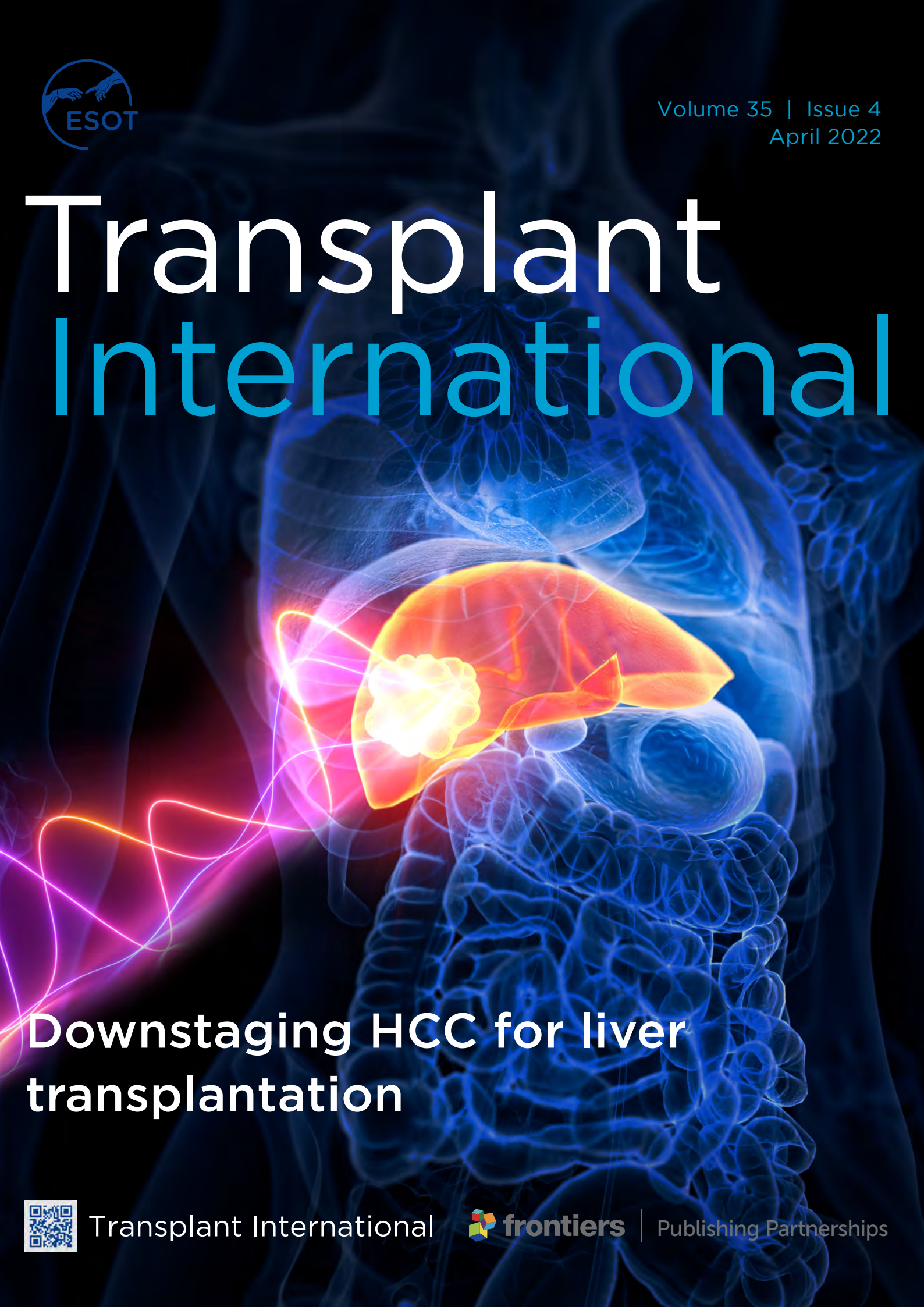
ESOT

Volume 35 | Issue 4
April 2022

Transplant International



Downstaging HCC for liver transplantation

Transplant International

frontiers | Publishing Partnerships

Transplant International

Editorial Office
Rebecca Smith
tieditorialoffice@frontierspartnerships.org

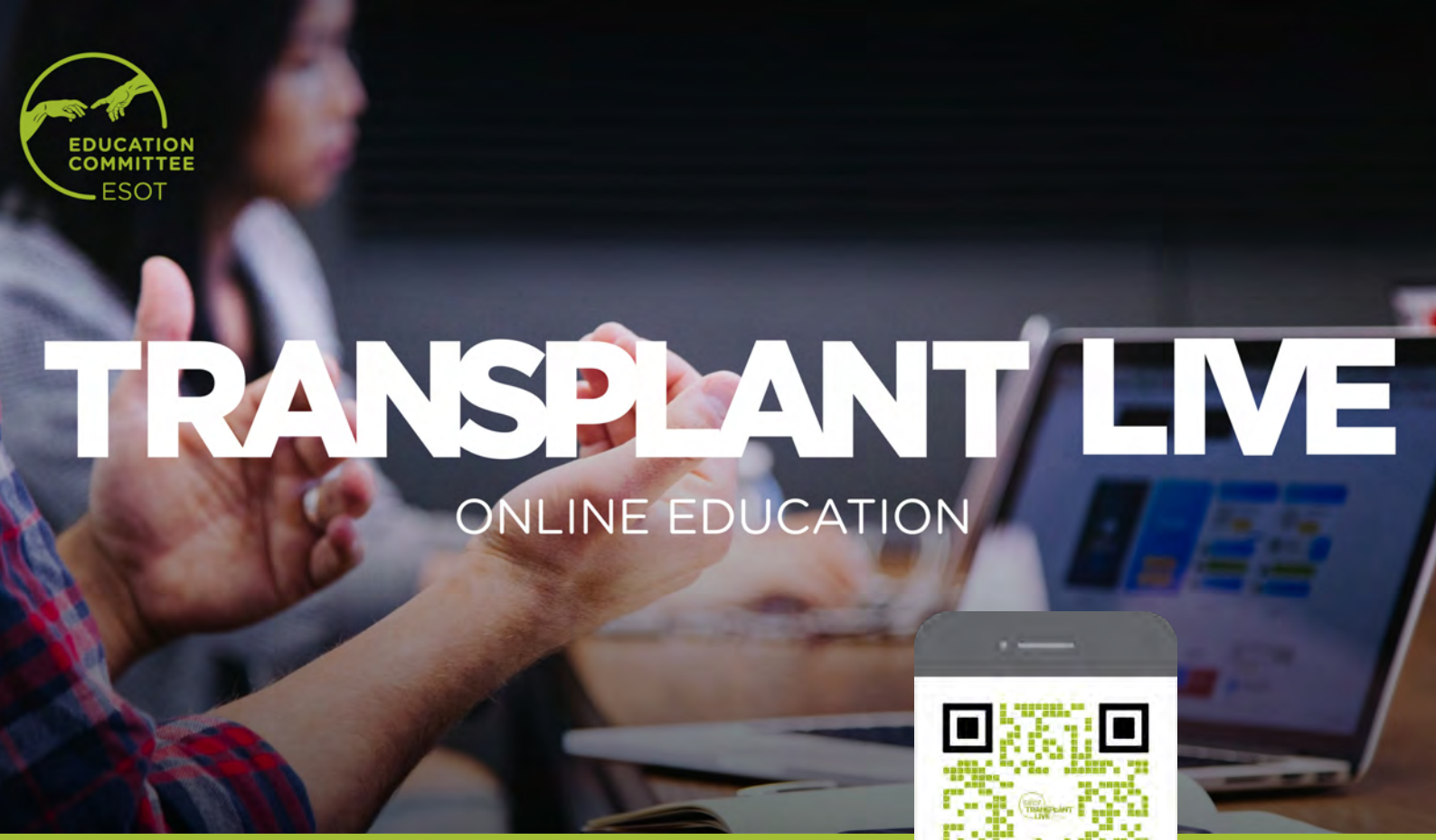
ESOT

Transplant International

Table of contents

Original Research

Protocol



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

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


ESOT
TLJ3.0
TRANSPLANTATION
LEARNING JOURNEY
PRAGUE, 13-15 NOV 2022
Consensus conference
Learning never stops
Join us at TLJ 3.0
And grow your knowledge in transplantation
esot.org
#ESOTTLJ

The course covers all aspects of liver transplantation, including indications and recipient evaluation, liver procurement and perfusion, hepatectomy and implantation, and peri- and post-operative management.

The programme, developed by the experts of the European Liver and Intestine Transplant Association (ELITA), includes a mix of state-of-the-art lectures, case discussions and interactive presentations.

"BCLT – the first and necessary step to the successful career in liver transplantation"

ECTORS
ESOT

ROTTERDAM

The Netherlands

3rd ECTORS meeting

29-30 September 2022

#ESOT_ECTORS #ECTORSmeeting

learn more

The main topics for 3rd ECTORS meeting will be:

- Stem cells
- organoids
- machine perfusion
- regeneration

Learning Objectives:

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

Target Group:
Researchers and clinicians from the transplant field interested in regenerative medicine

ESOT | Transplant International

EDITORIAL
published: 22 April 2022
doi: 10.3389/ti.2022.10529

Editorial: Rubies for ESOT!

