the Effect of Synbiotics on Infectious Complications Following Living Donor Liver Transplant—PREPRO Trial.

by Mallick, S., et al. Journal of Hepato biliary pancreatic Sciences 2022 [record in progress].

Aims

Participants were randomly assigned to receive either the synbiotic drug Prowel® or a placebo.

Interventions

Participants were randomised into two groups: the intervention group, in which the patients participated in a personalised exercise rehabilitation program in addition to standard care, or the control group where the patients received standard care alone.

Participants

100 recipients of live donor liver transplant (LDLT).

Outcomes

The primary outcome was the occurrence of culture-proven bacterial infection in blood, urine or drain fluid. The secondary outcomes included hospital stay, noninfectious complications, use of antibiotics and 30-day mortality.

Follow-Up

30 days.

CET Conclusion

The double-blinded, randomised controlled trial evaluated if 2-weeks of synbiotic therapy starting 2 days before living donor liver transplantation (LDLT) reduced infections in recipients. LDLT recipients were randomised according to a computer-generated sequence in sequentially numbered envelopes to the synbiotic drug or an identical looking placebo. The power calculation showed that 100 patients were needed. One hundred patients were randomised and all were included in the 30-day posttransplant analysis of primary and secondary outcomes. There were significantly less infections in the synbiotic group compared with placebo. Further analysis showed that blood stream infections were lower in the synbiotic group but there were no differences between groups for urinary tract and intra-abdominal infections. All secondary outcomes were similar between groups.

Jadad Score

5.

Data Analysis

Strict intention-to-treat analysis.

Allocation Concealment

Yes.

Trial Registration

CTRI/2017/09/009869.

Funding Source

No funding was received.

CLINICAL IMPACT SUMMARY

This is overall a well conducted randomised controlled trial in live donor liver transplantation. There are some slight weaknesses in the methodology on deeper assessment; The method of randomisation was computer-generated however the results were kept in sealed envelopes, so this is not completely free of bias potential. The study is described as double-blinded, with capsules used to convey the study symbiotic preparation, or emptied capsules for the placebo arm of the study. However, it is possible that patients or clinicians could then determine which arm of the study they were in by closely examining the capsules. Reassuringly the primary endpoint was well-defined, as the presence of culture-proven bacterial infection in the blood, urine or drain fluid.

The power calculation used to design the study was based on very low overall infection rates, assuming a reduction from 24% to 4% comparing placebo and study arms. The rate of infection found in the study was actually much higher than this, however with a large difference between the study groups, such that a statistically significant difference was still seen.

The study recorded a significant reduction in overall infection rate at 30-days with the Prepro symbiotic compared to placebo (22% versus 44%), *Klebsiella pneumoniae* being the most common organism. This seems to be particularly the case in blood-stream infections, but the data are not completely clear as some patients may have had more than one infection. There was no other significant difference in major complications seen, although the study had not been powered to detect small differences.

Despite the large reduction in infection rates, the study did not find that the use of probiotics reduced antibiotic use due to the low threshold for starting empirical treatment.

Previous, good quality, trials in liver transplantation have shown the benefit of probiotic and symbiotic preparations. This study adds significant supporting data to this.

AUTHOR CONTRIBUTIONS

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

CONFLICT OF INTEREST

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

ESOT | Transplant International

MEETING REPORT
published: 26 September 2022
doi: 10.3389/ti.2022.10843

Joining Forces in Basic Science: ITS Meeting 2.0

Nina Pilat[1]*, *Fadi Issa*[2], *Xunrong Luo*[3], *Anita Chong*[4], *Jonathan Bromberg*[5] and *Katja Kotsch*[6]

[1]Department of Cardiac Surgery, Center for Biomedical Research, Medical University of Vienna, Vienna, Austria, [2]Transplantation Research and Immunology Group, Nuffield Department of Surgical Sciences, University of Oxford, Oxford, United Kingdom, [3]Department of Medicine, Division of Nephrology, Duke University, Durham, NC, United States, [4]Section of Transplantation, Department of Surgery, The University of Chicago, Chicago, IL, United States, [5]Department of Surgery, University of Maryland School of Medicine, Baltimore, MD, United States, [6]Department for General and Visceral Surgery, Charité Universitätsmedizin Berlin, Berlin, Germany

The second International Transplant Science (ITS) meeting jointly organized by the European Society for Organ Transplantation (ESOT), the American Society of Transplantation (AST), and The Transplantation Society (TTS) took place in May 2022 in one of Europe's most iconic cities: Berlin, Germany. The ITS meeting 2022 was designed to serve as an international platform for scientific discussions on the latest ground-breaking discoveries in the field, while providing an excellent opportunity to present cutting-edge research to the scientific community. We think this is fundamental for the exchange of new ideas and establishment of collaborative work between advanced transplant experts, young professionals and early-stage researchers and students. Scientific sessions tackled hot topics in transplantation such as mechanisms of tolerance, biomarkers, big data and artificial intelligence. Our educational pre-meeting focused on the breakthrough and challenges in single-cell multimodal omics. The program included panel discussions illuminating various topics concerning conflicts and problems related to gender, such as challenges for female scientists. Attendees returned to their institutes with not only profound knowledge of the latest discoveries, technologies, and concepts in basic and translational science, but also inspired and excited after discussions and networking sessions with fellow scientists which have been duly missed during the pandemic.

Keywords: transplantation, basic science, translational science, B cells, innate immunity, tolerance, biomarkers, big data

OPEN ACCESS

Correspondence:
Nina Pilat
nina.pilat@meduniwien.ac.at

Received: *18 August 2022*
Accepted: *13 September 2022*
Published: *26 September 2022*

Citation:
Pilat N, Issa F, Luo X, Chong A, Bromberg J and Kotsch K (2022) Joining Forces in Basic Science: ITS Meeting 2.0. Transpl Int 35:10843. doi: 10.3389/ti.2022.10843

Basic transplantation science meetings have always been small expert meetings designed to foster networks and collaboration between established scientists in the field and experts from other fields. The goal has always been to provide in-depth, cutting-edge talks from leading experts in the field, focusing on addressing challenges that arise from both, fundamental discoveries in basic science as well as clinical problems and unexpected hurdles in translational science. Even though there has always been a strong connection between basic science researchers in the field, the three international multidisciplinary societies (TTS, AST and ESOT) decided to join forces in organizing annual International Transplantation Science (ITS) meetings. The first ITS meeting was held in November 2019 in Clearwater, Florida [1]. When we started planning the subsequent meeting at the end of 2019, nobody could envision a global pandemic postponing almost all in-person meetings for 2 years. While planning this meeting we remained flexible and ready to respond to new challenges related to the pandemic, ultimately

succeeding in creating a high-quality scientific program with cutting-edge topics, which the community acknowledged with a new record of 100+ abstract submissions.

The ITS meeting 2022 started with a pre-meeting workshop on "Single Cell Multimodal Omics," an exciting technology which has recently gained a lot of attention in transplantation. Single-cell multimodal omics technologies can provide a "holistic approach" to study cells and tissues at the genomic, transcriptional and epigenomic level. Technical advances allow for the simultaneous assessment of multiple modalities as well as spatial organization, providing new opportunities in the discovery of new cell types, cellular differentiation trajectories and communication networks across cells and tissues. The session was started by Ricardo Ferreira (University of Oxford) who presented a multi-omics approach for simultaneous and targeted protein quantification increasing the power of single-cell RNA sequencing (scRNA-seq) to investigate heterogeneity of human T cell populations [2]. In the following talk, Matthias Farlik (Medical University Vienna) explained how scRNA-seq profiling can be used to assess disease progression and help with the discovery of novel biomarkers for monitoring before clinical symptoms arise. By monitoring genetic stability on a single cell and single strand level, Ashley Sanders (Max-Delbrück-Center Berlin) taught us how genetic mutations form and change cell states in health and disease. The final talk of this interesting session was given by Xunrong Luo (Duke University), discussing how these techniques are used for the discovery of novel cellular pathways in the rejection of kidney allografts.

For the main scientific sessions of the ITS meeting 2022 we created an innovative format aiming to put young researchers into the spotlight. Instead of having only invited talks for the thematic sessions, we selected additional and matching talks from abstract presenters, which were of outstanding scientific quality. The mix of educational talks from experts in their field and ongoing projects from younger researchers resulted in stimulating and inspiring discussions at the end of every session.

Session 1 focused on B cells and the role of protective/pathogenic antibodies in humoral alloresponses. Dr. Anita Chong (University of Chicago) set the stage for this interesting session by presenting her work on innate-like autoreactive B cells infiltrating kidney allografts. Using single-cell RNA sequencing it was shown that graft-infiltrating B cells exhibit an innate cell transcriptional state resembling mouse peritoneal B1 cells, which drive tissue destruction mediated by antibody-mediated rejection [3]. In the following talk, we switched to another organ and Emmanuel Zorn (Columbia University) provided insights into intragraft antibody responses in human heart allograft rejection [4]. His data revealed different expression profiles in transcriptomes of endomyocardial biopsies, indicating different types of antibody-mediated rejection. Oriol Bestard (University Hospital Vall d'Hebron) gave an update on his work of alloreactive memory B cells in kidney transplantation and its impact and implementation in the clinics. Three abstracts on B cells and antibodies were selected and discussed 1) the role of IgE after cardiac transplantation, 2) a novel immunosuppressive Bcl6-targeting compound for prevention of humoral rejection, as well as 3) (intragraft) donor-HLA-specific B cells in renal transplant patients.

The next session was all about "Big data and Artificial Intelligence (AI)," which are important topics, especially with implementation of –omics and sequencing techniques, both generating huge amounts of data. Sophie Limou (Nantes University) started with a talk on genomics in kidney transplantation, introducing the term "fat data" and raised awareness for biases and systematic errors in—omics studies. The next presentation from Kathie Connor (University of Edinburgh) was about machine learning in clinical transplantation. Although machine learning is increasingly important for transplantation research due to an increase of (very) big data sets and the increase in computer processing power and development of algorithms, there are lots of challenges and limitations about machine learning. Finally, we learned from Ali Zarrinpar about big data and AI in liver transplantation and how non-invasive techniques may supplement or even 1 day replace biopsies, which are the current standard of care for rejection assessment. Taken together, these talks provided lots of information on how to generate and interpret big data, and how AI can assist on the implementation of biomarkers in the clinic.

The final session of the day was on Innate Immunity and was kicked off with a presentation from Jonathan Bromberg (University of Maryland) on lymph node fibroblastic reticular cells and how these cells are able to steer immune responses. Afterwards, Andreas Diefenbach (Charité-Universitätsmedizin Berlin) spoke on Innate Lymphoid Cells (ILC) and how these populations are influenced by the gut microbiome.

The second day started with Session 5: Marginal Organs and *ex-vivo* machine perfusion. In this context, Zoltan Czygany (Universitätsmedizin Charité) reviewed recent findings on clinical machine liver perfusion. This presentation was followed by Cyril Moers (University of Groningen), who gave an excellent speech about machine perfusion in kidney transplantation, including updates of state-of-the-art and latest developments in the field to futuristic scenarios using cryopreservation strategies that would allow organ storage for longer periods of time and the development of "organ banks" for on-demand use. Another exciting strategy to decrease alloimmunity is silencing of HLA expression on donor cells, creating so-called "invisible organs." Constanca Figueiredo (Medizinische Hochschule Hannover) reported on the success of her group with this approach using *ex vivo* machine perfusion to silence MHC transcription.

The following session focused on basic (T cell) immunology with the topic "Mechanisms of alloimmunity and tolerance." The first speaker Ludger Klein (Ludwig-Maximilians University Munich), an expert in T cell development in the thymic micro-environment presented the "holy grail" in tolerance research and how MHC/peptide ligands are important for T cell repertoire selection. Gilles Blancho (Nantes University) gave updates on selective costimulation blockade and progress in the development of antagonist anti-CD28 therapeutics [5]. Two excellent abstracts presentations about how human regulatory macrophages can induce Treg generation and how Tregs are recruited to human kidney transplants after ischemia reperfusion

injury were followed by a lively discussion among panelists and with the audience.

Session 7 was about COVID-19 and the immune response towards vaccination in transplant patients. Arne Sattler (Charite Universitätsmedizin Berlin) reported on findings about SARS-CoV2 vaccination of adolescent and adult kidney transplant recipients and their outcomes based on comprehensive humoral-, B- and T cell analyses. His data document that kidney transplant patients constitute a special patient group, which needs to be carefully evaluated after vaccination as the immune response is dampened mainly due to antimetabolite treatment [6, 7]. Afterwards Petra Bacher (Christian-Abbrechts University Kiel) discussed low-avidity $CD4^+$ T cell responses to SARS-CoV-2 in unexposed individuals and humans with severe COVID-19. She demonstrated that SARS-CoV-2-reactive $CD4^+$ memory T cells were present in unexposed individuals, displaying low functional avidity and multiple, highly variable cross-reactivities, e.g., towards common cold coronavirus, which were not present COVID-19 patients.

The keynote lecture was given by Florent Ginhoux (Singapore Immunology Network). He reported on single cell profiling strategies to characterize myeloid cells in health and disease [8].

The last day of the meeting started with "Basic mechanisms of organ regeneration and organoids" and an excellent talk given by Luc van der Laan (Erasmus University Medical Centre) on organoids and liver regenerative medicine.

The next session "A star is born" was designed to bring the very best young researchers on stage, so the best ranked abstracts had the chance of being presented in front of a big audience and the presenters showing off not only their data but also their presentation skills in front of a tough jury.

The last session was about Biobanks and Bioassays and our first speaker was Sarah Cross (University of Oxford), who is not only manager of the Oxford biobank but also National Coordinator for Quality in Organ Donation (QUOD), an initiative that aims to improve and facilitate the collection of biological samples in order to improve the understanding of all aspects of organ donation and transplantation. Jianing Fu (Columbia University) gave insights into her and Megan Sykes' work on the alloreactive T cell repertoire in transplant patients and how to track these cells by TCR sequencing. Federica Genovese (Nordic Bioscience Copenhagen) presented a novel bioassay to determine the activity of extracellular matrix remodeling and how this can be used for a more accurate prognosis in kidney transplant recipients in the future.

Overall, we had a vibrant and inspiring meeting after a long period of "scientific isolation." Hungry for more, we look therefore forward towards the next meeting which will be organized by TTS in 2023, to be held in North America.

AUTHOR CONTRIBUTIONS

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

FUNDING

This work was supported by the Austrian Science Fund (FWF; P31186-B28 to NP).

CONFLICT OF INTEREST

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

REFERENCES

1. Chong AS, Issa F, Leuvenink HGD, Alegre ML. The First ITS Meeting. *Transplantation* (2020) 104(6):1114–6. doi:10.1097/TP.0000000000003132
2. Trzupek D, Dunstan M, Cutler AJ, Lee M, Godfrey L, Jarvis L, et al. Discovery of CD80 and CD86 as Recent Activation Markers on Regulatory T Cells by Protein-RNA Single-Cell Analysis. *Genome Med* (2020) 12(1):55. doi:10.1186/s13073-020-00756-z
3. Asano Y, Daccache J, Jain D, Ko K, Kinloch A, Veselits M, et al. Innate-like Self-Reactive B Cells Infiltrate Human Renal Allografts during Transplant Rejection. *Nat Commun* (2021) 12(1):4372. doi:10.1038/s41467-021-24615-6
4. Mantell BS, Cordero H, See SB, Clerkin KJ, Vasilescu R, Marboe CC, et al. Transcriptomic Heterogeneity of Antibody Mediated Rejection after Heart Transplant with or without Donor Specific Antibodies. *J Heart Lung Transpl* (2021) 40(11):1472–80. doi:10.1016/j.healun.2021.06.012
5. Vanhove B, Poirier N, Soulillou JP, Blancho G. Selective Costimulation Blockade with Antagonist Anti-CD28 Therapeutics in Transplantation. *Transplantation* (2019) 103(9):1783–9. doi:10.1097/TP.0000000000002740
6. Sattler A, Schrezenmeier E, Weber UA, Potekhin A, Bachmann F, Straub-Hohenbleicher H, et al. Impaired Humoral and Cellular Immunity after SARS-CoV-2 BNT162b2 (Tozinameran) Prime-Boost Vaccination in Kidney Transplant Recipients. *J Clin Invest* (2021) 131(14):150175. doi:10.1172/JCI150175
7. Schrezenmeier E, Rincon-Arevalo H, Jens A, Stefanski AL, Hammett C, Osmanodja B, et al. Temporary Antimetabolite Treatment Hold Boosts SARS-CoV-2 Vaccination-specific Humoral and Cellular Immunity in Kidney Transplant Recipients. *JCI Insight* (2022) 7(9):e157836. doi:10.1172/jci.insight.157836
8. Mulder K, Patel AA, Kong WT, Piot C, Halitzki E, Dunsmore G, et al. Cross-tissue Single-Cell Landscape of Human Monocytes and Macrophages in Health and Disease. *Immunity* (2021) 54(8):1883–900. e5. doi:10.1016/j.immuni.2021.07.007

ESOT | Transplant International

FORUM
published: 05 September 2022
doi: 10.3389/ti.2022.10683

Adopting Individualized Strategies to Prevent Large-For-Size Syndrome in Adult Liver Transplant Recipients: The Graft Morphology Should Also Be Taken Into Account

Guang-Peng Zhou[1,2], *Lin Wei*[1,2] *and Zhi-Jun Zhu*[1,2]*

[1]*Liver Transplantation Center, National Clinical Research Center for Digestive Diseases, Beijing Friendship Hospital, Capital Medical University, Beijing, China,* [2]*Clinical Center for Pediatric Liver Transplantation, Capital Medical University, Beijing, China*

Keywords: large-for-size syndrome, *ex vivo* right posterior sectionectomy, size mismatch, graft morphology, right anteroposterior vertical distance

A Forum discussing:

A Novel Strategy for Preventing Posttransplant Large-For-Size Syndrome in Adult Liver Transplant Recipients: A Pilot Study
by Pu X, He D, Liao A, Yang J, Lv T, Yan L, Yang J, Wu H, Jiang L (2022). Transpl Int 35:10177. doi: 10.3389/ti.2021.10177

Large-for-size syndrome (LFSS) is a less common but life-threatening complication following adult liver transplantation during the early post-transplant period, characterized by postoperative liver necrosis, vascular complications, and primary nonfunction due to severe liver graft compression (1). In this issue of Transplant International, Pu et al. reported a novel surgical technique of *ex vivo* right posterior sectionectomy while preserving the right hepatic vein in the liver graft to prevent posttransplant LFSS in adult liver transplant recipients, which was successfully performed in all five recipients discharged without procedure-related complications (2). Pu et al. should be congratulated for describing a feasible intervention to save patients from the potential risk of LFSS. However, the graft morphology should also be taken into account when adopting this novel surgical treatment.

Pu et al. selected graft-recipient weight ratio (GRWR) combined with graft weight (GW)/right anteroposterior (RAP) as a new "LFSS predictor," in which both GRWR > 2.5% and GW/RAP > 100 g/cm indicated the need for reduction of the right liver graft. However, this new "LFSS predictor" has its intrinsic limitation, which only considers the graft weight and the depth of the lower right hemithorax of the recipient, but not the morphological parameters of the graft, especially the RAP vertical distance and the longest horizontal distance (3). The morphology of the liver grafts may differ among individuals. Some large-volume livers exhibit a short, "squat" shape (relatively short and thick right liver span). In contrast, others have a narrow, flat, and elongated morphology (relatively long and thin right liver span) (3). Therefore, both GRWR and GW/RAP could not fully indicate the possibility of severe compression of the right liver graft from the recipient's rib cage. Within the past several years, our center has also completed several *ex vivo* right posterior sectionectomy cases in both pediatric and adult liver transplant recipients. Despite the advantages of *ex vivo* right posterior sectionectomy, as described by Pu et al., it must be admitted that this surgical procedure still carries increased risks of surgical complications, especially in patients with decompensated cirrhosis with a high MELD score (1, 4). Based on

Forum

OPEN ACCESS

Correspondence:
Zhi-Jun Zhu
zhu-zhijun@outlook.com

Received: *31 May 2022*
Accepted: *17 August 2022*
Published: *05 September 2022*

Citation:
Zhou G-P, Wei L and Zhu Z-J (2022) Adopting Individualized Strategies to Prevent Large-For-Size Syndrome in Adult Liver Transplant Recipients: The Graft Morphology Should Also Be Taken Into Account. Transpl Int 35:10683. doi: 10.3389/ti.2022.10683

our experience and others, the size discrepancy between the anteroposterior dimensions of the graft and the longest RAP of the recipient should still be considered a first-line index for evaluating the occurrence of the LFSS. Thus, the choice of the liver segments to be resected should be based on the combination of the anthropometrics of the donor graft with that of the recipient (1, 5). The anteroposterior dimension of the graft can be accurately measured on the back table to provide a precise parameter for determining the necessity of right posterior sectionectomy. If the right liver graft vertical distance is less than the longest RAP vertical distance of the recipient, graft reduction with resection of the right posterior sector (segment 6–7) may not be necessary. In this condition, left lateral lobectomy or left hemihepatectomy may be more appropriate with their convenience and relatively low risk (6). Nevertheless, concerns remain that a limited graft reduction such as left lobectomy is very unlikely to avoid rib compression over the right liver (3). Recently, Paterno et al. provided a further solution named "bilateral marginal costotomy," which rescued a liver transplant recipient from severe graft compression from the bilateral narrow rib cages after the failed temporary abdominal closure (7). Thus, marginal costotomy can be performed either as a primary or adjunctive treatment to avoid graft compression due to the ribs after the implantation of a large-for-size liver graft or as a rescue treatment after conventional interventions failed to relieve allograft compression.

Collectively, combining the volumetric and morphological parameters of the donor liver with the anthropometrics of the recipient may be more beneficial in determining the individualized strategies to prevent the occurrence of LFSS in adult liver transplant recipients when facing donor-recipient mismatching.

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

Authors G-PZ, LW, and Z-JZ contributed equally to the preparation and editing of this letter to the editors. The final version was unanimously approved by all authors.

FUNDING

This study was supported by grants from the Capital's Funds for Health Improvement and Research (2020-1-2024) and Beijing Postdoctoral Research Foundation. The funding body had no role in the design of the study and writing of the manuscript.

CONFLICT OF INTEREST

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

REFERENCES

1. Addeo P, Noblet V, Naegel B, Bachellier P. Large-for-Size Orthotopic Liver Transplantation: a Systematic Review of Definitions, Outcomes, and Solutions. *J Gastrointest Surg* (2020) 24:1192–200. doi:10.1007/s11605-019-04505-5
2. Pu X, He D, Liao A, Yang J, Lv T, Yan L, et al. A Novel Strategy for Preventing Posttransplant Large-For-Size Syndrome in Adult Liver Transplant Recipients: A Pilot Study. *Transpl Int* (2021) 35:10177. doi:10.3389/ti.2021.10177
3. Allard MA, Lopes F, Frosio F, Golse N, Sa Cunha A, Cherqui D, et al. Extreme Large-For-Size Syndrome after Adult Liver Transplantation: A Model for Predicting a Potentially Lethal Complication. *Liver Transpl* (2017) 23: 1294–304. doi:10.1002/lt.24835
4. Nagatsu A, Yoshizumi T, Ikegami T, HaradaN, Soejima Y, et al. *In Situ* Posterior Graft Segmentectomy for Large-For-Size Syndrome in Deceased Donor Liver Transplantation in Adults: A Case Report. *Transpl Proc* (2017) 49:1199–201. doi:10.1016/j.transproceed.2017.03.036
5. Addeo P, Bachellier P, Noblet V. Combination of Donor Anthropometrics with Recipient Imaging to Improve Matching in Liver Transplantation. *Liver Transpl* (2022) 28:512–3. doi:10.1002/lt.26300
6. Kim YM, Kwak BJ, Shim DJ, Kwon YK, Yoon YC. *In Situ* Left Lateral Sectionectomy in Deceased Donor Liver Transplantation: Could This Be Another Solution for a Large-For-Size Graft? A Case Report. *Transpl Proc* (2019) 51:3116–9. doi:10.1016/j.transproceed.2019.07.005
7. Paterno F, Amin A, Lunsford KE, Brown LG, Pyrsopoulos N, Lee ES, et al. Marginal Costotomy: A Novel Surgical Technique to Rescue from "Large-For-Size Syndrome" in Liver Transplantation. *Liver Transpl* (2022) 28:317–20. doi:10.1002/lt.26252

ESOT | Transplant International

FORUM
published: 05 September 2022
doi: 10.3389/ti.2022.10805

How to Choose the Optimal Surgical Strategy to Predict and Prevent LFSS Following Liver Transplantation?

Diao He[1], *Xingyu Pu*[2] *and Li Jiang*[2,3]*

[1]Key Laboratory of Transplant Engineering and Immunology, Laboratory of Liver Transplantation, Frontiers Science Center for Disease-Related Molecular Network, West China Hospital, Sichuan University, Chengdu, China, [2]Department of Liver Surgery, West China Hospital, Sichuan University, Chengdu, China, [3]Department of General Surgery, West China Tianfu Hospital, Sichuan University, Chengdu, China

Keywords: LFSS, prediction, prevention, adult, reduced-size liver transplantation

A Forum discussing:

Adopting Individualized Strategies to Prevent Large-For-Size Syndrome in Adult Liver Transplant Recipients: The Graft Morphology Should Also Be Taken Into Account
by Zhou G-P, Wei L and Zhu Z-J (2022). Transpl Int 35:10683. doi: 10.3389/ti.2022.10683

We appreciate the positive feedback that Zhou GP and his colleagues provided on our article, "A Novel Strategy for Preventing Posttransplant Large-For-Size Syndrome in Adult Liver Transplant Recipients: A Pilot Study" (1). Their article raised several concerns on our published article. We are grateful to the Editor for allowing us to respond to these comments.

It is crucial to match donor and recipient sizes appropriately to prevent Large-for-Size Syndrome (LFSS). A valuable idea presented by Zhou et al. is the incorporation of graft morphological parameters, particularly the anteroposterior (RAP) vertical distance and the longest horizontal distance, into the LFSS indicator (2). By combining the morphological parameter of graft, graft-recipient weight ratio (GRWR) and graft weight (GW)/RAP, it is possible to more accurately indicate the need for reduction of the right graft (3).

The point is how to measure the morphological parameter of graft using an appropriate method. As of today, computed tomography (CT) scan is the most accurate method to measure the right RAP vertical distance and the largest horizontal distance of grafts in living donor liver transplantation (LDLT) (4). However, Donation after Citizens Death (DCD) donors need to receive treatment in the intensive care unit and should not be moved, which limits the use of CT scans in for measuring graft parameters in deceased donor liver transplantation (DDLT). Doppler ultrasonography can be performed at the bedside, but DCD donors may experience edema in their gastrointestinal tracts during maintenance periods, affecting the accuracy of the measurement results. Alternatively, measurements can be taken during graft procurement period, which has the advantage of being done under naked eye conditions. In view of the fact that the graft does not have blood filling *in vitro*, the *ex vivo* measurement value is smaller than the actual one *in vivo*. For a closer match between *in vitro* and *in vivo* measurement values, we propose to combine several transplant centers and develop a new calculation formula with a large sample size.

Paterno et al. recently proposed a new solution, "bilateral marginal costotomy," for rescuing a liver transplant recipient from severe graft compression caused by bilateral narrow rib cages after temporary abdominal closure failed (5). Yet, this method is more likely to be a salvage measure

Forum

OPEN ACCESS

***Correspondence:**
Li Jiang
jl339@126.com

Received: *28 July 2022*
Accepted: *17 August 2022*
Published: *05 September 2022*

Citation:
He D, Pu X and Jiang L (2022) How to Choose the Optimal Surgical Strategy to Predict and Prevent LFSS Following Liver Transplantation? Transpl Int 35:10805. doi: 10.3389/ti.2022.10805

for donor-recipient matching fails than a conventional treatment since the thoracic cavity needs to be changed, increasing the risk of postoperative complications. In contrast, according to our observations using the HuaXi-eRPS technique, all recipients had intact hepatic arteries, hepatic veins, and biliary tracts as well as good blood supply without any biliary complications. Thus, HuaXi-eRPS under the existing conditions should be considered a safe and effective procedure for the prevention of posttransplant LFSS. With the advancement of technology, we will also try new detection methods and incorporate new predictive indicators in order to make more effective control strategies for posttransplant LFSS.

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

DH, XP, and LJ wrote the paper. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by the Key R&D Support Plan of Chengdu Science and Technology Bureau (2021-YF05-00703-SN). The funding body had no role in the design of the study and writing of the manuscript.

CONFLICT OF INTEREST

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

REFERENCES

1. Pu X, He D, Liao A, Yang J, Lv T, Yan L, et al. A Novel Strategy for Preventing Posttransplant Large-For-Size Syndrome in Adult Liver Transplant Recipients: A Pilot Study. *Transpl Int* (2021) 35:10177. doi:10.3389/ti.2021.10177
2. Zhou GP, Wei L, Zhu ZJ. Adopting Individualized Strategies to Prevent Large-For-Size Syndrome in Adult Liver Transplant Recipients: The Graft Morphology Should Also Be Taken into Account. *Transpl Int* (2022) 35: 10683. doi:10.3389/ti.2022.10683
3. Allard MA, Lopes F, Frosio F, Golse N, Sa Cunha A, Cherqui D, et al. Extreme Large-For-Size Syndrome after Adult Liver Transplantation: A Model for Predicting a Potentially Lethal Complication. *Liver Transpl* (2017) 23: 1294–304. doi:10.1002/lt.24835
4. Cai L, Yeh BM, Westphalen AC, Roberts JP, Wang ZJ. Adult Living Donor Liver Imaging. *Diagn Interv Radiol* (2016) 22(3):207–14. doi:10.5152/dir.2016.15323
5. Paterno F, Amin A, Lunsford KE, Brown LG, Pyrsopoulos N, Lee ES, et al. Marginal Costotomy: A Novel SurgicalTechnique to Rescue from "Large-For-Size Syndrome" in Liver Transplantation. *Liver Transpl* (2022) 28:317–20. doi:10.1002/lt.26252

SYSTEMATIC REVIEW AND META-ANALYSIS
published: 08 September 2022
doi: 10.3389/ti.2022.10330

Physical Effects, Safety and Feasibility of Prehabilitation in Patients Awaiting Orthotopic Liver Transplantation, a Systematic Review

Wesley D. Jetten[1*†‡], *Rianne N. M. Hogenbirk*[2†‡], *Nico L. U. Van Meeteren*[1,3†], *Frans J. C. Cuperus*[4†], *Joost M. Klaase*[2†] and *Renate De Jong*[1†]

[1]Department of Anesthesiology, Erasmus University Medical Center, Erasmus University Rotterdam, Rotterdam, Netherlands, [2]Department of Surgery, Division of Hepatopancreatobiliary Surgery and Liver Transplantation, University Medical Center Groningen, University of Groningen, Groningen, Netherlands, [3]Top Sector Life Sciences and Health (Health~Holland), The Hague, Netherlands, [4]Department of Gastroenterology and Hepatology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands

Prehabilitation improves surgical outcomes in patients undergoing surgery. However, patients preparing for orthotopic liver transplantation (OLT) are physically "frail" and suffer from comorbidities that generally hamper physical activity. This systematic review aims to evaluate the physical effects, safety and feasibility of prehabilitation in OLT candidates. Relevant articles were searched, in Embase, Web of Science, Cochrane, Medline and Google Scholar, to December 2021. Studies reporting on specified preoperative exercise programs, including adult OLT candidates with end-stage liver disease, with a model for end-stage liver disease (MELD) score ≥12 or Child-Pugh classification B/C, were included. This resulted in 563 potentially eligible studies, out of which eight were selected for inclusion, consisting of 1,094 patients (male sex 68%; mean age 51–61 years; mean MELD score 12-21). Six of the included studies were classified as low-quality by the GRADE system, and three studies had high risk for ineffectiveness of the training program according to the i-CONTENT tool. Significant improvement was observed in VO2 peak, 6-minute walking distance, hand grip strength, liver frailty index and quality of life. Feasibility ranged from an adherence of 38%–90% in unsupervised-to >94% in supervised programs. No serious adverse events were reported. In conclusion, prehabilitation in patients awaiting OLT appears to improve aerobic capacity, and seems feasible and safe. However, larger clinical trials are required to accurately examine the preoperative and postoperative effects of prehabilitation in this specific patient population.

Keywords: prehabilitation, orthotopic liver transplantation, physical exercise training, aerobic capacity, safety, feasibility

OPEN ACCESS

Correspondence:
Wesley D. Jetten
wesley.jetten@gmail.com

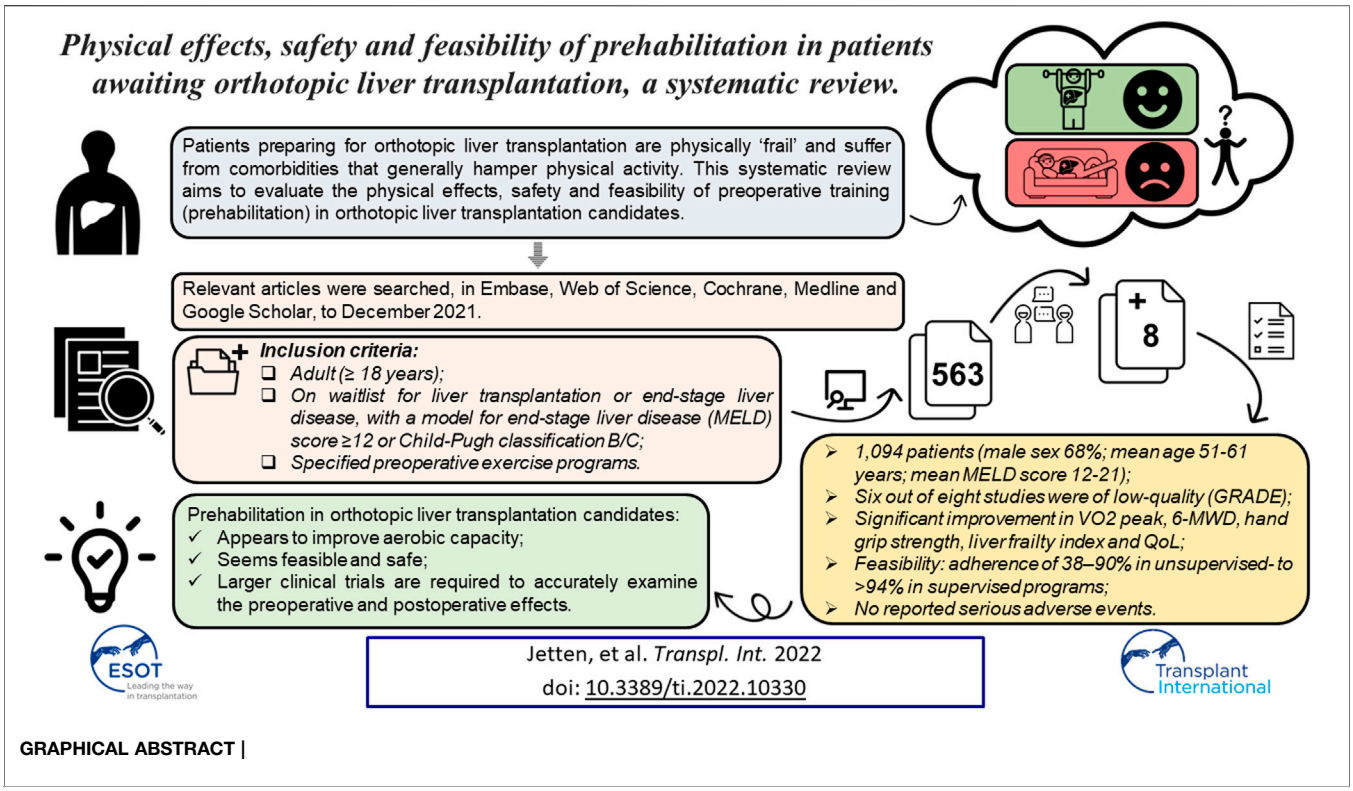
†ORCID:
Wesley D. Jetten
orcid.org/0000-0001-8896-6608
Rianne N. M. Hogenbirk
orcid.org/0000-0002-7723-0256
Nico L. U. Van Meeteren
orcid.org/0000-0002-3067-4167
Frans J. C. Cuperus
orcid.org/0000-0001-6742-1510
Joost M. Klaase
orcid.org/0000-0002-4409-2280
Renate De Jong
orcid.org/0000-0003-4847-0610

‡These authors have contributed equally to this work

Received: *31 December 2021*
Accepted: *24 August 2022*
Published: *08 September 2022*

Citation:
Jetten WD, Hogenbirk RNM, Van Meeteren NLU, Cuperus FJC, Klaase JM and De Jong R (2022) Physical Effects, Safety and Feasibility of Prehabilitation in Patients Awaiting Orthotopic Liver Transplantation, a Systematic Review. Transpl Int 35:10330. doi: 10.3389/ti.2022.10330

Abbreviations: 6MWD, six-minute walking distance; ERAS, enhanced recovery after surgery; ESLD, end-stage liver disease; F, delta point; GRADE, Grades of Recommendation, Assessment, Development and Evaluation for quality assessment of clinical studies; HR-QoL, health-related quality of life; i-CONTENT, international Consensus on Therapeutic Exercise aNd Training; ICU, intensive care unit; IQR, interquartile range; LFI, liver frailty index; MELD, model for end-stage liver disease; OLT, orthotopic liver transplantation; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; PROSPERO, prospective register of systematic reviews; RCT, randomized controlled trial; SD, standard deviation; VO2 peak, oxygen consumption at peak exercise.



GRAPHICAL ABSTRACT |

INTRODUCTION

Poor physical fitness and functional status compromise postoperative functional recovery and lead to adverse postoperative outcomes, including complications, prolonged length of in-hospital stay, and mortality (1).

In current practice, patients who undergo (major) abdominal surgery are postoperatively supported by physical therapists and dieticians as part of the Enhanced Recovery After Surgery (ERAS®) program to accelerate postoperative recovery by enhancing perioperative health and reducing the impact of hospitalization and surgical stress (2,3). In addition, preoperative physical fitness measured by cardiopulmonary exercise tests has shown to be an independent predictor for postoperative morbidity and mortality after major abdominal surgery (4). Therefore, in the recent years, an increasing amount of scientific evidence focusses on preoperative "rehabilitation," known as prehabilitation (5,6). Prehabilitation is aimed at strengthening the "psychophysiological reserve" and mitigating the postoperative surgical stress response to improve postoperative outcomes by enhancing preoperative general health and reducing individual risk factors (6).

Previous studies showed that prehabilitation programs are feasible, safe, and effective in patients scheduled for major abdominal surgery (7-9). However, the evidence regarding the beneficial effects of prehabilitation in patients awaiting orthotopic liver transplantation (OLT), a generally physically 'frail' patient population, is limited. OLT candidates not only exhibit key premorbid components of frailty, such as diminished functional capacity, sarcopenia, and decreased aerobic capacity, but may also suffer from cirrhosis-induced complications, such as ascites, hepatic encephalopathy, or variceal bleeding (10,11), which raises questions concerning their trainability. However, the waiting period for this procedure, on average 28 weeks in the Netherlands, 13–17 weeks in the United Kingdom, and 24 weeks in the United States of America, might be used to optimize physical condition by training prior to OLT (12-14).

Moreover, previous research in OLT candidates predicted a higher survival after OLT in patients with a higher anaerobic threshold (a submaximal exercise parameter of cardiorespiratory reserve) (15). Therefore, prehabilitation could possibly benefit patients in reducing morbidity and mortality during the waiting period or after OLT.

The primary objective of this systematic review is to evaluate the observed effects of preoperative training on physical and functional capacity, and to evaluate the effect of prehabilitation on postoperative surgical outcomes after OLT. The secondary objective is to determine the feasibility and safety of prehabilitation programs in patients awaiting OLT. In addition to the primary and secondary objectives, we aim to provide an overview of the studied prehabilitation programs, including their content and potential for success (16,17).

MATERIALS AND METHODS

Study Design

This systematic review was conducted and reported in accordance with the Preferred Reporting Items for Systematic

Reviews and Meta-Analyses (PRISMA) Statement (18,19), see **Online Supplementary S1**. Two authors (WJ, RH) independently reviewed the selected studies in EndNote X9© (Clarivate Analytics, Boston, MA, United States). Identified articles were screened on title, abstract, and, subsequently, on full-text. Disagreements during the selection process were discussed by the two reviewing authors (WJ and RH) and a third author (RJ) until consensus was reached.

Search Strategy

The search strategy was developed in collaboration with a clinical librarian and information specialist and was executed in Embase, Web of Science, Cochrane, Medline (PubMed) and Google Scholar. Free text words and MeSH terms related to prehabilitation and liver transplantation were used. Reference lists of relevant review articles and current treatment guidelines were screened for additional eligible articles. All studies published before 21 December 2021 were included for screening by title and abstract. The full literature database search strategy is described in **Online Supplementary S2**.

Eligibility Criteria

All peer-reviewed randomized, controlled, and cohort studies reporting a specified preoperative exercise program for adult (age ≥18 years) patients actively listed for OLT or with end-stage liver disease (ESLD). To assess ESLD, the model for end-stage liver disease (MELD) score, a disease severity scoring system used to improve organ allocation for patients on the liver transplantation waiting list, and the Child-Pugh classification were used. Studies that assessed patients with a laboratory or exception MELD score ≥12 or a Child-Pugh classification B or C were included. Animal studies, case-reports, systematic reviews, conference abstracts, duplicates and studies containing paediatric patients were excluded.

Quality Assessment

Quality assessment of included studies was executed by using the principles of the Grading of Recommendation, Assessment, Development, and Evaluation (GRADE) (20,21). For a transparent assessment of the potential effectiveness of the exercise therapy programs studied in trials, intervention programs were evaluated according to the international Consensus on Therapeutic Exercise Training (i-CONTENT) tool (17). The i-CONTENT is used to assess the therapeutic quality of exercise programs employed in clinical trials (17).