Thierry Berney[1]*, *Nuria Montserrat*[2], *Maarten Naesens*[2], *Stefan Schneeberger*[2], *Maria Irene Bellini*[3] and *Thomas Neyens*[4]

[1]Editor-in-Chief, Transplant International, [2]Deputy Editor-in-Chief, Transplant International, [3]Social Media Editor, Transplant International, [4]Statistical Editor, Transplant International

Forty years ago, on April 28th, 1982, ESOT was founded in Zürich, Switzerland by an assembly of 14 European transplant surgeons, to satisfy the "need for a society . . . which would represent the aims and needs of transplantation surgery and surgeons in Europe" (1). The founders had the wisdom to suggest that all scholars actively involved in organ transplantation should be included in such an organization rather than transplant surgeons only. This idea prevailed thanks to the visionary Sir Roy Calne who would become ESOT's first president (1). Thus, instead of a European Society of Transplant Surgeons (ESTS), as originally planned, the European Society for Organ Transplantation (ESOT) was born. While surgeons were driving (and dominating) the world of transplantation in the 1980's, Sir Roy had understood the need to build a broader European transplant community inclusive of physicians and scientists, meeting in a common forum, rather than at parallel events. This vision has endured and ESOT progressively engaged other categories of transplant professionals: transplant coordinators, allied healthcare professionals, and - more recently - patients and (bio)technology scientists. This last advancement is the response to the broadening of our field towards organ reconditioning, regenerative medicine, artificial and bioartificial organs (2).

The founding assembly of ESOT was a group of formidable individuals including Guy Alexandre, Max Dubernard, Carl Groth, Walter Land or Raimund Margreiter, to name only a few. One of these founding fathers was Gauke Kootstra, transplant surgeon in Maastricht, the Netherlands. Gauke had pioneered the development of machine perfusion for marginal organs (3), but his claim to fame has been the understanding of the value of "non heart-beating donors" for increasing the donor organ pool (4). His efforts contributed significantly to the standardization and categorization of donors with circulatory death (DCD), which led to the Maastricht classification (5).

The ESOT founders understood the need for the society to establish a scientific journal and Gauke Kootstra was appointed as the first editor-in-chief of *Transplant International* (1988–1998). As a result of the strong commitment of the ESOT community, the editorial board released the first issue containing 10 articles in 1988 (6). Since then, *Transplant International* has successfully established itself as a major and respected title and has consistently progressed under the stewardship of Ferdinand Mühlbacher (1999–2014) and the team of Thomas Wekerle and Rainer Oberbauer (2015–2021) in their roles as editors-in-chief. The current leading editorial board is grateful to have inherited such a high-quality journal from our forebears.

From its creation, *Transplant International* was committed to modernity. As written in the editorial of the first issue: "We are convinced that with such modern means of communication as the fax, it is possible to keep the processing and publication times in our journal to a minimum. This will be of great importance, especially in the field of transplantation which is, by its nature, a very dynamic science" (6). Thirty-four years later, the fax looks like a prehistoric communication tool, but the spirit of the current editorial board is pretty much the same, embracing all the tools brought by the information revolution, for the communication with our readers and ESOT members and for the dissemination of our authors' work (7). This has allowed to strengthen further the ties between ESOT and *Transplant International* and to work toward our common interests: the desire to address the new cutting-edge topics developing fast in the field of organ replacement (2, 8, 9). Anticipating, rather than adjusting to the modernization of scientific publication led us to adopt a gold open access model (10). The

Editorial

OPEN ACCESS

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

Received: 24 March 2022
Accepted: 25 March 2022
Published: 22 April 2022

Citation:
Berney T, Montserrat N, Naesens M, Schneeberger S, Bellini MI and Neyens T (2022) Editorial: Rubies for ESOT!. Transpl Int 35:10529. doi: 10.3389/ti.2022.10529

immediate -and huge-benefit is already palpable with the free access granted to all present and past *Transplant International* publications.

Traditionally, 40 years mark a ruby anniversary. As *Transplant International* has elected to go for gold, we offer (virtual) rubies to ESOT!

REFERENCES

1. ESOT (2022). Available at: https://esot.org/history-of-esot/ (Accessed March 19, 2022).
2. Berishvili E, Casiraghi F, Amarelli C, Scholz H, Piemonti L, Berney T, et al. Mini-Organs Forum: How to Advance Organoid Technology to Organ Transplant Community. *Transpl Int* (2021). 34:1588–93. doi:10.1111/tri.13988
3. Slooff MJ, van der Wijk J, Rijkmans BG, Kootstra G. Machine Perfusion Versus Cold Storage for Preservation of Kidneys before Transplantation. *Arch Chir Neerl* (1978). 30:83–90.
4. van der Vliet JA, Slooff MJH, Rijkmans BG, Kootstra G. Use of Non-Heart-Beating Donor Kidneys for Transplantation. *Eur Surg Res* (1981). 13:354–60. doi:10.1159/000128202
5. Kootstra G, Daemen JH, Oomen AP. Categories of Non-Heart-Beating Donors. *Transpl Proc* (1995). 27:2893–4.
6. Kootstra G. From the Editor's Desk. *Transpl Int* (1988). 1:51.
7. Bellini MI, Montserrat N, Naesens M, Neyens T, Schneeberger S, Berney T. The Power of Online Tools for Dissemination: Social Media, Visual Abstract, and Beyond. *Transpl Int* (2021). 34:1174–6. doi:10.1111/tri.13929
8. Berney T, Montserrat N, Naesens M, Schneeberger S, Bellini MI, Neyens T. Editorial: Changing of the Guard at Transplant International. *Transpl Int* (2021). 34:609. doi:10.1111/tri.13843
9. Montserrat N, Casiraghi F, Amarelli C, Bellini MI, Neyens T, Naesens M, et al. Task Force Groups of Transplant International: Working Together to Globally Connect the Transplant Community of Tomorrow. *Transpl Int* (2021). 34:767–8. doi:10.1111/tri.13869
10. Bellini MI, Montserrat N, Naesens M, Neyens T, Schneeberger S, Berney T. Editorial: Transplant International Goes for GOLD!. *Transpl Int* (2022). 35:10340. doi:10.3389/ti.2022.10340

TRANSPLANT TRIAL WATCH
published: 19 April 2022
doi: 10.3389/ti.2022.10434

Transplant Trial Watch

Simon R. Knight[1,2]*

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

Keywords: liver transplantation, hepatocellular carcinoma (HCC), cytomegalovirus, solid organ transplant, randomised controlled trial

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

Hepatocellular Carcinoma Progression during Bridging Before liver Transplantation

by Renner, P., et al. BJS Open 2021; 5(2): zrab005.

AIMS

This was a substudy of the SiLVER study which examined the link between pretransplant bridging therapy and long-term posttransplant survival.

INTERVENTIONS

Participants in the original trial were randomised to receive either a centre specific immunosuppressive regimen (mTOR inhibitor free), or a sirolimus based immunosuppressive regimen.

PARTICIPANTS

350 liver transplant patients from the SilVER study who underwent one or more hepatocellular carcinoma (HCC) bridging treatments.

OUTCOMES

The main outcomes of interest were disease-free survival and overall survival within and outside the Milan criteria.

OPEN ACCESS

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

Received: *15 February 2022*
Accepted: *03 March 2022*
Published: *19 April 2022*

Citation:
Knight SR (2022) Transplant Trial Watch. Transpl Int 35:10434. doi: 10.3389/ti.2022.10434

FOLLOW-UP

Median follow-up was 5.3 years (inter-quartile range (2.4–6.2 years)).

CET CONCLUSION

This manuscript reports a substudy of the SILVER trial (sirolimus in liver transplant candidates with HCC), investigating the relationship between pretransplant bridging therapy and post-transplant survival. The authors report that patients with progression despite bridging therapy had inferior survival, and that those patients with tumours downsized successfully with bridging therapy had inferior outcomes compared to those who had smaller tumours initially. This suggests that downstaging patients with tumours exceeding the Milan criteria with bridging therapy does not improve the probability of survival. Whilst these results are interesting, it is important to remember that the SILVER study was not designed or powered to test the effects of bridging therapy, and bridging therapy used was very variable. Future prospective studies would be needed to further assess the role of response to bridging therapy on post-transplant outcomes.

TRIAL REGISTRATION

EudraCT: 2005-005362-36; Clinicaltrials.gov: NCT00355862.

FUNDING SOURCE

Not reported.

RANDOMISED CONTROLLED TRIAL 2

Maribavir for Refractory Cytomegalovirus Infections With or Without Resistance Post-Transplant: Results from a Phase 3 Randomized Clinical Trial

by Avery, R. K., et al. Clinical Infectious Diseases 2021 [record in progress].

AIMS

This study aimed to investigate the safety and efficacy of maribavir compared to investigator-assigned therapy (IAT) for the treatment of with or without resistance cytomegalovirus (R/R CMV) infection in solid-organ transplant (SOT) and hematopoietic-cell transplant (HCT) recipients.

INTERVENTIONS

Participants were randomised to either the maribavir group or the IAT group.

PARTICIPANTS

352 HCT and SOT recipients.

OUTCOMES

The primary outcome was confirmed CMV viremia clearance. Secondary outcomes included achievement of CMV clearance and symptom control.

FOLLOW-UP

16 weeks.

CET CONCLUSIONS

This multicentre RCT investigated the use of Maribavir (a UL97 protein kinase inhibitor) in post-transplant (HCT or SOT) patients with refractory CMV infection. Maribavir was compared to investigator assigned treatment with either valganciclovir/ganciclovir, foscarnet, or cidofovir. CMV clearance was significantly more likely in the Maribavir group (55.7% vs. 23.9%) and demonstrated less nephrotoxicity than foscarnet, and less myelosuppression than valganciclovir/ganciclovir. Whilst unblinded, the study is pragmatic and well designed. There is some variability in included patients ("refractory" patients had to have failed to respond to one first line therapy, but this was not specified in detail) and in the investigator assigned comparator group, but this likely reflects real-world variations in practice. The results encouraging for the use of Maribavir as an alternative, potentially less toxic, alternative to existing therapies in this setting.

JADAD SCORE

3.

DATA ANALYSIS

Per protocol analysis.

ALLOCATION CONCEALMENT

Yes.

TRIAL REGISTRATION

ClinicalTrials.gov—NCT02931539.

FUNDING SOURCE

Industry funded.