Primary and Secondary Outcomes

The primary outcome was defined as the observed effects of preoperative training programs on physical and functional capacity and surgical outcome. Physical and functional capacity was assessed by comparing outcomes such as pre- and post-training oxygen consumption at peak exercise (VO2-peak), 6-minute walking distance (6MWD), hand grip strength, and quality of life (QoL). Surgical outcome was assessed by comparing data on post-OLT complications, length of in-hospital stay, length of intensive care unit (ICU) stay, and mortality.

Secondary outcomes were safety and feasibility of study- and training programs. The safety of training programs was assessed by comparing the occurrence and types of serious adverse outcomes during the training. The feasibility of studies was assessed by comparing patients identified as eligible for inclusion with the total number of included patients. The feasibility of training programs was assessed by an evaluation of the adherence to the training programs during the waiting period prior to OLT.

Data Collection and Definitions

Following the screening and selection of included studies, data was extracted by two independent authors (WJ, RH). Patient characteristics extracted included age; sex; body mass index (BMI); (lab and/or exception) MELD score; Child-Pugh classification and comorbidities, including diabetes mellitus, cardiac disease, pulmonary disease, ascites, gastroesophageal varices, and hepatic encephalopathy. Data regarding primary and secondary outcomes were extracted and tabulated. In addition, rationales, designs of the training programs, data on duration, frequency of training and exercises, training intensity and context, supervision of the training programs, and their potential for success were tabulated to provide a detailed overview of the prehabilitation programs. Normally distributed variables are presented as means with standard deviation (SD) and skewed variables as medians with interquartile range (IQR).

RESULTS

Search Results

The search of aforementioned databases provided a total of 892 articles possible for inclusion. After removing duplicates, 563 articles remained for screening by title and abstract. Of these, 510 were excluded based on titles and abstracts. The full-texts of the remaining 53 articles were assessed for eligibility and reviewed in detail, whereafter 47 papers were excluded and six papers were included (**Figure 1**). Eventually, another 12 potentially relevant articles were found by screening references from articles that were already included for analysis. Of this total of 18 remaining articles, another 10 were excluded (**Figure 1**), and eight full-text studies (11,22-28) remained for systematic analysis (**Table 1**).

Methodological Quality of Evidence Assessment

According to the GRADE system (20), two studies (24,28) were classified as moderate, while six (11,22-27) were classified as low-quality evidence, mainly due to the risk of bias and imprecision (**Table 1**). According to the i-CONTENT tool (17), five studies (23-28) were classified as low risk and three (11,22,27) as high risk for ineffectiveness of the training program. The main reason for high risk of ineffectiveness was due to unsupervised training (22,25,27,28) and missing reports on exercise-related adverse events (11,22,27,28) and adherence to the exercise program (11,22,23,27) (**Table 2**). For a detailed description of the

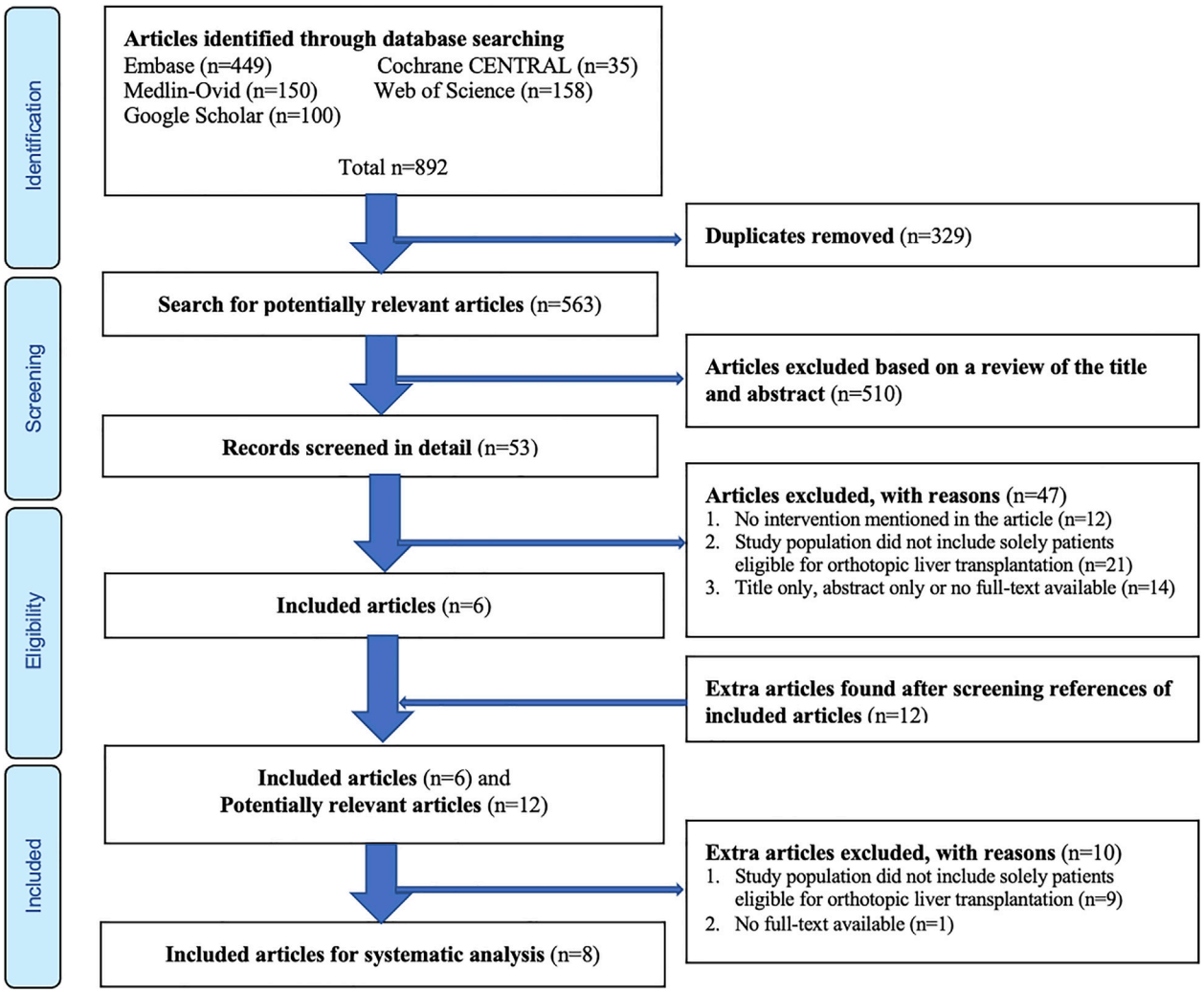


FIGURE 1 | Flow diagram of the article selection procedure based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline.

grading process with the GRADE system and i-CONTENT tool, see **Online Supplementary S3, S4**, respectively.

Included Studies

Eight studies investigating a total of 1,094 patients (median (IQR): 20 (17–139)) were included. A total of three randomized controlled trials (RCTs) (22,24,27), one ambispective cohort study (28), three prospective cohort studies (23,25,26), and one retrospective cohort study (11) were included. The contexts of the training programs varied between supervised in-hospital training (11,23,24,26) and unsupervised home-based training (11,22,24,25,27,28).

Demographics, Primary and Secondary Outcomes

The majority of patients were male (68%). The mean or median age of the patients included in the training programs and control groups ranged from 51 to 61 and 54 to 56, respectively. In the studies reporting BMI, mean BMI in the training groups was ranging from 25.4 to 31 (22,25-28), which was higher than in the control groups (range 27–29) (22,26,27). The mean and median MELD-scores differed between studies, with five studies reporting mean or median MELD-scores between 12 and 14 in the training group (23-26,28), while, in the other three studies, these scores were above 16 in both the intervention and control groups (11,22,27). Six studies reported on the presence of one or more cirrhosis-induced comorbidities as ascites, gastroesophageal varices and hepatic encephalopathy (22-25,27,28). The number of patients with ascites ranged from 15% to 78% in the training groups (22-25,27,28) compared to 67%–75% in the control groups (22,27). The reported prevalence of hepatic encephalopathy ranged from 33% to 100% in the training groups (25,27,28) and 100% in the control groups (27). Three studies reported a prevalence of gastro-oesophageal varices ranging from 56% to 81% in the training

TABLE 1 | Designs of included studies and patient demographics.

Author	Limongi (22)	Debette-Gratien (23)	Al-Judaibi (11)	Wallen (24)
Year	2014	2015	2019	2019
Study Design	Randomized controlled trial	Prospective cohort study	Retrospective cohort study	Randomized controlled trial
Study quality[a]	Low	Low	Low	Moderate
Population (n)				
Training group	5	13	258	11
Control group	12	NA	200	10
Demographics				
Age, years				49 (40–60)[b]
Training group	53.41 (8.42)	51 (12)	53.4 (9.6)	NR
Control group	56.2 (3.96)	NA	56.5 (10.7)	NR
Sex, male, %				81%
Training group	92%	77%	26%	NR
Control group	60%	NA	68%	NR
BMI, kg/m^2				
Training group	31 (7.4)	NR	NR	NR
Control group	28 (3.8)	NA	NR	NR
MELD-score				13.3 (4)
Training group	17.58 (4.46)	13 (6)	18 (6–40)[c]	NR
Control group	17 (3.93)	NA	21 (4–40)[c]	NR
Child Pugh-score				63%[d]
Training group	NR	B7 (3)	NR	NR
Control group	NR	NA	NR	NR
Comorbidities, n(%)				
Diabetes Mellitus				33%
Training group	3 (60%)	NR	90 (35.9%)	NR
Control group	3 (25%)	NA	43 (21.5%)	NR
Cardiac disease				0%
Training group	1 (20%)	0 (0%)	27 (10.8%)	NR
Control group	0 (0%)	NA	2 (1%)	NR
Pulmonary disease				
Training group	1 (20%)	NR	NR	NR
Control group	2 (17%)	NA	NR	NR
Ascites				
Training group	2 (40%)	2 (15%)	NR	NR
Control group	8 (67%)	NA	NR	NR
Gastroesophageal Varices				81%
Training group	NR	NR	NR	NR
Control group	NR	NA	NR	NR
Hepatic encephalopathy				
Training group	NR	NR	NR	NR
Control group	NR	NA	NR	NR

Author	Williams (25)	Morkane (26)	Chen (27)	Lin (28)
Year	2019	2019	2020	2021
Study Design	Prospective cohort study	Prospective cohort study	Randomized controlled trial	Ambispective cohort study
Study quality[a]	Low	Low	Low	Moderate
Population (n)				
Training group	18	16	9	517
Control group	NA	17	8	NA
Demographics				
Age, years				
Training group	55 (44-63)[b]	55.6 (7.8)	55 (7)	61 (53-66)[b]
Control group	NA	55.6 (7.8)	54 (11)	NA
Sex, male, %				
Training group	50%	88%	56%	59%
Control group	NA	82%	75%	NA
BMI				
Training group	25.4 (21-45) [b]	30.9 (5.6)	31 (8)	30 (25-34) [b]
Control group	NA	27 (4.7)	29 (4)	NA
MELD-score				
Training group	13 (12-26)[2]	13.7 (4.6)	16 (4)	12 (8-16) [b]
Control group	NA	13.2 (3.7)	19 (3)	NA
Child Pugh-score				

(Continued on following page)

TABLE 1 | (*Continued*) Designs of included studies and patient demographics.

Author	Williams (25)	Morkane (26)	Chen (27)	Lin (28)
Training group	NR	NR	9 (100%)[d]	NR
Control group	NR	NR	8 (100%)[d]	NR
Comorbidities, n (%)				
Diabetes Mellitus				
Training group	1 (5.6%)	NR	4 (45%)	227 (44%)
Control group	NA	NR	1 (13%)	NA
Cardiac disease				
Training group	1 (5.6%)	NR	NR	57 (11%)
Control group	NA	NR	NR	NA
Pulmonary disease				
Training group	NR	NR	NR	36 (7%)
Control group	NR	NR	NR	NA
Ascites				
Training group	6 (33%)	NR	7 (78%)	352 (69%)
Control group	NA	NR	6 (75%)	NA
Gastroesophageal Varices				
Training group	NR	NR	5 (56%)	340 (67%)
Control group	NR	NR	7 (88%)	NA
Hepatic encephalopathy				
Training group	6 (33.3%)	NR	9 (100%)	271 (53%)
Control group	NA	NR	8 (100%)	NA

[a]*Quality assessment according to the GRADE system for quality assessment of clinical studies (20).*
[b]*Data presented as median (IQR).*
[c]*Data presented as median (range).*
[d]*no of patients with Child Pugh B or C.*
Data are presented as mean (SD) unless stated otherwise.
BMI, body mass index; MELD, model for end-stage liver disease score; NA, not applicable; NR, not reported.

group (24,27,28) and 88% in the control group (27). Baseline study characteristics and demographics are displayed in **Table 1**.

The primary outcomes reported on in the included studies varied and included alterations in spirometry results (22), alterations in frailty metrics (28), readmissions within 90 days post-OLT (11), and the safety and feasibility of training (23-25). The secondary outcomes were more uniform between the studies and included general QoL assessments (22-24,27), aerobic functioning after training (23-28), and adverse events during the program (11,22-27).

Intervention

Three of six studies that implemented unsupervised home-based training programs provided once-to-thrice weekly telephone contact for supervision or motivational support (11,25,27). The duration of training programs varied from six (26) to eight (24) to 12 weeks (22,23,25,27) and until OLT (28). The frequency of training varied per study; Limongi et al. provided a manual for daily, non-supervised, home-based exercise training (22), while Debette-Gratien et al. implemented twice-weekly, supervised, in-hospital gym training (23). Thrice weekly supervised in-hospital training was utilized by Wallen, Morkane, and Al-Judaibi et al. (11,24,26), Williams, Chen and Lin et al. advised non-supervised training up to five times per week, dependent on pre-defined weekly targets (25,27,28).

Physical training programs mainly consisted of aerobic training by cycle ergometer or walking programs (11,22-28), and strength exercises (11,22-25,28), or coughing and breathing exercises (22). Except for the interval training described by Morkane et al., Debette-Gratien et al. and Williams' set goal to archive a work rate of 12–14 on the Borg scale of rate of perceived exertion (RPE-score) (23,25,26,29), no insight was provided into the intensity of the training programs in the other included studies. Al-Judaibi and Lin et al. provided education related to physical activity and dietary support in the training group (11,28), whereas, in Chen et al.'s study, both the intervention and control groups were provided with extra information regarding nutrition (27). Detailed information regarding the designs of the training programs, exclusion criteria, supervision, duration, and the risk of ineffectiveness is provided in **Table 2**.

Data-Analysis

Effect of Training on Physical Capacity

All the studies examining the physical effects of aerobic training reported some significant improvement in aerobic capacity (23-28) (**Table 3**). Debette-Gratien et al. reported a significant improvement in VO2 peak after training, from a mean VO2 peak of 21.5 (5.9) ml/kg/min before training to a mean VO2 peak value of 23.2 (5.9) ml/kg/min after training ($p = 0.008$) (23). In addition, Morkane et al. reported a significant VO2 peak improvement of 2.3 ml/kg/min in the training group ($p = 0.02$), while a decrease of 1.9 ml/kg/min was observed in the control group (p = 0.03) (26). Although Chen et al. found no significant improvement in VO2 peak after training (18 (7) before versus 17 (6) ml/kg/min after training, $p = 0.42$), they observed a decrease of 3 ml/kg/min ($p = 0.08$) in the control group (27). Debette-Gratien, Wallen, and Chen et al. reported significant

TABLE 2 | Details of included training programs.

Author	Limongi (22)	Debette-Gratien (23)	Al-Judaibi (11)	Wallen (24)
Exclusion criteria	Age <18; Acute liver failure	No prevention of esophageal bleeding (β-blockers or varices ligation); Ventricular ejection fraction <45%; Arrhythmia/cardiac decompensation during excercise	None	Previous LT; Listed for other organ transplantation; Current smoking; Adverse event during CPET; Uncontrolled diabetes; Orthopedic/ neurological limitation to exercise
Training details				
Training group	Physical training	Physical training	Physical training and nutritional support	Physical training
Control group	No exercises.	NA	Before implementation of training program.	No information regarding exercise training or physical activity provided.
Supervision training	Unsupervised training at home by manual.	Supervised in-hospital gym.	Supervised in hospital gym or unsupervised at home with twice/ thrice weekly supervision through phone calls	Supervised in hospital gym and unsupervised at home.
Duration training, weeks	12	12	Until suitable for transplantation Mean duration not reported	8
Frequency training	Daily	Twice weekly	1-5 times weekly	Thrice weekly
Type of training	1. Cough and breathing exercises	1. Aerobic training (cycle ergometer)	1. Aerobic training (cycle ergometer)	1. Aerobic training (cycle ergometer or walking)
	2. Isometric force exercises.	2. Muscle strength exercise (Press body building type)	2. Resistance strength exercise	2. Resistance strength exercise (circuit-based with weights)
			3. Education regarding activity.	
Risk of ineffectiveness of training program[a]	High	Low	High	Low

Author	Williams (25)	Morkane (26)	Chen (27)	Lin (28)
Exclusion criteria	Cardiovascular instability; CVA; ≥ grade 2 hepatic encephalopathy	Noncirrhotic liver disease; oncological diagnosis; contraindication for exercise	Large gastrointestinal varices without β-blocker use; HCC; hepatic encephalopathy; hydrothorax; pulmonary vascular complications of portal hypertension; cardiorespiratory contraindications for exercise	No exclusion criteria
Training details				
Training group	Physical training	Physical training	Physical training and nutritional support	Exercise prescription and one dietary consultation
Control group	NA	CPET at 0, 6 and 12 weeks, no exercise program	Nutritional support only	NA
Supervision training	Unsupervised at home	Supervised in hospital gym	Unsupervised training at home	Unsupervised home-based exercise workouts
	Once weekly supervision through phone calls		Weekly supervised counseling and daily motivational phone calls	Rarely: supervised home-based or outpatient physical therapy Once monthly phone follow-up and appointment after 90 - 120 days
Duration training (weeks)	12	6	12	Until LT
Frequency training	Twice weekly, 20 minutes exercise Thrice daily, 10 minutes walking.	Thrice weekly, 40 minutes.	Recommendation of 5 times weekly, 30 minutes.	Recommendation of 5 times weekly, 30 minutes
Type of training	1. Functional resistance exercises (video guide) 2. Aerobic exercises (video guide) 3. Walking program (daily step targets)	Aerobic training (cycle ergometer)	Walking training by increasing daily step-goal (Fitbit).	Home exercise program: 1. force: weights / resistance bands 2. aerobic: treadmills, elliptical or stationary bikes
Risk of ineffectiveness of training program[a]	Low	Low	High	Low

[a]*Risk of ineffectiveness of training program according to the i-CONTENT tool for assessing therapeutic quality of exercise programs employed in clinical trials (17)*
LT, liver transplantation; CPET, cardiopulmonary exercise test; NA, not applicable.

TABLE 3 | Physical effects of training in patients awaiting orthotopic liver transplantation.

Author	Aerobic capacity						Functional capacity		
	VO2 peak (ml/kg/min)			6MWD (m)			Handgrip strength (kg)		
	Before training	After training[a]	*p*-value	Before training	After training[a]	*p*-value	Before training	After training[a]	*p*-value
Debette-Gratien (23)	21.5 (5.9)	23.2 (5.9)	**0.008**	481 (69)	521 (64)	**0.02**	30 (10)	37 (13)	**0.008**
Wallen (24)									
Training/control[b]	NR	NR		NR	+103.8 (81.4)	**0.02**	NR	+6.3 (8.5)	0.24
Morkane (26)									
Training	16.2 (3.4)	18.5 (4.6)	**0.02**	NR	NR		26.4 (7.5)	29.4 (6.4)	**0.05**
Control	19.0 (6.1)	17.1 (6.0)	**0.03**	NR	NR		29.1 (10.7)	30.5 (13)	0.8
Chen (27)									
Training	18 (7)	17 (6)	0.42	423 (60)	482 (87)	**0.05**	NR	NR	
Control	18 (6)	15 (7)	0.08	418 (59)	327 (166)	0.21	NR	NR	
	GST (m/s)						**LFI**		
Lin (28) [c]	Before training	After training	*p*-value	Before training	After training	*p*-value	Before training	After training	*p*-value
Training (all patients)	1.0 (0.8–1.2)	*F* = 1.53	0.20	326 (244–390)	*F* = 1.88	0.13	3.8 (3.3–4.5)	*F* = 3.45	**0.01**
Training (full adherence group)[d]	1.0 (0.8–1.2)	*F* = 1.20	0.32	326 (244–390)	*F* = 2.64	0.07	3.8 (3.3–4.5)	*F* = 8.10	<**0.001**
Control	NR	NR	NR	NR	NR	NR	NR	NR	NR
				ISWT (m)			**SPPBT**		
				Before training	After 12 weeks	*p*-value	Before training	After 6 weeks	*p*-value
Williams (25)	NR	NR	NR	260 (70–1020)	470 (190–880)	<**0.01**	9.5 (6-12)	11.5 (9-12)	**0.02**
	FVC (%)			**FEV1 (%)**					
	Before training	After training[a]	p-value	Before training	After training[a]	p-value			
Limongi (22)									
Training	82.8 (13.1)	87 (7.9)	NR	76 (17)	82 (14.5)	NR	NR	NR	
Control	84.3 (12.2)	87 (19.2)	NR	84.3 (12.8)	85.4 (15.2)	NR	NR	NR	
Al-Judaibi (11)	NR	NR		NR	NR		NR	NR	

[a]*The control group did not receive any training.*
[b]*Only between-group changes (intervention vs. control) were reported in the study.*
[c]*This study did not mention after-training outcomes as absolute numbers, but as delta points (F).*
[d]*Full adherence: study patients who completed >80% of workout sessions.*
Data are presented as mean (SD) or median (IQR).
VO2 peak, oxygen consumption at peak exercise; 6MWD, 6-minute walking distance; F, delta points; GST, gait speed test; LFI, liver frailty index; FVC, forced vital capacity; FEV_1 = forced expiratory volume in one second; ISWT, incremental shuttle walk test; SPPBT, short physical performance battery test; NR, not reported.

improvements in walking distance after training (+40 m, $p = 0.02$; +16 m, p = 0.02 and +59m, p = 0.05, respectively) (23,24,27), while Lin et al. did not report a significant improvement in walking distance after training (F = 2.64, $p = 0.07$) (28). Furthermore, Debette-Gratien and Morkane et al. reported a significant improvement in grip strength (+7 kg, $p = 0.008$ and +3 kg, $p = 0.05$, respectively) after 12 weeks of training (23,26). However, in the study of Wallen et al., there was no significant improvement in grip strength after training (+0.4 kg, $p = 0.24$) (24). Regarding 6MWD and hand grip strength, no significant improvement or decline was observed in the control groups. Although Williams et al. did not report on VO2 peak or 6MWD, they did observe a significant improvement in aerobic capacity, measured by the incremental shuttle walk test (ISWT) (260 (70–1020) meter to 470 (190–880) meter, p < 0.01), and functional capacity, measured by the Short Physical Performance Battery Test (SPPBT) (9.5 (6–12) to 11.5 (9–12), p = 0.02), after 12 weeks of training. Lin et al. found a significant improvement of the liver frailty index (LFI) for all patients after training (F = 3.45, p = 0.01), and found an even larger effect in patients who adhered to >80% of the workout sessions until OLT (F = 8.10; p < 0.001) (28). Thereby, Lin et al. found a significant correlation with an improvement of the LFI and a survival advantages among included patients (28).

Perceived Health-Related Quality of Life Before and After Training

Four studies examined QoL before and after the training program while awaiting OLT (**Table 4**) (23-25,27). Williams et al. found an increase of 18% reported in the EuroQol visual analogue scale

TABLE 4 | Effect of training on quality of life in patients awaiting orthotopic liver transplantation.

Author	Tool	Quality of life		
		Before training	After training[a]	*p*-value
Debette-Gratien (23)	SF-36	36 (4)	39 (3)	0.46
Wallen (24)				
Training/control[b]	HR-QoL	NR	−0.3 (−1.3,0.8)	0.67
Williams (25)	EQ-VAS	NR	"Improvement of 18%"	**0.04**
	EQ-5D	NR	Improvement in: 44% - Mobility	
	No-problems reported		56% - Pain/discomfort	
	HADS	10 (1–26)	7 (0–22)	0.13
Chen (27)				
Training	SIP	11.2 (7.3)	7 (6.4)	0.10
Control[a]	SIP	11.5 (13)	15.7 (17.3)	0.07
Limongi (22)	NR			
Al-Judaibi (11)	NR			
Morkane (26)	NR			
Lin (28)	NR			

[a]*The control group did not receive any training.*
[b]*Only between-group changes (intervention vs. control) were reported in the study.*
Data are presented as mean (SD) or median (IQR).
SF-36, Short Form 36; HR-QoL, health related quality of life; EQ-VAS, EuroQol visual analogue scale; EQ-5D, european quality of life five dimensions; HADS, hospital anxiety and depression score; SIP, sickness impact profile; NR, not reported.

(EQ-VAS) questionnaire (p = 0.04) (25,30). And, although Williams et al. found no differences in median hospital anxiety and depression score (HADS) (10 (1–26) before training versus 7 (0–22) after training, p = 0.13), an increase of proportion of patients reporting no problems with mobility (44%) and pain/discomfort (56%) in the EuroQol 5-Dimension 5-Level (EQ-5D-5L) instrument was found after 12 weeks of prehabilitation (25). Debette-Gratien, Wallen, and Chen et al. found no differences in QoL between the training and control groups or between pre- and post-training on the SF-36 (24) or the HR-QoL (23,24,27). However, in Chen et al.'s study, an improvement was observed on the sickness impact profile (SIP) in the training group (−4.2, p = 0.10), while the SIP in the control group worsened (+4.2, p = 0.07) (27).

Effects of Training on Length of Hospital Stay After OLT

Two studies (11,26) described differences in the length of in-hospital stay after OLT between the training groups and control groups (**Table 5**). Al-Judaibi et al. found a significantly shorter median length of ICU stay before transplantation in the intervention group compared to the control group (n = 458, 2 vs. 3 days, p = 0.01), however, no significant difference was observed in the length of in-hospital stay after OLT (11). Morkane et al. found no difference in the median length of ICU stay between the intervention and control groups (2 (4) versus 4 (5.5), p = 0.77), but found a significant difference in postoperative median length of hospital stay between the training group and control group (13 (7–19) versus 30 (17–43), p = 0.02) (26).

Feasibility of the Studies Performed

Three studies reported on the participants identified for possible inclusion and the reasons for exclusion. Wallen et al. identified 38 patients, of whom 15 declined to participate; one patient was transplanted before the start of the training program, and another was delisted before commencement, leaving 21 (55%) suitable for inclusion (24). Chen et al. identified 227 OLT candidates and excluded 210 (93%) for various reasons: 85 because of the presence of a hepatocellular carcinoma, 73 due to logistic or transport issues, 35 because of cardiopulmonary or metabolic diseases, 14 because of being delisted as OLT candidates, two due to repeated hospitalization, and one because that patient already walked more than 10,000 steps per day (27). Williams et al. randomly selected 46 patients from the OLT waiting list: 32 (70%) were eligible for study entry, with patients awaiting a re-transplantation being the most common reason for exclusion (5 out of 46; 11%). Of the 32 patients deemed eligible, six (18.8%) declined participation and eight (25%) underwent OLT prior to study visit one. Therefore, a total 18 out of 32 eligible patients (56.2%) were enrolled in the study (25). Al-Judaibi et al. Debette-Gratien et al. and Lin et al. included consecutive patients and had a study feasibility of 100% (11,23,28). Limongi et al. identified 42 patients and included 17 (40%) in their study without listing reasons for exclusion (22), and Morkane et al. did not report on patients eligible for inclusion (26). No studies excluded patients with gastro-oesophageal varices treated with β-blockers (**Table 2**).

Feasibility and Safety of Training Programs

Outcomes regarding safety, feasibility, and adherence to the training programs are displayed in **Table 6**. Three author groups reported the feasibility and safety of their training programs as their primary outcome (24-26). Williams et al. defined feasibility as the absence of training-related serious adverse events; the eligibility of 66% or more of patients who are actively listed on the OLT waiting list; and more than 66% adherence to the daily step count and resistance exercises and completion of 6 weeks training (25). In their study, 82% of the patients adhered to daily step targets and 90% to the twice-weekly

TABLE 5 | Effect of training on postoperative surgical outcome after orthotopic liver transplantation.

Author	Length of hospital stay (days)	*p-value*	Length of ICU stay (days)	*p-value*	90-day readmission rate	*p-value*
Williams (25)	10 (5–41)		4 (1)		NR	
Al-Judaibi (11)						
Training	14 (3–150)	0.69	NR		17%	0.58
Control	17 (5–161)		NR		20%	
Morkane (26)						
Training	13 (7–19)	**0.02**	2 (4)	0.77	NR	
Control	30 (17–43)		4 (5.5)		NR	
Debette-Gratien (23)	NR		NR		NR	
Limongi (22)	NR		NR		NR	
Wallen (24)	NR		NR		NR	
Chen (27)	NR		NR		NR	
Lin (28)	NR		NR		NR	

Data are presented as mean (SD), median (IQR) or n (%).
ICU, intensive care unit; NR, not reported.

TABLE 6 | Feasibility and safety of prehabilitation in patients awaiting orthotopic liver transplantation.

Author	Feasibility/Adherence to the program	Safety and adverse events	No. patients lost to follow up intervention group
Debette-Gratien (23)	NR	1 – worsening hepatorenal syndrome	2 – moved to another region
		No cardiovascular events No cirrhotic decompensation No variceal bleeding or ascites)	2 –transplanted before 12 weeks 1 – deterioration of clinical condition
Wallen (24)	95% adherence to supervised exercise training 75% adherence to unsupervised exercise training	1 – adverse event (knee injury) No serious adverse events No variceal bleeding or hepatic encephalopathy	5 – transplanted before 8 weeks 1 – delisted and noncompliant
Williams (25)	82% adherence to step-targets 90% adherence to twice weekly exercises	No adverse events	1 – non-study related trauma
Morkane (26)	94% of total exercise sessions were completed	No adverse events No worsening cirrhotic decompensation	1 – transplanted before 12 weeks
Chen (27)	NR	NR	1 – other surgery 1 – transplanted before 12 weeks 1 – lost to follow-up
Lin (28)	Adherence to minimally 1 follow up: 211 (69%) of 305 LT-candidates Self-reported adherence: 4–5 day/week: 146 (38%) 1–3 day/week: 198 (51%) 0 days/week: 41 (11%)	NR	24 – failed to visit follow up sessions; unknown reason
Limongi (22)	NR	NR	NR
Al-Judaibi (11)	NR	NR	NR

LT, liver transplantation; NR, not reported

exercises (25). Morkane et al. reported a 94% adherence with all exercises (26), and Wallen et al. reported a 95% and 75% adherence to supervised and unsupervised exercise training, respectively (24). Lin et al. reported an adherence to minimally one follow-up physical therapy session of 69% (28). Patients' self-reported adherence varied from adherence of 4–5 days/week in 38% of the patients, to 1–3 days/week in 51% of the patients and 0 days/week in 11% of the patients (28).

Four studies (23-26) described the potential of serious adverse events resembling cardiovascular events, cirrhosis decompensation, variceal bleeding or hepatic encephalopathy, but none of the authors reported any of these events occurring during the study. Wallen et al. reported on one adverse event (knee injury, one out of 11 patients (9.1%)) that occurred during training (24). In the study of Debette-Gratien et al., one patient (one out of 13 patients (7.7%)) stopped training due to worsening of their hepatorenal syndrome (23). Most common reason for dropping out of the program was because of transplantation before the end of the study period. All reasons why patients were lost to follow-up are listed in **Table 6**.

DISCUSSION

The aim of this systematic review was to evaluate the effect of prehabilitation on physical capacity and surgical outcome in patients actively waiting for OLT. Six out of eight studies demonstrated significant improvements in aerobic or physical capacity (23-28). Adherence to the training programs was 69% or higher, and none of the included studies reported any serious adverse events. Therefore, these findings imply that prehabilitation programs are safe, feasible, and, potentially, effective for OLT-candidates.

In the past, one other review and one meta-analysis have been conducted in patients with chronic liver disease to assess the effect of training on their physical capacity (31,32). And although this current review shows resemblance to these previously conducted reviews, the majority of their included studies excluded potential OLT candidates and patients with MELD score ≥12, while this current review solely focussed on patients with ESLD awaiting OLT (31,32). For example, in the review conducted by Williams et al., the authors concluded that moderate-to-high intensity exercise can improve the physical components of frailty and QoL in patients with chronic liver disease, but that it remained to be elucidated whether this also applies to patients with Child Pugh B/C decompensated cirrhosis (33-39). In the review of Brustia et al., where not solely patients awaiting OLT were included, no adverse events were caused by the training, but neither an improvement in physical capacity was observed (32).

When elaborating on the physical effects of prehabilitation in OLT candidates, previous literature has shown that preoperative VO2 peak and MELD score are independent prognostic factors of mortality and duration of hospitalization during both the pre- and post-transplantation periods (15,40-42). Hence, it can be hypothesized that increased VO2 peak due to training, could improve surgical outcome for the OLT candidate. The ability to increase this physical capacity with training was shown by several studies included in this review (23-27). The studies of Debette-Gratien, Morkane and Williams et al. all found a significant improvement in aerobic capacity after training (23,25,26). Their results, however, differed from the study by Chen et al., who found no difference in VO2 peak after training (27). This difference in results might be explained by the differences in design of the training programs of the three studies: Debette-Gratien and Morkane et al. provided specified supervised aerobic training with a cycle ergometer, Williams et al. used video guided exercises and non-supervised walking training, and Chen et al. solely implemented non-supervised walking training (23,25-27). Thereby, only three out of eight studies outlined the aerobic intensity of the exercises (23,25,26). Debette-Gratien et al. and Morkane et al. based their patient-adjusted aerobic training protocol on VO_2 peak and on the anaerobic threshold which was objectified by CPET (23,26). Williams et al. used a subjective scale where patients were asked to achieve a work rate of 12–14 on the Borg scale (25). To speculate, these results should be interpreted with caution, but suggest that supervised aerobic cycling training by use of a patient-adjusted protocol could be more beneficial than unsupervised walking training. The hypothesis that physical training improves postoperative recovery was only described in three out of the eight included studies (11,25,26), and seems to be consistent with the findings of Lin et al., who found a significant correlation between survival advantage with improvement of the LFI score (28), and Morkane et al., who found a significant median difference of 17 days in the length of in-hospital stay between the intervention and control groups (26). However, in contrast with Morkane et al.'s finding, Al-Judaibi et al. found no difference in the length of in-hospital stay or 90-day readmission rate (11). The differences between the studies of Morkane and Al-Judaibi et al. may be explained by the studies' population sizes ($n = 17$ vs. $n = 458$, respectively) and the significantly older population with more comorbidities in the training group compared to the control group in the study of Al-Judaibi et al. (11), while in the study of Morkane et al. no significant differences in baseline demographics of the two groups were reported (26).

Debette-Gratien et al. were able to include 100% of eligible candidates in their study (23), while Chen et al. only included 7% of eligible candidates. This discrepancy between eligible and eventually included patients could be caused by tight inclusion criteria, but results in a questionable feasibility of the study and could increase the risk of potential attrition bias. However, when evaluating feasibility of the training programs, all studies that mentioned adherence to the program reported a 38–90% adherence in unsupervised exercise training (24,25,28) and 94% or higher adherence to supervised exercise training (24,26), suggesting a high feasibility of prehabilitation programs in OLT candidates. These findings are somewhat surprising since the psychological burden on the OLT candidate is high (43), and the long waiting time and presence of symptoms related to liver cirrhosis possibly corrode compliance and motivation (34). Nonetheless, the dropout rate was low in all studies, and the most common reason for dropout was because patients were transplanted before the end of the study period.

This review has several limitations. First of all, this review was not pre-registered on the PROSPERO database, which could have caused reduced transparency of the applied search strategy of this review. Secondly, there are certain limitations regarding the studied evidence: most included studies consisted of small patient populations, and focused on different primary outcomes, which made the comparison and analysis of the studies challenging. In addition, most of the studies were non-randomized, which leads to a reduction in the analysis strength of this review. Finally, as the values of the baseline and post-training outcomes are not independent of each other, and correlations were not reported by the individual studies, meta-analyses were not possible (44). The high heterogeneity and lack of high-quality trials make it difficult to draw conclusions on the true effect of prehabilitation, when taking infrastructural differences, waiting time and clinical status as prognostic factors of success of the training programs in account. However, by strictly including only studies with patients having ESLD who are actively waitlisted for OLT, a bias of representing a "healthier" study group is prevented. Therefore, the strength of this review is, therefore, to represent the "most physically frail" patients, namely the OLT candidates with ESLD.

In our opinion, home-based training, which is supervised by a dedicated physical therapist and is combined with nutritional and

educational support by a dietician, could be suitable for preoperative optimization until OLT. Patients might make some progress during these weeks of training, but, most importantly, deterioration of aerobic capacity could be prevented (27) and the number of hospital admissions due to decompensated liver disease during the waiting period could be reduced (45). To the best of our knowledge, the economic burden of the implementation of a prehabilitation program in this patient population has not been studied yet. One can imagine that the supervision and provision of a personalized training program for this frail population requires professional health-care workers as physiotherapists and dieticians. However, since previous studies showed cost-effectiveness for prehabilitation in patients undergoing abdominal surgery (46), we think that investing in personalized training programs for this specifically frail population could be beneficial. However, the effects of physical training in this patient population are still not decisive, and objectively measured effects of structured training programs on days of hospitalization, presence of complications and functional evolution after transplantation are scarce. Therefore, this review emphasizes the need for large (multicentre) longitudinal trials that not only study the physical effects, but also focus on possible improvement of surgical outcomes after a longer duration of training during the waiting period prior to OLT. Randomizing between training and no training is, in our opinion, not ethically justifiable, because various studies (45,47,48) have shown the benefits of improved physical capacity, activity, and muscle status with surgical outcome.