CLINICAL IMPACT SUMMARY

Treatment of refractory cytomegalovirus (CMV) infection in solid organ transplant recipients is challenging, with existing therapies limited by toxicity and drug resistance. Ganciclovir resistance is frequently seen, and foscarnet is associated with renal dysfunction in around 50% of patients treated (1). Safer, more effective treatments are needed to improve outcomes.

Avery et al. (2) have recently reported the outcomes of a multicentre, phase 3 randomised controlled trial of Maribavir, a novel UL97 protein kinase inhibitor that interferes with CMV DNA replication and encapsidation. The study randomised solid organ or stem cell transplant recipients with refractory CMV infection to Maribavir or investigator assigned treatment (IAT; valganciclovir/ganciclovir, foscarnet or cidofovir). Maribavir-treated patients demonstrated significantly higher clearance of viraemia after 8 weeks of treatment compared to IAT (55.7% vs. 23.9%). This response also appeared more sustained with Maribavir, with more patients achieving viraemia clearance and symptom control through to week 16.

Perhaps as importantly, Maribavir also appeared to have an improved safety profile compared to other agents. Incidence of renal dysfunction was lower than with foscarnet, and neutropenia was less frequent than valganciclovir/ganciclovir. Dysguesia was the most frequently reported side effect in Maribavir-treated patients. Overall, fewer patients discontinued therapy due to side effects than in the IAT group.

The study is pragmatic and well designed. It is not blinded, although this would be challenging given the different routes of administration of the various agents. There is some variability in included patients ("refractory" patients had to have failed to respond to one first line therapy, but this was not specified in detail) and in the investigator assigned comparator group, but this likely reflects real-world variations in practice.

Overall, the results are very encouraging and suggest that Maribavir offers an effective, better tolerated alternative to existing therapies for refractory CMV.

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.

REFERENCES

1. Avery RK, Arav-Boger R, Marr KA, Kraus E, Shoham S, Lees L, et al. Outcomes in Transplant Recipients Treated with Foscarnet for Ganciclovir-Resistant or Refractory Cytomegalovirus Infection. *Transplantation* (2016) 100:e74–e80. doi:10.1097/tp.0000000000001418
2. Avery RK, Alain S, Alexander BD, Maribavir for Refractory Cytomegalovirus Infections with or without Resistance Post-Transplant: Results from a Phase 3 Randomized Clinical Trial. *Clin Infect Dis* (2021). 1:ciab988. doi:10.1093/cid/ciab988

ESOT | Transplant International

POINT OF VIEW
published: 07 April 2022
doi: 10.3389/ti.2022.10339

Risk Aversion, Organ Utilization and Changing Behavior

Adnan Sharif[1,2]*

[1]Department of Nephrology and Transplantation, University Hospitals Birmingham, Birmingham, United Kingdom, [2]Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, United Kingdom

Improving organ acceptance and utilization rates is critical to ensure we maximize usage of donated organs as a scarce resource. Many factors underlie unnecessary discard of viable organs. Declined transplantation opportunities for candidates is associated with increased wait-list mortality. Technological advancements in organ preservation may help bridge the gap between donation and utilization, but an overlooked obstacle is the practice of risk aversion by transplant professionals when decision-making under risk. Lessons from behavioral economics, where experimental work has outlined the impact of loss or risk aversion on decision-making, have not been translated to transplantation. Many external factors can influence decision-making when accepting or utilizing organs, which are potentially amendable if external conditions are improved. However, attitudes and perceptions to risk for transplant professionals can pervade decision-making and influence behaviour. If we wish to change this behavior, then the underlying nature of decision-making under risk when accepting or utilizing organs must be studied to facilitate the design of targeted behavior change interventions to convert risk aversion to risk tolerance. To ensure optimal use of donated organs, we need more research into decision-making under risk.

Keywords: decision making, organ utilization, psychology, risk aversion, risk tolerance, discard

INTRODUCTION

Due to continued disparity between the supply versus demand for organs, maximizing usage of available organs is critically important. Strategies to increase both organ acceptance and utilization have been published, with the United Kingdom one example (1), acknowledging wide disparities in organ acceptance and/or utilization across national transplant programs. Some of this heterogeneity is unavoidable, relating to center-specific or cohort-specific factors, and multi-stakeholder calls to action acknowledge these barriers (2). However, another important variable is risk aversion. Specifically, risk aversion from transplant professionals when they receive viable organ offers but decision-making is skewed *against* acceptance and/or utilization. Risk aversion may occur due to infrastructural constraints, resource pressures or organ quality concerns. While the latter concern may be attenuated with development of novel techniques (e.g., normothermic perfusion), current financial realities limit the possibility of significant monetary investment into staffing and/or resources. Wide heterogeneity *between* centers can be explained by these confounders and is well documented. However, *within* center heterogeneity exists but is poorly described. Disparate practice by individuals is influenced by

Abbreviations: BCT, behavior change therapy.