In conclusion, this systematic review found that prehabilitation in patients actively listed for OLT may improve aerobic and functional capacity, and, more importantly, that deterioration in aerobic and functional capacity could be countered by prehabilitation. Thereby, since no serious adverse events were reported and adherence to the training programs was high, we conclude that prehabilitation is safe and feasible in the OLT candidate. Thus, from our point of view, all patients awaiting OLT, especially the most physically frail ones, should be enrolled in predefined prehabilitation programs.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

WJ and RH were involved in the review of the concept, literature search, design, manuscript preparation and review/editing of final manuscript. NM, FC, JK, and RJ were involved in the review of the concept and review/editing of final manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

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

ACKNOWLEDGMENTS

The authors would like to thank Wichor M. Bramer, PhD, as a biomedical information specialist from the Erasmus University Medical Center medical library for the collaboration in developing and updating the literary search strategy. The authors would also like to thank Sanne E. Hoeks, PhD as a clinical epidemiologist from the Erasmus University Medical Center for assistance with the statistical analyses of the results.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10330/full#supplementary-material

REFERENCES

1. Dronkers JJ, Chorus AMJ, van Meeteren NLU, Hopman-Rock M. The Association of Pre-operative Physical Fitness and Physical Activity with Outcome after Scheduled Major Abdominal Surgery. *Anaesthesia* (2013) 68(1):67–73. doi:10.1111/anae.12066
2. Mortensen K, Nilsson M, Slim K, Schäfer M, Mariette C, Braga M, et al. Consensus Guidelines for Enhanced Recovery after Gastrectomy: Enhanced Recovery after Surgery (ERAS®) Society Recommendations. *Br J Surg* (2014) 101(10):1209–29. doi:10.1002/bjs.9582
3. Lee Y, Yu J, Doumouras AG, Li J, Hong D. Enhanced Recovery after Surgery (ERAS) versus Standard Recovery for Elective Gastric Cancer Surgery: A Meta-Analysis of Randomized Controlled Trials. *Surg Oncol* (2020) 32:75–87. doi:10.1016/j.suronc.2019.11.004
4. Prentis JM, Trenell MI, Vasdev N, French R, Dines G, Thorpe A, et al. Impaired Cardiopulmonary reserve in an Elderly Population Is Related to Postoperative Morbidity and Length of Hospital Stay after Radical Cystectomy. *BJU Int* (2013) 112(2):E13–9. doi:10.1111/bju.12219
5. Bongers BC, Dejong CHC, den Dulk M. Enhanced Recovery after Surgery Programmes in Older Patients Undergoing Hepatopancreatobiliary Surgery: what Benefits Might Prehabilitation Have? *Eur J Surg Oncol* (2021) 47(3):551–9. doi:10.1016/j.ejso.2020.03.211
6. Durrand J, Singh SJ, Danjoux G. Prehabilitation.. *Clin Med* (2019) 19(6):458–64. doi:10.7861/clinmed.2019-0257
7. van Wijk L, Berkel AEM, Buis CI, Bongers BC, Klaase JM. Preoperative home-based Exercise Prehabilitation in Patients Scheduled for Liver or Pancreatic Resection: The First Results. *HPB (Oxford)* (2020) 46(2):S231. doi:10.1016/j.hpb.2020.04.086
8. van Wijk L, van der Snee L, Buis CI, Hentzen JEKR, Haveman ME, Klaase JM. A Prospective Cohort Study Evaluating Screening and Assessment of Six Modifiable Risk Factors in HPB Cancer Patients and Compliance to Recommended Prehabilitation Interventions. *Perioper Med* (2021) 10(1):5. doi:10.1186/s13741-020-00175-z
9. Hughes MJ, Hackney RJ, Lamb PJ, Wigmore SJ, Christopher Deans DA, Skipworth RJE. Prehabilitation before Major Abdominal Surgery: A Systematic Review and Meta-Analysis. *World J Surg* (2019) 43(7):1661–8. doi:10.1007/s00268-019-04950-y

10. Beyer N, Aadahl M, Strange B, Kirkegaard P, Hansen BA, Mohr T, et al. Improved Physical Performance after Orthotopic Liver Transplantation. *Liver Transpl Surg* (1999) 5(4):301–9. doi:10.1002/lt.500050406
11. Al-Judaibi B, Alqalami I, Sey M, Qumosani K, Howes N, Sinclair L, et al. Exercise Training for Liver Transplant Candidates. *Transpl Proc* (2019) 51(10): 3330–7. doi:10.1016/j.transproceed.2019.08.045
12. Tieleman M, van den Berg AP, Hoek B, Polak WG, Dubbeld J, Porte RJ, et al. Komt mijn nieuwe lever wel op tijd?" Overlevingskansen van patiënten op de wachtlijst voor levertransplantatie. *Ned Tijdschr Geneeskd* (2018) 162: D2159.
13. National Health Services (NHS). *Blood and Transplant. Liver Transplantation Data [Internet]. [cited 2022 Jun 17]* (2020). Available from: https://www.nhsbt.nhs.uk/organ-transplantation/liver/receiving-a-liver/how-long-is-the-wait-for-a-liver/ (Accessed June 15, 2022).
14. U.S. Department of Health and Human Services (HHS). *OPTN/SRTR 2019 Annual Data Report: Liver [Internet]. [cited 2022 Jun 17]* (2019). Available from: https://srtr.transplant.hrsa.gov/annual_reports/2019/Liver.aspx (Accessed June 15, 2022).
15. Prentis JM, Manas DMD, Trenell MI, Hudson M, Jones DJ, Snowden CP. Submaximal Cardiopulmonary Exercise Testing Predicts 90-day Survival after Liver Transplantation. *Liver Transpl* (2012) 18(2):152–9. doi:10.1002/lt.22426
16. Hoogeboom TJ, Dronkers JJ, Hulzebos EHJ, van Meeteren NLU. Merits of Exercise Therapy before and after Major Surgery. *Curr Opin Anaesthesiol* (2014) 27(2):161–6. doi:10.1097/ACO.0000000000000062
17. Hoogeboom TJ, Kousemaker MC, van Meeteren NL, Howe T, Bo K, Tugwell P, et al. I-CONTENT Tool for Assessing Therapeutic Quality of Exercise Programs Employed in Randomised Clinical Trials. *Br J Sports Med* (2020) 55:1153–60. doi:10.1136/bjsports-2019–10163010.1136/bjsports-2019-101630
18. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 Statement. *Syst Rev* (2015) 4(1):1. doi:10.1186/2046-4053-4-1
19. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JPA, et al. The PRISMA Statement for Reporting Systematic Reviews and Meta-Analyses of Studies that Evaluate Health Care Interventions: Explanation and Elaboration. *Plos Med* (2009) 6(7):e1000100. doi:10.1371/journal.pmed.1000100
20. Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, et al. GRADE: an Emerging Consensus on Rating Quality of Evidence and Strength of Recommendations. *BMJ* (2008) 336(7650):924–6. doi:10.1136/bmj.39489.470347.AD
21. Guyatt GH, Oxman AD, Sultan S, Glasziou P, Akl EA, Alonso-Coello P, et al. GRADE Guidelines: 9. Rating up the Quality of Evidence. *J Clin Epidemiol* (2011) 64(12):1311–6. doi:10.1016/j.jclinepi.2011.06.004
22. Limongi V, Dos Santos DC, Da Silva AMO, Ataide EC, Mei MFT, Udo EY, et al. Effects of a Respiratory Physiotherapeutic Program in Liver Transplantation Candidates. *Transpl Proc* (2014) 46(6):1775–7. doi:10.1016/j.transproceed.2014.05.044
23. Debette-Gratien M, Tabouret T, Antonini MT, Dalmay F, Carrier P, Legros R, et al. Personalized Adapted Physical Activity before Liver Transplantation: Acceptability and Results. *Transplantation* (2015) 99(1):145–50. doi:10.1097/TP.0000000000000245
24. Wallen MP, Keating SE, Hall A, Hickman IJ, Pavey TG, Woodward AJ, et al. Exercise Training Is Safe and Feasible in Patients Awaiting Liver Transplantation: A Pilot Randomized Controlled Trial. In: *Liver Transplantation*, 25. John Wiley & Sons (2019). p. 1576–80. doi:10.1002/lt.25616
25. Williams FR, Vallance A, Faulkner T, Towey J, Durman S, Kyte D, et al. Home-based Exercise in Patients Awaiting Liver Transplantation: A Feasibility Study.. *Liver Transpl* (2019) 25(7):995–1006. doi:10.1002/lt.25442
26. Morkane CM, Kearney O, Bruce DA, Melikian CN, Martin DS. An Outpatient Hospital-Based Exercise Training Program for Patients with Cirrhotic Liver Disease Awaiting Transplantation: A Feasibility Trial. *Transplantation* (2019) 104(1):97–103. doi:10.1097/TP.0000000000002803
27. Chen HW, Ferrando A, White MG, Dennis RA, Xie J, Pauly M, et al. Home-Based Physical Activity and Diet Intervention to Improve Physical Function in Advanced Liver Disease: A Randomized Pilot Trial. *Dig Dis Sci* (2020) 65(11): 3350–9. doi:10.1007/s10620-019-06034-2
28. Lin FP, Visina JM, Bloomer PM, Dunn MA, Josbeno DA, Zhang X, et al. Prehabilitation-driven Changes in Frailty Metrics Predict Mortality in Patients with Advanced Liver Disease. *Am J Gastroenterol* (2021) 116(10):2105–17. doi:10.14309/ajg.0000000000001376
29. Borg GA. Psychophysical Bases of Perceived Exertion. *Med Sci Sports Exerc* (1982) 14(5):377–81. doi:10.1249/00005768-198205000-00012
30. Janssen MF, Pickard AS, Golicki D, Gudex C, Niewada M, Scalone L, et al. Measurement Properties of the EQ-5D-5L Compared to the EQ-5D-3L across Eight Patient Groups: a Multi-Country Study. *Qual Life Res* (2013) 22(7): 1717–27. doi:10.1007/s11136-012-0322-4
31. Williams FR, Berzigotti A, Lord JM, Lai JC, Armstrong MJ. Review Article: Impact of Exercise on Physical Frailty in Patients with Chronic Liver Disease. *Aliment Pharmacol Ther* (2019) 50(9):988–1000. doi:10.1111/apt.15491
32. Brustia R, Savier E, Scatton O. Physical Exercise in Cirrhotic Patients: Towards Prehabilitation on Waiting List for Liver Transplantation. A Systematic Review and Meta-Analysis. *Clin Res Hepatol Gastroenterol* (2018) 42(3):205–15. doi:10.1016/j.clinre.2017.09.005
33. Berzigotti A, Albillos A, Villanueva C, Genescá J, Ardevol A, Augustín S, et al. Effects of an Intensive Lifestyle Intervention Program on portal Hypertension in Patients with Cirrhosis and Obesity: The SportDiet Study. *Hepatology* (2017) 65(4):1293–305. doi:10.1002/hep.28992
34. Hallsworth K, Jopson L, Jones DE, Trenell MI. Exercise Therapy in Primary Biliary Cirrhosis: the Importance of Moving while Sitting on a Surgical Waiting List-A Case Study. *Frontline Gastroenterol* (2016) 7(3):167–9. doi:10.1136/flgastro-2015-100672
35. Kruger C, McNeely ML, Bailey RJ, Yavari M, Abraldes JG, Carbonneau M, et al. Home Exercise Training Improves Exercise Capacity in Cirrhosis Patients: Role of Exercise Adherence. *Sci Rep* (2018) 8(1):99. doi:10.1038/s41598-017-18320-y
36. Nishida Y, Ide Y, Okada M, Otsuka T, Eguchi Y, Ozaki I, et al. Effects of home-based Exercise and Branched-Chain Amino Acid Supplementation on Aerobic Capacity and Glycemic Control in Patients with Cirrhosis. *Hepatol Res* (2017) 47(3):E193–200. doi:10.1111/hepr.12748
37. Román E, García-Galcerán C, Torrades T, Herrera S, Marín A, Doñate M, et al. Effects of an Exercise Programme on Functional Capacity, Body Composition and Risk of Falls in Patients with Cirrhosis: A Randomized Clinical Trial. *PLoS One* (2016) 11(3):e0151652. doi:10.1371/journal.pone.0151652
38. Román E, Torrades MT, Nadal MJ, Cárdenas G, Nieto JC, Vidal S, et al. Randomized Pilot Study: Effects of an Exercise Programme and Leucine Supplementation in Patients with Cirrhosis. *Dig Dis Sci* (2014) 59(8): 1966–75. doi:10.1007/s10620-014-3086-6
39. Zenith L, Meena N, Ramadi A, Yavari M, Harvey A, Carbonneau M, et al. Eight Weeks of Exercise Training Increases Aerobic Capacity and Muscle Mass and Reduces Fatigue in Patients with Cirrhosis. *Clin Gastroenterol Hepatol* (2014) 12(11):1920–6. doi:10.1016/j.cgh.2014.04.016
40. Epstein SK, Freeman RB, Khayat A, Unterborn JN, Pratt DS, Kaplan MM. Aerobic Capacity Is Associated with 100-day Outcome after Hepatic Transplantation. *Liver Transpl* (2004) 10(3):418–24. doi:10.1002/lt.20088
41. Trojetto T, Elliott RJ, Rashid S, Wong S, Dlugosz K, Helm D, et al. Availability, Characteristics, and Barriers of Rehabilitation Programs in Organ Transplant Populations across Canada. *Clin Transpl* (2011) 25(6):E571–8. doi:10.1111/j.1399-0012.2011.01501.x
42. Dharancy S, Lemyze M, Boleslawski E, Neviere R, Declerck N, Canva V, et al. Impact of Impaired Aerobic Capacity on Liver Transplant Candidates. *Transplantation* (2008) 86(8):1077–83. doi:10.1097/TP.0b013e318187758b
43. Yadav A, Chang YH, Carpenter S, Silva AC, Rakela J, Aqel BA, et al. Relationship between Sarcopenia, Six-Minute Walk Distance and Health-Related Quality of Life in Liver Transplant Candidates. *Clin Transpl* (2015) 29(2):134–41. doi:10.1111/ctr.12493

44. Cuijpers P, Weitz E, Cristea IA, Twisk J. Pre-post Effect Sizes Should Be Avoided in Meta-Analyses. *Epidemiol Psychiatr Sci* (2017) 26(4):364–8. doi:10.1017/S2045796016000809
45. Coca-Martinez M, Lopez-Hernandez A, Montane-Muntane M, Arguis MJ, Gimeno-Santos E, Navarro-Ripoll R, et al. Multimodal Prehabilitation as Strategy for Reduction of Postoperative Complications after Cardiac Surgery: a Randomised Controlled Trial Protocol. *BMJ Open* (2020) 10(12):e039885. doi:10.1136/bmjopen-2020-039885
46. Dholakia J, Cohn DE, Straughn JM, Dilley SE. Prehabilitation for Medically Frail Patients Undergoing Surgery for Epithelial Ovarian Cancer: a Cost-Effectiveness Analysis. *J Gynecol Oncol* (2021) 32(6):e92. doi:10.3802/jgo.2021.32.e92
47. Snowden CP, Prentis J, Jacques B, Anderson H, Manas D, Jones D, et al. Cardiorespiratory Fitness Predicts Mortality and Hospital Length of Stay after Major Elective Surgery in Older People. *Ann Surg* (2013) 257(6):999–1004. doi:10.1097/SLA.0b013e31828dbac2
48. Shulman MA, Cuthbertson BH, Wijeysundera DN, Pearse RM, Thompson B, Torres E, et al. Using the 6-minute Walk Test to Predict Disability-free Survival after Major Surgery. *Br J Anaesth* (2019) 122(1):111–9. doi:10.1016/j.bja.2018.08.016

ESOT | Transplant International

ORIGINAL RESEARCH
published: 15 September 2022
doi: 10.3389/ti.2022.10728

Dysbiosis and Depletion of Fecal Organic Acids Correlate With the Severity of Rejection After Rat Liver Transplantation

Siyuan Yao[1], Shintaro Yagi[2]*, Eri Ogawa[1], Masaaki Hirata[1], Yosuke Miyachi[1], Sena Iwamura[1], Ryuji Uozumi[3], Takuya Sugimoto[4], Takashi Asahara[4], Shinji Uemoto[5] and Etsuro Hatano[1]*

[1]Department of Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan, [2]Department of Surgery, Graduate School of Medicine, Kanazawa University, Ishikawa, Japan, [3]Department of Biomedical Statistics and Bioinformatics, Graduate School of Medicine, Kyoto University, Kyoto, Japan, [4]Yakult Central Institute, Yakult Honsha Co., Ltd., Tokyo, Japan, [5]Shiga University of Medical Science, Otsu, Japan

The impact of T cell-mediated rejection (TCMR) after liver transplantation (LT) on the alterations in the gut microbiota (GM) and associated intestinal environment represented by fecal organic acids (OAs) require further elucidation. A rat allogeneic LT model was prepared without immunosuppressants or antibiotics, and a syngeneic model was used as a control. Qualitative and quantitative analyses of fecal samples at fixed time points were performed. Correlation analyses were also performed between liver function and GMs and OA levels. In the allogeneic TCMR group, the number of predominant obligate anaerobes decreased as liver function declined. *Clostridioides difficile*, *Enterobacteriaceae*, *Enterococcus*, *Streptococcus*, and *Staphylococcus* were significantly increased. Regarding fecal OA concentration, short-chain fatty acid (SCFA) concentrations were depleted as liver function declined. In contrast, in the syngeneic group, GM and OAs exhibited only slight, transient, and reversible disturbances. In addition, alanine aminotransferase and total bilirubin were positively correlated with the number of *Enterobacteriaceae* and *Enterococcus,* and negatively correlated with the fecal concentration of SCFAs. The allogeneic TCMR model demonstrated distinct dysbiosis and depletion of fecal OAs as TCMR progressed after LT. The degree of graft injury was closely related to the number of specific bacterial strains and the concentrations of fecal SCFAs.

Keywords: T cell-mediated rejection, dysbiosis, predominant obligate anaerobes, ribosomal RNA-targeted reverse-transcription quantitative polymerase chain reaction, short-chain fatty acid

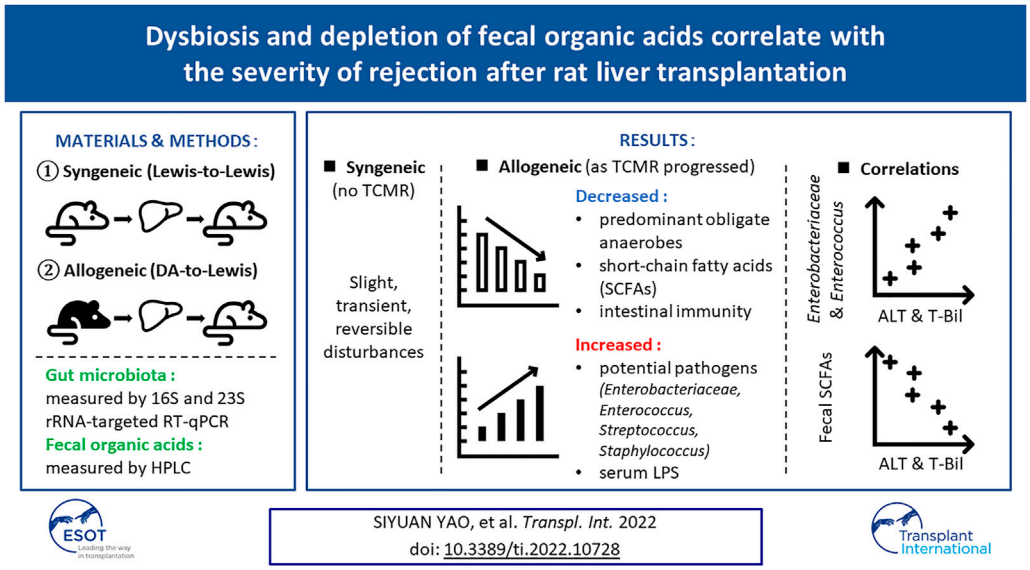
OPEN ACCESS

Correspondence:
Siyuan Yao
siyuan@kuhp.kyoto-u.ac.jp
Shintaro Yagi
yagi@med.kanazawa-u.ac.jp

Received: *27 June 2022*
Accepted: *05 September 2022*
Published: *15 September 2022*

Citation:
Yao S, Yagi S, Ogawa E, Hirata M, Miyachi Y, Iwamura S, Uozumi R, Sugimoto T, Asahara T, Uemoto S and Hatano E (2022) Dysbiosis and Depletion of Fecal Organic Acids Correlate With the Severity of Rejection After Rat Liver Transplantation. Transpl Int 35:10728. doi: 10.3389/ti.2022.10728

Abbreviations: ALT, alanine aminotransferase; BT, bacterial translocation; ELISA, enzyme-linked immunosorbent assay; ESLD, end-stage liver disease; GM, gut microbiota; IQR, interquartile range; LDLT, living donor liver transplantation; LPS, lipopolysaccharide; LT, liver transplantation; NLR, neutrophil/lymphocyte ratio; OA, organic acid; OLT, orthotopic liver transplantation; POAs, predominant obligate anaerobes; RT-qPCR, reverse-transcription quantitative polymerase chain reaction; SCFA, short-chain fatty acid; T-Bil, total bilirubin; TCMR, T cell-mediated rejection.



GRAPHICAL ABSTRACT |

INTRODUCTION

It is well recognized that the gut microbiota (GM) plays an important role in the development of complications of end-stage liver disease (ESLD) including bacterial infections and hepatic encephalopathy (1,2,3), and knowledge has gradually accumulated with regard to the GM composition in liver transplantation (LT) candidates (4,5,6,7). However, accurate interpretation of human GM and the associated intestinal environment, particularly in the peri-LT period, is difficult because they are influenced by miscellaneous factors including surgical stress, perioperative fasting, immunosuppressant use, and antibiotic administration. Therefore, animal experiments that exclude such confounders are required to understand the true traits of the GM.

T cell-mediated rejection (TCMR) is common early after LT. Although mild TCMR does not adversely affect the clinical course when adequately treated, severe TCMR still carries deleterious effects with an associated risk of graft loss and decreased survival (8). Since the target organ of TCMR in LT is the liver, severe TCMR, like other ESLDs, could cause secondary structural and functional changes in the intestine. Although previous experimental studies demonstrated that TCMR induced a structural shift of the GM in rats (9, 10), the clinical impact of graft function on specific strains, and vice versa, has never been investigated. Therefore, existing evidence needs to be updated using the latest technology. Herein, we introduce the 16S and 23S ribosomal RNA (rRNA)-targeted reverse-transcription quantitative polymerase chain reaction (RT–qPCR) system for the detection of microorganisms, which enables more sensitive qualitative and quantitative analyses than conventional real-time qPCR.

Advances in technology have made it possible to visualize the intestinal environment by evaluating not only GM but also fecal organic acids (OAs). Fecal OAs, especially short-chain fatty acids (SCFAs), including acetic acid, butyric acid, and propionic acid, produced by the GM are known to have beneficial physiological effects on host immunity through the suppression of the overgrowth of harmful microorganisms (11), protection of the intestinal epithelium (12), and regulation of intestinal immune function (13). Therefore, SCFAs would have a direct and decisive effect on maintaining host immunity and minimizing bacterial translocation (BT) (14, 15). Chronological changes in OA as a decisive consequence of GM alterations have never been investigated in the TCMR model.

To answer these clinical questions, a rat allogeneic LT model was prepared without fasting, antibiotic treatment, or immunosuppressant administration to monitor perioperative time-series changes in both GM and fecal OA levels complicated by impaired liver function caused by TCMR. A rat syngeneic LT model, which showed different transitions during liver function recovery, was concurrently prepared as a control. The goals of the current study were 3-fold:

(1) To observe the dynamic alterations of both the GM and fecal concentrations of OAs in the syngeneic and allogeneic LT model.
(2) To elucidate the interactions between graft liver function and these two variables (the GM and fecal OAs).
(3) To better assess the causal relationship between TCMR and BT.

MATERIALS AND METHODS

Experimental Protocol

Male Lewis rats (9–12 weeks old) weighing 270–320 g and male Dark Agouti (DA) rats (12–16 weeks old) weighing 260–290 g

were prepared. The whole liver graft was transplanted after 1 h of cold storage in phosphate-buffered saline. The median weight of grafts from Lewis and Dark Agouti rats was 10.465 g (range, 9.250–11.600) and 8.001 g (range, 7.510–8.888), respectively. Rats were divided into two groups after orthotopic liver transplantation (OLT): 1) the syngeneic group (n = 6), in which both the donors and recipients were Lewis rats; and 2) the allogeneic group (n = 6), in which the donors were DA rats and the recipients were Lewis rats. Fecal, blood, and histological (liver and small intestine) samples were obtained at four fixed time points (days 1, 3, 7, and 10) after OLT. In total, 48 OLTs were performed for 24 individuals in each group (6 individuals × 4 time points). Six healthy Lewis rats were used as the controls. As the present basic research is an exploratory study, a power calculation was not performed. We selected this relatively small sample size empirically because the GM and fecal OAs were measured simultaneously for rats for the first time, and therefore, the initial intention was to gather basic evidence regarding the transitions of these variables that could be utilized in future human studies. All OLT procedures were performed under inhalation anesthesia using 1.5% isoflurane with endotracheal intubation and artificial respiration, according to our previous techniques, with hepatic artery reconstruction (16) and without fasting, intravenous drip, antibiotic administration, or immunosuppression. Animals were housed under specific pathogen-free conditions in a temperature- and humidity-controlled environment under a 12-h light/dark cycle. The rats were fed a standard diet (F-2; Oriental Bio Service, Kyoto, Japan) and tap water *ad libitum*.

All experiments were conducted in accordance with the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) Guidelines. The institutional ethics committee of Kyoto University approved the experimental protocol (MedKyo18537).

Sample Collection

Under inhalation anesthesia, portal venous pressure (PVP) was measured and monitored *via* a pressure transducer using the following procedure: a segment of the mesenteric branch vein was cannulated with a 24-g cannula needle, and the tip of the cannula was advanced into the trunk of the superior mesenteric vein. Blood samples were collected from the inferior vena cava and feces from the rectum. Each individual was euthanized at each time point after sample collection.

The fecal samples were placed directly into two tubes (~1.0 g/tube); one tube contained 2 ml of RNAlater® (Ambion, Austin, TX, United States), and the other was empty. The samples with RNAlater® were held at room temperature for 10 min before storage at 4°C (for the analysis of GM), and the others were placed in a freezer at −80°C (for the analysis of fecal OA concentrations) within 30 min of excretion. Samples were sent to the Yakult Central Institute at −20°C for analysis.

Determination of Fecal Microbiota Counts

GM composition was analyzed by the 16S and 23S rRNA-targeted RT–qPCR system using Yakult Intestinal Flora-SCAN (YIF-SCAN®). The mechanisms and advantages of YIF-SCAN® for measuring bacterial counts in fecal and blood samples have been previously described elsewhere (17,18,19). Briefly, three serial dilutions of the extracted RNA sample were used for bacterial rRNA-targeted RT-qPCR, and threshold cycle values in the linear range of the assay were applied to the standard curve to obtain the corresponding bacterial cell count in each nucleic acid sample. These data were then used to calculate bacterial counts per sample. The specificity of the RT-qPCR assay using group-, genus-, or species-specific primers was determined as previously described (**Supplementary Table S1**).

The bacteria examined included obligate anaerobes (*Clostridium coccoides* group, *C. leptum* subgroup, *Bacteroides fragilis* group, genus *Bifidobacterium*, *Atopobium* cluster, genus *Prevotella*, *Clostridioides difficile*, and *C. perfringens*), facultative anaerobes (family *Enterobacteriaceae*, genus *Enterococcus*, genus *Streptococcus*, and genus *Staphylococcus*), and aerobes (genus *Pseudomonas*).

Determination of Fecal OA Concentrations

A portion of the feces was homogenized in four volumes of 0.15 mol/L perchloric acid and stored at 4°C for 12 h. The homogenate was centrifuged at 4°C at 20,400 ×g for 10 min, and the resulting supernatant was passed through a membrane filter with a pore size of 0.45-μm (Millipore Japan Ltd., Tokyo, Japan). The sample was analyzed by high-performance liquid chromatography using a Waters system with Waters 432 Conductivity Detector (Waters Co., Milford, MA) equipped with two columns (Shodex RS pack KC-811; Showa Denko Co. Ltd., Tokyo, Japan).

In this study, the SCFAs included acetic acid, butyric acid, and propionic acid.

Biochemical Assays

Blood tests, including complete blood count, peripheral neutrophil/lymphocyte ratio (NLR), aspartate aminotransferase, alanine aminotransferase (ALT), total bilirubin (T-Bil), serum albumin, and serum creatinine, were performed in a professional clinical laboratory (Japan Clinical Laboratories, Kyoto, Japan).

To evaluate the immune function, the CD4/CD8 T-cell ratio was analyzed. The conjugated mouse anti-rat monoclonal antibodies used for flow cytometry, APC-conjugated CD3, FITC-conjugated CD4, and PE-conjugated CD8a, were commercially available (BD Biosciences, San Josè, CA, United States). Samples were acquired using a BD Accuri C6 (BD Biosciences).

The fecal IgA content was determined to evaluate intestinal barrier function by enzyme-linked immunosorbent assay (ELISA) using a rat IgA ELISA kit (Bethyl Laboratories, Inc., Montgomery, TX, United States). Serum lipopolysaccharide (LPS) levels were evaluated using a rat LPS ELISA kit (CUSABIO, Wuhan, China).

Histological Analysis

Formalin-fixed, paraffin-embedded sections (4-μm thickness) of rat liver grafts and small intestines were stained with hematoxylin and eosin (H&E). For electron microscopy, rat

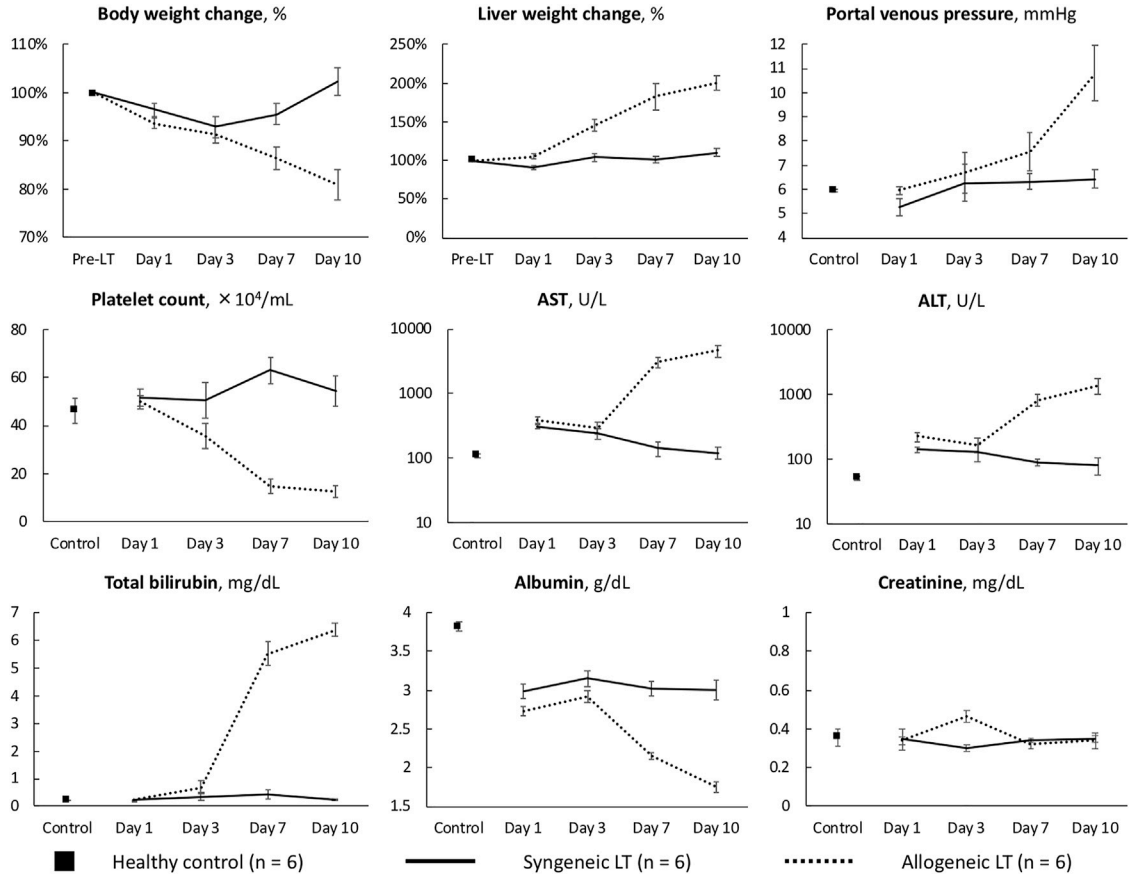


FIGURE 1 | Characteristics of the syngeneic and allogeneic LT models AST, aspartate aminotransferase; ALT, alanine aminotransferase.

small intestines were perfused through the aorta with a mixture of 2% glutaraldehyde and 4% paraformaldehyde and then extracted. The intestines were cut into small pieces and stored overnight at 4°C. The sections were stained with saturated uranyl acetate and lead citrate and observed using a Hitachi H-7650 electron microscope (Hitachi, Tokyo, Japan) for transmission electron microscopy (TEM). Two independent investigators examined all the tissue sections in a blinded manner. The severity of TCMR was evaluated in accordance with the Banff classification (20).

Statistical Analysis

The results of the fecal GM and OA analyses are expressed as the mean ± standard error. For statistical calculation, a value of half of the detection limit was assigned when the count or concentration was below the detection limit. Longitudinal data of these variables were analyzed using a linear mixed-effects model, which included the study group, time after LT, and interaction of the study group with the time after LT. Other continuous variables were presented as the median and range or interquartile range (IQR), as appropriate. Categorical variables were presented as numbers and percentages. Correlations between two variables were determined using Spearman's rank correlation coefficient. Statistical significance was set at a p value < 0.05. JMP 14.0 (SAS Institute, Cary, NC, United States) was used for all statistical analyses.

RESULTS

Experimental Characteristics of Rat LT Models

Representative biochemical analyses (**Figure 1**) and histopathological findings (**Figures 2**, **3**) are presented. Briefly, after OLT, the hepatic graft suffered from ischemia/reperfusion injury in both syngeneic and allogeneic groups on day 1, with elevated liver enzyme levels (**Figure 1**) and histological periportal edema (**Figures 2A,E**). However, the graft recovered to nearly normal levels both functionally and histologically in the syngeneic group (**Figures 2B–D**), whereas progressive TCMR led to irreversible graft failure after day 7 and by day 10 in the allogeneic group (**Figures 2G,H**). **Figures 2F–H** represent "mild," "moderate," and "severe" by Banff classification, respectively. As liver enzyme levels increased and cholestasis progressed, synthetic ability decreased, and portal venous pressure increased (**Figure 1**). Small intestine histology in the allogeneic group showed worsening submucosal edema and

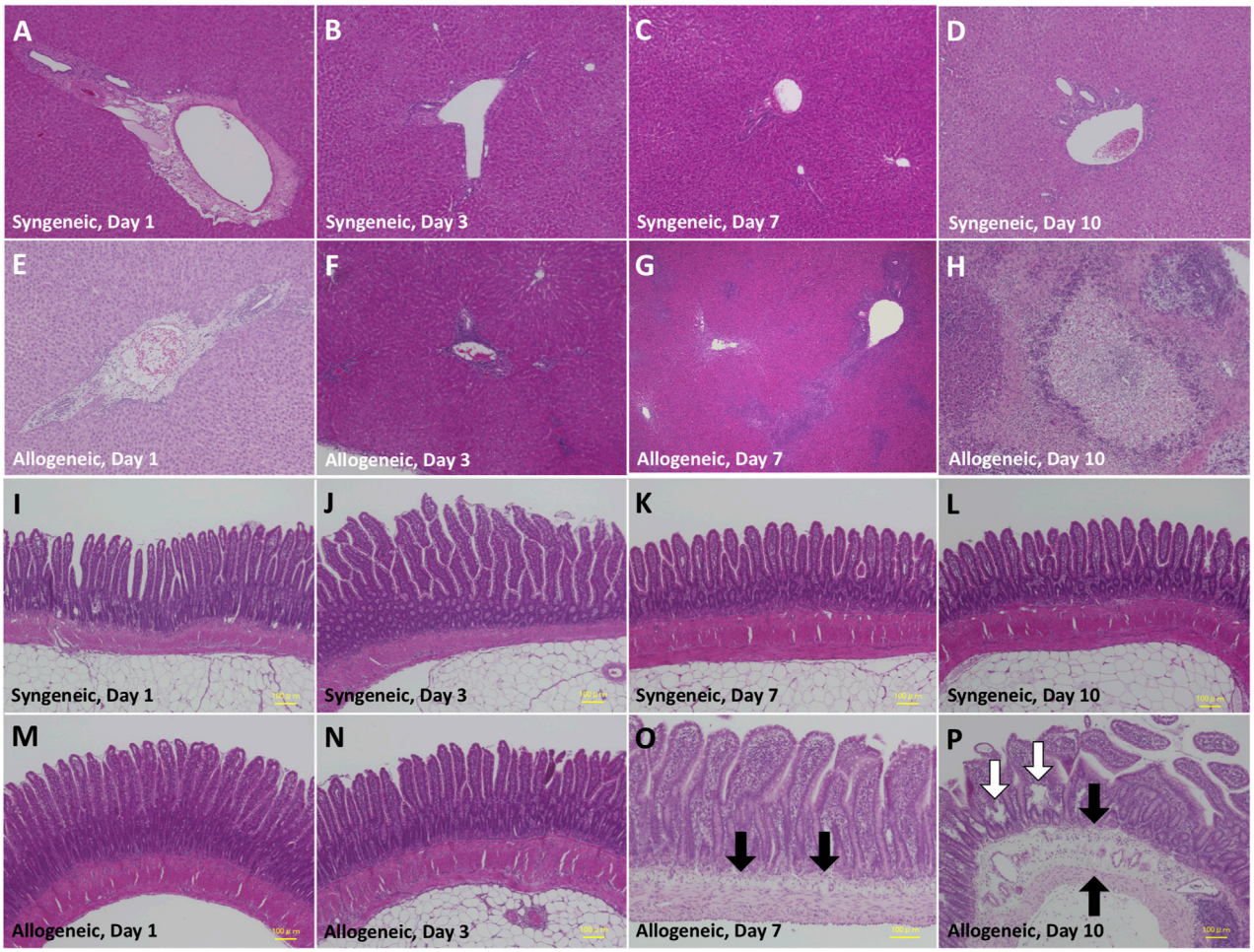


FIGURE 2 | Histopathological alteration of the liver **(A–D)** and small intestine **(E–H)** by hematoxylin-eosin staining. **(A)** Periportal edema due to ischemia/reperfusion injury on day 1. **(B)** Attenuated periportal edema on day 3. **(C)** Almost normal histology on day 7. **(D)** Almost normal histology on day 10. **(E)** Periportal edema due to ischemia/reperfusion on day 1. **(F)** Mild inflammatory cell infiltration in Glisson's capsule on day 3, with preservation of the structure of the portal vein, hepatic artery, and bile duct.**(G)** Massive inflammatory cell infiltration in Glisson's capsule and degeneration of hepatocytes in the hepatic lobule on day 7. **(H)** Extensive inflammatory cell infiltration and hepatocyte necrosis on day 10. **(I–N)** Almost normal histology. **(O)** Mild submucosal edema (black arrow) and swollen villi. **(P)** Severe submucosal edema with apparent vascular dilation (black arrow) and disarrangement of the epithelium (white arrow).

disarrangement of the epithelium as TCMR progressed from day 7 to day 10 (**Figures 2M–P**). Transmission electron microscopy images of the small intestine demonstrated epithelial cell structural destruction as TCMR progressed from day 7, indicating disruption of barrier function (**Figure 3**).

The median survival period of allogeneic liver grafts was 11 days (range, 10–13), whereas all syngeneic liver grafts survived.

Time-Series Changes in the GM and OA Concentrations

Dynamic comparisons of the representative fecal microbiota are shown in **Figure 4A**. Dysbiosis progressed as the liver function declined in the allogeneic group, mainly on days 7 and 10, whereas it recovered as the liver function improved in the syngeneic group. The number of predominant obligate anaerobes (POAs), such as the *C. coccoides* group, *B. fragilis* group, and *Bifidobacterium,* decreased as liver function declined in the allogeneic group. These changes were more remarkable in obligate and facultative anaerobes, some of which are responsible for opportunistic infections. Total *lactobacilli* and its subgroup showed a significant decrease, and *Clostridioides difficile*, *Enterobacteriaceae*, *Enterococcus*, *Streptococcus*, and *Staphylococcus* showed a significant increase in the allogeneic group on days 7 and 10, as liver function declined. Meanwhile, GM seemed to be restored to normal by day 10 in the syngeneic group as liver function improved. *C. perfringens*, *Lacticaseibacillus*, and *Pseudomonas* were below the detection limits.

Dynamic comparisons of representative OAs are shown in **Figure 4B**. Overall, the allogeneic group showed significantly lower OA concentrations as the liver function declined. More specifically, in the allogeneic group, SCFA concentrations were depleted by day 10, with a slight recovery trend from days 1–3. In

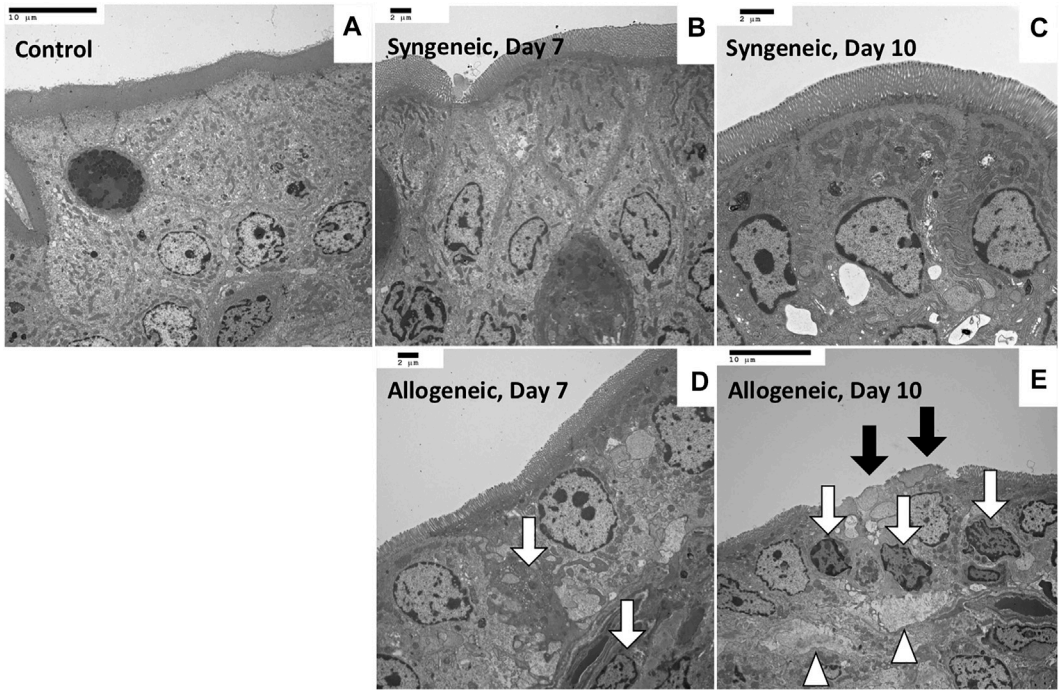


FIGURE 3 | Transmission electron microscopy images of rat small intestine samples **(A)** Control, ×1,200. **(B)** Maintenance of the whole structure, with a clear cell boundary, on day 7, ×1,500. **(C)** Maintenance of the whole structure on day 10, ×2,000. **(D)** Vague boundary of epithelial cells because of partial necrosis (white arrow), ×1,500. **(E)** Microvilli shedding (black arrow), epithelial cell necrosis (arrow), and divergence of intercellular spaces (arrowhead) on day 10, ×1,200.

contrast, the concentration of SCFAs in the syngeneic group recovered to nearly normal levels after depletion on day 1. The remaining values are listed in **Supplementary Table S2**. The transitions in these values were similar between the groups.

In short, although the disturbance was slight, transient, and reversible in the syngeneic model, the allogeneic TCMR model demonstrated distinct dysbiosis and depletion of fecal OAs.

Immune Function and Intestinal Barrier Function

In the syngeneic group, all measured values gradually returned to normal by day 10 after LT (**Figure 4C**). In contrast, immune function, represented by the NLR and CD4/CD8 ratio, and intestinal barrier function, represented by the fecal IgA level, decreased as liver function declined in the allogeneic group. On days 7 and 10, the allogeneic group showed a significantly higher NLR, a lower CD4/CD8 ratio, and decreased fecal IgA levels than the syngeneic group. Consequently, extremely high LPS levels were observed from days 7–10, implying the occurrence of BT.

Correlations Between Liver Function and the GM and OA Concentrations

Spearman's rank correlation coefficient was calculated using the data of all 48 OLTs because no single individual was sampled multiple times. ALT was significantly negatively correlated with *C. coccoides* group and *Prevotella* and positively correlated with *Enterobacteriaceae* and *Enterococcus* (**Figure 5A**). With regard to OA, there were significant negative correlations between ALT and fecal concentrations of total OA, butyric acid, and propionic acid. T-Bil was significantly negatively correlated with total *lactobacilli* and *Lactobacillus* and positively correlated with *Enterobacteriaceae* and *Enterococcus* (**Figure 5B**). With regard to OA, there were significant negative correlations between T-Bil and fecal concentrations of total OA, acetic acid, butyric acid, and propionic acid.

In summary, liver function affected the counts of *Enterobacteriaceae* and *Enterococcus,* and the fecal concentration of SCFAs.

DISCUSSION

The valuable strength of this experimental study is the new insight that dysbiosis of the GM and depletion of fecal OAs progressed in proportion to deteriorating graft function caused by TCMR in the absence of intervention, including fasting, immunosuppressant, and antibiotic use. We also demonstrated that severe TCMR could become critically complicated by BT due to impaired intestinal barrier function, while the function was maintained in mild to moderate TCMR. Although these data would help us comprehend GM in the context of liver disease, several issues require discussion.

TCMR is known to induce a structural shift of the GM in rats (9, 10), possibly as a consequence of cholestasis caused by bile

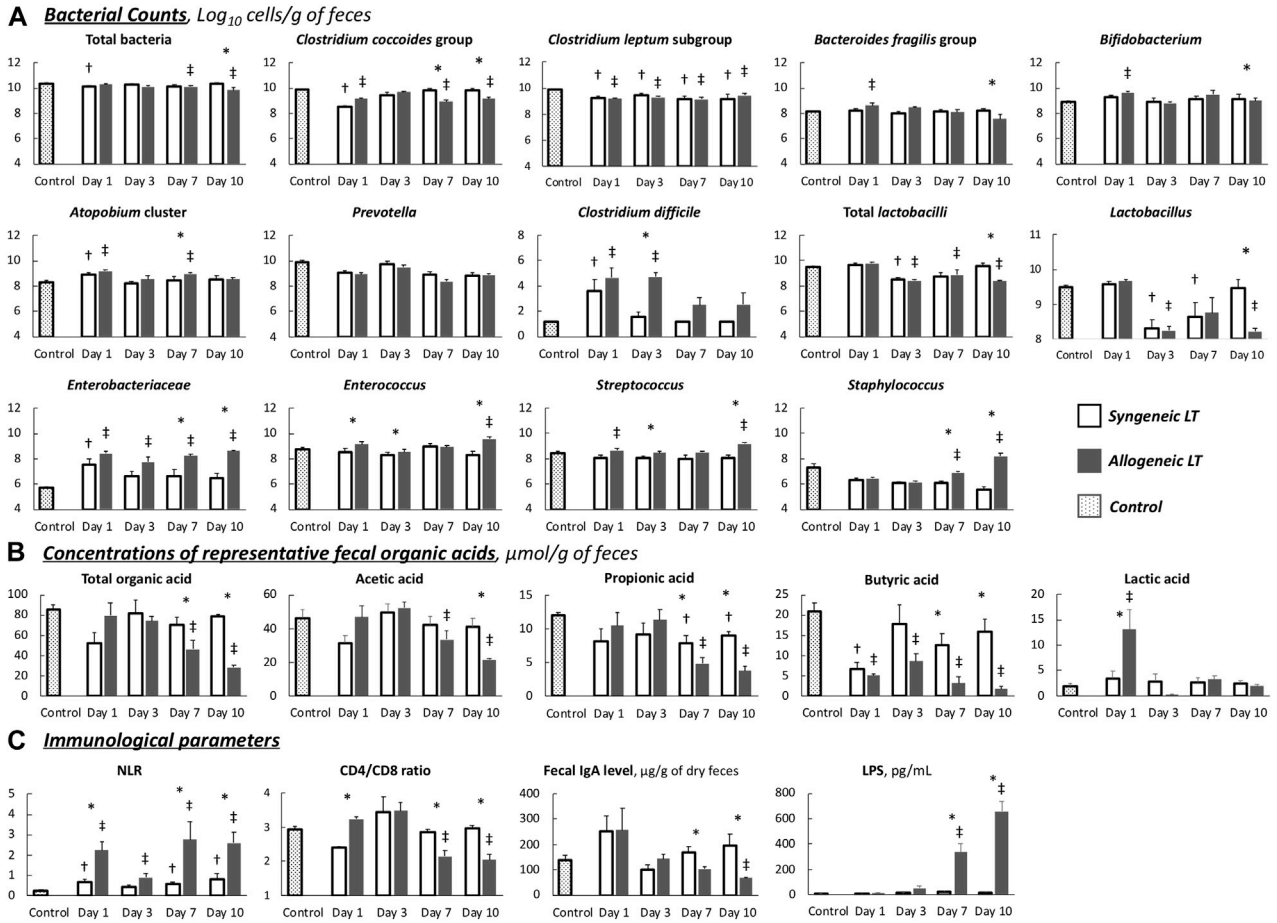


FIGURE 4 | Chronological changes in parameters **(A)** Gut microbiota. **(B)** Organic acids. **(C)** Immunological parameters. Values are the mean (SE), $n = 6$ per group. $^{*}p < 0.05$, syngeneic group compared to allogeneic group. $^{\dagger}p < 0.05$, syngeneic group compared to control group. $^{\ddagger}p < 0.05$, allogeneic group compared to control group. NLR, neutrocyte/lymphocyte ratio; LPS, lipopolysaccharide.

duct injury characterizing TCMR (21). A previous study using conventional qPCR showed that *Faecalibacterium prausnitzii* and *Lactobacillus* were significantly reduced in a rat TCMR model with enrichment of *Clostridium bolteae* (10), these microorganisms were not the key pathogens relevant to the clinical practice of LT cited in past studies (7, 22). Our results demonstrated significant alterations in more strains. The number of POAs, such as *C. coccoides* group, *B. fragilis* group, and *Bifidobacterium,* decreased as liver function declined. The changes were more remarkable in obligate and facultative anaerobes: total *lactobacilli* and its subgroup showed a significant decrease, and *C. difficile, Enterobacteriaceae*, *Enterococcus*, *Streptococcus*, and *Staphylococcus* showed a significant increase. This high testing capability is due to the sensitivity of the YIF-SCAN®. YIF-SCAN® targets rRNA molecules that are abundant in bacteria (approximately 10^4 copies per actively growing cell), and its sensitivity is 100 times higher than that of qPCR assays that target rRNA genes (more than 10 copies/bacterial genome) (23). It can only measure live bacteria that are highly associated with infectivity, inflammation induction, and pathogenicity. In contrast, conventional qPCR targets DNA that remains even in dead bacteria. Therefore, this method was able to capture the dynamic changes in the GM more accurately.

In addition to the disturbance in the GM, depletion of OAs, especially SCFAs, was observed under TCMR. Since most POAs, such as *C. coccoides* group, *Bifidobacterium,* and *Lactobacilli* have been reported to produce SCFAs in the intestine (11,24,25,26), the decrease in the number of POAs and other beneficial bacteria might lead to a decreased fecal concentration of SCFAs, which reflects the condition of intestinal dysbiosis and impaired intestinal barrier function. In such conditions, BT tends to occur, and these events can subsequently lead to bacteremia and postoperative infectious complications (15).

In the clinical setting, differentiating between acute TCMR and infection remains a clinical challenge during the early post-LT period. The two diagnoses can even coexist and lead to unfavorable outcomes. Although the GM and OAs were maintained under mild to moderate TCMR, our data showed an increased risk of BT as TCMR progressed, with worsening

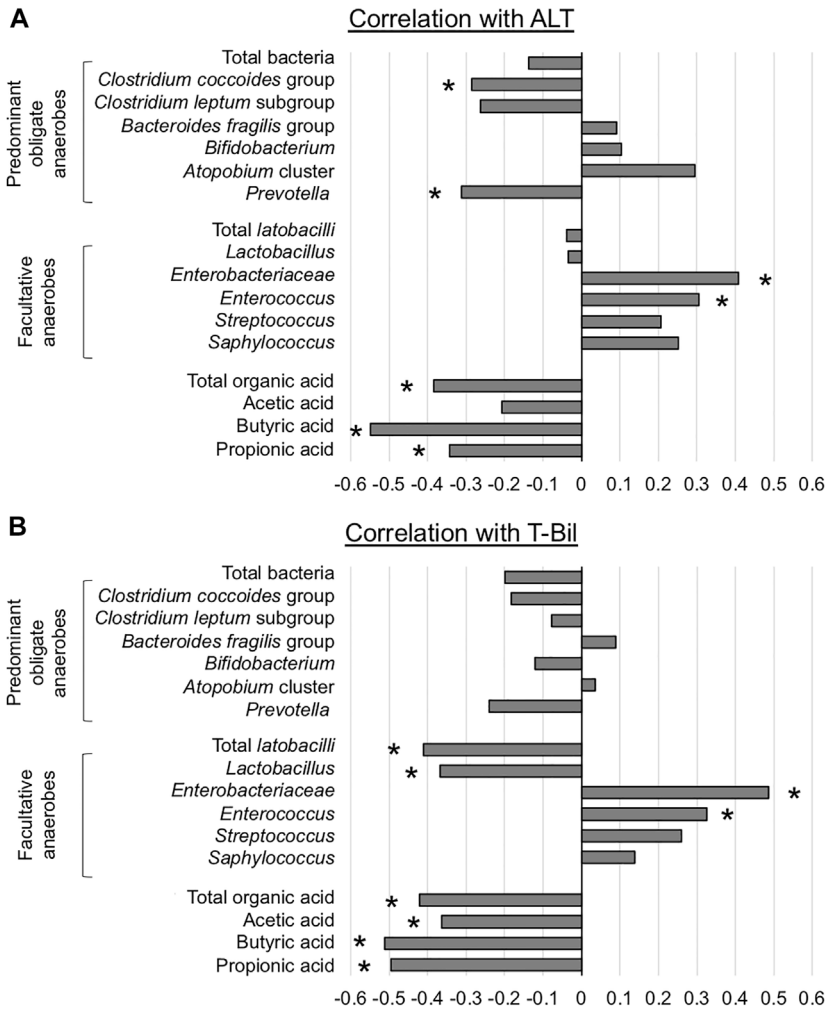


FIGURE 5 | Association between liver function and representative microorganisms and fecal organic acids **(A)** Alanine aminotransferase (ALT) **(B)** Total bilirubin (T-Bil) *$p < 0.05$, Spearman's rank correlation coefficient.

dysbiosis, depleting SCFAs, and increasing LPS levels. Since the treatment options for TCMR and infection are diametrically opposed, severe TCMR cases may develop infection and have an irreversible course. These results explain one of the reasons why mild TCMR is treatable, while severe TCMR is refractory to treatment with potent immunosuppressants (8). However, administrating therapeutic antibiotics to all patients with suspicious TCMR is unreasonable because antibiotics can further agitate the gut microbiota. Ideally, if TCMR occurs, we will watch for the onset of BT and consider antibiotic administration referring to other objective test results including inflammatory maker levels and pathology by liver biopsy. Although a comprehensive decision is needed in the clinical setting, speculation can be raised based on our results that if TCMR is pathologically mild to moderate, antibiotics are not necessary, and if it is moderate to severe, they might be required to prevent BT.

According to the correlation analyses, the counts of *Enterobacteriaceae* and *Enterococcus* were positively correlated with ALT and T-Bil, and consequently, the fecal concentration of SCFAs was negatively correlated. These interactions suggest that impaired liver function would provoke increases in potential pathogens and intestinal barrier dysfunction, which is an important aspect of the gut-liver axis. In clinical practice, *Enterobacteriaceae* and *Enterococcus* are the dominant pathogens in LT recipients (7, 22). Moreover, *Enterobacteriaceae* and *Enterococcus* have been reported to be enriched in various chronic liver diseases, including viral hepatitis, alcoholic abuse, non-alcoholic steatohepatitis, and cholestatic liver disease (27,28,29,30,31). In addition, considering that the correlation with SCFAs was stronger for T-Bil than for ALT, cholestasis might be the main contributor to the pathogenesis of these phenomena rather than the hepatocellular damage itself. Although bile acids are known to play an essential role in regulating the intestinal immune system (32,33,34), our findings confirmed that SCFAs depletion is a key mechanism connecting dysbiosis caused by reduced amounts of bile acids within the intestine and intestinal barrier dysfunction in liver disease.

This experimental model also provides a clue to comprehend the impact of surgical invasiveness on the intestinal environment. Our results demonstrated that the alterations in both GM and OAs until day 3 were slight, transient, and reversible without TCMR. These changes reflect the true influence of surgical stress because perioperative fasting and antibiotic use, which could impact GM, were not conducted. While surgical procedures are reported to cause large alterations in the GM and OAs in human subjects (35, 36), we assume that it would largely account for concomitant long-term fasting or antibiotic use.

The current study has several limitations. First and foremost, a concurrent allogeneic LT model with immunosuppressants was not prepared. Since many immunosuppressive drugs have been reported to induce dysbiosis in rodent models (37), we intended to exclude the potential confounder in interpreting the GM. Besides, our results demonstrated that the GM and OAs strongly correlate with graft function; thus, the fluctuations would be minimal as long as graft function is maintained with the aid of immunosuppressants. Second, we could not provide data on the underlying mechanisms of the altered intestinal environment. More specific analysis based on individual strains and their metabolites is demanding. Finally, experimental findings implicating individual organisms or genera in animal models are less valuable until they are validated in humans. Although our next step is to investigate perioperative changes in GM in human LT recipients, it is expected that miscellaneous confounders would make interpretation difficult in human subjects. Hopefully, the findings of this experimental study will provide clues for interpreting the results of future research.

In conclusion, distinct characteristics of both GM and fecal concentrations of OAs in the TCMR model were visualized. While successful LT would have little influence on the GM and intestinal environment, TCMR could increase pathogenic strains, weaken intestinal immune function, and elevate the potential risk of BT. In addition, the degree of graft injury is closely related to the counts of some specific bacterial strains and the concentrations of fecal SCFAs; thus, rejection and infection may coexist when rejection is uncontrollable.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The animal study was reviewed and approved by the Animal Experimentation Committee of Kyoto University.

AUTHOR CONTRIBUTIONS

SYY, SY, EO, and TA designed the study. SYY performed the animal experiments, collected samples, and wrote the manuscript. MH, YM, and SI collected samples. SYY, TS, and TA processed the data. RU reviewed the manuscript and was an expert statistician. SU and EH critically reviewed and proofread the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by "Grant-in-Aid for Scientific Research (C) (R.O. No. 18K08567)" from the Japan Society for the Promotion of Science, Tokyo, Japan, and "Grant-in-Aid for Young Researcher" from the Fujiwara Memorial Foundation, Kyoto, Japan.

CONFLICT OF INTEREST

Authors TS and TA were employed by the company Yakult Honsha Co., Ltd.

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

ACKNOWLEDGMENTS

The authors thank Yukiko Kado, Akira Takahashi, Satomi Atobe, Rie Date, and Takako Ishiduka (Yakult Central Institute) for their cooperation in performing microbiota analyses. We also thank Keiko Okamoto-Furuta and Haruyasu Kohda (Division of Electron Microscopic Study, Center for Anatomical Studies, Graduate School of Medicine, Kyoto University) for technical assistance with electron microscopy and Dr. Masato Nagahama (Department of Pathology, Handa City Hospital) for offering advice on histopathological review.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10728/full#supplementary-material

REFERENCES

1. Chen Y, Yang F, Lu H, Wang B, Lei D, Wang Y, et al. Characterization of Fecal Microbial Communities in Patients with Liver Cirrhosis. *Hepatology* (2011) 54: 562–72. doi:10.1002/hep.24423
2. Riordan SM, Williams R. Gut Flora and Hepatic Encephalopathy in Patients with Cirrhosis. *N Engl J Med* (2010) 362:1140–2. doi:10.1056/NEJMe1000850
3. Wiest R, Krag A, Gerbes A. Spontaneous Bacterial Peritonitis: Recent Guidelines and beyond. *Gut* (2012) 61:297–310. doi:10.1136/gutjnl-2011-300779
4. Wu ZW, Ling ZX, Lu HF, Zuo J, Sheng JF, Zheng SS, et al. Changes of Gut Bacteria and Immune Parameters in Liver Transplant Recipients. *Hepatobiliary Pancreat Dis Int* (2012) 11:40–50. doi:10.1016/s1499-3872(11)60124-0
5. Sun LY, Yang YS, Qu W, Zhu ZJ, Wei L, Ye ZS, et al. Gut Microbiota of Liver Transplantation Recipients. *Sci Rep* (2017) 7:3762. doi:10.1038/s41598-017-03476-4

6. Bajaj JS, Kakiyama G, Cox IJ, Nittono H, Takei H, White M, et al. Alterations in Gut Microbial Function Following Liver Transplant. *Liver Transpl* (2018) 24:752–61. doi:10.1002/lt.25046
7. Annavajhala MK, Gomez-Simmonds A, Macesic N, Sullivan SB, Kress A, Khan SD, et al. Colonizing Multidrug-Resistant Bacteria and the Longitudinal Evolution of the Intestinal Microbiome after Liver Transplantation. *Nat Commun* (2019) 10:4715. doi:10.1038/s41467-019-12633-4
8. Jadlowiec CC, Morgan PE, Nehra AK, Hathcock MA, Kremers WK, Heimbach JK, et al. Not All Cellular Rejections Are the Same: Differences in Early and Late Hepatic Allograft Rejection. *Liver Transpl* (2019) 25:425–35. doi:10.1002/lt.25411
9. Xie Y, Luo Z, Li Z, Deng M, Liu H, Zhu B, et al. Structural Shifts of Fecal Microbial Communities in Rats with Acute Rejection after Liver Transplantation. *Microb Ecol* (2012) 64:546–54. doi:10.1007/s00248-012-0030-1
10. Ren Z, Jiang J, Lu H, Chen X, He Y, Zhang H, et al. Intestinal Microbial Variation May Predict Early Acute Rejection after Liver Transplantation in Rats. *Transplantation* (2014) 98:844–52. doi:10.1097/TP.0000000000000334
11. Asahara T, Shimizu K, Takada T, Kado S, Yuki N, Morotomi M, et al. Protective Effect of Lactobacillus Casei Strain Shirota against Lethal Infection with Multi-Drug Resistant *Salmonella enterica* Serovar Typhimurium DT104 in Mice. *J Appl Microbiol* (2011) 110:163–73. doi:10.1111/j.1365-2672.2010.04884.x
12. Feng Y, Wang Y, Wang P, Huang Y, Wang F. Short-Chain Fatty Acids Manifest Stimulative and Protective Effects on Intestinal Barrier Function through the Inhibition of NLRP3 Inflammasome and Autophagy. *Cell Physiol Biochem* (2018) 49:190–205. doi:10.1159/000492853
13. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly- YM, et al. The Microbial Metabolites, Short-Chain Fatty Acids, Regulate Colonic Treg Cell Homeostasis. *Science* (2013) 341:569–73. doi:10.1126/science.1241165
14. Kamiya S, Nagino M, Kanazawa H, Komatsu S, Mayumi T, Takagi K, et al. The Value of Bile Replacement during External Biliary Drainage: an Analysis of Intestinal Permeability, Integrity, and Microflora. *Ann Surg* (2004) 239:510–7. doi:10.1097/01.sla.0000118594.23874.89
15. Yokoyama Y, Mizuno T, Sugawara G, Asahara T, Nomoto K, Igami T, et al. Profile of Preoperative Fecal Organic Acids Closely Predicts the Incidence of Postoperative Infectious Complications after Major Hepatectomy with Extrahepatic Bile Duct Resection: Importance of Fecal Acetic Acid Plus Butyric Acid Minus Lactic Acid gap. *Surgery* (2017) 162:928–36. doi:10.1016/j.surg.2017.04.022
16. Nagai K, Yagi S, Uemoto S, Tolba RH. Surgical Procedures for a Rat Model of Partial Orthotopic Liver Transplantation with Hepatic Arterial Reconstruction. *J Vis Exp* (2013) e4376. doi:10.3791/4376
17. Matsuda K, Tsuji H, Asahara T, Kado Y, Nomoto K. Sensitive Quantitative Detection of Commensal Bacteria by rRNA-Targeted Reverse Transcription-PCR. *Appl Environ Microbiol* (2007) 73:32–9. doi:10.1128/AEM.01224-06
18. Yokoyama Y, Nishigaki E, Abe T, Fukaya M, Asahara T, Nomoto K, et al. Randomized Clinical Trial of the Effect of Perioperative Synbiotics versus No Synbiotics on Bacterial Translocation after Oesophagectomy. *Br J Surg* (2014) 101:189–99. doi:10.1002/bjs.9385
19. Yokoyama Y, Fukaya M, Mizuno T, Ebata T, Asahara T, Nagino M. Clinical Importance of "Occult-Bacterial Translocation" in Patients Undergoing Highly Invasive Gastrointestinal Surgery: A Review. *Surg Today* (2020) 51:485–92. doi:10.1007/s00595-020-02126-z
20. Demetris AJ, Bellamy C, Hübscher SG, O'Leary J, Randhawa PS, Feng S, et al. 2016 Comprehensive Update of the Banff Working Group on Liver Allograft Pathology: Introduction of Antibody-Mediated Rejection. *Am J Transpl* (2016) 16:2816–35. doi:10.1111/ajt.13909
21. Ben-Ari Z, Pappo O, Mor E. Intrahepatic Cholestasis after Liver Transplantation. *Liver Transpl* (2003) 9:1005–18. doi:10.1053/jlts.2003.50212
22. Abad CL, Lahr BD, Razonable RR. Epidemiology and Risk Factors for Infection after Living Donor Liver Transplantation. *Liver Transpl* (2017) 23:465–77. doi:10.1002/lt.24739
23. Niikura M, Atobe S, Takahashi A, Kado Y, Sugimoto T, Tsuji H, et al. Development of a Rapid and Sensitive Analytical System for *Pseudomonas aeruginosa* Based on Reverse Transcription Quantitative PCR Targeting of rRNA Molecules. *Emerg Microbes Infect* (2021) 10:677–86. doi:10.1080/22221751.2021.1906164
24. Asahara T, Takahashi A, Yuki N, Kaji R, Takahashi T, Nomoto K. Protective Effect of a Synbiotic against Multidrug-Resistant Acinetobacter Baumannii in a Murine Infection Model. *Antimicrob Agents Chemother* (2016) 60:3041–50. doi:10.1128/AAC.02928-15
25. Duncan SH, Louis P, Flint HJ. Lactate-utilizing Bacteria, Isolated from Human Feces, that Produce Butyrate as a Major Fermentation Product. *Appl Environ Microbiol* (2004) 70:5810–7. doi:10.1128/AEM.70.10.5810-5817.2004
26. Reichardt N, Duncan SH, Young P, Belenguer A, McWilliam Leitch C, Scott KP, et al. Phylogenetic Distribution of Three Pathways for Propionate Production within the Human Gut Microbiota. *ISME J* (2014) 8:1323–35. doi:10.1038/ismej.2014.14
27. Zhu L, Baker SS, Gill C, Liu W, Alkhouri R, Baker RD, et al. Characterization of Gut Microbiomes in Nonalcoholic Steatohepatitis (NASH) Patients: a Connection between Endogenous Alcohol and NASH. *Hepatology* (2013) 57:601–9. doi:10.1002/hep.26093
28. Sabino J, Vieira-Silva S, Machiels K, Joossens M, Falony G, Ballet V, et al. Primary Sclerosing Cholangitis Is Characterised by Intestinal Dysbiosis Independent from IBD. *Gut* (2016) 65:1681–9. doi:10.1136/gutjnl-2015-311004
29. Wang J, Wang Y, Zhang X, Liu J, Zhang Q, Zhao Y, et al. Gut Microbial Dysbiosis Is Associated with Altered Hepatic Functions and Serum Metabolites in Chronic Hepatitis B Patients. *Front Microbiol* (2017) 8:2222. doi:10.3389/fmicb.2017.02222
30. Dubinkina VB, Tyakht AV, Odintsova VY, Yarygin KS, Kovarsky BA, Pavlenko AV, et al. Links of Gut Microbiota Composition with Alcohol Dependence Syndrome and Alcoholic Liver Disease. *Microbiome* (2017) 5:141. doi:10.1186/s40168-017-0359-2
31. Tang R, Wei Y, Li Y, Chen W, Chen H, Wang Q, et al. Gut Microbial Profile Is Altered in Primary Biliary Cholangitis and Partially Restored after UDCA Therapy. *Gut* (2018) 67:534–41. doi:10.1136/gutjnl-2016-313332
32. Vavassori P, Mencarelli A, Renga B, Distrutti E, Fiorucci S. The Bile Acid Receptor FXR Is a Modulator of Intestinal Innate Immunity. *J Immunol* (2009) 183:6251–61. doi:10.4049/jimmunol.0803978
33. Isaacs-Ten A, Echeandia M, Moreno-Gonzalez M, Brion A, Goldson A, Philo M, et al. Intestinal Microbiome-Macrophage Crosstalk Contributes to Cholestatic Liver Disease by Promoting Intestinal Permeability in Mice. *Hepatology* (2020) 72:2090–108. doi:10.1002/hep.31228
34. Blesl A, Stadlbauer V. The Gut-Liver Axis in Cholestatic Liver Diseases. *Nutrients* (2021) 13:1018. doi:10.3390/nu13031018
35. Kanazawa H, Nagino M, Kamiya S, Komatsu S, Mayumi T, Takagi K, et al. Synbiotics Reduce Postoperative Infectious Complications: a Randomized Controlled Trial in Biliary Cancer Patients Undergoing Hepatectomy. *Langenbecks Arch Surg* (2005) 390:104–13. doi:10.1007/s00423-004-0536-1
36. Sugawara G, Nagino M, Nishio H, Ebata T, Takagi K, Asahara T, et al. Perioperative Synbiotic Treatment to Prevent Postoperative Infectious Complications in Biliary Cancer Surgery: a Randomized Controlled Trial. *Ann Surg* (2006) 244:706–14. doi:10.1097/01.sla.0000219039.20924.88
37. Gabarre P, Loens C, Tamzali Y, Barrou B, Jaisser F, Tourret J. Immunosuppressive Therapy after Solid Organ Transplantation and the Gut Microbiota: Bidirectional Interactions with Clinical Consequences. *Am J Transpl* (2022) 22:1014–30. doi:10.1111/ajt.16836

ESOT | Transplant International

ORIGINAL RESEARCH
published: 31 August 2022
doi: 10.3389/ti.2022.10364

Timing of Organ Procurement From Brain-Dead Donors Associates With Short- and Long-Term Outcomes After Liver Transplantation

*Verner Eerola, Ilkka Helanterä, Fredrik Åberg, Marko Lempinen, Heikki Mäkisalo, Arno Nordin, Helena Isoniemi and Ville Sallinen **

Department of Transplantation and Liver Surgery, Helsinki University Hospital and the University of Helsinki, Helsinki, Finland

Brain death-induced cytokine storm is thought to harm transplantable organs. However, longer procurement times have been associated with non-inferior or better outcomes in kidney, heart, and lung transplants, while optimal procurement time for liver allografts is unknown. Our aim was to analyze the association of time interval from brain death to organ procurement with liver allograft outcomes in two nationwide cohorts. The association of procurement interval with graft survival and short-term complications was analysed in multivariable models. Altogether 643 and 58,017 orthotopic liver transplantations from brain-dead donors were included from Finland between June 2004 and December 2017 and the US between January 2008 and August 2018, respectively. Median time from brain death to organ procurement was 10.5 h in Finland and 34.6 h in the US. Longer interval associated with better graft survival (non-linearly, p = 0.016) and less acute rejections (OR 0.935 95% CI 0.894–0.978) in the US cohort, and better early allograft function (p = 0.005; Beta –0.048 95% CI –0.085 –(–0.011)) in the Finnish cohort, in multivariable models adjusted with Donor Risk Index, recipient age, Model for End-Stage Liver Disease and indication for transplantation. Progressive liver injury after brain death is unlikely. Rushing to recover seems unnecessary; rest and repair might prove beneficial.

Keywords: graft survival, brain death, organ allocation, donor hepatectomy, liver allograft function, liver transplant dysfunction, procurement surgery

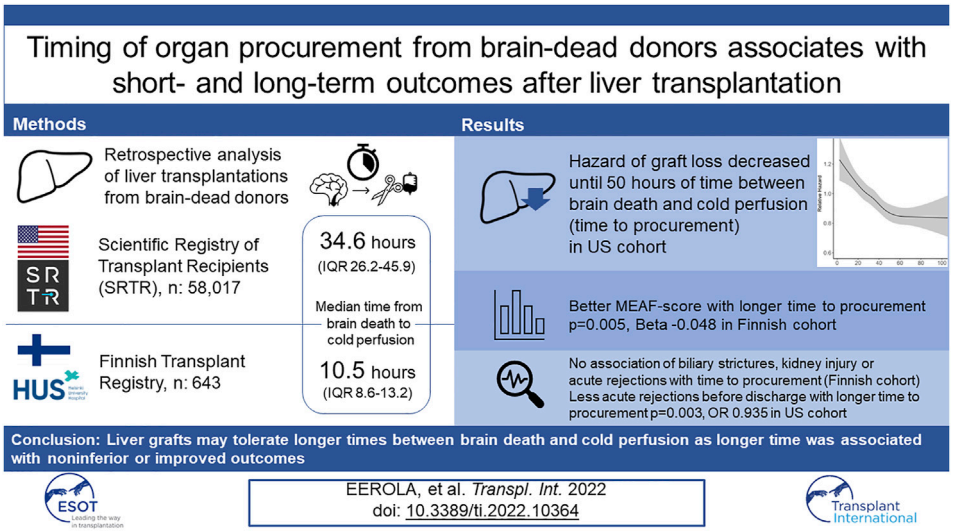
OPEN ACCESS

Correspondence:
Ville Sallinen
ville.sallinen@helsinki.fi

Received: *17 January 2022*
Accepted: *12 July 2022*
Published: *31 August 2022*

Citation:
Eerola V, Helanterä I, Åberg F, Lempinen M, Mäkisalo H, Nordin A, Isoniemi H and Sallinen V (2022) Timing of Organ Procurement From Brain-Dead Donors Associates With Short- and Long-Term Outcomes After Liver Transplantation. Transpl Int 35:10364. doi: 10.3389/ti.2022.10364

Abbreviations: DRI, donor risk index; HR, hazard ratio; IQR, interquartile range; MEAF, Model for Early Allograft Function; OR, odds ratio; SRTR, Scientific Registry of Transplant Recipients.



GRAPHICAL ABSTRACT |

INTRODUCTION

Majority (86%–100%) of liver transplants are still obtained from brain dead donors (1). The so called "cytokine storm" that follows brain death causes hemodynamic and blood coagulation changes leading to well described cell damage and ischaemia in various organs (2). Animal studies suggest organs from brain dead donors are harmed during and after brain death (2), and longer procurement interval (i.e., time interval from brain death to procurement) has led to increased inflammation, immune activation, and organ dysfunction (3–5). However, brain death is a continuous process and donor stabilization—"storm settling"—is usually achieved in a manner of hours (6) as care for the donor has been perfected over the decades up to nearly a routine. Effects of brain death and recovery of damage to the organs related to time of brain death are not well understood, and some transplant centers aim to procure as fast as possible. However, procurement intervals in US centers have grown gradually longer, without apparent harm in retrospective studies of transplanted kidneys, hearts and lungs (7–10). Of note, effect of procurement interval on liver allografts has not been studied.

Consequences of brain death may differ between organs and so might the optimal time-point of procurement, which for lungs and heart seems as long as possible, but for the kidneys between 20 and 50 h (9–11). Identifying the optimal time for procurement of liver grafts has implications both in transplantation logistics and outcomes. The simultaneous nature of abdominal organ procurement demands this effect to be studied in all organs.

This study aimed to examine the association of procurement interval with early liver allograft function and graft survival in two different transplant populations with different median times from brain death to organ procurement (Finland and the US). Associations with other important endpoints, such as acute rejections, biliary strictures, and post-operative kidney injury, available for the Finnish cohort, were also studied.

MATERIALS AND METHODS

Donors and Patients

Finnish Cohort

All orthotopic liver transplantations from deceased donors performed in Finland between June 2004 and December 2017 were included and followed until death, retransplantation, or October 2020. The data were extracted from the Finnish Transplant Registry and donor medical records. Organs exchanged internationally were excluded from the study. All included organs were procured within Finland by the same team of transplant surgeons from Helsinki Transplantation and Liver Surgery Unit, and all transplantations in Finland were performed at Helsinki University Hospital. All liver grafts were donations after brain death (DBD) in Finland during the study period.

US Cohort

This study used data from the Scientific Registry of Transplant Recipients (SRTR). The SRTR data system includes data on all donors, wait-listed candidates, and transplant recipients in the US, submitted by the members of the Organ Procurement and Transplantation Network (OPTN). The Health Resources and Services Administration (HRSA), U.S. Department of Health and Human Services provides oversight to the activities of the OPTN and SRTR contractors. Orthotopic liver transplantations recorded in SRTR database in the US between January 2008 to August 2018 were included. Follow-up consisted of the same time-period. Only livers transplanted from DBD donors were included, and livers from donation after circulatory death (DCD) or living donors were excluded.

The clinical and research activities being reported are consistent with the Principles of the Declaration of Istanbul as outlined in the "Declaration of Istanbul on Organ Trafficking and Transplant Tourism" and the Declaration of Helsinki.

Variables

The following donor variables were collected for both Finnish and US cohorts: donor age and gender, the time of declaration of brain death, the start time of cold perfusion in organ procurement surgery, cause of death, body mass index, donor history of hypertension, diabetes and hepatitis C status. Regarding the recipient and transplantation, recipient age and gender, cause of end-stage liver disease, Model for End-Stage Liver Disease (MELD) score at listing and before transplantation, body mass index, history of hypertension, human leukocyte antigen mismatches, graft cold ischemia time, anhepatic time, use of partial graft, organ location, acute rejection episodes, and graft survival were collected. For the Finnish cohort usage of Molecular Adsorbent Recirculating System (MARS), hemodialysis prior to transplantation and additional follow-up data of post-operative dialysis, post-operative laboratory results, and biliary complications were collected. Donor Risk Index (DRI) was calculated from donor variables according to formula by Feng et al. (12) for both Finnish and US donors. Variables used to calculate DRI are donor age, cause of death, race, graft splitting, donor height, organ location, and cold ischemia time. All organs in Finland were defined as local. Race was not available for the Finnish cohort due to Finnish legislation, but as overwhelming majority of the Finnish population is Caucasian, all Finnish donors were considered Caucasian. Because the models included DRI, donor factors used to calculate DRI were left out from the multivariable models due to possible multi-collinearity. Procurement interval was defined as the time from the declaration of brain death to the start of *in situ* cold perfusion.

Endpoints

Model of Early Allograft Function (MEAF)-score was selected as the primary short-term outcome measure (13). Based on alanine aminotransferase, international normalized ratio, and bilirubin, MEAF-score defines liver function numerically from 0 to 10, 3 days after transplantation. Acute liver failures, transplantations for under 18-year-olds and split transplantations were excluded, because MEAF is validated only for full liver grafts, adults and for non-acute liver failures. Beta in the results is given by one MEAF point per 1 hour change in procurement interval. Missing International Normalized Ratio values for 42 cases were calculated from prothrombin time with a conversion table supplied by the laboratory (HUSLAB) responsible for the blood tests. Post-operative kidney injury was assessed with post-operative need of dialysis and also by any grade of kidney injury defined by the Kidney Disease Improving Global Outcomes (KDIGO) -guidelines within the first 7 days (14).

For the Finnish cohort, acute rejections were defined as the need for rejection treatment in a biopsy-proven borderline, or acute cellular, or antibody-mediated rejection. The risk of intrahepatic biliary strictures was also assessed since this complication is strongly associated with ischemia-reperfusion injury (15). Strictures were diagnosed with either endoscopic retrograde cholangiography (ERC) or with magnetic imaging where ERC was not possible or not done.

Acute rejections in the US cohort were recorded to the SRTR database by accuracy of whether patient had an acute rejection before discharge or before a follow-up date. Consequently, early acute rejections were defined as a rejection before discharge time. Acute rejections during first year were analyzed by patient having an acute rejection episode before discharge or before 1-year follow-up after transplantation. In the Finnish cohort, 30 days was considered the cut-off for early acute rejection.

Graft survival, in which graft failure was defined as a composite outcome of retransplantation or recipient death, was chosen as the long-term dependent outcome measure.

Statistical Analysis

Transplantations were divided into tertiles based on procurement interval for graphical purposes. Characteristics of data and groups are reported with median and interquartile range (IQR) for continuous data and frequencies with percentages for categorical data in the tables. Number of patients with missing values are stated in **Table 1**.

Potential confounders to analysis were identified by a directed acyclic graph (DAG) (16). The DAG presentation (**Supplementary Figure S1**) explains our team's understanding of factors affecting the analysis, which were considered the same for all endpoints. From the DAG we identified DRI, patient age, patient MELD and indication of acute liver failure as confounders. The association between procurement interval (hours) and MEAF were assessed with a linear regression model (ordinary least squares). Cox proportional hazards models were used to analyze association of procurement interval on graft survival. The association of procurement interval with post-operative kidney injury and kidney injury requiring dialysis was assessed with logistic regression models after excluding preoperatively dialyzed patients. Logistic regression was used to analyze the association of procurement interval with biliary strictures and with acute rejections. Potential confounders were controlled with complete-cases data in all analyses and cases with missing variables were excluded.

Restricted cubic spline functions were used to account for potentially non-linear association between the outcome of interest and procurement interval and confounders, as the linear regression, logistic regression and Cox models involve the assumption of linearity for continuous data. Non-linearity was tested for, and the associations were modelled either as linear or non-linear. Linear associations between procurement interval and the outcome of interest were reported using the beta, odds ratio (OR) or hazard ratio (HR) with 95% confidence interval (CI), as appropriate. Non-linear results are reported with *p*-values and figures for clarity. The associations analyzed with spline functions were reported by plotting the predicted relative hazard of graft survival or endpoint as a function of procurement interval. The proportional hazards assumption for procurement interval was checked using Schoenfeld residuals, and no violations were detected. Effort to limit bias was addressed

TABLE 1 | Characteristics and outcomes of liver transplantations in Finland from June 2004 to December 2017 and the US from January 2008 to August 2018.

Variable	Finland N: 643	US N: 58 017	Missing FIN	Missing US
Donor				
Procurement interval, hours	10.5 (8.6–13.2)	34.6 (26.2–45.9)	0	0
Donor age, years	53 (41–61)	38 (24–52)	0	0
Donor BMI, kg/m^2	24.5 (22.7–26.9)	26.1 (22.6–30.4)	0	0
Donor gender, male	342 (53.2%)	34,591 (59.6%)	0	0
Donor medical history				
Hypertension	170 (26.4%)	18,403 (31.7%)	0	339 (0.6%)
Diabetes	37 (5.8%)	5,729 (9.9%)	0	0
Donor cause of death:			0	0
Anoxia	16 (2.5%)	17,773 (30.6%)		
Cerebrovascular accident	443 (68.9%)	18,833 (32.5%)		
Trauma	163 (25.3%)	19,985 (34.4%)		
Other	21 (3.3%)	1,426 (2.5%)		
Donor Risk Index (DRI)[a]	1.46 (1.22–1.68)	1.27 (1.08–1.52)	7 (1.1%)	557 (1.0%)
Donor organ yield[b]	3 (3–4)	3 (3–4)	0	0
More than liver and kidney donor[c]	258 (40.1%)	31,664 (54.6%)	0	0
Thoracic organ donor	204 (31.7%)	29,806 (51.4)	0	0
Donor cardiac arrest prior to brain death	98 (15.2%)	3,868 (6.7%)	0	0
Donor race, caucasian	NA	44,764 (77.2%)	NA	0
Recipient				
Partial/split graft	55 (8.6%)	1,465 (2.5%)	0	0
Cold ischemia, hours	4.9 (4.3–5.7)	6.1 (4.8–7.8)	7 (1.1%)	557 (1.0%)
Recipient age at transplantation, years	52 (37–60)	56 (47–62)	0	0
Recipient gender, male	350 (54.4%)	37,885 (65.3%)	0	0
Retransplantation	57 (8.9%)	3,637 (6.3%)	0	0
Combination transplantation, kidney	28 (4.4%)	5,917 (10.2%)	0	0
Median waiting time, days	24 (6–61)	83 (15–274)	0	0
Usage of MARS	50 (9.3%)	NA	105 (16.3%)	NA
Anhepatic time, minutes	57 (51–65)	NA	6 (0.9%)	NA
Total bleeding, litres	2.5 (1.5–4.5)	NA	7 (1.1%)	NA
MELD at transplantation	15.2 (10.5–21.4)	21 (13–31)	82 (12.8%)	0
Indication for transplantation			0	10 (0.0%)
Acute liver disease	80 (12.4%)	3,002 (5.2%)		
Chronic liver disease	463 (72.0%)	45,924 (79.2%)		
Metabolic liver disease[d]	22 (3.4%)	1,767 (3.0%)		
Tumor	78 (12.1%)	7,314 (12.6%)		
Primary liver pathology			0	0
Acute liver failure	80 (12.4%)	2,633 (4.5%)		
Primary sclerosing cholangitis	108 (16.8%)	2,496 (4.3%)		
Primary biliary cirrhosis	50 (7.8%)	1,335 (2.3%)		
Malignancy[e]	101 (15.7%)	10,392 (17.9%)		
Alcoholic liver disease	104 (16.2%)	10,730 (18.5%)		
HCV cirrhosis	16 (2.5%)	11,983 (20.7%)		
NASH	19 (3.0%)	5,857 (10.1%)		
Other	168 (26.1%)	12,591 (21.7%)		
Graft survival[f]			0	0
1-year	91.6%	88.1%		
2-year	87.8%	83.7%		
3-year	85.2%	80.3%		
5-year	80.8%	74.4%		
10-year	71.5%	59.5%		
15-year	55.1%	NA		
Model of Early Allograft Function-score	3.2 (1.9–4.4)	NA	7 (1.4%)	NA
Intrahepatic biliary stricture	31 (4.8%)	NA	2 (0.3%)	NA
Anastomotic biliary stricture	91 (14.2%)	NA	0	NA
Biliary leak	18 (2.8%)	NA	1 (0.2%)	NA
Early acute rejection	152 (23.6%)	3,102 (5.4%)	0	51 (0.1%)
Acute rejection during first year	231 (35.9%)	3,418 (14.6%)[g]	0	0[g]
Dialysis after transplantation[h]	146 (22.7%)	NA	0	NA
Post-operative kidney injury[i]	366 (68.0%)	NA	1 (0.2%)	NA
Grade 1	151 (28.1%)			
Grade 2	88 (16.4%)			
Grade 3	127 (23.6%)			
Follow-up time, years	6.6 (3.3–10.7)	2.9 (1.0–5.8)	0	0

[a]*Formula by Feng et al. (12).*

[b]*Number of organs donated per donor.*

[c]*Donor donated organs besides liver and kidneys.*

[d]*Metabolic liver disease by definition of Scientific Registry of Transplant Recipients (e.g., Wilson's disease, hemochromatosis, alpha-1 antritrypsin deficiency, thyrosinemia, primary oxalosis, hyperlipidemia; does not include nonalcoholic fatty liver disease).*

[e]*Malignancy in removed liver, indication in some cases has been other (e.g., PSC or alcoholic cirrhosis) prior to transplantation, overrules other primary diagnoses.*

[f]*Graft survival defined as combination outcome of death or retransplantation.*

[g]*Sub-cohort of 23,430 patients with sufficient data from 2013 to 2018.*

[h]*Includes all patients after transplantation.*

[i]*Acute kidney injury defined by KDIGO guidelines, 104 patients excluded from analysis because of preoperative dialysis.*

All values are stated as median (interquartile range) or categorical data as exact number (percentage of all) unless otherwise indicated.