Point of View

OPEN ACCESS

Correspondence:
Adnan Sharif
adnan.sharif@uhb.nhs.uk
orcid.org/0000-0002-7586-9136

Received: *05 January 2022*
Accepted: *18 March 2022*
Published: *07 April 2022*

Citation:
Sharif A (2022) Risk Aversion, Organ Utilization and Changing Behavior. Transpl Int 35:10339. doi: 10.3389/ti.2022.10339

risk psychology, but estimating its true prevalence is difficult without internal audit and governance measures.

While this issue has not been completely overlooked in the transplant literature (3), targeted research pales in comparison to other areas. However, even with better tools like real-time risk calculators, biomarkers, artificial intelligence algorithms, etc., decision-making for some transplant professionals will still favor risk aversion over risk tolerance due to individualized cognitive biases. After summarizing the problem, I hope to argue for a proactive way forward to tackle the risk psychology component in organ offer decision-making.

Organ Utilization Data

Many viable organs are discarded. Using kidneys as an example, Mohan et al. observed 17.3% of procured kidneys in the United States between 2000 and 2015 were discarded, with considerable geographical variation (4). Donor kidneys with multiple unfavorable characteristics were more likely to be discarded. However, some unilaterally discarded kidneys had favorable donor characteristics, evidenced by recipients of the non-discarded partner kidneys experiencing 1-year death-censored graft survival rates >90%. Exploring the last 2 decades in the United States, Stewart et al. observed >80% of kidney discard rates between 1987 and 2015 could be explained by the broadening donor pool, but the presence of unexplained residual factors suggested behavioral factors at play (5).

Organ discard rates in European countries are lower than the United States (6). If deceased donor kidney acceptance in the United States mirrored the French model (discard rate 17.9% versus 9.1%, respectively, $p < 0.001$), then Aubert et al. hypothesize 62% of discarded kidneys ($n = 17,435$) could generate 132,445 allograft life-years (7). Efforts to address this imbalance have been initiated. In the United States, new metrics for performance monitoring of transplant programs were approved in December 2021 (8). Compared to only post-transplant factors previously monitored (1-year patient/graft survival alone), new metrics include two additional post-transplant measures (90-day graft survival and 1-year graft survival conditional on 90-day graft survival) and importantly two new pre-transplant measures for each transplant program: 1) the rate of pre-transplant deaths, and; 2) the ratio of organ offers made to and accepted for candidates. These metrics are important as, while death or removal from the waiting-list is an unfortunate outcome for anyone awaiting a solid organ offer, for such a waiting list outcome to occur after refusal of a viable organ offer (i.e., accepted by another center on behalf of another wait-list candidate) is a travesty.

Outcomes for Candidates of Declined Offers

Declined organ offers is not a benign event for wait-listed candidates. Husain et al. studied a United States cohort of 280,041 wait-listed kidney transplant candidates (9). They observed approximately 30% of candidates who received at least one deceased-donor offer that was declined on their behalf eventually died or were removed from the waiting list.

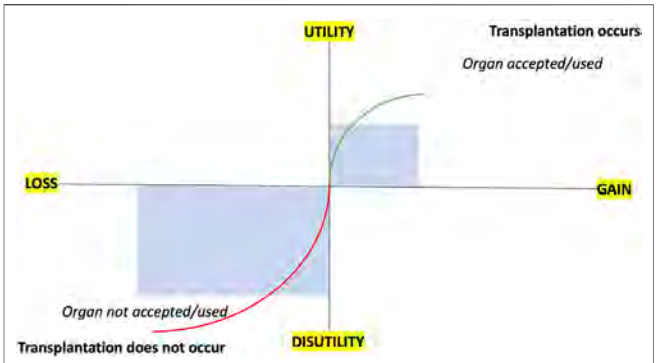


FIGURE 1 | Schematic outlining prospect theory and loss aversion in relation to organ acceptance/utilization and transplantation.

Odds for death on the waiting-list varied significantly across the country. Choi et al. studied a United States cohort of 9,628 wait-listed heart transplant candidates between 2007 and 2017 (10). They observed every 10% increase in center-adjusted acceptance rate for organ offers made to the highest-priority candidates was associated with a 27% reduction in the mortality rate among patients on the waitlist, with no detriment in 5-year adjusted post-transplant patient or graft failure. Center variability was dramatic, with acceptance rates to first-rank candidates varying nationally between 12.3% and 61.5% after adjustment for donor, candidate and geographical variables. Among 19,703 unique organ offers, only 6,302 hearts (32.0%) were accepted for first-rank candidates. Similar acceptance rates are observed after liver transplantation. Goldberg et al., in another cohort study undertaken in the United States, observed 8,882 out of 23,740 unique organ offers (37.4%) were accepted for first-ranked liver transplant candidates (11). After adjustment for organ quality and burden of illness in wait-listed candidates, the adjusted center-specific organ acceptance rates varied nationally between 15.7% and 58.1% ($p < 0.001$). In multivariable models, there was 27% increased odds of waitlist mortality for every 5% absolute decrease in center-adjusted organ offer acceptance rate (adjusted Odds Ratio 1.27, 95% Confidence Interval 1.20–1.32). While there may be genuinely valid clinical reasons for declining organs for first-ranked candidates, the influence of non-clinical factors for some declines cannot be ignored.

Lessons From Behavioral Economics

Perhaps the most difficult challenge in organ transplantation is deciding to accept or decline an offered organ. Risks associated with the donor or organ must be balanced against the survival prospects of the wait-listed candidate. Translating national statistics and population-level data to the individual for personalized decision-making is fraught with challenges. Transplant professionals will complement objective evidence with their subjective perception and experience, which can result in markedly variable assessment of risk versus benefit. If we translate classic economic theory to transplantation, we can speculate that transplant professionals will make choices that

TABLE 1 | Spectrum of risk attitudes applied to transplantation.

Attribute	Risk avoiding	Risk averse	Risk neutral	Risk tolerant	Risk seeking
Focus	Focus mainly on negative risk and avoiding loss at all costs	Focus on managing or avoiding negative risk drives most decisions	Focus on managing risk balance between negative and positive	Focus is on positive risk, but negative risk is also considered	Focus on positive risk and maximising gain—*all-or-nothing* philosophy
Attitude	Risk is very bad and to be avoided at all costs	Risk is bad but acceptable in some circumstances	Risk is seen as both bad and good to be managed equally	Risk is good but unacceptable in some circumstances	Risk is very good and to be embraced at all costs
Transplant example	Declining all organ offers as '*no organ is ever risk-free*'	Declining most organ offers as '*no organ is risk-free*'	Accepting some organ offers but declining some as '*not every organ offer is better than no offer*'	Accepting most organ offers as '*any organ is better than no organ*' in majority of cases	Accepting all organ offers as '*any organ is better than no organ*'
Risk versus benefit scale	Risk >>> Benefit	Risk > Benefit	Risk = Benefit	Risk < Benefit	Risk <<< Benefit
Optimal attitude[a]	Problematic	Questionable	Good	Ideal	Problematic

[a]*Author opinion.*

facilitates the greatest expected value (12). If an organ offer is declined, it is implied that the perceived costs (adverse outcomes) outweigh the benefits and we believe the recipient would be better off without accepting that particular organ offer.

However, it is not that simple or straightforward. Prospect theory, popularized by the Nobel Laureates Daniel Kahneman and Amos Tversky, would suggest individuals give more weight to factors framed as potential losses (risk) than to potential gains (benefits) (13). A transplant professional may overweigh the losses associated with accepting an organ and reject it even if the benefits outweigh the costs. This behavior is termed loss aversion and, when translated to transplantation, will manifest as usable organs being discarded (see **Figure 1**). A related behavioral factor that influences decision-making is risk aversion, where individuals choose a certain outcome over an outcome with less certainty. For transplant professionals, risk aversion will be the fear of larger loss (adverse outcome) resulting in settling for an unfavorable settlement (declining the kidney). Subjectively this attitude seems common, and we lack objective data about its true prevalence, but disparities in organ utilization data (either between (2, 4) or within centers) would support this assumption.

Decision-Making Under Risk

The implanting surgeon is considered to be ultimately accountable for the use of a donated organ. However, while surgeons taking primary organ offers is the most common system, some centers and/or countries have physicians (14) or other transplant professionals as first contact. Many decisions are made outside working hours, often with limited information about the donor, working under significant stress and scrutiny. Time-pressured decision-making could introduce a perception that the penalty of accepting an organ may outweigh the penalty of declining the offer. Experiments undertaken in the setting of financial transactions show time-pressured decision-making has no effect on risk attitude for gains, but increased risk aversion for losses (15).

While shared decision-making with wait-listed candidates can attenuate some of this burden, this is challenging after hours or with time pressures to genuinely obtain informed consent. Shared decision-making with other members of the transplant professional team, either another surgeon or multi-disciplinary colleagues such as physicians or anesthetists, may absorb clinical responsibility across a wider team than the operating surgeon alone. However, the success or failure of this approach will be influenced by the overall risk appetite of the unit. Wider consultation may paradoxically lead to higher decline rates due to a dilution of clinical responsibility and a form of "*regression towards the mean*" (16).

Clinical Decision-Making and Perception of Risk

Transplant professionals are willing to take risk to varying degrees, which is dependent upon their internal attitudes and perceptions (see **Table 1**) and external factors. While opinions will differ, and depend on personal bias, I suggest both extreme attitudes (risk avoidance and risk seeking) are undesirable for accepting organ offers, with risk tolerance the optimal "middle ground" with external factors all being equal.

Risk perception varies among surgeons, and other transplant professionals, but has never been empirically studied. In the surgical literature, Dilaver et al. undertook a systematic review of surgeons' perception of post-operative outcomes and risk (17). Twenty-seven studies comprising 20,898 patients undergoing a range of surgical procedures (but not solid organ transplantation) were included. Surgeons consistently overpredicted 30-day mortality rates and were outperformed by risk scoring tools in 6/7 studies comparing area under receiver operating characteristic curves (AUC). While surgeons' prediction of general morbidity was good, being equivalent or better than risk prediction models, long-term outcomes were poorly predictive with AUC values ranging from 0.51 to 0.75.