NA, data not available for US cohort.

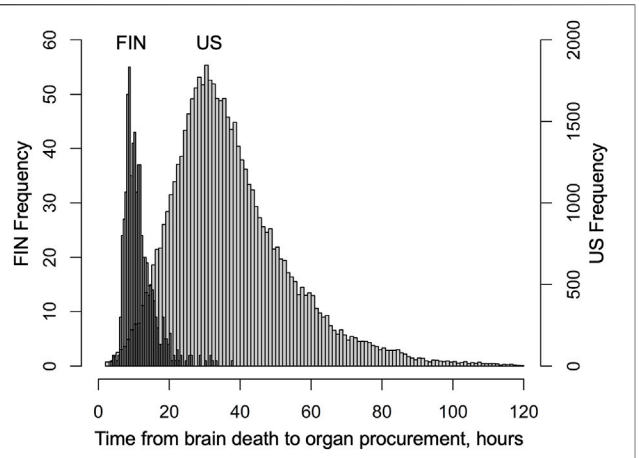


FIGURE 1 | Distribution of time from declaration of brain death to organ procurement (procurement interval) in Finnish liver donors from June 2004 to December 2017 and SRTR liver donors from January 2008 to August 2018.

by sparse exclusion criteria, testing all endpoints for non-linearity and adjusting for possible confounders. Sensitivity analyses by donor organ yield and year of transplantation were conducted to account for possible confounding.

The significance level was set at 5% and analyses were carried out as two-tailed. All analyses were performed using either IBM SPSS version 27 for Windows (Armonk, NY), or R software, including survival and rms packages (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Patients

Altogether 721 and 73,222 orthotopic liver transplantations were performed during the time periods in Finland and the US, respectively. In the Finnish cohort, 77 transplantations were excluded as the graft was received from another country and one was lost to follow-up resulting in 643 transplantations in the final Finnish cohort. From the US cohort, 3,104 living and 3,737 DCD donors were excluded from the analysis. In addition, extreme procurement interval values of over 120 h (203 donors) and under 2 hours (eight donors) were excluded for unreliability of brain death time. Also, transplantations with missing time of brain death, follow-up time or status (8,153 transplantations) were excluded, leaving 58,017 transplantations in the US cohort altogether.

Median interval from brain death to cold perfusion was 10.5 h in Finland and 34.6 h in the US. Distribution of these procurement intervals are presented in **Figure 1**. During follow-up, 131 and 11,396 patients died, and 42 and 1,509 were retransplanted in Finland and the US, respectively. Characteristics of donors, transplantations and patients in both cohorts are summarised in **Table 1**, which also includes follow-up data of complications in the Finnish cohort and numbers of missing values. Characteristics are divided by procurement interval tertiles in **Table 2** and outcomes in **Table 3**.

Short-Term Clinical Outcomes

Biliary Strictures, Acute Rejections and Kidney Injury as Outcome in the Finnish Cohort

In the Finnish cohort, 31 patients had intrahepatic biliary strictures during follow-up. 18 of these occurred in patients with primary sclerosing cholangitis (PSC), five with acute liver failure, two with alcoholic liver disease, one with liver malignancy, and five in patients with other liver pathologies as the indication for liver transplantation. In a univariable logistic regression model with spline, procurement interval was not associated with intrahepatic strictures (p = 0.65 for non-linearity in univariable analysis, p = 0.78 for linear component, OR 1.08 95% CI 0.76–1.54). No association was found in a multivariable logistic regression model (p = 0.36 for non-linearity, linear OR 0.99 95% CI 0.67–1.46).

During the first year after transplantation, 231 of 643 (36%) patients had an acute rejection episode. In a univariable logistic regression model with spline, the association of procurement interval to acute rejection during first year was not significantly non-linear (p = 0.31) and in a linear model failed to show statistical significance (OR 1.15 95% CI 0.98–1.36). In the adjusted model the association stayed insignificant (p = 0.29, OR 1.11 95% CI 0.92–1.34). Early acute rejections in the first 30 post-operative days were in a linear univariable model associated with longer procurement interval (p = 0.024, OR 1.23 95% CI 1.03–1.47). This association was lost in a multivariable model (p = 0.16, OR 1.16 95% CI 0.94–1.42).

From the kidney injury analysis, 104 (16.2%) patients were excluded having been dialyzed preoperatively. 85 patients required dialysis during the first seven post-operative days after transplantation. In a univariable logistic regression model with spline, the association of procurement interval to kidney injury requiring dialysis failed to show non-linearity (p = 0.62) or significant linear association (OR 1.02 95% CI 0.79–1.31), which was the case for the multivariable model as well (linear model OR 1.09 95% CI 0.82–1.44). Similarly, when defined by acute kidney injury (AKI) grade 1, 2 or 3 of KDIGO guidelines, kidney injury was not associated with procurement interval (non-linearity p = 0.64 and p = 0.70, linearly OR 0.99 95% CI 0.81–1.21 and OR 1.03 95% CI 0.82–1.30 in univariable and multivariable model, respectively). Univariable logistic regression model probabilities of endpoints are represented with a spline function by procurement interval in **Supplementary Figure S2**, which sums the results regarding the Finnish cohort short-term logistic regression results.

MEAF-Score as Outcome in the Finnish Cohort

MEAF-score could not be calculated for six (0.9%) patients due to missing laboratory results and 4 patients due to death before third post-operative day. For this analysis, 65 underaged, 4 partial grafts and 68 acute liver failures were excluded for lack of validation of MEAF in these cohorts. Median MEAF in the remaining 496 complete cases was 3.19 (IQR 1.93–4.39). Longer procurement interval associated with better MEAF-scores (p = 0.021, Beta −0.018 95% CI −0.079 −(−0.006)) in a

TABLE 2 | Characteristics of liver transplantations in Finland and the US by tertiles of time between brain death and organ procurement (interval).

Variable	Finland 6/2004–12/2017			US 1/2008–8/2018		
Donor						
Tertile of procurement interval	1st < 9.2 h n:214	2nd 9.2–12.0 h n:215	3rd > 12.0 h n:214	1st < 29.0 h n:19,333	2nd 29.0–41.3 h n:19,326	3rd > 41.3 h n:19,358
Procurement interval, hours	8.1 (7.2–8.6)	10.5 (9.9–11.3)	14.9 (13.2–17.9)	22.8 (18.2–26.2)	34.6 (31.8–37.7)	52.1 (45.9–62.2)
Donor age, years	59 (51–64)	51 (37–59)	47 (33–56)	45 (26–56)	38 (23–51)	35 (23–48)
Donor BMI, kg/m^2	24.8 (23.4–27.8)	24.3 (22.0–26.3)	24.2 (22.5–26.6)	26.4 (22.8–30.7)	26.0 (22.4–30.4)	26.0 (22.6–30.2)
Donor gender, male	113 (52.8%)	109 (50.7%)	120 (56.1%)	11,136 (57.6%)	11,548 (59.8%)	11,907 (61.5%)
Donor medical history						
Hypertension	77 (36.0%)	47 (21.9%)	46 (21.5%)	7,343 (38.1%)	5,939 (30.9%)	5,121 (26.7%)
Diabetes	21 (9.8%)	6 (2.8%)	10 (4.7%)	2,329 (12.0%)	1,821 (9.4%)	1,579 (8.2%)
Donor cause of death						
Anoxia	0 (0.0%)	4 (1.9%)	12 (5.6%)	5,668 (29.3%)	5,823 (30.1%)	6,282 (32.5%)
Cerebrovascular accident	163 (76.2%)	143 (66.5%)	137 (64.0%)	7,393 (38.2%)	6,149 (31.8%)	5,291 (27.3%)
Trauma	44 (20.6%)	62 (28.8%)	57 (26.6%)	5,885 (30.4%)	6,874 (35.6%)	7,226 (37.3%)
Other	7 (3.3%)	6 (2.8%)	8 (3.7%)	387 (2.0%)	480 (2.5%)	559 (2.9%)
Donor Risk Index[a]	1.52 (1.38–1.77)	1.42 (1.16–1.65)	1.35 (1.16–1.52)	1.33 (1.11–1.61)	1.26 (1.08–1.52)	1.22 (1.08–1.47)
More than liver and kidney donor[b]	29 (13.6%)	84 (39.1%)	145 (67.8%)	7,240 (37.4%)	11,204 (58.0%)	13,220 (68.3%)
Thoracic donor	12 (5.6%)	68 (31.6%)	124 (57.9%)	6,542 (33.8%)	10,511 (54.4%)	12,753 (65.9%)
Recipient						
Partial/split graft	11 (5.1%)	19 (8.8%)	25 (11.7%)	312 (1.6%)	525 (2.7%)	628 (3.2%)
Cold ischemia, hours	4.74 (4.22–5.70)	4.93 (4.28–5.70)	4.95 (4.38–5.68)	6.0 (4.7–7.7)	6.3 (5.0–8.0)	6.0 (4.8–7.7)
Recipient age at transplantation, years	55 (44–60)	51 (32–60)	50 (34–59)	56 (48–61)	56 (47–62)	56 (46–62)
Recipient MELD at transplantation	16 (11–23)	15 (11–21)	15 (10–20)	21 (14–30)	21 (13–30)	22 (13–33)
Liver pathology						
Acute liver failure	31 (14.5%)	24 (11.2%)	17 (7.9%)	952 (4.9%)	881 (4.6%)	978 (5.1%)
Malignancy	33 (15.4%)	36 (16.7%)	32 (15.0%)	4,885 (25.3%)	4,788 (24.8%)	4,829 (24.9%)
PSC	25 (11.7%)	39 (18.1%)	45 (21.0%)	788 (4.1%)	799 (4.1%)	852 (4.4%)
Alcoholic liver disease	44 (20.6%)	29 (13.5%)	38 (17.8%)	3,137 (16.2%)	3,227 (16.7%)	3,596 (18.6%)
Other	81 (37.9%)	87 (40.5%)	82 (38.3%)	9,571 (49.5%)	9,631 (49.8%)	9,103 (47.0%)
Year of Transplant	2011 (2007–2014)	2010 (2007–2014)	2012 (2009–2016)	2011 (2009–2014)	2013 (2011–2016)	2015 (2013–2017)
Follow-up time, years	7.15 (3.97–11.78)	6.65 (3.38–10.89)	5.34 (3.11–9.06)	4.1 (1.3–7.0)	3.0 (1.0–5.9)	1.9 (0.6–3.9)

[a]*Formula by Feng et al (12).*

[b]*Organs donated besides liver and kidneys, categorical.*

All values are stated as median (interquartile range) or exact number (percentage of all) unless otherwise indicated.

univariable linear model and in a multivariable model (p = 0.005, Beta −0.048 95% CI −0.085 −(−0.011)). A linear regression curve with confidence intervals is portrayed with a scatter plot of MEAF over procurement interval in **Supplementary Figure S3**.

Acute Rejections as Outcome in the US Cohort

Of 57,966 transplants 3,102 (5.4%) suffered an early acute rejection before discharge time, which was median 10 days (IQR 7–18 days). Longer procurement interval was linearly associated with lower risk for early acute rejection in univariable analysis (p = 0.005, OR 0.939 per 1 hour longer interval, 95% CI 0.899–0.981) and in multivariable model (p = 0.003, OR 0.935, per 1 hour longer interval, 95% CI 0.894–0.978) (**Figure 2**).

Acute rejections during the first year were analysed only from 2013 forward due to missing data. Restricting the cohort to transplantations from 2013 forward and to cases with complete 1-year follow-up of acute rejections, a total 23,430 transplantations were included for this sub-group analysis. 3,418 (14.6%) patients had an acute rejection episode before 1-year follow-up. No significant association of procurement interval with acute rejections during first year was detected (p = 0.36 for non-linearity, OR = 1.01 95% CI 0.97–1.06 for univariable model, OR 1.00 95% CI 0.95–1.04 for multivariable model).

Graft Survival

Finnish Cohort

In the Finnish cohort, procurement interval was not significantly associated with graft survival. In a univariable spline model, procurement interval was not associated with graft survival non-linearly (p = 0.21) or linearly (p = 0.44, HR 0.99 95% CI 0.95–1.02). The relative hazards of both univariable and multivariable model are presented in **Figure 3**. Non-linear association of procurement interval with graft survival did not reach statistical significance in a multivariable model (p = 0.07) and no linear association was found (p = 0.45, HR 1.01 95% CI 0.98–1.05). Non-proportionality was tested for and held in a univariable

TABLE 3 | Outcomes of liver transplantations in Finland and the US by tertiles of time between brain death and organ procurement (interval).

Variable	Finland 6/2004–12/2017			US 1/2008–8/2018		
Tertile of procurement interval	**1st <9.2 h n:214**	**2nd 9.2–12.0 h n:215**	**3rd >12.0 h n:214**	**1st <29.0 h n:19,333**	**2nd 29.0–41.3 h n:19,326**	**3rd >41.3 h n:19,358**
Graft survival						
1-year	92.1%	90.2%	92.5%	87.0%	88.2%	89.3%
3-year	84.6%	83.1%	87.8%	78.5%	80.3%	82.5%
5-year	81.4%	77.0%	84.4%	72.6%	74.7%	76.5%
10-year	74.6%	64.2%	77.0%	57.2%	61.9%	58.9%
15-year	57.2%	46.4%	66.0%	NA	NA	NA
Intrahepatic biliary stricture	7 (3.3%)	13 (6.1%)	11 (5.2%)	NA	NA	NA
Anastomotic biliary stricture	33 (15.4%)	27 (12.6%)	31 (14.5%)	NA	NA	NA
Biliary leak	4 (1.9%)	7 (3.3%)	7 (3.3%)	NA	NA	NA
Discharge time	NA	NA	NA	10 (7–18)	10 (7–18)	11 (7–19)
Early acute rejection[a]	44 (20.5%)	56 (26.2%)	52 (24.3%)	1,116 (5.8%)	1,012 (5.3%)	970 (5.1%)
Acute rejection during first year[b]	73 (34.1%)	84 (39.1%)	74 (34.6%)	1,122 (14.4%)	1,137 (14.6%)	1,159 (14.8%)
MEAF[c]	3.3 (2.1–4.6)	3.3 (2.0–4.5)	2.9 (1.6–4.1)	NA	NA	NA
Post-operative dialysis[d]	25 (14.1%)	30 (16.7%)	30 (16.5%)	NA	NA	NA
Post-operative kidney injury[e]	124 (70.5%)	122 (67.8%)	121 (66.5%)	NA	NA	NA
Difference in creatinine[f]	57 (18–131)	40 (14–121)	45 (15–113)	NA	NA	NA

[a]*In Finnish cohort acute rejection before 30 days and in the US cohort before discharge.*
[b]*For US in sub-cohort of transplantations performed 2013 onwards (middle-tertile of 33–46 h of procurement interval).*
[c]*Model for Early Allograft Function (13), median (interquartile range).*
[d]*AKI requiring dialysis within 7 post-operative days.*
[e]*Acute kidney injury defined by KDIGO guidelines, grades 1–3. 104 patients (16.2%) were dialysed preoperatively and were excluded from post-operative kidney injury and dialysis analysis.*
[f]*Difference between highest creatinine in 7 post-operative days and pretransplantation creatinine in mmol/l.*

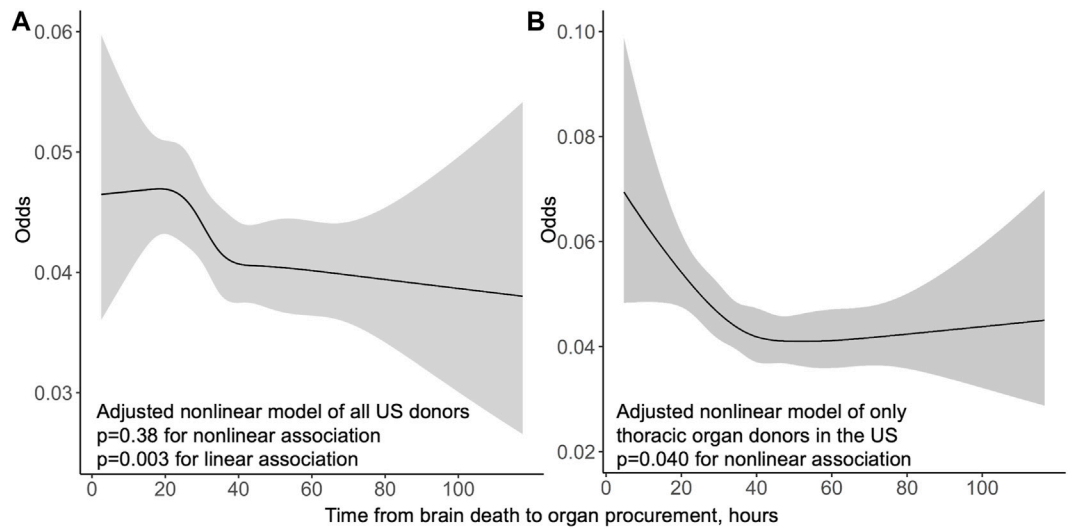


FIGURE 2 | Odds of acute rejection before discharge by procurement interval from multivariable logistic regression models in the US whole cohort **(A)** and thoracic organ donors only **(B)** with 95% confidence intervals in grey.

model ($p = 0.76$) and in the multivariable model ($p = 0.72$) for procurement interval.

US Cohort

Median follow-up period in the US was 3 years. In a univariable model, the association of procurement interval with graft survival showed strong non-linearity ($p < 0.001$) and is presented by a cubic spline function of relative hazard in **Figure 4**. Longer interval associated non-linearly ($p = 0.016$) with better graft survival also in multivariable models adjusted with Donor Risk Index (DRI) and recipient factors (age, MELD and acute liver failure) (**Figure 4**). Proportional hazards assumption held true for procurement interval ($p = 0.20$). Kaplan-Meier curves of both cohorts are presented in **Figure 5**.

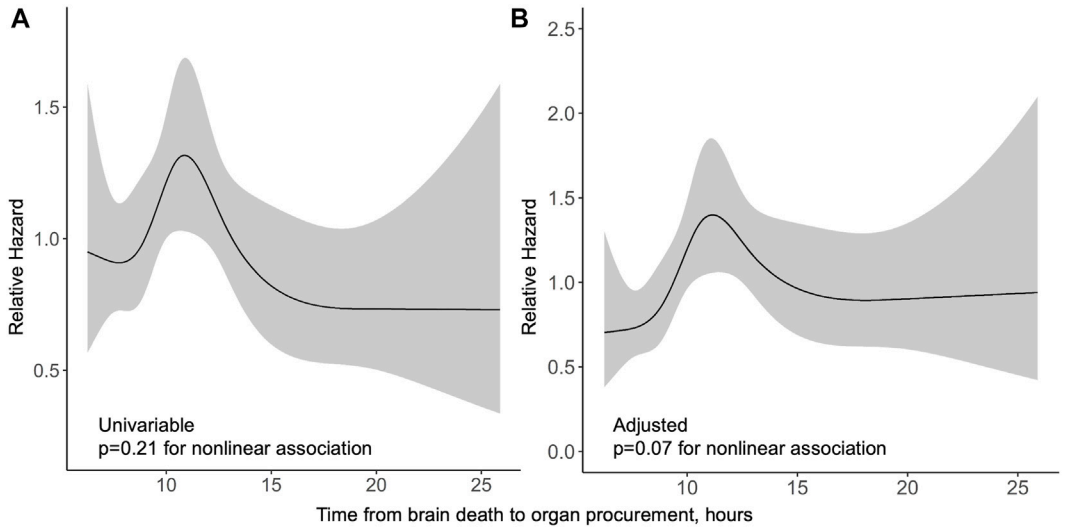


FIGURE 3 | Relative hazard of graft loss or death by procurement interval from univariable **(A)** and multivariable **(B)** Cox regression models in the Finnish cohort with 95% confidence intervals in grey.

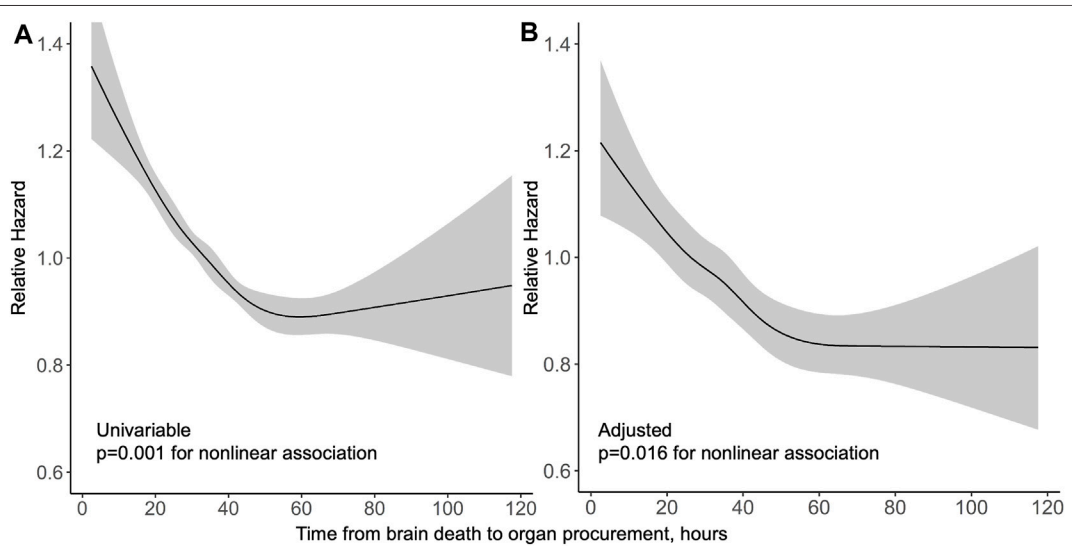


FIGURE 4 | Relative hazard of graft loss or death by procurement interval from univariable **(A)** and multivariable **(B)** Cox regression models in the US cohort with 95% confidence intervals in grey.

Sensitivity Analyses

Organ Yield

All analyses were repeated with stratification to donor organ yield. Cohorts were separated by whether thoracic organs were donated or not (thoracic donor) resulting in two sensitivity analyses by organ yield. In the Finnish cohort, no new associations of short-term outcomes were detected. Linear association of longer procurement interval with decreasing MEAF-score was barely lost in thoracic donors (univariable p = 0.072, Beta −0.060 95% CI −0.125–0.005) and multivariable model (p = 0.070 Beta −0.051 95% CI −0.115–0.013), and no significant association was detected for donors who donated only abdominal organs (p = 0.23). Otherwise, the sensitivity analyses by organ yield concurred with results for the Finnish cohort.

In the US cohort, stratification to thoracic donors resulted in increasing variance in short and very long procurement interval associations with graft survival, leading to dissipated non-linearity (p = 0.22) (**Supplementary Figure S4**). Linear decreasing hazard remained (HR 0.910 95% CI 0.880–0.942) with longer procurement interval with stratification to thoracic donors in a multivariable model. The non-linear association of

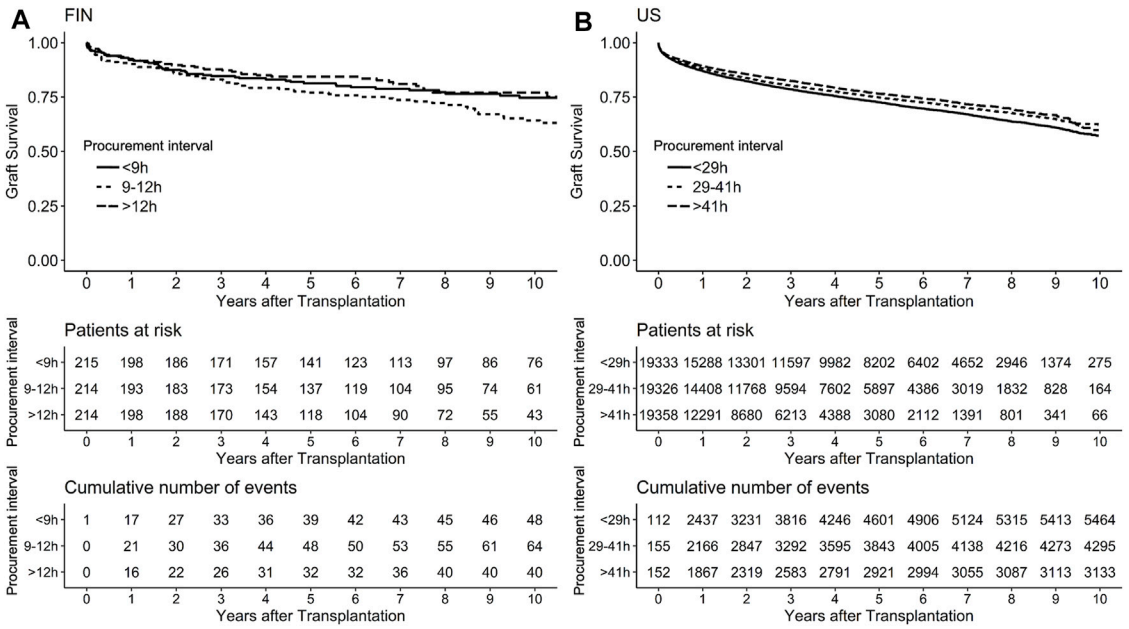


FIGURE 5 | Kaplan-Meier curves with survival tables in Finnish **(A)** and the US **(B)** cohort with cases divided by procurement interval tertiles for graphical purposes with 95% confidence intervals in grey.

procurement interval with graft survival observed in the whole cohort persisted with non-thoracic donors (p = 0.008). Stratification to thoracic donors yielded a non-linear association of longer procurement interval with less acute rejections before discharge (p = 0.040) (**Figure 2**). This association of procurement interval with acute rejections disappeared entirely when the cohort was restricted to non-thoracic donors (adjusted non-linear association p = 0.99, linear p = 0.15).

Transplant Year

Procurement intervals grew longer during the follow-up in both cohorts (**Table 2**). A sensitivity analysis was conducted in the US cohort by dividing transplantations to two groups: 2008–2012 and 2013–2018 to account for this possible confounding. For both sub-cohorts of US cohort, non-linearity of association of procurement interval with graft survival was lost due to growing of confidence intervals in shorter procurement intervals (**Supplementary Figure S5**). Linear decreasing adjusted hazard of graft loss or death was significant in both sub-cohorts (earlier transplantations p = 0.002, HR 0.962 95% CI 0.939–0.985, and later p = 0.028, HR 0.959 95% CI 0.924–0.995). The association of procurement interval with acute rejections before discharge disappeared for both earlier and later sub-cohorts (adjusted linear association p = 0.051 and p = 0.77, respectively).

In the Finnish cohort, sensitivity analyses by transplantation year groups were 2004–2011 and 2012–2017. Longer procurement interval was associated with better MEAF-scores (p = 0.002, Beta −0.074 95% CI −0.120 −(−0.028)) only in the later years reflecting the change to longer procurement intervals (in earlier transplantations adjusted p = 0.80). When the Finnish cohort was divided to earlier and later transplantations, new associations of procurement interval with other outcomes were not detected concurring with whole cohort analyses. In **Supplementary Figure S6** a spline function represents the association of procurement interval with relative hazard of graft loss or death for earlier and later transplantations in the Finnish cohort.

1-year Graft and Patient Survival

When the follow-up was restricted to 1 year after transplantation, the results concerning the composite endpoint of graft and patient survival remained the same. In the Finnish cohort, the association remained insignificant (**Supplementary Figure S7**). In the US cohort, the association of the composite endpoint with procurement interval was non-linear (p = 0.0036) in the multivariable Cox model—the relative hazard diminishing until 60 h after brain death (**Supplementary Figure S8**). The sample size of US cohort enabled us to analyze separately solely graft- and patient survival 1 year after transplantation. For both separate endpoints—solely graft and patient survival—the association of procurement interval remained non-linear in the multivariable analysis (p = 0.0030 and p = 0.0023) (**Supplementary Figures S9, S10**).

DISCUSSION

This study shows that longer procurement interval is associated with better liver graft survival and early function. The association with graft survival was only detected in the US cohort, where

procurement intervals were considerably longer compared to the Finnish cohort. The shorter procurement intervals in Finland possibly fail to grasp this beneficial association seen in the US cohort. In addition, longer procurement interval showed no negative association with short-term outcomes. In contrast, a slight but significant association of longer procurement interval with better early allograft function was detected and also 1-year graft and patient survival showed a similar decreasing hazard. These results imply, that longer interval is not detrimental to the allograft and instead, it may benefit early function and longevity of the liver graft.

These short- and long-term results provide support to the trend of increasing procurement intervals over the years, which was observed in both cohorts. The reasons to lengthening procurement intervals seem logistics-driven. Due to improved donor management, the need of urgent procurement from an unstable donor has undoubtedly decreased and thus, also contributes to longer intervals. In addition, earlier studies negating harm to other organs may also have contributed.

In human studies, no organ has benefited from a very short procurement interval. In kidney allografts, four studies have reported improved graft survival with longer interval while one smaller study found no association either way (7, 8, 11, 17, 18). In heart allografts, longer procurement interval has not benefited nor harmed graft survival (10, 19). While lung transplants showed no association of procurement interval with graft survival, they benefited from longer interval with less acute rejections and bronchiolitis-obliterans-free survival (9). In these other studies on kidneys and hearts, procurement interval has not associated with acute rejections.

Unquestionably brain death is harmful for organs. Although changes in blood coagulation, cytokine profiles, and gene transcription (20) are widely recognised, time-dependent changes in relation to brain death have rarely been reported. Danobeitia et al showed in rhesus macaques that the massive catecholamine storm dies down after 6 h from brain death (21). In a novel human study, Schwartz et al showed for the first time how different cytokines fluctuate several hours after brain death (22). In their study, procurement was performed at median time of 15 h. Cytokines Interleukin-1B and Interleukin-10 increased until 7 h after brain death and stayed level until procurement. Tumour Necrosis Factor peaked at 7 h, while Interferon-gamma in turn started increasing only after 7 h after brain death. Cytokine storm seems to continue after catecholamine storm subsides, although no explicit serial data on humans exist. These and earlier studies concerning procurement intervals have led to the two-hit theory of brain death, with a catecholamine storm followed by "storm cooling," and recovery before the second hit of cold ischemia, for which the organ is probably more prepared for after a longer procurement interval. This study is in line with this theory presented first by Kunzendorf et al (8, 17). The mechanisms are beyond the scope of this study but could be related to the upregulation of cytokines and cytoprotective genes caused by brain death similarly to the theory behind remote ischemic preconditioning, which is also being actively investigated in the field of transplantation (23).

A concern in waiting for long periods prior to procurement has been the possible loss of unstable donors and hence valuable organs. Donor management protocols have in recent decades however made this concern practically irrelevant as few potential donors are lost due to cardiovascular collapse (6, 24–28).

This study has some limitations. Firstly, causality cannot be concluded from an observational registry analysis. Due to the retrospective nature of the study, it is also susceptible to confounding and non-random allocation, which concern all the previous studies as well. Confounding is most evident in possibly changed clinical practices over the years with simultaneous lengthening of procurement intervals, which cannot be adjusted for in expense of follow-up time. This possible confounding was negated by the sensitivity analysis conducted, although limiting the association to only linear connections. Finnish cohort sample size and narrow distribution of procurement intervals, especially concerning non-multiorgan donors, also limits our ability to adjust our model and divide to sensitivity analyses.

As only the time of declaration of brain death was available to us from the cohorts, we chose to use this time for the start of the interval, although the exact time of the brain insult is unknown. Practises in different systems and countries may also differ on the urgency of diagnosing brain death. In some cases, a suspected donor will need to be stabilised before attaining the diagnosis and this can create a delay in the start of the interval. The procurement interval presented here serves therefore as the best available marker of the physiologic interval. A selection bias is unavoidable as better quality organs were distributed to longer procurement times possibly due to allocation and testing of thoracic organs. We sought to limit this with a multivariable analysis and sensitivity analyses.

The strengths of this study are the comprehensive multivariable analyses, which account for better quality organs distributing unevenly between procurement intervals, great sample size as a whole, and two cohorts with different procurement intervals offering a wider scope to the associations. One of the strengths of this research is that the findings to the same direction were found in two different populations. Since associations or effects in nature are seldom linear, spline functions were used to account for non-linearity. The peaking hazard in Finnish cohort graft survival, although insignificant, is interesting since most organs are procured exactly at the peak. Bias in this peak cannot be ruled out. Differences in patient characteristics between the cohorts were evident and practises to declare time of brain death may differ between countries; thus cohorts were analyzed separately and meant to complement each other rather than compare the cohorts. Also, practices in diagnosing and treating acute rejections may vary greatly between centers.

This analysis is to our knowledge the first to show that liver grafts may tolerate longer procurement intervals, as longer time from brain death to procurement was associated with improved outcomes. Our findings do not support a progressive organ injury induced by the cytokine storm.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because restrictions issued by the authorities in Finland apply to the availability of the data from transplant patients for sharing. Restrictions apply to the availability of the US data based on the current data use agreements with SRTR. Other data are available from the corresponding author upon reasonable request. Requests to access the datasets should be directed to ville.sallinen@hus.fi.

ETHICS STATEMENT

According to local legislation, ethics committee approval or patients/participants (legal guardian/next of kin) consent is not required for retrospective registry studies. This study was approved by the Institutional Review Board of Helsinki University Hospital (HUS/459/2018) and SRTR.

AUTHOR CONTRIBUTIONS

Study design: IH, VS, ML, HM, AN, and HI. Acquisition of data: VE, IH, VS, ML, HM, AN, and HI. Interpretation and analysis: VE, FÅ, VS, and IH. Drafting and revising critically: VE, IH, VS, FÅ, ML, HM, AN, and HI. Final version was approved by all authors.

FUNDING

This study was funded by grants from Helsinki University Hospital (VTR to HI, VS, and IH) and Finska Läkaresällskapet (to IH and VS).

AUTHOR DISCLAIMER

The interpretation and reporting of these data are the responsibility of the author(s) and in no way should be seen as an official policy of or interpretation by the SRTR or the U.S. Government.

CONFLICT OF INTEREST

VE reports receiving funding from grants awarded to IH. VE reports receiving a grant from Munuaissäätiö (Finnish Kidney Association). VS reports receiving grants from Academy of Finland, Sigrid Juselius Foundation, Cancer Foundation Finland, Vatsatautien tutkimussäätiö Foundation and Mary and Georg Ehrnrooth's Foundation. IH reports receiving consulting fees from Novartis and Hansa Biopharma outside the submitted work. FÅ received research grants from Mary and Georg Ehrnrooth Foundation, Finska Läkaresällskapet, and Sigrid Jusélius Foundation outside the submitted work.

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

ACKNOWLEDGMENTS

We thank the transplantation coordinators Siv Ansa, Eero Hartikka, Heikki Norio, Carola Schauman and Leena Toivonen from Helsinki University Hospital and Nina Ask for their help with the transplantation registry of Finland. The data reported here have been supplied by the Hennepin Healthcare Research Institute (HHRI) as the contractor for the Scientific Registry of Transplant Recipients (SRTR).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10364/full#supplementary-material

REFERENCES

1. Kwong AJ, Kim WR, Lake JR, Smith JM, Schladt DP, Skeans MA, et al. OPTN/SRTR 2019 Annual Data Report: Liver. *Am J Transpl* (2021) 21(S2):208–315. doi:10.1111/ajt.16494
2. Van der Hoeven JAB, Ter Horst GJ, Molema G, de Vos P, Girbes ARJ, Postema F, et al. Effects of Brain Death and Hemodynamic Status on Function and Immunologic Activation of the Potential Donor Liver in the Rat. *Ann Surg* (2000) 232(6):804–13. doi:10.1097/00000658-200012000-00009
3. Olinga P, Van der Hoeven JAB, Merema MT, Freund RL, Ploeg RJ, Groothuis GMM. The Influence of Brain Death on Liver Function. *Liver Int* (2005) 25(1):109–16. doi:10.1111/j.1478-3231.2005.01035.x
4. Van der Hoeven JAB, Lindell S, Van Schilfgaarde R, Molema G, Ter Horst GJ, Southard JH, et al. Donor Brain Death Reduces Survival after Transplantation in Rat Livers Preserved for 20hr. *Transplantation* (2001) 72(10):1632–6. doi:10.1097/00007890-200111270-00009
5. Van der Hoeven JAB, Moshage H, Schuurs T, Nijboer M, Van Schilfgaarde R, Ploeg RJ. Brain Death Induces Apoptosis in Donor Liver of the Rat. *Transplantation* (2003) 76(8):1150–4. doi:10.1097/01.TP.0000080983.14161.95
6. Lytle FT, Afessa B, Keegan MT. Progression of Organ Failure in Patients Approaching Brain Stem Death. *Am J Transpl* (2009) 9:1446–50. doi:10.1111/j.1600-6143.2009.02575.x
7. Nijboer WN, Moers C, Leuvenink HGD, Ploeg RJ. How Important Is the Duration of the Brain Death Period for the Outcome in Kidney Transplantation? *Transpl Int* (2010) 24(1):14–20. doi:10.1111/j.1432-2277.2010.01150.x
8. Ergün M, Özdemir-van Brunschot DMD, Donders RART, Hilbrands LB, Hoitsma AJ, Warlé MC. Prolonged Duration of Brain Death Was Associated with Better Kidney Allograft Function and Survival: A Prospective Cohort Analysis. *Ann Transpl* (2019) 24:147–54. doi:10.12659/AOT.913869
9. Jawitz OK, Raman V, Barac Y, Mulvihill MS, Moore C, Choi AY, et al. Impact of Donor Brain Death Duration on Outcomes after Lung Transplantation. *Ann Thorac Surg* (2019) 108:1519–26. doi:10.1016/j.athoracsur.2019.05.026

10. Jawitz OK, Raman V, Barac YD, Anand J, Patel CB, Mentz RJ, et al. Influence of Donor Brain Death Duration on Outcomes Following Heart Transplantation: A United Network for Organ Sharing Registry Analysis. *J Thorac Cardiovasc Surg* (2019) 159:1345–53. doi:10.1016/j.jtcvs.2019.04.060
11. Eerola V, Helanterä I, But A, Lempinen M, Makisalo H, Nordin A, et al. The Association of Time to Organ Procurement on Short-And Long-Term Outcomes in Kidney Transplantation. *Clin J Am Soc Nephrol* (2021) 16(3): 427–36. doi:10.2215/CJN.11420720
12. Feng S, Goodrich NP, Bragg-Gresham JL, Dykstra DM, Punch JD, DebRoy MA, et al. Characteristics Associated with Liver Graft Failure: The Concept of a Donor Risk index. *Am J Transpl* (2006) 6(4):783–90. doi:10.1111/j.1600-6143.2006.01242.x
13. Pareja E, Cortes M, Hervás D, Mir J, Valdivieso A, Castell JV, et al. A Score Model for the Continuous Grading of Early Allograft Dysfunction Severity. *Liver Transpl* (2015) 21(1):38–46. doi:10.1002/lt.23990
14. Khwaja A. KDIGO Clinical Practice Guidelines for Acute Kidney Injury. *Nephron Clin Pract* (2012) 120(4):c179–84. doi:10.1159/000339789
15. Taner CB, Bulatao IG, Willingham DL, Perry DK, Sibulesky L, Pungpapong S, et al. Events in Procurement as Risk Factors for Ischemic Cholangiopathy in Liver Transplantation Using Donation after Cardiac Death Donors. *Liver Transpl* (2012) 18(1):100–11. doi:10.1002/lt.22404
16. Suttorp MM, Siegerink B, Jager KJ, Zoccali C, Dekker FW. Graphical Presentation of Confounding in Directed Acyclic Graphs. *Nephrol Dial Transpl* (2015) 30(9):1418–23. doi:10.1093/ndt/gfu325
17. Kunzendorf U, Hohenstein B, Oberbarnscheid M, Muller E, Renders L, Schott GE, et al. Duration of Donor Brain Death and its Influence on Kidney Graft Function. *Am J Transpl* (2002) 2(3):292–4. doi:10.1034/j.1600-6143.2002.20316.x
18. Muruve NA, Helling TS, Luger AM, Martinez J, Nelson PW, Pierce GE, et al. Effect of Donor Brain-Death Duration on Graft Outcome. *Transpl Proc* (2001) 33(6):2980–1. doi:10.1016/S0041-1345(01)02279-5
19. Cantin B, Kwok BWK, Chan MCY, Valantine HA, Oyer PE, Robbins RC, et al. The Impact of Brain Death on Survival after Heart Transplantation: Time is of the Essence. *Transplantation* (2003) 76(9):1275–9. doi:10.1097/01.TP.0000093445.50624.5A
20. Weiss S, Kotsch K, Francuski M, Reutzel-Selke A, Mantouvalou L, Klemz R, et al. Brain Death Activates Donor Organs and is Associated with a Worse I/R Injury after Liver Transplantation. *Am J Transpl* (2007) 7(6):1584–93. doi:10.1111/j.1600-6143.2007.01799.x
21. Danobeitia JS, Sperger JM, Hanson MS, Park EE, Chlebeck PJ, Roenneburg DA, et al. Early Activation of the Inflammatory Response in the Liver of Brain-Dead Non-human Primates. *J Surg Res* (2012) 176(2):639–48. doi:10.1016/J.JSS.2011.10.042
22. Schwarz P, Custódio G, Rheinheimer J, Crispim D, Leitão CB, Rech TH. Brain Death-Induced Inflammatory Activity is Similar to Sepsis-Induced Cytokine Release. *Cel Transpl* (2018) 27(10):1417–24. doi:10.1177/0963689718785629
23. Uutela A, Helantera I, Lemstrom K, Passov A, Syrjala S, Aberg F, et al. Randomised Sham-Controlled Double-Blind Trial Evaluating Remote Ischaemic Preconditioning in Solid Organ Transplantation: A Study Protocol for the RIPTRANS Trial. *BMJ Open* (2020) 10(11):e038340. doi:10.1136/bmjopen-2020-038340
24. Inaba K, Branco BC, Lam L, Salim A, Talving P, Plurad D, et al. Organ Donation and Time to Procurement: Late is Not Too Late. *J Trauma* (2010) 68(6):1362–6. doi:10.1097/TA.0b013e3181db30d3
25. Branco BC, Inaba K, Lam L, Salim A, Barmparas G, Teixeira PGR, et al. Donor Conversion and Procurement Failure: The Fate of Our Potential Organ Donors. *World J Surg* (2011) 35(2):440–5. doi:10.1007/s00268-010-0870-0
26. Westphal GA, Slaviero TA, Montemezzo A, Lingiardi GT, de Souza FCC, Carnin TC, et al. The Effect of Brain Death Protocol Duration on Potential Donor Losses Due to Cardiac Arrest. *Clin Transpl* (2016) 30(11):1411–6. doi:10.1111/ctr.12830
27. Salim A, Velmahos GC, Brown C, Belzberg H, Demetriades D. Aggressive Organ Donor Management Significantly Increases the Number of Organs Available for Transplantation. *J Trauma* (2005) 58(5):991–4. doi:10.1097/01.TA.0000168708.78049.32
28. Meyfroidt G, Gunst J, Martin-Loeches I, Smith M, Robba C, Taccone FS, et al. Management of the Brain-Dead Donor in the ICU: General and Specific Therapy to Improve Transplantable Organ Quality. *Intensive Care Med* (2019) 45(3):343–53. doi:10.1007/s00134-019-05551-y

ESOT | Transplant International

ORIGINAL RESEARCH
published: 12 September 2022
doi: 10.3389/ti.2022.10618

Survival After Simultaneous Pancreas-Kidney Transplantation in Type 1 Diabetes: The Critical Role of Early Pancreas Allograft Function

*Mengmeng Ji[1], Mei Wang[1], Wenjun Hu[1], Mohamed Ibrahim[1], Krista Lentine[2], Massini Merzkani[1], Haris Murad[1], Yazen Al-Hosni[1], Ronald Parsons[3], Jason Wellen[1], Su-Hsin Chang[1] and Tarek Alhamad[1]**

[1]School of Medicine, Washington University in St. Louis, St. Louis, MO, United States, [2]Division of Nephrology, Department of Internal Medicine, Saint Louis University School of Medicine, St. Louis, MO, United States, [3]Division of Transplantation, Department of Surgery, Emory University School of Medicine, Atlanta, GA, United States

Simultaneous pancreas-kidney transplantation (SPK) carries about a 7%–22% risk of technical failure, but the impact of early pancreas allograft loss on subsequent kidney graft and patient survival is not well-defined. We examined national transplant registry data for type 1 diabetic patients who received SPK between 2000 and 2021. Associations of transplant type (i.e., SPK, deceased-donor kidney transplant [DDKA], living-donor kidney transplant [LDKA]) with kidney graft failure and patient survival were estimated by multivariable inverse probability of treatment-weighted accelerated failure-time models. Compared to SPK recipients with a functioning pancreas graft 3 months posttransplant (SPK,P+), LDKA had 18% (Time Ratio [TR] 0.82, 95%CI: 0.70–0.95) less graft survival time and 18% (TR 0.82, 95%CI: 0.68–0.97) less patient survival time, DDKA had 23% (TR 0.77, 95%CI: 0.68–0.87) less graft survival time and 29% (TR 0.71, 95%CI: 0.62–0.81) less patient survival time, and SPK with early pancreas graft loss had 34% (TR 0.66, 95%CI: 0.56–0.78) less graft survival time and 34% (TR 0.66, 95%CI: 0.55–0.79) less patient survival time. In conclusion, SPK,P+ recipients have better kidney allograft and patient survival compared with LDKA and DDKA. Early pancreas graft failure results in inferior kidney and patient survival time compared to kidney transplant alone.

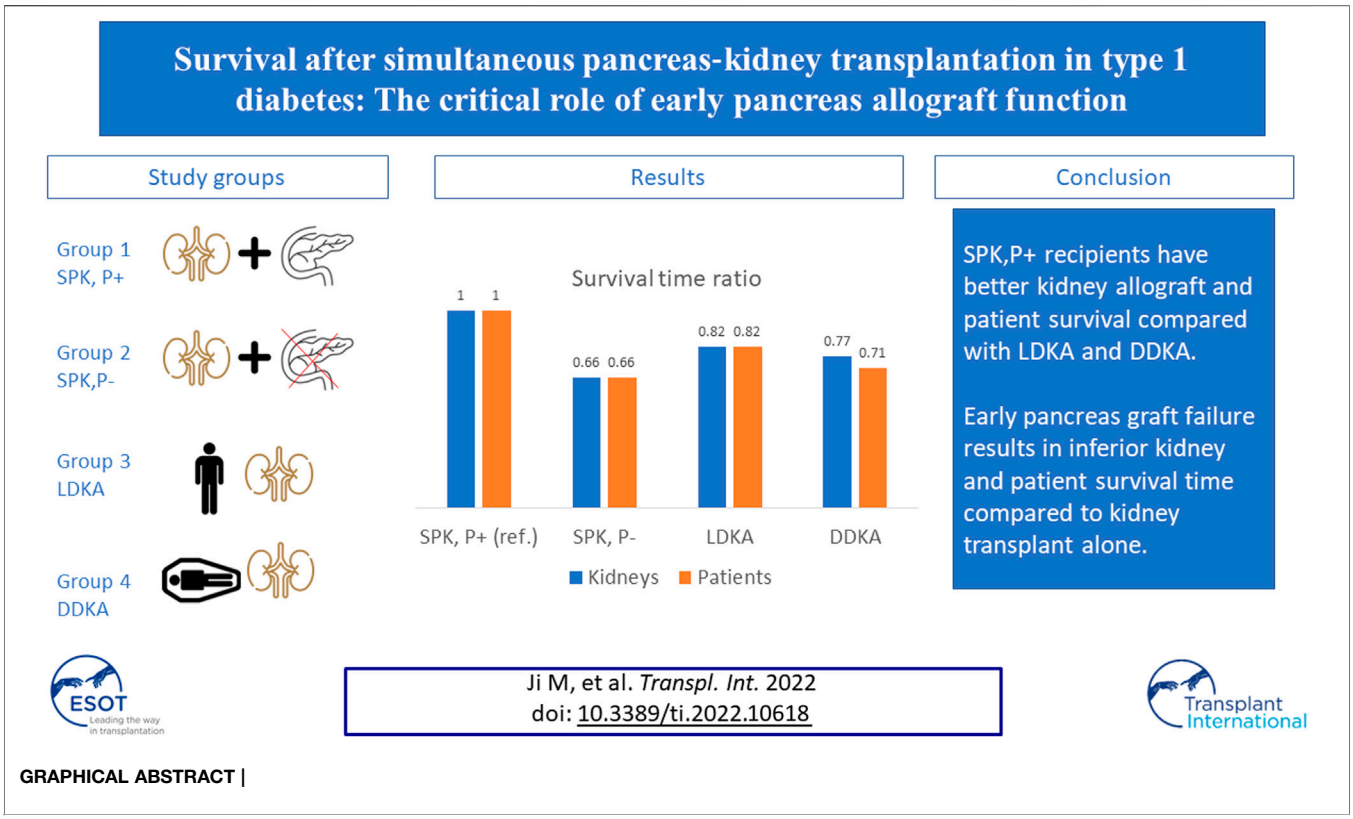
Keywords: allograft failure, kidney transplant, simultaneous pancreas-kidney transplantation, type 1 diabetes mellitus, allograft survival

OPEN ACCESS

***Correspondence:**
Tarek Alhamad
talhamad@wustl.edu

***Received:** 04 May 2022*
***Accepted:** 24 August 2022*
***Published:** 12 September 2022*

Citation:
Ji M, Wang M, Hu W, Ibrahim M, Lentine K, Merzkani M, Murad H, Al-Hosni Y, Parsons R, Wellen J, Chang S-H and Alhamad T (2022) Survival After Simultaneous Pancreas-Kidney Transplantation in Type 1 Diabetes: The Critical Role of Early Pancreas Allograft Function. Transpl Int 35:10618. doi: 10.3389/ti.2022.10618



GRAPHICAL ABSTRACT |

INTRODUCTION

In the United States, 60.6% of incident patients with end-stage kidney disease (ESKD) have diabetes mellitus (DM) (1). Mortality rates of patients with ESKD and DM vary depending on treatment choice. Those on dialysis have a 15%–20% mortality rate within 1 year of treatment initiation and a 5-year survival rate of under 50% (1). Compared to diabetic patients on dialysis, those who receive kidney transplants have significantly higher 5-year survival rates of 85% for type 1 diabetes (T1DM) and 77% for type 2 diabetes (T2DM) (2). However, poor glycemic control after transplantation remains an important challenge and contributes to excessive morbidity and mortality among diabetic recipients (3–5). Simultaneous pancreas-kidney (SPK) transplantation is a well-established treatment for patients with T1DM to restore normoglycemia and ameliorate diabetic complications (6). Owing to improved surgical technique, immunosuppression, donor and recipient selection, and graft surveillance, the 5- and 10-year patient survival rates for SPK transplantation have reached 87% and 70%, respectively (7).

Previous studies reported conflicting results about whether long-term kidney allograft and patient survival in SPK recipients is superior to that of kidney transplant alone recipients, especially as compared with a living donor kidney transplant alone (LDKA) (8–10). The mixed results may partially attribute to the higher rate of postoperative complications associated with SPK and the long-term benefits of euglycemia afforded by a functioning pancreas allograft. Despite improvements in surgical technique, the recent Scientific Registry of Transplant Recipients (SRTR) report showed that roughly 7% of pancreas grafts after SPK transplant are lost within 3 months of transplant (11). Early pancreas graft loss was historically associated with reduced kidney allograft function and inferior survival outcomes (12–16). Given improvements in immunosuppression management of complications and comorbidities, studies of contemporary cohorts have reported better outcomes and excellent kidney allograft function following early pancreas loss compared to earlier studies (17). The question remains whether SPK with and without early pancreas graft function has better survival in comparison to kidney transplant alone in the current transplant era.

This study aims to determine whether conditional 3-month pancreas graft survival is associated with long-term kidney allograft survival and patient survival in patients with T1DM who received SPK, compared to deceased donor kidney transplant alone (DDKA) and LDKA recipients, in a large, contemporary national U.S. cohort.

PATIENTS AND METHODS

Study Population

Data was obtained from the Organ Procurement and Transplantation Network (OPTN), a data system that contains all national data on the candidate waiting list, organ donation and matching, and transplantation. We performed a retrospective

TABLE 1 | Baseline recipient, donor, and transplant factors of the study cohort, stratified by transplant type.

	SPK	SPK,P+	SPK,P-	DDKA	LDKA	*p*-value
	n = 10,383	*n* = 9,832	*n* = 551	*n* = 6,202	*n* = 5,673	
	46.65%	44.17%	2.48%	27.86%	25.49%	
Recipient factors						
Age (years)[a,b]						<0.0001
18–50	84.11	84.07	84.75	47.55	62.08	
>50	15.89	15.93	15.25	52.45	37.92	
Gender[a,b]						<0.0001
Female	39.20	39.10	41.02	42.62	42.73	
Male	60.80	60.90	58.98	57.38	57.27	
Race[a,b]						<0.0001
White	65.70	65.57	68.06	57.59	78.48	
Black	19.91	19.97	18.87	24.22	8.81	
Hispanic	11.62	11.70	10.16	13.74	10.31	
Other	2.77	2.77	2.90	4.45	2.40	
BMI (kg/m^2)[a,b,c]						<0.0001
<18.5	1.96	1.97	1.81	1.47	1.89	
18.5–24.9	50.11	50.47	43.74	30.81	40.67	
25–29.9	36.25	36.26	36.12	32.65	32.15	
>30	11.53	11.16	18.15	34.76	24.55	
PRA%[a,b]						<0.0001
0	70.29	70.36	68.97	54.11	58.12	
1–19	12.87	12.89	12.52	13.03	11.9	
20–80	12.62	12.56	13.61	16.37	10.59	
>80	3.40	3.36	4.17	15.41	3.21	
Missing	0.83	0.83	0.73	1.08	16.18	
Dialysis time[a,b]						<0.0001
0	20.47	20.38	21.96	12.24	33.83	
<24	37.73	25.52	25.59	34.41	12.06	
24–60	25.52	37.93	34.3	17.28	30.79	
>60	5.63	5.6	6.17	25.19	2.12	
Missing	10.64	10.57	11.98	10.88	21.21	
CMV[a,b]						<0.0001
D + R+	18.67	18.66	18.87	13.87	0	
D-R-	12.67	12.6	13.97	9.88	0	
R+	29.79	29.58	33.58	41.23	36.59	
Missing	38.86	39.16	33.58	35.02	63.41	
Donor factors						
Age (years)[a,b,c]						<0.0001
<18	19.65	19.98	13.79	9.06	0	
18–50	79.64	79.38	84.21	65.64	73.17	
>50	0.71	0.64	2.00	25.30	26.83	
Gender[a,b]						
Female	30.14	30.19	29.22	39.62	62.10	
Male	69.86	69.81	90.78	60.38	37.90	
Race[a,b]						<0.0001
White	63.14	63.12	63.52	71.56	79.76	
Black	18.72	18.70	19.06	12.46	7.65	
Hispanic	14.32	14.31	14.52	12.98	10.17	
Other	3.81	3.86	2.90	3.00	3.00	
BMI (kg/m^2)[a,b,c]						<0.0001
<18.5	6.45	6.43	7.99	6.9	0.93	
18.5–24.9	56.93	57.30	50.27	34.15	32.22	
25–29.9	29.91	29.65	34.66	30.89	40.86	
>30	6.62	6.54	7.99	29.76	22.60	
Hypertension[a,b,c]						<0.0001
No	95.58	95.72	93.1	73.49	97.17	
Yes	4.42	4.28	6.9	26.51	2.83	
Transplant factor						
HLA Mismatch[a,b]						<0.0001
0	0.72	0.71	0.91	12.16	8.5	
1–2	3.57	3.52	4.54	7.79	18.58	
3–6	95.70	95.77	94.56	79.49	71.81	

(Continued on following page)

TABLE 1 | (*Continued*) Baseline recipient, donor, and transplant factors of the study cohort, stratified by transplant type.

	SPK	SPK,P+	SPK,P-	DDKA	LDKA	*p*-value
	n = 10,383	*n* = 9,832	*n* = 551	*n* = 6,202	*n* = 5,673	
	46.65%	44.17%	2.48%	27.86%	25.49%	
Cold ischemic time (hours)[a,b,c]						<0.0001
<12	60.59	60.91	54.81	24.83	80.2	
12–24	33.81	33.51	39.2	53.92	1.23	
>24	1.32	1.32	1.27	17.98	0.76	
Missing	4.28	4.25	4.72	3.27	17.8	

[a]p < 0.05 for chi-squared tests comparing differences between SPK,P+, SPK,P-, DDKA, and LDKA groups.
[b]p < 0.05 for chi-squared tests comparing differences between SPK, DDKA, and LDKA groups.
[c]p < 0.05 for chi-squared tests comparing differences between SPK,P+ and SPK,P- groups.
p-value was reported for testing differences between SPK, DDKA, and LDKA groups.
BMI, body mass index; CMV, cytomegalovirus; DDKA, deceased-donor kidney transplant alone; HLA, human leukocyte antigen; LDKA, living-donor kidney transplant alone; PRA, panel reactive antibody; SPK, simultaneous pancreas-kidney transplantation; SPK,P+, simultaneous pancreas-kidney transplant recipients with a functioning pancreas graft at 3-month post-SPK; SPK,P-, simultaneous pancreas-kidney transplant recipients with loss of pancreas graft at 3-month post-SPK.

cohort study of all adult ESKD patients with T1DM who received transplants (i.e., deceased donor kidney transplant [DDKA], LDKA, and SPK) between January 1, 2000 and May 31, 2021. ESRD due to T1DM was defined based on the diagnosis as reported by transplant centers to UNOS, where the diabetic status of recipients was categorized into six groups: no diabetes, type 1, type 2, other type, type unknown, and missing. The proportion of unknown type and missing data is small (<1%). Exclusion criteria included: 1) younger than 18 years of age at the time of transplant, 2) multiorgan transplants aside from SPK, and 3) previous KT recipients. In addition, patients who died or developed kidney allograft failure within 3 months of transplant were excluded. Surviving SPK recipients with kidney allograft function at 3 months were further categorized into two groups: (1) SPK with a functioning pancreas graft at 3 months posttransplant (SPK, P+); and (2) SPK recipients with a loss of pancreas graft at 3 months posttransplant (SPK, P-). We additionally evaluated the study population without excluding patients who died or developed kidney allograft failure within 3 months of transplant in sensitivity analyses.

Outcomes

The outcomes were kidney allograft failure and patient death. For kidney allograft failure, the survival time was calculated from the date of transplantation to the date of irreversible graft failure signified by a return to dialysis, kidney re-transplantation, or patient death. For patient survival, patients were followed until death or being censored. Patient outcomes were followed-up until September 2021.

Statistical Analysis

Descriptive data were summarized as percentage (%) for categorical variables, and differences across transplant groups were compared using the chi-squared test. Accelerated failure time (AFT) models were performed in this study, because Scaled Schoenfeld Residuals indicated a violation of proportional hazards assumption in the Cox proportional hazards models (18). The Weibull distribution was selected for AFT models based on the minimum Akaike Information Criterion (AIC) among different survival distributions (i.e., exponential, loglogistic, Weibull, and lognormal). A multivariable AFT model was adjusted for recipient factor (age, gender, race, body mass index [BMI], dialysis time, panel reactive antibody [PRA], donor/recipient cytomegalic virus [CMV] serostatus), donor (age, gender, race, BMI, hypertension status), and transplant factors (cold ischemia time, human leukocyte antigen [HLA] mismatch). The results of AFT models were expressed in acceleration coefficients, which explain how much faster or slower the event of interest occurred in each group. For interpretability, the results of AFT models are exponentiated to calculate time ratio (TR), which was interpreted as the expected time to graft failure or patient death in one category relative to the referent group. Unlike the interpretation of proportional hazard model results where hazard ratios larger than 1 are equal to higher risk, TRs larger than 1 were considered to have a longer survival time compared to the reference group.

To account for the potential bias arising from confounding variables that affect the selection of patients into different groups and the outcomes, generalized boosted regression with covariates (see **Table 1**) was performed to predict a patient's propensity score of receiving a certain type of transplant, which was then used to generate the weights for the inverse probability of treatment weighted (IPTW) Kaplan-Meier curves and IPTW AFT models. Covariate balance was assessed by comparing the absolute standardized mean differences (ASMD) between the treatment groups on the pretreatment covariates before and after weighting (see **Supplementary Figure S1**). In addition, Bonferroni correction was applied to adjust for multiple comparisons.

All statistical analyses were performed using STATA 15.1 version (StataCorp, College Station, TX) and TWANG package in R statistical software version 4.0.2 (R Project for Statistical Computing).

Ethical Statements

Exemptions for study approval and informed consent were obtained for this cohort study from the Washington University in St. Louis School of Medicine Institutional Review

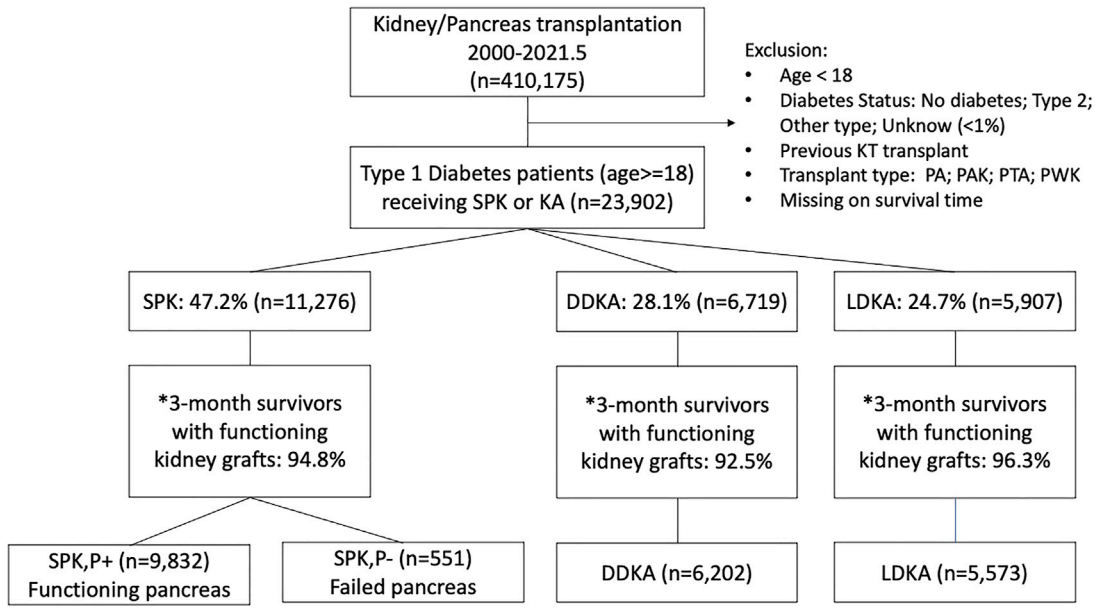


FIGURE 1 | Sampling scheme for identification of kidney transplants in recipients with kidney disease secondary to type 1 diabetes from 2000 to 2021.

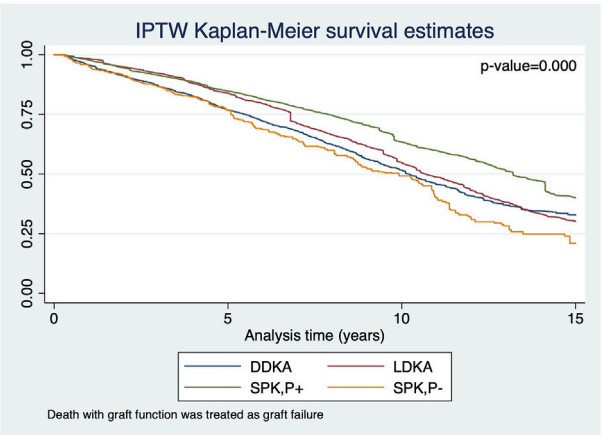


FIGURE 2 | IPTW Kaplan-Meier curves for kidney allograft survival in recipients who survived the first 3 months of transplant with functioning kidney allograft.

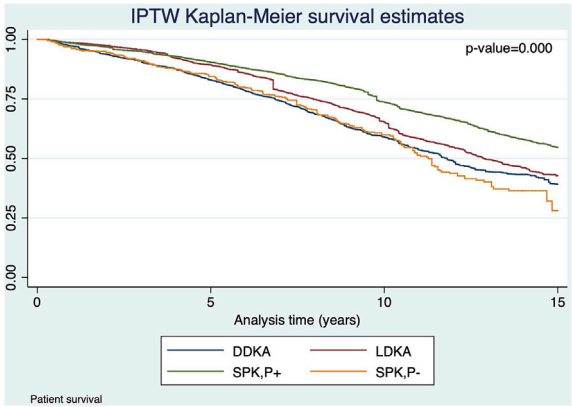


FIGURE 3 | IPTW Kaplan-Meier curves for patient overall survival in recipients who survived the first 3 months of transplant with functioning kidney allograft.

Board because the study was secondary analyses of deidentified data.

Results

Figure 1 shows the sampling scheme for identification of adult (age ≥18 years) patients with T1DM who received SPK (n = 11,276), DDKA (n = 6,719), or LDKA (n = 5,907) between 2000 and 2021. Among them, 94.8% of the SPK patients (n = 10,383), 92.5% of the DDKA patients (n = 6,202), and 96.3% of the LDKA patients (n = 5,673) survived with functioning kidney grafts within 3 months following transplantation. Early pancreas loss within 3 months occurred in 6.4% of SPK recipients. Among these 10,383 SPK recipients with functioning kidney at 3 months, 5.3% had pancreas allograft failure within 3 months (SPK,P-, n = 551), and the remaining 94.7% had a functioning pancreatic graft at 3 months (SPK,P+, n = 9,832).

Table 1 shows the baseline recipient, donor, and transplant factors of 22,258 transplants stratified by transplant type. These were statistically significantly different across transplant types. Notably, SPK recipients were more likely to be younger (age ≤50), male, and with a normal weight, but less likely to have zero HLA mismatches than kidney transplant alone recipients. DDKA recipients had higher PRA (PRA>80) and longer dialysis time

TABLE 2 | Model results of multivariable inverse probability of treatment-weighted (IPTW) weibull accelerated failure time (AFT) for graft failure and patient death.

Event	Time ratio [95% bonferroni-adjusted CI]	
	Kidney graft failure	**Patient death**
	Main analyses (References group: SPK,P+)	
LDKA	0.82 [0.70, 0.95]**	0.82 [0.68, 0.97]*
DDKA	0.77 [0.68, 0.87]***	0.71 [0.62, 0.81]***
SPK,P-	0.66 [0.56, 0.78]***	0.66 [0.55, 0.79]***
	Sensitivity analyses: without excluding patients who died or developed kidney allograft failure within 3 months of transplant (References group: SPK,P+)	
LDKA	0.75 [0.59, 0.95]***	0.72 [0.58, 0.90]**
DDKA	0.64 [0.51, 0.79]***	0.62 [0.51, 0.76]***
SPK,P-	0.35 [0.25, 0.49]***	0.36 [0.27, 0.49]***

*Note: The results of AFT models are exponentiated to calculate time ratios, which was interpreted as the expected time to graft failure or patient death in one category relative to the referent group. Multivariate analysis was adjusted for recipients' factors (age, gender, race, BMI, dialysis time, panel reactive antibody, donor/recipient cytomegalic virus serostatus), donors' factors (age, gender, race, BMI, hypertension status), and transplant factors (cold ischemia time, human leukocyte antigen mismatch). *p < 0.05; **p < 0.01; ***p < 0.001.*

(>60 months) compared to SPK and LDKA recipients. Donors for SPK were more likely to be younger (age ≤50), male, within the normal weight, and have shorter cold ischemia times (<24 h). Donors for DDKA were more likely to be hypertensive. Compared with SPK,P- recipients, SPK,P+ recipients were more likely to have normal BMI. Donors of SPK,P- recipients were more likely to be older, obese, hypertensive, and had longer cold ischemia times than donors of SPK,P+.

The Kaplan-Meier curves of SPK,P+ and LDKA recipients crossed during early years post-transplant. In the long-term, SPK,P+ recipients showed better kidney allograft survival and patient survival than LDKA, SPK,P- and DDKA recipients (**Figures 2**, **3**). **Table 2** presented results from multivariable-adjusted AFT models. Compared to SPK,P+ recipients, LDKA had 18% less graft survival time (TR 0.82, 95% Confidence Interval [CI]: 0.70, 0.95) and 18% less patient survival time (TR 0.82, 95% CI: 0.68, 0.97), DDKA had 23% less graft survival time (TR 0.77, 95% CI: 0.68, 0.87) and 29% less patient survival time (TR 0.71, 95% CI: 0.62, 0.81), and SPK,P- had 34% less graft survival time (TR 0.66, 95% CI: 0.56, 0.78) and 34% less patient survival time (TR 0.66, 95% CI: 0.55, 0.79). When including patients who died or developed kidney allograft failure within 3 months of transplant, the results for kidney and patient survival were similar.

DISCUSSION

This study analyzed the characteristics and outcomes of patients with kidney failure from T1DM who received kidney transplants from 2000 to 2021 in the United States. Overall, 47% received SPK transplants, 25% received LDKA, and 28% received DDKA. Early pancreas loss occurred in 6.4% of SPK recipients. After adjusting for propensities of the type of transplant and recipient, donor, and transplant factors captured in the transplant registry, we observed superior outcomes for kidney graft and patient survival with SPK,P+ over LDKA and DDKA. SPK recipients with early pancreas graft failure was associated with inferior kidney and patient survival compared to kidney transplant alone.

Our study found that Kaplan-Meier survival curves crossed over in the early posttransplant years and the proportional hazard assumption was violated. The survival curves showed that LDKA was associated with the best initial graft and patient survival, but long-term follow-up beyond 5 years after transplantation showed highest kidney graft and patient survival among the SPK,P+ group. This time-dependent difference in the relative survival advantage of SPK and LDKA was also found in previous studies of large transplant registries. A prior 72-month follow-up study found that LDKA was associated with significantly lower risks of kidney graft failure and patient death, while another study using the same database with longer follow-up demonstrated equivalent patient survival in SPK and LDKA recipients (9, 16). The initially better graft survival in LDKA compared with SPK may be attributed to a lower rate of delayed graft function, better HLA matching, a shorter dialysis time, a lower rate of technical problems, and lower early mortality. This result added to the evidence that differences in outcome between SPK and kidney transplantation alone can be evaluated in a valid manner only with >5 years of post-transplantation follow-up (8, 19).

Our findings of superior outcomes of SPK,P+ are similar to prior studies (12, 20). Weiss et al. found best patient survival in SPK recipients with functioning pancreas graft at 12 months posttransplant in the 1997–2005 US cohort (12). Barlow et al. found best patient survival in SPK recipients with a functioning pancreas graft at 3 months posttransplant in the 2001–2014 UK cohort (20). The survival advantage of SPK,P+ is potentially due to the long-term euglycemic effects of a functional pancreas graft. In addition, studies have found that recipients of SPK with a functioning pancreas have a lower risk of long-term cardiovascular mortality, which is the leading cause of death in kidney transplant recipients (21, 22). Furthermore, in this study, SPK recipients were on average younger, leaner, and less likely to have hypertension. These favorable recipient and donor factors may reflect an inherent bias by transplant centers to list candidates for SPK with lower preoperative risks. Noteworthy, we performed IPTW survival analyses to control for the nonrandom assignment to different transplant types and the aforementioned factors, such as age and BMI, have been accounted for, which advances the existing studies. Nonetheless, the unobserved confounding may impact this assignment and thus overestimate the benefit of SPK.

Another main finding of this study was that SPK recipients with early technical failure of the pancreas have significantly inferior kidney graft and patient survival outcomes compared to LDKA and DDKA, although the negative influence was mitigated in subgroup recipients with >5 years of post-transplantation follow-up. The difference can be explained in large part because of complications associated or resulting from loss of the pancreas graft, including hemorrhage, sepsis, pancreatitis, thrombosis, and systemic inflammatory response (23). In SPK recipients, avoiding early technical failure of the pancreas is of great importance to avoid associated kidney graft loss, which may

be achieved through improved surgical technique, proper immunosuppression, appropriate donor and recipient selection, and early detection of graft failure.

Most previous studies have concluded that living kidney donation shows better graft survival than those from deceased donors (24, 25). Surprisingly, this study did not find a significant difference in kidney graft survival between LDKA and DDKA. There are several possible explanations. First, the frequency of acute rejection episodes is lower among LDKA, which reduces chronic rejection and thereby increases long-term graft survival (26). By excluding those patients who died or developed kidney allograft failure within 3 months posttransplant, this study likely underestimated the long-term survival benefit of LKDA. Second, this analysis has controlled for recipient age, pretransplant diabetes mellitus, pretransplant PRA, donor race, sex, hypertension, and preservation-related factors such as long cold ischemia, all of which are major risk factors of graft survival rates for LDKA and DDKA (27). Additionally, we performed IPTW survival analyses to control for the nonrandom assignment to different transplant types and thus the potential bias caused by healthier LDKA recipients and donors is reduced.

Several limitations of our study should be noted. First, this was a retrospective study that can identify associations but not prove causation; therefore, the results should be interpreted carefully. Second, there was a small amount of missing data for certain factors, although we attempted to reduce their impact by adjusting for "missing" status. Third, selection bias may have occurred at the time of listing, especially as candidates with higher surgical risks may have been more suitable for kidney transplant. Although generalized boosted regression was used to estimate propensity scores to improve covariate balance across the groups, sufficient overlap of the scores across groups is not guaranteed. Last, despite the long follow-up of this study, this database did not allow for the tracking of diabetic complications, such as retinopathy and neuropathy, and their consequential impact on quality of life. These complications may be curtailed by a functioning pancreas graft as long-term benefits in addition to graft and patient survival. Future studies addressing these issues are warranted.

In conclusion, SPK recipients with early functioning pancreas had superior kidney allograft and patient survival compared with SPK,P-, LDKA, and DDKA recipients. Our findings highlight the long-term benefits of SPK utilization; however, the benefits are dependent on early pancreas allograft function. More research is needed to identify surgical and medical factors that increase the risks for early pancreas graft loss, such as high pancreas donor risk index and longer preservation time, to optimize patient outcomes.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: STAR (Standard Transplant Analysis and Research) files. https://optn.transplant.hrsa.gov/data/view-data-reports/request-data/data-request-instructions/.

ETHICS STATEMENT

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

AUTHOR CONTRIBUTIONS

MJ analyzed and interpreted data, drafted the paper, revised it critically, gave final approval of the version to be published and agrees to be accountable for all aspects of the work with regard to its accuracy and integrity. TA and SC designed the study, acquired data, interpreted data, revised the paper critically, gave final approval of the version to be published, and agree to be accountable for all aspects of the work with regard to its accuracy and integrity. MW and WH analyzed data, interpreted data, gave final approval of the version to be published, and agree to be accountable for all aspects of the work with regard to its accuracy and integrity. KL, MM, HM, MI, and JW interpreted data, revised the paper critically, gave final approval of the version to be published, and agree to be accountable for all aspects of the work with regard to its accuracy and integrity. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by grant 4746 from the Foundation for Barnes-Jewish Hospital.

AUTHOR DISCLAIMER

The data reported here have been supplied by the United Network for Organ Sharing (UNOS) as the contractor for the Organ Procurement and Transplantation Network (OPTN). The interpretation and reporting of these data are the responsibility of the author(s) and in no way should be seen as an official policy of or interpretation by the OPTN or the US government.

CONFLICT OF INTEREST

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

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10618/full#supplementary-material

Supplementary Figure S1 | Balance before and after weighting.

REFERENCES

1. United States Renal Data System (USRDS). *USRDS Annual Data Report: Epidemiology of Kidney Disease in the United States*. Bethesda, MD: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases (2018).
2. Harding JL, Pavkov M, Wang Z, Benoit S, Burrows NR, Imperatore G, et al. Long-term Mortality Among Kidney Transplant Recipients with and without Diabetes: a Nationwide Cohort Study in the USA. *BMJ Open Diabetes Res Care* (2021) 9:e001962. doi:10.1136/bmjdrc-2020-001962
3. Cosio FG, Hickson LJ, Griffin MD, Stegall MD, Kudva Y. Patient Survival and Cardiovascular Risk after Kidney Transplantation: the challenge of Diabetes. *Am J Transpl* (2008) 8(3):593–9. doi:10.1111/j.1600-6143.2007.02101.x
4. Giorda CB, Carnà P, Salomone M, Picariello R, Costa G, Tartaglino B, et al. Ten-year Comparative Analysis of Incidence, Prognosis, and Associated Factors for Dialysis and Renal Transplantation in Type 1 and Type 2 Diabetes versus Non-diabetes. *Acta Diabetol* (2018) 55(7):733–40. doi:10.1007/s00592-018-1142-y
5. Taber DJ, Meadows HB, Pilch NA, Chavin KD, Baliga PK, Egede LE. Pre-existing Diabetes Significantly Increases the Risk of Graft Failure and Mortality Following Renal Transplantation. *Clin Transpl* (2013) 27(2):274–82. doi:10.1111/ctr.12080
6. Jiang AT, BHScRowe N, Sener A, Luke P. Simultaneous Pancreas-Kidney Transplantation: The Role in the Treatment of Type 1 Diabetes and End-Stage Renal Disease. *Can Urol Assoc J* (2014) 8(3-4):135–8. doi:10.5489/cuaj.1597
7. McCullough KP, Keith DS, Meyer KH, Stock PG, Brayman KL, Leichtman AB. Kidney and Pancreas Transplantation in the United States, 1998-2007: Access for Patients with Diabetes and End-Stage Renal Disease. *Am J Transpl* (2009) 9(4):894–906. doi:10.1111/j.1600-6143.2009.02566.x
8. Morath C, Zeier M, Döhler B, Schmidt J, Nawroth PP, Schwenger V, et al. Transplantation of the Type 1 Diabetic Patient: the Long-Term Benefit of a Functioning Pancreas Allograft. *Clin J Am Soc Nephrol* (2010) 5(3):549–52. doi:10.2215/CJN.03720609
9. Young BY, Gill J, Huang E, Takemoto SK, Anastasi B, Shah T, et al. Living Donor Kidney versus Simultaneous Pancreas-Kidney Transplant in Type I Diabetics: an Analysis of the OPTN/UNOS Database. *Clin J Am Soc Nephrol* (2009) 4(4):845–52. doi:10.2215/CJN.02250508
10. Morath C, Zeier M, Döhler B, Schmidt J, Nawroth PP, Opelz G. Metabolic Control Improves Long-Term Renal Allograft and Patient Survival in Type 1 Diabetes. *J Am Soc Nephrol* (2008) 19(8):1557–63. doi:10.1681/ASN.2007070804
11. Kandaswamy R, Stock PG, Gustafson SK, Skeans MA, URban R, Fox A, et al. OPTN/SRTR 2017 Annual Data Report: Pancreas. *Am J Transpl* (2019) 19(2):124–83. doi:10.1111/ajt.15275
12. Weiss AS, Smits G, Wiseman AC. Twelve-month Pancreas Graft Function Significantly Influences Survival Following Simultaneous Pancreas-Kidney Transplantation. *Clin J Am Soc Nephrol* (2009) 4(5):988–95. doi:10.2215/CJN.04940908
13. Norman SP, Kommareddi M, Ojo AO, Luan FL. Early Pancreas Graft Failure Is Associated with Inferior Late Clinical Outcomes after Simultaneous Kidney-Pancreas Transplantation. *Transplantation* (2011) 92(7):796–801. doi:10.1097/TP.0b013e31822dc36b
14. Hill M, Garcia R, Dunn T, Kandaswamy R, Sutherland DE, Humar A. What Happens to the Kidney in an SPK Transplant when the Pancreas Fails Due to a Technical Complication? *Clin Transpl* (2008) 22(4):456–61. doi:10.1111/j.1399-0012.2008.00809.x
15. Viglietti D, Serrato T, Abboud I, Antoine C, Pillebout E, Busson M, et al. Kidney Graft Dysfunction in Simultaneous Pancreas-Kidney Recipients after Pancreas Failure: Analysis of Early and Late Protocol Biopsies. *Clin Transpl* (2013) 27(3):E249–55. doi:10.1111/ctr.12095
16. Reddy KS, Stablein D, Taranto S, Stratta RJ, Johnston TD, Waid TH, et al. Long-term Survival Following Simultaneous Kidney-Pancreas Transplantation versus Kidney Transplantation Alone in Patients with Type 1 Diabetes Mellitus and Renal Failure. *Am J Kidney Dis* (2003) 41:464–70. doi:10.1053/ajkd.2003.50057
17. Das DM, Huskey JL, Harbell JW, Heilman RL, Singer AL, Mathur A, et al. Early Technical Pancreas Failure in Simultaneous Pancreas-Kidney Recipients Does Not Impact Renal Allograft Outcomes. *Clin Transpl* (2021) 35(1):e14138. doi:10.1111/ctr.14138
18. Zare A, Hosseini M, Mahmoodi M, Mohammad K, Zeraati H, Holakouie Naieni K. A Comparison between Accelerated Failure-Time and Cox Proportional Hazard Models in Analyzing the Survival of Gastric Cancer Patients. *Iran J Public Health* (2015) 44(8):1095–102.
19. Fioretto P, Steffes MW, Sutherland DE, Goetz FC, Mauer M. Reversal of Lesions of Diabetic Nephropathy after Pancreas Transplantation. *N Engl J Med* (1998) 339(69–75):69–75. doi:10.1056/NEJM199807093390202
20. Barlow AD, SaebParsy K, Watson CJ. An Analysis of the Survival Outcomes of Simultaneous Pancreas and Kidney Transplantation Compared to Live Donor Kidney Transplantation in Patients with Type 1 Diabetes: a UK Transplant Registry Study. *Transpl Int* (2017) 30(9):884–92. doi:10.1111/tri.12957
21. Biesenbach G, Konigsrainer A, Gross C, Margreiter R. Progression of Macrovascular Diseases Is Reduced in Type 1 Diabetic Patients after More Than 5 Years Successful Combined Pancreas-Kidney Transplantation in Comparison to Kidney Transplantation Alone. *Transpl Int* (2005) 18:1054–60. doi:10.1111/j.1432-2277.2005.00182.x
22. Orsenigo E, Socci C, Fiorina P, Zuber V, Secchi A, Di Carlo V, et al. Cardiovascular Benefits of Simultaneous Pancreas-Kidney Transplant versus Kidney Alone Transplant in Diabetic Patients. *Transpl Proc* (2005) 37(8):3570–1. doi:10.1016/j.transproceed.2005.09.059
23. Mittal S, Johnson P, Friend P. Pancreas Transplantation: Solid Organ and Islet. *Cold Spring Harb Perspect Med* (2014) 4(4):a015610. doi:10.1101/cshperspect.a015610
24. Tarantino A. Why Should We Implement Living Donation in Renal Transplantation? *Clin Nephrol* (2000) 53(4):suppl 55-63–63.
25. Davis CL, Delmonico FL. Living-donor Kidney Transplantation: a Review of the Current Practices for the Live Donor. *J Am Soc Nephrol* (2005) 16(7):2098–110. doi:10.1681/ASN.2004100824
26. Naderi GH, Mehraban D, Kazemeyni SM, Darvishi M, Latif AH. Living or Deceased Donor Kidney Transplantation: a Comparison of Results and Survival Rates Among Iranian Patients. *Transpl Proc* (2009) 41(7):2772–4. doi:10.1016/j.transproceed.2009.07.041
27. Zhang H, Wei Y, Liu L, Li J, Deng R, Xiong Y, et al. Different Risk Factors for Graft Survival between Living-Related and Deceased Donor Kidney Transplantation. *Transpl Proc* (2018) 50(8):2416–20. doi:10.1016/j.transproceed.2018.03.047

ESOT | Transplant International

ORIGINAL RESEARCH
published: 15 September 2022
doi: 10.3389/ti.2022.10714

Recoverability of Diabetic Nephropathy of Donor Kidney After Kidney Transplantation

*Kyo Won Lee[1†], Jongmin Sim[2†], Sean S. W. Park[1], Junseok Jeon[3], Gyuri Kim[4], Min Jung Kim[5], Ghee Young Kwon[6], Hye Ryoun Jang[3], Wooseong Huh[3] and Jae Berm Park[1]**

[1]Department of Surgery, Samsung Medical Center, Sungkyunkwan University, Seoul, South Korea, [2]Department of Pathology, Anam Hospital, Korea University, Seoul, South Korea, [3]Division of Nephrology, Department of Medicine, Samsung Medical Center, Sungkyunkwan University, Seoul, South Korea, [4]Division of Endocrinology and Metabolism, Department of Medicine, Samsung Medical Center, Sungkyunkwan University, Seoul, South Korea, [5]Department of Surgery, Seoul Medical Center, Seoul, South Korea, [6]Department of Pathology, Samsung Medical Center, Sungkyunkwan University, Seoul, South Korea

Some kidney donors have diabetes, and little of their natural course of diabetic nephropathy (DN) is known. The aim of this study was to analyze the changes in pathologic lesions in the diabetic donor kidney after KT by performing protocol biopsy two weeks and one year after KT. This retrospective study included 103 patients who underwent KT, with kidneys from donors with a history of diabetes mellitus (DM). Among them, data of 34 patients who underwent biopsy two weeks and one year after KT were reviewed. Biopsy specimens were reviewed using light microscopy and electron microscopy. Glomerular basement membrane (GBM) thickness at 2 weeks and 1 year was compared. Biopsy showed that DN occurred in 29 of the 34 patients. Only trivial histological changes were observed in 22 patients (64.7%), including 5 patients who did not show DN. At one year after transplantation, there was no change in the DN histologic class in 26 patients (76.5%), and there was no statistically significant difference in the change in GBM thickness. This pattern was observed regardless of the recipient's DM or glycemic control. With this understanding, clinicians can use kidneys from DM donors with more comfort, thereby reducing the kidney discard rate.

Keywords: kidney transplantation, biopsy, donor, diabetic nephropathy, glomerulus basement membrane

OPEN ACCESS

***Correspondence:**
Jae Berm Park
jbparkmd@gmail.com

†These authors have contributed equally to this work

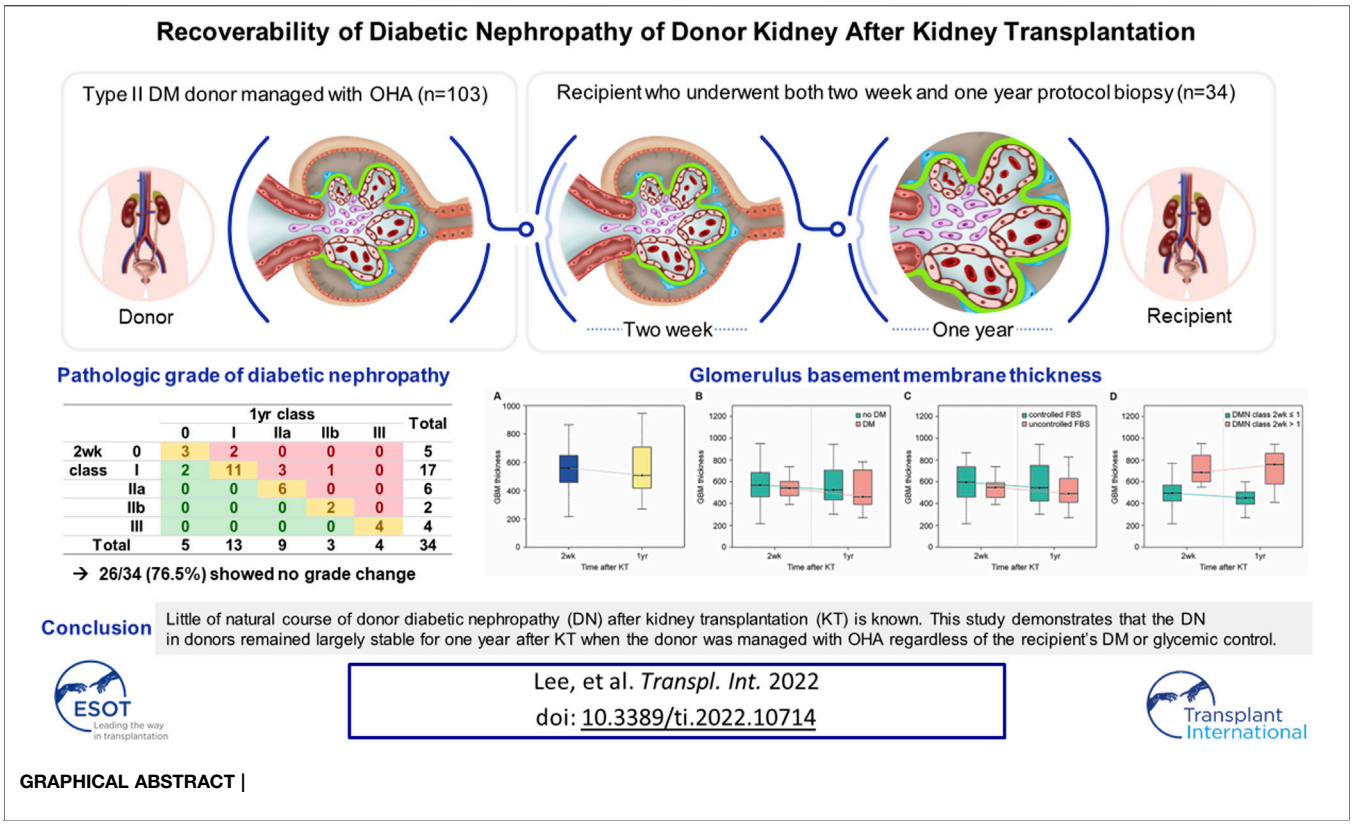
Received: *19 June 2022*
Accepted: *05 September 2022*
Published: *15 September 2022*

Citation:
Lee KW, Sim J, Park SSW, Jeon J, Kim G, Kim MJ, Kwon GY, Jang HR, Huh W and Park JB (2022) Recoverability of Diabetic Nephropathy of Donor Kidney After Kidney Transplantation. Transpl Int 35:10714. doi: 10.3389/ti.2022.10714

INTRODUCTION

Several studies have demonstrated that kidney transplantation is the treatment of choice in patients with end-stage renal disease. Therefore, attempts have been made to implement more kidney transplantations (KTs) and expand the donor criteria. With the expansion of the donor criteria, the number of KT with a diabetic donor kidney is also increasing. The effect of diabetic donors on the outcome of KT is controversial. A study by Mohan et al showed that diabetes mellitus (DM) alone in donors did not appear to have any effect on death-censored graft survival (1). but in a study by

Abbreviations: EM, electron microscopy; DM, diabetes mellitus; DN, diabetic nephropathy; FBS, fasting blood sugar; GBM, glomerular basement membrane; KT, kidney transplantation; LM, light microscopy; MGN, membranous glomerulonephritis; PTDM, post-transplant diabetes mellitus.



GRAPHICAL ABSTRACT |

Ahmad et al, there was a significant difference in death-censored graft survival depending on the presence or absence of DM in the donor. Although statistically significant, the 10-year death-censored graft survival was not considerably different, with 57.1% in the DM group and 54.6% in the non-DM group (2). By analyzing the United Network for Organ Sharing (UNOS) registry data, Cohen et al confirmed that allograft survival was significantly lower when a kidney from a diabetic donor was used, and reported that the difference in allograft survival was also significantly affected by the presence or absence of DM in the recipient (3). These results suggest that diabetic nephropathy (DN) is affected by glycemic control.

DN in patients with type 1 DM may be reversible when diabetes is cured by pancreas transplantation (4). However, it is not well documented whether pathologic changes in DN in type 2 DM can also be reversible, as in type 1 DM. It is difficult to evaluate the reversibility of pathologic lesions of DN in patients with type 2 DM because there is no established treatment that cures type 2 DM, and these patients often have several comorbidities that can affect kidney disease, including metabolic syndrome.

In recent years, many efforts have been made to reduce the donor kidney discard rate, and understanding the natural course of donor DN is essential to reduce the discard rate in the reality that more than 40% of kidneys from diabetic donors are discarded (5). However, only few studies have evaluated the pathologic status and changes in the kidneys of DM donors and these studies included a small number of patients with DN (6,7,8,9). Therefore, to reach a more robust conclusion, biopsy results at regular intervals are needed in a larger number of patients.

The aim of this study was to analyze the changes in pathologic lesions in the DM donor kidney after KT in a large number of patients and for the same 1-year duration by performing biopsy at 2 weeks and 1 year after KT. In addition, the difference in the change according to the recipient's DM status, glycemic control, and severity of donor kidney DN was also determined.

MATERIALS AND METHODS

Study Design

Among the patients who underwent KT between January 2013 and December 2018 at Samsung Medical Center, 103 recipients received kidneys from donors with a history of DM but only 37 recipients completed full sets of post-transplant protocol biopsies. A retrospective review of those patients was carried out, and three patients were excluded as their graft tissue samples were inappropriate for assessment, leaving 34 patients for the final analysis. In our center, we perform post-transplant protocol biopsy at 2 weeks and 1 year. The biopsy tissue at 2 weeks is considered to reflect the donor's DN status, and the tissue at 1 year the recipient's glycemic control status over the first year after KT. The protocol biopsy is contraindicated if the patient does not consent or if percutaneous coronary intervention had been performed within the preceding year of surgery, requiring ongoing anticoagulation. Pediatric cases and donation after circulatory death

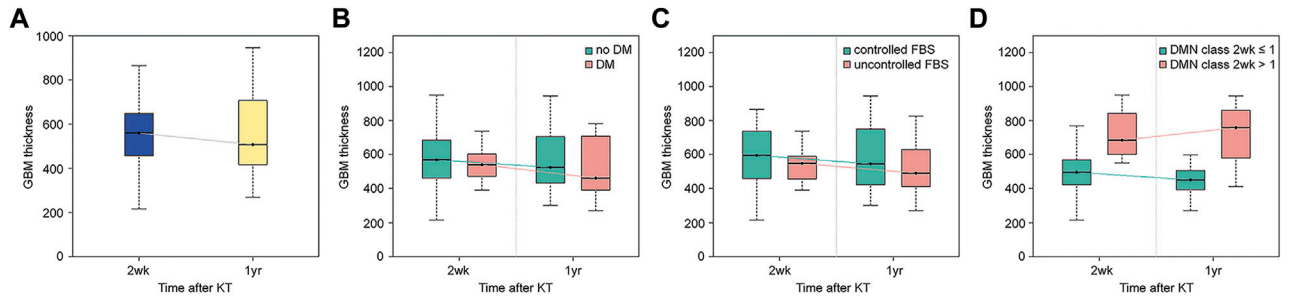


FIGURE 1 | Representative light microscopic images of renal biopsy specimens obtained from patients with kidney transplantation (periodic acid-Schiff stain, ×400). **(A)** Nonspecific change, diabetic nephropathy (DN) class I. Minimal mesangial change was seen. **(B)** Mild mesangial expansion and hypercellularity, DN class IIa. **(C)** Marked mesangial expansion and hypercellularity without nodule formation, DN class IIb. **(D)** Marked mesangial expansion and nodule formation, DN class III.

were excluded from the study as they are not routinely included in our protocol biopsy.

Recipient DM was defined as a history of DM or DM medication requirement after transplantation. Uncontrolled fasting blood sugar (FBS) was defined as an FBS level of ≥126 mg/dl, which was observed two times or more from 2 months after KT when the maintenance steroid dose (methylprednisolone 4mg per day) was being administered.

The institutional review board of Samsung Medical Center approved this study protocol (SMC 2020–12-139) and waived the requirement for obtaining patients' written informed consent because of the retrospective nature of the study and as the data used were anonymized.

Post-Transplant Management

For induction immunosuppression, basiliximab (20 mg/day, 2 days) and rabbit antithymocyte globulin (1.5 mg/kg, 3 days) were used. For maintenance immunosuppression, all patients were treated with a triple immunosuppressive regimen of tacrolimus, mycophenolate mofetil, and methylprednisolone. For therapeutic monitoring of tacrolimus, the tacrolimus trough level was monitored and the dosage was adjusted to maintain a target concentration of 8–10 ng/ml during 1 month post-KT, 5–8 ng/ml during 1 month to 1 year, and 3–7 ng/ml afterward. Methylprednisolone was started on the day of surgery at an intravenous dose of 500 mg/day and administered for 2 days and then tapered by half every day to 60 mg/day. Oral methylprednisolone was administered at 32 mg/day for 7 days, 16 mg/day for the next 2 weeks, 8 mg/day for the next month, and 4 mg/day for maintenance. Post-transplant steroids were gradually tapered off and totally withdrawn 6 months after KT.

Blood glucose measurements were continued using a glucometer 4 times a day in patients who underwent kidney transplantation. In most cases, after administration of high-dose steroids, there was a rapid rise in blood glucose level, and when pre-meal blood glucose levels continued to exceed 200 mg/dl, multiple daily insulin injections were started and the insulin dose was titrated according to the blood glucose level. If the pre-meal blood glucose level was 150–200 mg/dl, oral hypoglycemic agents were used. Subsequent reductions in the steroid dose according to the immunosuppressive protocol resulted in a decrease in insulin requirements and a 10%–20% reduced insulin dose was administered. When the low-dose steroid was maintained and the blood glucose was well controlled, insulin administration was switched to oral hypoglycemic agents or discontinued.

TABLE 1 | Patient characteristics.

	N
Donor age (years, mean ± SD)	60.38 ± 9.53
Male donor (n, %)	24 (70.6)
Donor BMI (kg/m^2, mean ± SD)	24.27 ± 3.7
Donor HTN (n, %)	20 (58.8)
Terminal creatinine (mg/dL, mean ± SD)	1.6 ± 0.9
Donor DM duration (years, median [range])	7.5 [1.0, 22.0]
Donor DM medication	
OHA (n, %)	31 (91.2)
No treatment (n, %)	2 (5.9)
Unknown (n, %)	1 (2.9)
Donor HbA1c (%, mean ± SD)	6.9 ± 1.4
Donor proteinuria[a] (n, %)	13 (30.2)
LD/DD	2/32
Recipient age (years, mean ± SD)	53.82 ± 10.68
Male recipient (n, %)	20 (58.8)
Recipient BMI (kg/m^2, mean ± SD)	22.87 ± 2.97
Recipient diabetes (n, %)	11 (32.4)
Recipient HTN (n, %)	29 (85.3)
Cause of ESRD (n, %)	
DM	10 (29.4)
HTN	3 (8.8)
GN	4 (11.8)
Others	17 (50.0)
Patients with previous transplants (n, %)	3 (8.8)
dialysis duration (day, mean ± SD)	2337.09 ± 1032.23

SD, standard deviation; BMI, body mass index; HTN, hypertension; LD, living donor; DD, deceased donor; ESRD, end-stage renal disease; GN, glomerulonephritis.
[a]Donor proteinuria was defined when dipstick ≥2+.

Histologic Assessment of DN

The assessment of renal biopsy specimens was undertaken by a specialist renal pathologist, who was blinded to the clinical details. Biopsy specimens were reviewed by light microscopy (LM) and electron microscopy (EM). Immunofluorescence staining results

TABLE 2 | Change in diabetic nephropathy histologic class.

		1-Year class					Total
		0	I	IIa	IIb	III	
2-Week class	0	3	2	0	0	0	5
	I	2	11	3	1	0	17
	IIa	0	0	6	0	0	6
	IIb	0	0	0	2	0	2
	III	0	0	0	0	4	4
Total		5	13	9	3	4	34

were reviewed with the pathology reports. Hematoxylin and eosin staining, and periodic acid-Schiff staining were performed for LM sections. The details of the histopathological features examined are as follows: the number of total glomeruli/globally and segmentally sclerotic glomeruli, as well as mesangial expansion. The histological examinations were performed twice under LM before reaching the final classification of DN. During the process, we encountered a discrepancy in only one case, where an additional independent assessment was performed to resolve the inconsistency. The thickness of the glomerular basement membrane (GBM) was measured through EM, and samples with a relatively uniform thickness were measured at five locations, and non-uniform cases were measured at up to 21 locations; the average of the measured values was used for analysis.

The criteria suggested in previous studies were used for histological classification of DN (10, 11). Class I was defined as a change in the LM that was insignificant and when the GBM was thickened upon observation under EM (by definition, exceeding the average thickness of 430 nm in males and 395 nm in females). Class II was defined as mesangial expansion seen in >25% of the glomeruli upon observation with LM (mild mesangial expansion was referred to as IIa and severe mesangial expansion was referred to as IIb). Class III was defined as the mesangial expansion to form a nodule, and class IV was defined as global sclerosis in more than half of the glomeruli (**Figure 1**).

Statistical Analysis

Statistical analysis was performed using SAS version 9.4 (SAS Institute, Cary, NC, United States) and R 4.0.3 (Vienna, Austria; http://www.R-project.org/) software. GBM thickness at 2 weeks and that at 1 year were compared using paired t-test and Wilcoxon signed-rank test, and logistic regression test was used for the DN progression risk-factor analysis. Cox regression test was used for the graft failure risk-factor analysis. Statistical significance was defined as a P-value < 0.05.

RESULTS

The donor and recipient information of the 34 cases is summarized in **Table 1**. The mean age of donors was 60.4 years, 94% (32/34) were brain-dead donors, and the pre-transplant serum creatinine level was 1.6 ± 0.9 mg/dl. All donors had insulin independent type 2 DM, 31 (91.2%) of whom were on oral hypoglycemic agent (OHA). The mean age of recipients was 53.8 years, and 32.4% (11/34) had a history of DM.

One patient was found to have stage I membranous glomerulonephritis (MGN), with concurrent DN (12). This patient was diagnosed with Class IIa DN on both protocol biopsies at 2 weeks and 1 year. The rest of the patients were negative for immunoglobulin G (IgG), IgA, IgM, complement 1q (C1q), C3, and C4. Furthermore, electron dense deposit was not present in all other patients except for the one with MGN.

Changes in the DN Histologic Class

Table 2 summarizes the changes in the DN histologic class in the biopsy at 2 weeks and 1 year. At the 2-week biopsy, five patients were classified as having class 0 (no DN), 17 as having class I, 6 as having class IIa, two as having class IIb, 4 as having class III, and none as having class IV. None of the donor characteristics including age, duration of DM, HbA1c were not associated with the DN class (**Supplementary Table S1**). The donors whose DN class was III were found to have had DM for more than 6 years (**Supplementary Figure S1**). At the 1-year biopsy, 5 patients were classified as having class 0, 13 as having class I, 9 as

TABLE 3 | Change in diabetic nephropathy histologic class according to recipient DM status.

		Non-DM recipient						DM recipient					
		1-Year class					Total	1-Year class					Total
		0	I	IIa	IIb	III		0	I	IIa	IIb	III	
2-Week class	0	2	2	0	0	0	4	1	0	0	0	0	1
	I	1	6	2	1	0	10	1	5	1	0	0	7
	IIa	0	0	5	0	0	5	0	0	1	0	0	1
	IIb	0	0	0	2	0	2	0	0	0	0	0	0
	III	0	0	0	0	2	2	0	0	0	0	2	2
Total		3	8	7	3	2	23	2	5	2	0	2	11

DM, diabetes mellitus.

TABLE 4 | Change in diabetic nephropathy histologic class according to recipient FBS control status.

		Controlled FBS						Uncontrolled FBS					
		1-Year class					Total	1-Year class					Total
		0	I	IIa	IIb	III		0	I	IIa	IIb	III	
2-Week class	**0**	2	2	0	0	0	4	1	0	0	0	0	1
	I	0	3	0	1	0	4	2	8	3	0	0	13
	IIa	0	0	5	0	0	5	0	0	1	0	0	1
	IIb	0	0	0	2	0	2	0	0	0	0	0	0
	III	0	0	0	0	1	1	0	0	0	0	3	3
Total		2	5	5	3	1	16	3	8	4	0	3	18

FBS, fasting blood sugar.

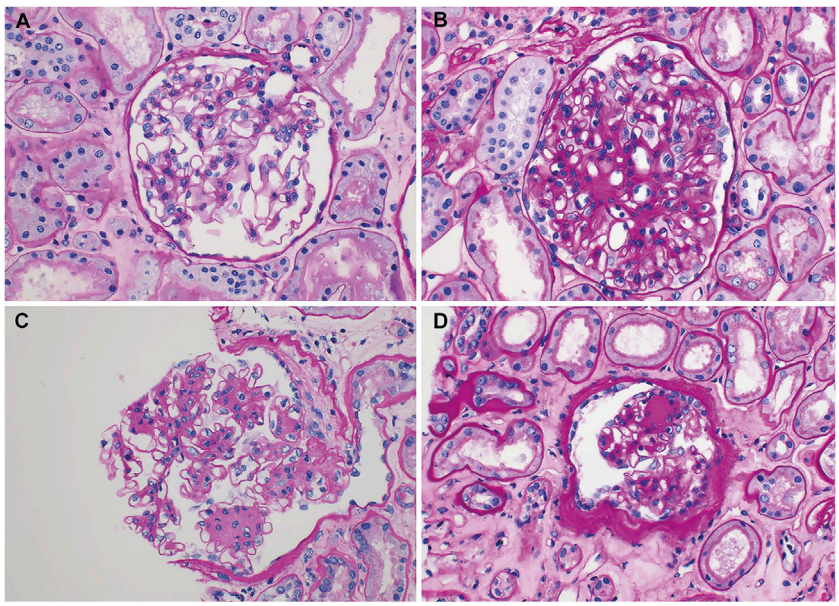


FIGURE 2 | Representative electron microscopic images of renal biopsy specimens obtained from kidney transplantation patients with nonspecific changes (diabetic nephropathy, grade 0 or I) in the light microscope and a change in the electron microscope. In addition to the typical measurement values shown in the images, the average value was obtained by additionally measuring for up to 16 points. **(A,B)** Progression; **(A)** segmental and mild thickening of the glomerular basement membrane (GBM), measuring 318–511 nm (375 nm in mean) (original magnification, ×4000) and **(B)** uniformly thickened GBM, measuring 395–611 nm (536 nm in mean) (original magnification, ×3500). **(C,D)** Regression; **(C)** marked thickening with segmental normal thickness of the GBM, measuring 254–767 nm (600 nm in mean) (original magnification, ×5000) and **(D)** marked, but segmental thickening of the GBM, measuring 208–562 nm (372 nm in mean) (original magnification, ×6000).

having class IIa, 3 as having class IIb, 4 as having class III, and none as having class IV.

Between 2 weeks and 1 year, the histologic class regressed in two patients (6.9%) and progressed in 6 patients (17.6%), and there was no change in the histologic class in 26 patients (76.5%). In most cases, there was no change in histologic class, and this pattern did not change even when classifying recipients according to DM or FBS control status. There was no change in histologic class in 9 out of 11 recipients with DM (81.8%), and no change in histologic class in 17 out of 23 recipients without DM (73.9%). The number of patients who showed regression was one in each group, and the number of patients who showed progression was 5 (21.7%) in the non-DM group and one (9.1%) in the DM group (**Table 3**). The same pattern was observed when the patients were classified according to their FBS control status. Class change was not observed in 13 (81.3%) of the 16 patients with controlled FBS, and there was no change in class in 13 (72.2%) of the 18 patients with uncontrolled FBS. Regression occurred in only two patients with uncontrolled FBS, and progression occurred in three patients in each group (controlled FBS, 23.1% and uncontrolled FBS, 16.7%) (**Table 4**). The status of recipient DM or uncontrolled FBS was not evaluated as a risk factor of the histologic grade progression of DN (**Supplementary Table S2**).

Change in GBM Thickness

Compared with the GBM thickness measured at the 2-week biopsy, that at 1-year biopsy decreased in 21 patients (**Figures 2A,B**) and increased in 13 patients (**Figures 2C,D**). The mean GBM thickness

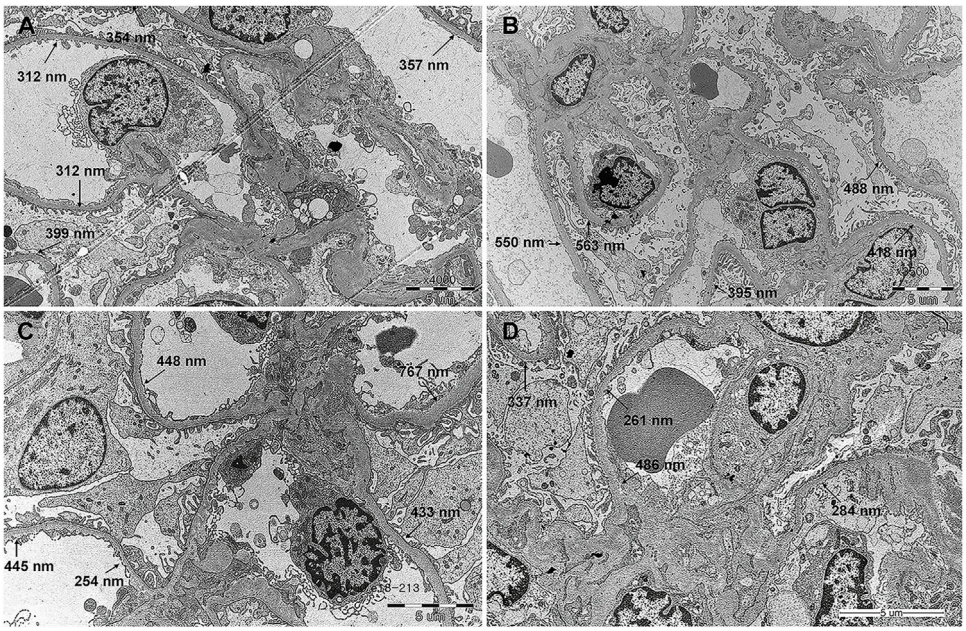


FIGURE 3 | Change in glomerulus basement membrane thickness from 2 weeks to 1 year after kidney transplantation. **(A)** All patients. **(B)** Patients were divided according to recipient diabetes status. **(C)** Patients were divided according to fasting blood sugar control status. **(D)** Patients were divided according to diabetic nephropathy histologic class at 2 weeks after kidney transplantation.

showed a decreasing trend, but the difference was not statistically significant (**Figure 3A**, $p = 0.29$). When patients were classified based on the presence or absence of recipient DM, the thickness increased in 3 out of 11 patients (27.3%) with DM and decreased in 8 (72.7%). The average thickness at both time points decreased in both groups, but the difference was not statistically significant (**Figure 3B**, $p = 0.21$ and 0.73, respectively).

When the patients were classified based on recipient FBS control status, the thickness increased in 5 out of 18 (27.8%) patients with uncontrolled FBS and decreased in 13 (72.2%), and it increased in 8 out of 16 (50%) patients with controlled FBS and decreased in 8 (50%). The average thickness at both time points appeared to decrease in both groups, but the difference was not statistically significant (**Figure 3C**, $p = 0.24$ and 0.85, respectively). When the patients were classified based on the severity of DN, the thickness increased in 8 of the 22 patients with class I or lower disease and decreased in 14. Thickness increased in five of the 12 patients with class II or higher disease and decreased in 7. The average thickness decreased in patients with class I or lower disease during both time points and increased in patients with class II or higher disease, but the difference between the mean values was not statistically significant (**Figure 3D**, $p = 0.10$ and 0.81, respectively).

Clinical Outcomes After Kidney Transplantation

The median follow-up of the patients was 48.5 months. No patient died during this period, but 6 patients (17.6%) lost their grafts. The mean GFRs at 2 weeks and 1 year were 49.1 ± 22.5, and 51.2 ± 15.3 (mL/min/1.73 m^2), respectively (**Supplementary Table S3**). The cause of graft failure was attributed to recurrent rejection and septic shock in two cases, cardiovascular shock after aortic dissection in one case, DN progression in one case. Two cases did not have any obvious cause identified. Among the 6 patients who lost their graft, three patients showed class IV DN at 2-week protocol biopsy and the other three class I DN. A risk factor analysis for graft failure demonstrated that an evidence of class III DN at 2-week biopsy was the only independent risk factor ($p < 0.001$) even though DN was a cause of graft failure in only one patient. However, progression of DN was not a significant risk factor for graft failure with p value of 0.61 (**Supplementary Table S4**).

DISCUSSION

We have analyzed how donor DN changes over the year after KT. Pathological biopsy of patients who received KT from 34 DM donors showed that DN occurred in 29 of the 34 patients. However, 17 of them (50% of the total patients) were classified as having class I, a mild case with only an increase in GBM thickness observed under EM. Minor histological changes were observed in 22 patients (64.7% of the total), including 5 patients who did not show DN. At 1 year after transplantation, there was no change in the DN histologic class in 26 patients (76.5%), and there was no statistically significant difference in the change in GBM thickness. This pattern was observed regardless of the recipient's DM or FBS control status.

Based on a study by Fioretto et al that reported improved DN after pancreas transplantation, DN is known to improve with good glycemic control (4, 13, 14). In this study, the histological findings of DN improved when the blood sugar levels were normalized by

pancreas transplantation; this was not a short-term phenomenon, and histological improvements occurred 10 years after the transplantation. Abouna et al reported a case in which histological improvements occurred following KT with a donor's kidney with DN in a non-DM patient (15). Similarly, Harada et al investigated how histological lesions changed over a year after good glycemic control in three non-diabetic recipients who underwent KT with donor kidneys showing early diabetic nephropathy (two class I patients and one class IIa patient). The recipients who had pre-existing DM, or who developed post-transplant DM (PTDM) or new-onset diabetes after transplantation (NODAT), were excluded. The study demonstrated that the early diabetic changes in the graft improved in all patients after good glycemic control post KT. However, in this study, even in patients with no history of DM or PTDM (n = 23), the class of DN was stable or progressed after 1 year of KT (**Table 3**), and the change in GBM thickness was also not significant (**Figure 3B**). The class of DN was found to remain stable or progress (**Table 4**) even in the group that was selected more stringently, which excluded those with uncontrolled FBS (n = 16), and the change in GBM thickness was not significant (**Figure 3C**). This may be because the period of 1 year was short, as changes in glycemic control for a sufficient period are required to induce histologic changes in DN, as stated by Fioretto et al.

The incidence of PTDM is quite high owing to the use of immunosuppressants after KT (16, 17). In a multicenter study, Porrini et al conducted an oral glucose tolerance test every year in 672 patients for up to 5 years after KT and confirmed that PTDM occurred in 32% of the patients, and in nearly half of the patients when prediabetes was included (16). Therefore, it is difficult to generalize the results of Harada et al in the field of KT. Truong et al confirmed that DN was stable or progressed slowly through post-perfusion and follow-up biopsies. Three patients were confirmed to be stable, and four patients who were confirmed to have disease progression had PTDM (8). By analyzing the UNOS registry data, Cohen et al confirmed that allograft survival was significantly lower when a kidney of a diabetic donor was used, and reported that the difference in allograft survival was also significantly affected by recipient DM (3). These results suggest that DN is affected by glycemic control. However, the results of this study showed that the changes in the histologic class of DN after 1 year of KT did not differ depending on the status of DM or FBS control (**Tables 3**, **4**), and changes in GBM thickness did not show any different patterns depending on the status of DM or FBS control (**Figures 3B,C**). This could be simply due to the previously mentioned insufficient duration of follow up. But It is also possible that the poor outcome of the recipient with DM when diabetic donor kidney was used is caused by reasons other than the histological evidence of deterioration alone. Therefore, long-term data on the natural course of donor DN are required to verify this.

Hsu et al reported that donor DN is transmissible to recipients (9). DN was transmitted in five of the six cases with donor DN, and the histologic class of DN progressed in three of the five cases. The recipient in whom DN was not transmitted had no DM history, no PTDM, and a level of HbA1c maintained below 6% after transplantation. In the 5 cases in which DN transmission occurred, the recipients had a high histologic class of DN (one class IIa patient, two class IIb patients, and two class III patients). In this study, the changing pattern of GBM thickness also showed different patterns depending on the histologic findings at 2 weeks after KT. If the tissue class was I or lower at 2 weeks, the average thickness decreased, similar to the overall pattern, but if the class was II or higher, the average thickness showed an increasing pattern (**Figure 3D**). Although only one out of three graft failures in the study was directly caused by DN, while the other two by recurrent rejection complicated by sepsis and cardiovascular shock after aortic dissection, the risk of graft failure was higher if the DN class was III at 2 weeks (**Supplementary Table S4**). And the DM donors with DN of class III had DM duration of at least 6 years (**Supplementary Figure S1**). This suggests that identifying the class of DN in DM donors through donor kidney biopsy can potentially help predict the prognosis of non-diabetic recipients with DM donor kidneys, especially when the duration of DM was longer than 6 years. This should be confirmed through further research as statistical significance was not demonstrated in this study.

This study has a few limitations. First, given that we only used data from a single institution, the sample size was small. Second, there could be a selection bias as the donors with severe DN would have been clinically unsuitable for KT, and consequently excluded from the study. Hence, the findings from this study are primarily applicable to the insulin independent diabetic donors who are on OHA treatment. Third, we followed up the histological changes for 1 year only. Finally, the possibility of combined idiopathic nodular glomerulosclerosis secondary to smoking, obesity, or other reasons, cannot be completely excluded (18). However, in the current state, where less is known about the course of donor DN after KT, this study will provide important clues in understanding the natural course of donor DN as it monitored the changes during the same period of 1 year using the highest number of tissue findings of DN reported till date. More long-term data of histological changes are needed to improve our understanding of the natural course of donor DN after KT.

CONCLUSION

This study demonstrates that the DN in donors remained largely stable for 1 year after KT when the donor with the type 2 DM donor was only managed with OHA. This finding was true, regardless of the recipient's DM status or how well FBS control was achieved. With this understanding, clinicians may feel more comfortable accepting kidneys from donors with diabetes mellitus, thereby reducing the kidney discard rate. However, long-term follow up data are warranted to better understand the natural course of DN present in donors.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the SMC 2020-12-139. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

KL contributed to the design of the study and writing of the manuscript. JS contributed to histologic analysis and revising the manuscript. SP and MK contributed to revising the manuscript. JJ contributed to data analysis. GK, HJ, and WH contributed to the design of the study. GYK contributed to histologic analysis. JP contributed to the design of the study and revising the manuscript.

FUNDING

This research was supported by the SungKyunKwan University and the BK21 FOUR (Graduate School Innovation) funded by the Ministry of Education (MOE, Korea) and National Research Foundation of Korea (NRF).

CONFLICT OF INTEREST

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

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10714/full#supplementary-material

REFERENCES

1. Mohan S, Tanriover B, Ali N, Crew RJ, Dube GK, Radhakrishnan J, et al. Availability, Utilization and Outcomes of Deceased Diabetic Donor Kidneys; Analysis Based on the UNOS Registry. *Am J Transpl* (2012) 12:2098–105. doi:10.1111/j.1600-6143.2012.04167.x
2. Ahmad M, Cole EH, Cardella CJ, Cattran DC, Schiff J, Tinckam KJ, et al. Impact of Deceased Donor Diabetes Mellitus on Kidney Transplant Outcomes: a Propensity Score-Matched Study. *Transplantation* (2009) 88:251–60. doi:10.1097/TP.0b013e3181ac68a9
3. Cohen JB, Bloom RD, Reese PP, Porrett PM, Forde KA, Sawinski DL. National Outcomes of Kidney Transplantation from Deceased Diabetic Donors. *Kidney Int* (2015) 89:636–47. doi:10.1038/ki.2015.325
4. Fioretto P, Steffes MW, Sutherland DE, Goetz FC, Mauer M. Reversal of Lesions of Diabetic Nephropathy after Pancreas Transplantation. *N Engl J Med* (1998) 339:69–75. doi:10.1056/NEJM199807093390202
5. Hart A, Lentine KL, Smith JM, Miller JM, Skeans MA, Prentice M, et al. OPTN/SRTR 2019 Annual Data Report: Kidney. *Am J Transpl* (2021) 21:21–137. doi:10.1111/ajt.16502
6. Harada S, Ushigome H, Nishimura A, Nakao T, Nakamura T, Koshino K, et al. Histological Reversibility of Diabetic Nephropathy after Kidney Transplantation from Diabetic Donor to Non-diabetic Recipient. *Nephrology (Carlton)* (2015) 20:40–4. doi:10.1111/nep.12451
7. Truong LD, Gaber LW, Khan F. Donor-related Diabetic Nephropathy: a Comprehensive Clinicopathological Study. *Hum Pathol* (2019) 85:136–44. doi:10.1016/j.humpath.2018.10.032
8. Truong LD, Suki WN, Gaber LW, Gaber OA, Khan F. Kidney Donors with Diabetes: Renal Biopsy Findings at Time of Transplantation and Their Significance. *Transpl Direct* (2019) 5:e465. doi:10.1097/TXD.0000000000000903
9. Hsu CT, Wen MC, Chiu HF, Tsai SF, Yu TM, Yang CK, et al. Ongoing Donor-Transmitted Diabetic Kidney Disease in Kidney Transplant Recipients with Fair Sugar Control: a Single center Retrospective Study. *BMC Nephrol* (2020) 21:458. doi:10.1186/s12882-020-02132-w
10. Tervaert TW, Mooyaart AL, Amann K, Cohen AH, Cook HT, Drachenberg CB, et al. Pathologic Classification of Diabetic Nephropathy. *J Am Soc Nephrol* (2010) 21:556–63. doi:10.1681/ASN.2010010010
11. Qi C, Mao X, Zhang Z, Wu H. Classification and Differential Diagnosis of Diabetic Nephropathy. *J Diabetes Res* (2017) 2017:8637138. doi:10.1155/2017/8637138
12. Ehrenreich T, Porush JG, Churg J, Garfinkel L, Glabman S, Goldstein MH, et al. Treatment of Idiopathic Membranous Nephropathy. *N Engl J Med* (1976) 295:741–6. doi:10.1056/NEJM197609302951401
13. Fioretto P, Mauer SM, Bilous RW, Goetz FC, Sutherland DE, Steffes MW. Effects of Pancreas Transplantation on Glomerular Structure in Insulin-dependent Diabetic Patients with Their Own Kidneys. *Lancet* (1993) 342:1193–6. doi:10.1016/0140-6736(93)92183-t
14. Fioretto P, Mauer M. Reversal of Diabetic Nephropathy: Lessons from Pancreas Transplantation. *J Nephrol* (2012) 25:13–8. doi:10.5301/jn.5000061
15. Abouna GM, Al-Adnani MS, Kremer GD, Kumar SA, Daddah SK, Kusma G. Reversal of Diabetic Nephropathy in Human Cadaveric Kidneys after Transplantation into Non-diabetic Recipients. *Lancet* (1983) 2:1274–6. doi:10.1016/s0140-6736(83)91151-0
16. Porrini EL, Diaz JM, Moreso F, Delgado Mallen PI, Silva Torres I, Ibernon M, et al. Clinical Evolution of post-transplant Diabetes Mellitus. *Nephrol Dial Transpl* (2016) 31:495–505. doi:10.1093/ndt/gfv368
17. Jenssen T, Hartmann A. Post-Transplant Diabetes Mellitus in Patients with Solid Organ Transplants. *Nat Rev Endocrinol* (2019) 15:172–88. doi:10.1038/s41574-018-0137-7
18. Eadon MT, Lampe S, Baig MM, Collins KS, Melo Ferreira R, Mang H, et al. Clinical, Histopathologic and Molecular Features of Idiopathic and Diabetic Nodular Mesangial Sclerosis in Humans. *Nephrol Dial Transpl* (2021) 37:72–84. doi:10.1093/ndt/gfaa331

ESOT | Transplant International

ORIGINAL RESEARCH
published: 06 September 2022
doi: 10.3389/ti.2022.10648

"A Delicate balance"—Perceptions and Experiences of ICU Physicians and Nurses Regarding Controlled Donation After Circulatory Death. A Qualitative Study

Matthieu Le Dorze[1,2]*, *Sara Martouzet*[3], *Etienne Cassiani-Ingoni*[1], *France Roussin*[1], *Alexandre Mebazaa*[1,4], *Lucas Morin*[5] *and Nancy Kentish-Barnes*[6]

[1]AP-HP, Hôpital Lariboisière, Department of Anesthesia and Critical Care Medicine, Paris, France, [2]Université Paris-Saclay, UVSQ, INSERM, CESP, U1018, Villejuif, France, [3]Université de Tours, EA 7505 Éducation, Éthique et Santé, Tours, France, [4]Université de Paris, Inserm, UMRS 942 Mascot, Paris, France, [5]INSERM CIC 1431, University Hospital of Besançon, Besançon, France, [6]AP-HP, Saint Louis University Hospital, Famiréa Research Group, Medical Intensive Care Unit, Paris, France

Controlled donation after circulatory death (cDCD) is considered by many as a potential response to the scarcity of donor organs. However, healthcare professionals may feel uncomfortable as end-of-life care and organ donation overlap in cDCD, creating a potential barrier to its development. The aim of this qualitative study was to gain insight on the perceptions and experiences of intensive care units (ICU) physicians and nurses regarding cDCD. We used thematic analysis of in-depth semi-structured interviews and 6-month field observation in a large teaching hospital. 17 staff members (8 physicians and 9 nurses) participated in the study. Analysis showed a gap between ethical principles and routine clinical practice, with a delicate balance between end-of-life care and organ donation. This tension arises at three critical moments: during the decision-making process leading to the withdrawal of life-sustaining treatments (LST), during the period between the decision to withdraw LST and its actual implementation, and during the dying and death process. Our findings shed light on the strategies developed by healthcare professionals to solve these ethical tensions and to cope with the emotional ambiguities. cDCD implementation in routine practice requires a shared understanding of the tradeoff between end-of-life care and organ donation within ICU.

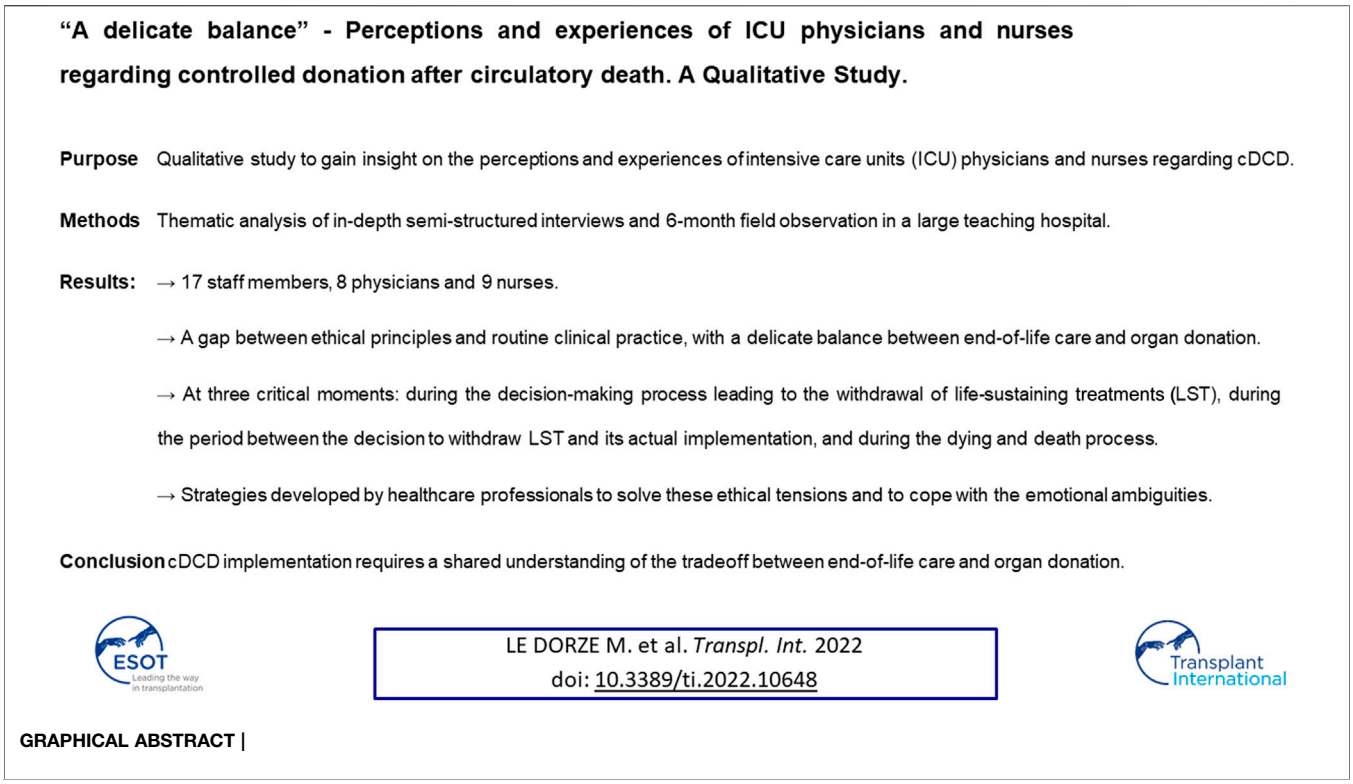
Keywords: organ donation, qualitative research, end of life, controlled donation after circulatory death, withdrawal of life-sustaining treatments

OPEN ACCESS

***Correspondence:**
Matthieu Le Dorze
matthieu.ledorze@aphp.fr

***Received:** 16 May 2022*
***Accepted:** 23 August 2022*
***Published:** 06 September 2022*

Citation:
Le Dorze M, Martouzet S, Cassiani-Ingoni E, Roussin F, Mebazaa A, Morin L and Kentish-Barnes N (2022) "A Delicate balance"—Perceptions and Experiences of ICU Physicians and Nurses Regarding Controlled Donation After Circulatory Death. A Qualitative Study. Transpl Int 35:10648. doi: 10.3389/ti.2022.10648



GRAPHICAL ABSTRACT |

INTRODUCTION

Controlled donation after circulatory death (cDCD) refers to organ donation from patients whose death is defined using circulatory criteria after the planned withdrawal of life-sustaining treatments (WLST) (1). The scarcity of donor organs and the good transplantation outcomes (2–4) legitimately support the development of this type of donation (5–7) in a context where WLST decisions occur more and more frequently in intensive care units (ICU) worldwide (8–10).

cDCD reshapes end-of-life care by introducing the issue of organ donation before the time of death. Thus, cDCD may potentially affect not only the decision-making process leading to WLST but also other end-of-life care practices (11–14). The French cDCD protocol explicitly states that decision to withdraw LST must be made in the patient's best interest, independently from any consideration regarding organ donation, and that cDCD must not alter end-of-life care (15). Yet, healthcare professionals can feel particularly uncomfortable when, in practice, end-of-life care and organ donation overlap (16–18). The challenge is not only to identify potential cDCD donors, but also to give healthcare professionals a reassuring ethical framework. Research has shown that physicians and nurses working in ICUs are not always at ease with organ donation after brain death (19).

Developing knowledge on the perceptions and experiences of healthcare professionals regarding cDCD is crucial to improve the quality of the process but remains rarely investigated (16, 17, 18, 20, 21). We conducted a cross-sectional qualitative study to better understand ICU physicians' and nurses' experience of cDCD. This will enable to develop interventions to support and guide them throughout this practice, which in turn should not only improve their experience but also the experience of patients' relatives.

MATERIALS AND METHODS

To carry out this monocentric qualitative study in an optimal way, we brought together a multidisciplinary research team, which included an ICU physician involved in organ donation (MLD), a graduate student in anthropology student (SM), and a sociologist (NKB).

Objectives

Our objectives were to understand how healthcare professionals perceived WLST decision-making process and how they experienced end-of-life care in this particular context, and finally how their relationship with the patient's relatives was affected.

Design

In-Depth Interviews

Between May and November 2019, we conducted in-depth interviews with healthcare professionals working in the ICU of a large teaching hospital in central Paris (610 beds overall, including 29 ICU beds). In this ICU, cDCD is implemented according to the ethical and technical requirements of the

TABLE 1 | Characteristics of the study participants.

Code	Role	Age range	Sex	ICU experience	cDCD experience
P01	Senior physician	31–40 years	Man	5–10 years	5 to 10 procedures
P02	Senior physician	31–40 years	Man	5–10 years	5 to 10 procedures
P03	Senior physician	31–40 years	Man	10–15 years	>10 procedures
P04	Senior physician	41–50 years	Man	15–20 years	>10 procedures
P05	Senior physician	31–40 years	Man	5–10 years	5 to 10 procedures
P06	Senior physician	51–60 years	Man	>20 years	5 to 10 procedures
P07	Senior physician	31–40 years	Man	10–15 years	5 to 10 procedures
P08	Senior physician	31–40 years	Woman	5–10 years	1 to 5 procedures
N01	Nurse assistant	41–50 years	Woman	>20 years	5 to 10 procedures
N02	Nurse	21–30 years	Man	5–10 years	1 to 5 procedures
N03	Nurse	21–30 years	Woman	0–5 years	1 to 5 procedures
N04	Nurse	31–40 years	Woman	5–10 years	5 to 10 procedures
N05	Nurse	51–60 years	Man	>20 years	>10 procedures
N06	Nurse	31–40 years	Woman	5–10 years	5 to 10 procedures
N07	Nurse	21–30 years	Man	0–5 years	1 to 5 procedures
N08	Nurse	21–30 years	Woman	0–5 years	1 to 5 procedures
N09	Nurse	31–40 years	Man	10–15 years	5 to 10 procedures

TABLE 2 | The decision-making process leading to the withdrawal of life-sustaining treatments in a context of potential organ donation Domains and Quotes.

A gap between theory and practice

Quote 1: "When we decide to withdraw life sustaining treatments, the intention is completely schizophrenic. We are told that the two processes must be totally sealed. In practice, this is impossible! All the doctors, everyone will tell you . . . it's impossible to dissociate the two. It's the same team who decides to withdraw life sustaining treatments and who calls the coordination office to start the organ procurement process. It's rather hypocritical" (Physician interview P03)

Quote 2: "Of course there is porosity between the two. cDCD is something we have in mind before, and it is a difficulty" (Physician interview P01)

Formal and informal communication

Quote 3: "We know the patients who are potentially Maastricht 3 donors. We talk about it among ourselves, not in an official, written way, but we know that a decision to withdraw treatment can lead to a M3" (Nurse interview N06)

Quote 4: "The nursing staff attends the collegial procedure meetings. It's extremely important for them that we make a clear and complete distinction between withdrawal of life sustaining treatments and Maastricht 3 organ donation process" (Physician interview P02)

Quote 5: "It's important that everyone adheres to the project, it allows us to feel comfortable. In any case, that everyone is clear with the situation and that everyone has been able to express themselves. It's very important that it goes well between us. Because if it all goes well, people will agree to do it again" (Physician interview P04)

Quote 6: "In practice, we are not going to delude ourselves: we tend to anticipate, at least among ourselves (physicians), the possibility of a cDCD" (Physician interview P05)

End-of-life care as a process, organ donation as a procedure

Quote 7: "End of life and Maastricht 3 are really dissociated. What's most important is the patient's end of life. Maastricht 3, when you understand that it's just a procedure –and therefore it's a technique and an organization – then it's no longer a problem, in fact. What's important is what is upstream" (Physician interview P04)

Making sense of the ethical dilemma

Quote 8: "There's nothing more we can do, the patient is going to die, and it may save someone else's life. I like this way of looking at things. I find that it de-dramatizes the situation. It breaks the tragic image of death. In the end, he didn't die for nothing. It gives a meaning to death" (Nurse interview N06)

Quote 9: "There is a real social benefit behind the process and a true purpose for the recipients"(Physician interview P03)

TABLE 3 | The period between the decision to withdraw life-sustaining treatment and its actual implementation. Domains and Quotes.

A difficult compromise between end-of-life care and organ preservation

Quote 1: "Do we resuscitate to preserve the organs, or do we let this patient die because there is no therapeutic plan?" We shouldn't resuscitate someone who doesn't have a therapeutic plan. It's not clear at all. This time period is what we find the most disturbing; we know the patient is going to die but how far should we go to preserve his organs?"(Physician interview P07)

Quote 2: "I asked myself whether it is ethically acceptable to keep the patient alive for his organs"(Nurse interview N06)

Quote 3: "It's really invasive, it may seem really aggressive, but I think it's the right solution for organ preservation" (Nurse interview N05)

A time to support relatives

Quote 4: "You have to explain again and again, you have to try to be as clear and simple as possible, you have to make them understand that it will be long and difficult. It requires relational skills" (Nurse interview N08)

Quote 5: "As the family is here just waiting it gives us a little more time together. This is the moment to give them (the family) space, to give them as much time as possible with their loved one, and to give them time to accept the situation" (Nurse interview N04)

Quote 6: "It also gives us the opportunity to prepare the patient and to focus on the person in the bed" (Nurse interview N08)

TABLE 4 | Dying, death and organ procurement. Domains and Quotes.

The pressure for organ donation success and its potential impact on end-of-life practices
Quote 1: "There is a form of pressure because we know the patient can donate his organs and save lives" (Nurse interview N03)
Quote 2: "The doctor in charge is caught between two injunctions: to ensure a dignified end of life for the patient, and to respect the deadlines imposed by the procedure" (Nurse interview N09)
Quote 3: "There's this idea like… 'hurry, he must die'" (Physician interview P08)
Quote 4: "There is a strong temptation to push what needs to be pushed in order to be within the deadlines" (Physician interview P03)
Quote 5: "I don't feel comfortable with this possibility. Indeed we know that sometimes there is transgression" (Physician interview P02)
Procedural failures as a positive ethical signal
Quote 6: "I want things to go well so that the organs can go to people who need them and who can get better. That, for me, is a positive issue. If organs can't be transplanted, well for me it's a negative experience" (Physician interview P01)
Quote 7: "The institution puts a lot of pressure on us. We have to resist. We must accept that sometimes the procedure fails. We're all convinced that the team will be more at ease with this activity if we screw up a situation once in a while" (Physician interview P03)
A modified experience of dying and death
Quote 8: "Family members don't know where to put themselves, it's complicated for us" (Physician interview P04)
Quote 9: "There were 15 of us in the room, and the patient was already halfway through the surgery before the cDCD procedure. On the one hand, there was the surgeons' timeframe; they were practically in their sterile clothes with a scalpel in each hand, ready. And on the other, there were the family members and I could see that they weren't able to say goodbye to their loved one because there were too many people in the room, there was no possible intimacy" (Physician interview P02)
Quote 10: "There was no care or support. It was really very technical. It wasn't a peaceful or just a normal dying atmosphere at all. The patient died so it's "OK he's dead, that's it, let's start the clock" (Nurse interview N03)
Quote 11: "With everyone watching it's just like a show. You want to say 'come on, this isn't a show, it's a man dying'. I find it very difficult" (Physician interview P03)
Quote 12: "It all went well, technically it all went very well . . . But, in fact, we had forgotten that we were caring for a dying patient, as though he wasn't there in a way" (Nurse interview N03)
Quote 13: "A patient who dies decently is just as important as a patient who heals" (Nurse interview N06)

nationwide protocol, particularly with the systematic use of normothermic regional perfusion (15). The WLST take place preferentially in the ICU, which facilitates the support of relatives by clinicians. When lung retrieval is considered, WLST is exceptionally done in the operating room. In all cases, the ICU team takes care of the patient until death and presence of family members is encouraged if they wish. After the declaration of death, the organ procurement team and a surgical team collaborate on the cannulation and the start of the normothermic regional perfusion.

The semi-structured interview guide was developed *a priori* by the investigators (**Supplementary Table S1**). Questions were open-ended, which allowed participants to describe their experience in their own words and to broach specific issues that they considered relevant.

Field Observation

In addition, one investigator (SM) immersed herself full-time in the ICU for a 6-month field observation to better understand the professional culture and the institutional context in which the interviews were conducted (22).

Data Collection

In-Depth Interviews

We used purposeful sampling based on professional status (physicians/nurses) and number of cDCD experiences (23). Participants were recruited through e-mail and personal solicitations. Interviews were conducted individually and in-person by a single investigator (SM) and lasted between 1 and 2 h. All interviews were audio recorded, pseudonymized, and then transcribed verbatim for analysis. Data collection was interrupted when we reached data saturation, namely when no new themes emerged from the interviews (24).

Field Observation

Detailed descriptive notes were taken in the form of a daily research journal. Reflective field notes were also taken. These notes go beyond descriptions to include the researcher's problems, impressions, analyses, clarifications, syntheses, connections, and other ideas about the research project.

Data Analysis

Primary Data, Interviews

Three researchers (MLD, NKB, and SM) read all the transcripts. Using an inductive approach, they identified initial key themes and concepts that occurred throughout the first three interviews using thematic analysis (25). Then they developed a codebook through an iterative process that ended when the three authors had achieved consensus (26). These authors then coded the same three interviews independently to check for intercoder reliability, after which they convened as a group to discuss potential disagreements and refine the initial themes and categories. Using this consolidated codebook, one researcher (SM) then coded the remaining interviews, adding or modifying codes as necessary given the content of subsequent interviews. Any difficulties or uncertainties were discussed with NKB and MLD during research meetings.

Secondary Data, Observation

Field notes were coded by SM and then discussed and analyzed by NKB and MLD. Field notes allowed us to develop a comprehensive and richer understanding of the interviews and helped confirm thematic analysis of interviews.

RESULTS

A total of 20 staff members were interviewed but due to saturation, a total of 17 were analyzed, including interviews

with 8 physicians and 9 nurses (**Table 1**). No clinician approached refused an interview. Qualitative analysis highlighted the ethical tensions experienced by clinicians at different stages of the process. We identified three key phases in the process, each with specific tensions. These phases and their associated perceived ethical tensions are described below. For each phase, we derived a sample of representative quotes is provided in **Tables 2**, **3**, **4**.

Ethical Tensions During the Decision-Making Process Leading to the Withdrawal of Life-Sustaining Treatments in a Context of Potential Organ Donation

A Gap Between Theory and Practice

In theory, the decision to withdraw LST should only be made in the patient's best interest, must comply with the legal requirements, and should be independent of any subsequent consideration (including organ donation). However, in practice, physicians and nurses expressed their inability to set aside the potentiality of cDCD during the WLST decision-making process (**Table 2**, quote 1). This gap between theory and practice is experienced as a difficulty (quote 2).

Formal and Informal Communication

One strategy for dealing with this difficulty is to adopt a dual approach combining formal and informal communication (quote 3). Formal communication asserts official recommendations, namely the independence between WLST decision and organ donation possibility. For this purpose, a formal multidisciplinary meeting is organized by the medical team to explicitly reaffirm the priority of the patient's best interest over the potentiality of organ donation. Field observation revealed that physicians set the scene in order to show to the other ICU staff members that organ donation has not been considered and that attention is focused solely on the WLST decision (quote 4). Physicians explained how, during the meeting, this dissociation between the WLST decision and the possibility of subsequent organ donation helps healthcare professionals to understand and accept the decision (quote 5). They also believed that it legitimated the WLST decision by removing doubt concerning a possible conflict of interest. In contrast, backstage informal communication allowed to consider organ donation as a possibility during the WLST decision-making process (quote 6).

End-of-Life Care as a Process, Organ Donation as a Procedure

Another strategy for dealing with this difficulty is one the hand to define end-of-life care as a process and an ethical priority and, on the other, to define organ donation as a strict procedure (quote 7).

Making Sense of the Ethical Dilemma

Participants perceived the gap between theory and practice as "impossible," "hypocritical," and "schizophrenic." The ethical tension appeared to be partly resolved by considering organ donation as a way to give meaning to the patient's death (quote 8). This consideration is not restricted to the patients themselves but is in fact extended to the future transplant recipients. This utilitarian approach allows healthcare professionals to consider cDCD in a broader benefit-risk balance (quote 9).

Ethical Tensions During the Period Between the Decision to Withdraw Life-Sustaining Treatments and Its Actual Implementation

The tension between end-of-life care and organ donation is particularly evident during this period. Combining taking care of the patient during end of life and organizing the organ donation procedure, with its technical and operational requirements, can be challenging for healthcare professionals.

Experience of Dual Objectives: An Example From the Field Observation

A particular situation led to intense debates within the ICU team. A 36-year-old patient was identified as a potential cDCD donor. During the 48 h required to organize the cDCD procedure, he developed a heparin-induced thrombocytopenia with pulmonary embolism. The question for the team was how to deal with a potential worsening of the situation. Some members of the ICU team felt uncomfortable with this double objective: on the one hand providing end-of-life care and avoiding unnecessary treatments and, on the other hand, preserving the organs before they were retrieved. Each new complication that occurred during this period was an opportunity to discuss the tensions they experienced.

A Difficult Compromise Between End-of-Life Care and Organ Preservation

For half of the interviewed ICU staff members, the introduction or the increase of treatments that are no longer necessary for the patient but that are useful to preserve organ viability raises ethical questions and discomfort (**Table 3**, quotes 1 and 2). For the other half, and as in the situation described above, a compromise is possible and severe complications should be treated on two conditions: first they should not compromise the organ procurement proposal, and second the patient should be kept under deep and continuous sedation until death (quote 3).

A Time to Support Relatives

The participating nurses were adamant to use this time period to reword the physicians' explanations and to provide emotional support to the relatives (quote 4). They insisted that special attention was given to the dying patient, which enables the organization of end-of-life rituals (quote 5). Last, this time period also allowed healthcare professionals and relatives to provide active verbal and non-verbal support to the patient, thus encouraging patient-centered care (quote 6).

Ethical Tensions During Dying, Death, and Organ Procurement Procedure

French regulation specifies that, following WLST, the agonic phase—that is, the time running from treatment withdrawal to

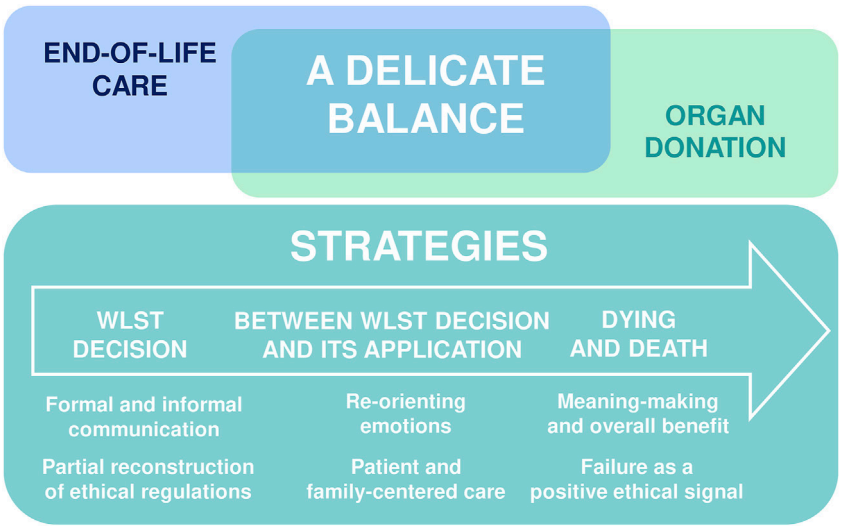


FIGURE 1 | Experienced and perceived solutions and strategies.

death—has to be less than 180 min in order to allow organ procurement.

The pressure for Organ Donation Success and Its Potential Impact on End-of-Life Practices

Participants reported increased stress during the implementation of decisions to withdraw LST (**Table 4**, quote 1), related to the fact that circulatory death must occur within the timeframe required for organ donation to be successful (quotes 2 and 3). This pressure on success can lead to changes in end-of-life practices, particularly regarding sedative practices (quote 4). This potential impact of the cDCD procedure on sedative practices is experienced as difficult for many healthcare professionals (quote 5).

Procedural Failures as a Positive Ethical Signal

A strategy for dealing with this pressure is to define a successful organ donation procedure as one that results in effective organ procurement (quote 6). However, another strategy exists to feel ethically comfortable: many physicians reported that they were reassured when a cDCD procedure failed because the patient didn't die within the allowed timeframe. This procedural "failure" gives an opportunity to place the patient—rather than the organ donation—at the heart of their practice (quote7).

A Modified Experience of Dying and Death

The systematic use of normothermic regional perfusion offers logistic advantages to the relatives, especially the continuation of end-of-life care in the ICU. However, our field observations showed that end-of-life support was not always optimal and that he atmosphere in the room was deemed as being not appropriate for providing support (quote 8). This difficulty is even more acute when WLST occurs in the operating room where relatives are unable to support the patient and to say goodbye (quote 9). Several participants highlighted the fact that organ procurement is an exceptionally technical procedure (quote 10). Healthcare professionals sometimes take the opportunity to attend the procedure although they are not directly involved in the patient's care, which was perceived as a form of voyeurism that may further desacralize the patient's end of life (quote 11). Last, healthcare professionals often felt that they were unable to care for the dying patient as they would have liked to (quote 12). Hence, they felt that they were "stealing the patient's death" from both the patient him/herself and from the relatives. This was problematic for healthcare professionals who described quality of dying as a major criterion for the quality of their work (quote 13).

DISCUSSION

National policies and guidelines have attempted to shape the process of cDCD into a routine activity for healthcare professionals so that it can become an accepted practice (15, 27). Ethical frameworks imply that healthcare professionals should not experience a moral tension between caring for the dying patient and altering his/her care for the purpose of donation. The interviews conducted during our study show that in practice the situation is more complex for both ICU physicians and nurses with a delicate balance between, on the one hand, end-of-life care and, on the other, organ donation (**Figure 1**). Indeed there is a gap between ethical theories and practice (28, 29) that clinicians seek to fill the best they can at all stages of the process.

Concerned simultaneously about end-of-life care and organ donation, healthcare professionals do not want to act against their moral principles and thus develop five types of strategies to solve the ethical and emotional tensions they experience (**Figure 1**). The first strategy used relies on virtue-centered communication (29). Physicians learn to be demonstrative by staging a distinct temporality between the WLST decision and the organ donation discussion in order to internalize the ethical principal at the basis of cDCD: the separation between WLST decision-making and organ

donation decision-making. The second strategy is partial reconstruction of ethical regulations: once the demonstration described above has operated, clinicians can more openly express the intellectual and emotional limits of this practice. The third strategy concerns re-orientating emotions: at the time of WLST decision, some healthcare professionals focus on the WLST decision-making process by relegating organ donation to a secondary organizational and logistical issue. Once the decision to WLST has been made, healthcare professionals may experience important discomforts concerning end-of-life care vs. organ preservation strategies, or tensions concerning the direct exposure of relatives to the organizational dimensions of death. Instead of dwelling on the ethical tensions surrounding the patients' treatment in anticipation of organ donation, they seek to use the extra time to provide quality support and care to the relatives and to ensure that healthcare professionals and relatives accept and adhere to both the WLST decision and the organ donation project. For some physicians and nurses, this delay may contribute to the quality of the patient's death by allowing time for the relatives to be at the patient's side and to say goodbye. The fourth strategy implies defending the principal of "overall benefit". Indeed when confronted with death in the context of cDCD clinicians can experience moral distress and the feeling of "robbing" the patient's death. To overcome this tension, the overall benefit of organ donation serves to maintain motivation. The fifth and last strategy implies necessary failures of the cDCD end-of-life procedure: ensuring that failure can happen (i.e., the patient doesn't die within the timeframe) is a comfort for clinicians in that the quality of the person's end-of-life takes precedence over the technical procedure.

One important finding of our study is that cDCD procedures are the result of several days of emotional and ethical tension between healthcare professionals, most often shared with the patient's relatives. cDCD reshapes end of life in ICU, as end-of-life care is not only followed by death but also by organ donation. Despite the above-mentioned strategies, none of the stages of the process are black or white and there are no undisputable solutions to the complexity of the moral tensions experienced. Clinicians navigate in "grey areas," juggling with official guidelines and ethical dilemmas, as well as with concrete moral, intellectual and emotional difficulties. Their task is to give meaning to the process, a meaning that can be shared with the patients' family members and among the team (30). These concerns around "ethics in practice" take place within an ICU and, each time, within a specific ethical climate (31).

Healthcare professionals are vital for the implementation of cDCD and their attitudes can influence their participation. Satisfaction with end-of-life care impacts on physicians' and nurses' well-being (32) as well as on relatives' well-being both during and after the patient's death (33–35). Quality of communication between team members (36), adapted leadership and involvement of nurses (37) at all stages of the process are important elements that will help clinicians overcome these ethical tensions as a group—left alone to deal with these tensions, clinicians could develop moral distress and burnout leading to leaving the ICU (38).

Our study has some limitations. First, it was conducted in a single country (France), with specific end-of-life legislation (13) and cDCD protocols (15). Moreover, it was conducted in a single ICU, one of the first to have implemented this procedure, with a potential impact of the unit culture on the results. However, results of this exploratory single-centre study provide insights into healthcare professionals' experience that may help design future multicentre studies (39). Last, although our purposive sampling strategy was designed to maximize the diversity of ICU clinicians who participated in the study, our results are—by definition—not entirely generalizable to all healthcare professionals working in ICU. Participation in qualitative interviews was voluntary, creating a possible selection bias: clinicians with difficulties in (or reluctance to) expressing themselves or their experiences concerning the cDCD process may have been omitted. Last, only one researcher coded the interviews. However, to reduce the risk of bias, two other researchers independently coded 3 transcripts for intercoder reliability that proved to be good. Any difficulties or uncertainties encountered by the main coder were discussed and resolved during team meetings.

This qualitative study provides in-depth understanding of the experience of ICU clinicians of the cDCD process. Despite clear and transparent national guidelines, the process remains entangled in a variety of ethical and emotional ambiguities that they strive to solve using various strategies. Overall, ICU clinicians believe that the implementation of cDCD is ethically reasonable as long as end-of-life care is preserved. Taken together, our results indicate that although national guidelines for cDCD are warranted to create a common legal, clinical and ethical framework, the implementation of cDCD in routine practice requires a shared understanding of the difficult compromises experienced by ICU clinicians between end-of life care and organ donation among ICU clinicians.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

All participants provided informed consent, and interview data were anonymized during transcription. The appropriate institutional review board (Comité d'Ethique pour la Recherche en Anesthésie-Réanimation, CERAR No. 2020-127) approved this study.

AUTHOR CONTRIBUTIONS

ML and NK-B contributed to the conception of the study protocol, the analysis of the data, the draft of the manuscript, approved the manuscript prepared for publication, and agreed to be accountable for all aspects of the work. SM contributed to the conception of the study protocol, the acquisition and the analysis of the data, approved the manuscript prepared for publication, and agreed to be accountable for all aspects of the work. EC-I contributed to the analysis of data, approved the manuscript prepared for publication, and agreed to be accountable for all aspects of the work. FR contributed to the conception of the study

protocol, approved the manuscript prepared for publication, and agreed to be accountable for all aspects of the work. AM critically revised the work for important intellectual content, approved the manuscript prepared for publication, and agreed to be accountable for all aspects of the work. LM critically revised the work for important intellectual content, approved the manuscript prepared for publication, and agreed to be accountable for all aspects of the work.

FUNDING

A source of funding was provided by the Fondation de France and was used for the transcription of the interviews. The funding source had no role in the design and conduct of the study.

CONFLICT OF INTEREST

ML and FR are members of the steering committee of the French cDCD program.

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

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10648/full#supplementary-material

REFERENCES

1. Thuong M, Ruiz A, Evrard P, Kuiper M, Boffa C, Akhtar MZ, et al. New Classification of Donation after Circulatory Death Donors Definitions and Terminology. *Transpl Int* (2016) 29(7):749–59. doi:10.1111/tri.12776
2. Savier E, Lim C, Rayar M, Orlando F, Boudjema K, Mohkam K, et al. Favorable Outcomes of Liver Transplantation from Controlled Circulatory Death Donors Using Normothermic Regional Perfusion Compared to Brain Death Donors. *Transplantation* (2020) 104(9):1943–51. doi:10.1097/TP.0000000000003372
3. Antoine C, Jasseron C, Dondero F, Savier E. Liver Transplantation from Controlled Donors after Circulatory Death Using Normothermic Regional Perfusion: An Initial French Experience. *Liver Transpl* (2020) 26(11):1516–21. doi:10.1002/lt.25818
4. Savoye E, Legeai C, Branchereau J, Gay S, Riou B, Gaudez F, et al. Optimal Donation of Kidney Transplants after Controlled Circulatory Death. *Am J Transpl* (2021) 18:2424–36. doi:10.1111/ajt.16425
5. Weiss J, Elmer A, Mahíllo B, Dominguez-Gil B, Avsec D, Nanni Costa A, et al. Evolution of Deceased Organ Donation Activity versus Efficiency over a 15-year Period: An International Comparison. *Transplantation* (2018) 102(10):1768–78. doi:10.1097/TP.0000000000002226
6. Smith M, Dominguez-Gil B, Greer DM, Manara AR, Souter MJ. Organ Donation after Circulatory Death: Current Status and Future Potential. *Intensive Care Med* (2019) 45(3):310–21. doi:10.1007/s00134-019-05533-0
7. Lomero M, Gardiner D, Coll E, Haase-Kromwijk B, Procaccio F, Immer F, et al..Donation after Circulatory Death Today: an Updated Overview of the European Landscape. *Transpl Int* (2020) 33(1):76–88. doi:10.1111/tri.13506
8. Myburgh J, Abillama F, Chiumello D, Dobb G, Jacobe S, Kleinpell R, et al. End-of-life Care in the Intensive Care Unit: Report from the Task Force of World Federation of Societies of Intensive and Critical Care Medicine. *J Crit Care* (2016) 34:125–30. doi:10.1016/j.jcrc.2016.04.017
9. Lesieur O, Herbland A, Cabasson S, Hoppe MA, Guillaume F, Leloup M. Changes in Limitations of Life-Sustaining Treatments over Time in a French Intensive Care Unit: A Prospective Observational Study. *J Crit Care* (2018) 47:21–9. doi:10.1016/j.jcrc.2018.05.018
10. Sprung CL, Ricou B, Hartog CS, Maia P, Mentzelopoulos SD, Weiss M, et al..Changes in End-Of-Life Practices in European Intensive Care Units from 1999 to 2016. *JAMA* 322:1692–704. doi:10.1001/jama.2019.14608
11. Puybasset L, Bazin JE, Beloucif S, Bizouarn P, Crozier S, Devalois B, et al. Critical Appraisal of Organ Procurement under Maastricht 3 Condition. *Ann Fr Anesth Reanim* (2014) 33(2):120–7. doi:10.1016/j.annfar.2013.11.004
12. Graftieaux JP, Bollaert PE, Haddad L, Kentish-Barnes N, Nitenber G, Robert R, et al. Contribution of the Ethics Committee of the French Society of Intensive Care Medicine to a Scenario for the Implementation of Organ Donation after Maastricht III-type Cardiac Death in France. *Ann Fr Anesth Reanim* (2014) 33(2):128–34. doi:10.1016/j.annfar.2014.01.003
13. Le Dorze M, Kandelman S, Veber B, SFAR's Ethics Committee. End-of-life Care in the French ICU: Impact of Claeys-Leonetti Law on Decision to Withhold or Withdraw Life-Supportive Therapy. *Anaesth Crit Care Pain Med* (2019) 38(6):569–70. doi:10.1016/j.accpm.2019.10.013
14. Le Dorze M, Kandelman S, Veber B, SFAR Ethics Committee. Deep Continuous Sedation Maintained until Death, in French Intensive Care Units. *Anaesth Crit Care Pain Med* (2019) 39:115–6. doi:10.1016/j.accpm.2019.07.008
15. Corinne A, Bronchard R, Durin L. Direction générale médicale et scientifique de l'Agence de la Biomédecine, Paris, France. Conditions for Recovery of Organs after Circulatory Arrest (Maastricht Category 3) in a Health Care Facility Authorized to Recover Organs (2019). Available from: https://www.agence-biomedecine.fr/IMG/pdf/v7_guide_ddac_miii_novembre_2019_eng.pdf (Accessed March 5, 2022).
16. Mandell MS, Zamudio S, Seem D, McGaw LJ, Wood G, Liehr P, et al. National Evaluation of Healthcare Provider Attitudes toward Organ Donation after Cardiac Death. *Crit Care Med* (2006) 34(12):2952–8. doi:10.1097/01.CCM.0000247718.27324.65
17. D'Alessandro AM, Peltier JW, Phelps JE. Understanding the Antecedents of the Acceptance of Donation after Cardiac Death by Healthcare Professionals. *Crit Care Med* (2008) 36(4):1075–81. doi:10.1097/CCM.0b013e3181691b2b
18. Bastami S, Matthes O, Krones T, Biller-Andorno N. Systematic Review of Attitudes toward Donation after Cardiac Death Among Healthcare Providers and the General Public. *Crit Care Med* (2013) 41(3):897–905. doi:10.1097/CCM.0b013e31827585fe
19. Kentish-Barnes N, Duranteau J, Montlahuc C, Charpentier J, Martin-Lefevre L, Joseph L, et al. Clinicians' Perception and Experience of Organ Donation from Brain-Dead Patients. *Crit Care Med* (2017) 45(9):1489–99. doi:10.1097/CCM.0000000000002581
20. Hart JL, Kohn R, Halpern SD. Perceptions of Organ Donation after Circulatory Determination of Death Among Critical Care Physicians and Nurses: a National Survey. *Crit Care Med* (2012) 40(9):2595–600. doi:10.1097/CCM.0b013e3182590098
21. Lee YY, Ranse K, Silvester W, Mehta A, Van Haren F. Attitudes and Self-Reported End-Of-Life Care of Australian and New Zealand Intensive Care Doctors in the Context of Organ Donation after Circulatory Death. *Anaesth Intensive Care* (2018) 46(5):488–97. doi:10.1177/0310057X1804600510
22. Jones J, Smith J. Ethnography: Challenges and Opportunities. *Evid Based Nurs* (2017) 20(4):98–100. doi:10.1136/eb-2017-102786
23. Malterud K. Qualitative Research: Standards, Challenges, and Guidelines. *Lancet* (2001) 358(9280):483–8. doi:10.1016/S0140-6736(01)05627-6
24. Saunders B, Sim J, Kingstone T, Baker S, Waterfield J, Bartlam B, et al. Saturation in Qualitative Research: Exploring its Conceptualization and Operationalization. *Qual Quant* (2018) 52(4):1893–907. doi:10.1007/s11135-017-0574-8
25. Braun V, Clarke V. Using Thematic Analysis in Psychology. *Qual Res Psychol* (2006) 3(2):77–101. doi:10.1191/1478088706qp063oa
26. DeCuir-Gunby J, Marshall P, Mcculloch A. Developing and Using a Codebook for the Analysis of Interview Data: An Example from a Professional

Development Research Project. *Field Methods* (2011) 23:136–55. doi:10.1177/1525822X10388468
27. Cooper J. Problematising the Ethics of Organ Donation after Circulatory Death in the UK. *Crit Public Health* (2017) 27(4):499–505. doi:10.1080/09581596.2016.1225948
28. Madani M, Larijani B, Madani E, Ghasemzadeh N. Establishment of Medical Education upon Internalization of Virtue Ethics: Bridging the gap between Theory and Practice. *J Med Ethics Hist Med* (2017) 10:3.
29. Madani M, Vedadhir A, Larijani B, Khazaei Z, Gharamaleki AF. Bridging the Gap between Ethical Theory and Practice in Medicine: A Constructivist Grounded Theory Study. *Sci Eng Ethics* (2020) 26(4):2255–75. doi:10.1007/s11948-020-00217-1
30. Kentish-Barnes N, Davidson JE, Cox CE. Family Presence during Cardiopulmonary Resuscitation: an Opportunity for Meaning-Making in Bereavement. *Intensive Care Med* (2014) 40(12):1954–6. doi:10.1007/s00134-014-3396-3
31. Van den Bulcke B, Metaxa V, Reyners AK, Rusinova K, Jensen HI, Malmgren J, et al. Ethical Climate and Intention to Leave Among Critical Care Clinicians: an Observational Study in 68 Intensive Care Units across Europe and the United States. *Intensive Care Med* (2020) 46(1):46–56. doi:10.1007/s00134-019-05829-1
32. Boissier F, Seegers V, Seguin A, Legriel S, Cariou A, Jaber S, et al. Assessing Physicians' and Nurses' Experience of Dying and Death in the ICU: Development of the CAESAR-P and the CAESAR-N Instruments. *Crit Care* (2020) 24(1):521. doi:10.1186/s13054-020-03191-z
33. Kentish-Barnes N, Chaize M, Seegers V, Legriel S, Cariou A, Jaber S, et al. Complicated Grief after Death of a Relative in the Intensive Care Unit. *Eur Respir J* (2015) 45(5):1341–52. doi:10.1183/09031936.00160014
34. Kentish-Barnes N, Azoulay E. The Challenge of Addressing Family Needs at the End of Life. *Am J Respir Crit Care Med* (2016) 193(2):108–10. doi:10.1164/rccm.201509-1913ED
35. Kentish-Barnes N, Seegers V, Legriel S, Cariou A, Jaber S, Lefrant JY, et al. CAESAR: a New Tool to Assess Relatives' Experience of Dying and Death in the ICU. *Intensive Care Med* (2016) 42(6):995–1002. doi:10.1007/s00134-016-4260-4
36. Azoulay E, Forel JM, Vinatier I, Truillet R, Renault A, Valade S, et al. Questions to Improve Family-Staff Communication in the ICU: a Randomized Controlled Trial. *Intensive Care Med* (2018) 44(11):1879–87. doi:10.1007/s00134-018-5423-2
37. Kentish-Barnes N, Chevret S, Azoulay E. Guiding Intensive Care Physicians' Communication and Behavior towards Bereaved Relatives: Study Protocol for a Cluster Randomized Controlled Trial (COSMIC-EOL). *Trials* (2018) 19(1):698. doi:10.1186/s13063-018-3084-7
38. Vincent H, Jones DJ, Engebretson J. Moral Distress Perspectives Among Interprofessional Intensive Care Unit Team Members. *Nurs Ethics* (2020) 27(6):1450–60. doi:10.1177/0969733020916747
39. Assistance Publique - Hôpitaux de Paris. *Experience Of Relatives And Caregivers Of Death In ICU After The Withdrawal Of Life-Sustaining Therapies, Associated With An Organ Procurement Procedure (Controlled Donation after Circulatory Death).* clinicaltrials.Gov (2021). Available at: https://clinicaltrials.gov/ct2/show/NCT05041023 (Accessed March 5, 2022).

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