There are limited data with regards to how surgical decision-making is linked to risk taking behavior (18). Sacks et al. conducted a randomized controlled trial exploring surgeons' judgement and clinical decision-making to recommend surgery based upon four clinical vignettes (19). They were asked to assess risks (probability of serious complications or death) and benefits (recovery) of operative versus non-operative management and to rate their likelihood of recommending surgery. A national sample

of surgeons were randomized into usage of clinical vignettes alone (control group; $n = 384$) versus supplementation by data from a risk calculator (risk calculator group; $n = 395$). The results demonstrated exposure to risk calculator data led to more homogenous and accurate judgments of operative risk among surgeons. However, while risk calculators may facilitate more informed discussions of various treatment options, they did not alter the likelihood of the surgeon recommending an operation on a 5-point scale (3.7 versus 3.7 per randomized arm, $p = 0.76$).

Given the same clinical scenarios in a different study with 767 participants, surgeons' perceptions of treatment risks and benefits varied significantly and was highly predictive of their decision to operate (20). Analyzing hypothetical clinical vignettes, surgeons varied markedly in their assessment of the risks and benefits of operative and nonoperative management (range 4%–100%) and in their decision to operate (range 49%–85%). Surgeons were less likely to operate as their perception of operative risk increased and their perception of nonoperative benefit increased. By contrast, they were more likely to operate as their perception of operative benefit increased and their perception of nonoperative risk increased. Difference in risk/benefit perceptions explained 39% of the observed variation in decision to operate.

Some of this heterogeneity may be due to underlying personality traits of the operating surgeon. For example, Contessa et al. analyzed the association between personality factors (measured by the Myers-Briggs Type Indicator personality inventory), risk tolerance (measured by the Revised Physicians' Reactions to Uncertainty) and Physician Risk Attitude scales in 27 surgeons at a single campus (21). From their analysis, surgeons with personality factors E (Extravert), T (Thinking), and P (Perception) demonstrated higher tolerance for risk, while surgeons with personality factors I (Introvert), F (Feeling), and J (Judgment) demonstrated risk aversion on the same measures. Factors such as gender, seniority and age may also play a role, with an increase in rationality and decrease in risk-readiness examples of profession-specific personality trait shifts (22).

External Influences on Decision-Making Under Risk

Risk attitude will be influenced by external factors. Organ utilization will be sub-optimal if professionals accepting organ offers do not feel confident in the environment to perform surgery. Unfavorable environments lead to defensive medicine being practiced, even if contrary to evidence-based findings (23). In a cross-sectional survey of 220 physicians working in surgical specialities, defensive medicine was widely encountered with no correlation to age or experience (24). Transplantation occurs under regulatory oversight to ensure transplant centers achieve benchmark outcomes. However, pressure to achieve normative outcomes creates bias against accepting transplant risk (25). Center-specific factors weight heavily in decisions to accept organs. Their attenuation may alter risk perception, and improve organ acceptance/utilization in some cases, but will not totally overcome individual cognitive biases.

Explaining Risk to the Wait-Listed Candidate

Wait-listed candidates also make decisions under stress, reliant upon good communication from the transplant professional for informed choice (26). Risk communication to patients about organ offers should incorporate discussion of risk, benefit and uncertainty that acknowledges the health literacy of the transplant candidate. However, risk communication to patients can be flawed. Objective evidence can be subjectively framed using different tricks to influence consent, with different examples of framing bias influencing decision-making (27). Therefore, even if organs are accepted, they may not be utilized after refusal by the wait-listed candidate during consent. While this may be appropriate in some cases, there will be scenarios where decision-making has been skewed towards risk aversion rather than risk tolerance by the inappropriate framing of risk by transplant professionals.

Solutions: Targeting Behaviour Change for Improved Decision-Making

Before interventions can be developed, we must first define what optimal decision-making means. This can be subjective or heterogenous dependent upon individualized clinical scenarios. As described by Milkman et al., normative models provided by economic theorists offer a reasonable benchmark for how optimal decision-making is defined (28). According to these models, decision-making should be transitive, insensitive to minor changes in context, revealed preferences should be consistent with stated preferences, no systematic mathematical errors in judgment should arise, and a decision maker should remain satisfied after making a choice that their decision was correct after reflection. Most importantly, an optimal decision is one that a decision maker regards as the right choice regardless of whether they were evaluating their own decision or someone else's.

To change decision-making behavior for organ offers, we must follow evidence-based methodology to firstly understand the underlying behavior and then utilize the correct intervention(s) for application. Systematic methods to understand behavior change exist, with a hierarchically-structured, taxonomy of 93 techniques used in behavior change therapy (BCT) clustered into 16 groups (29). Combining adequate assessment of the behavior to be changed (i.e., risk aversion), and application of the relevant theoretical constructs, a toolkit to design behavior change interventions to convert risk aversion to tolerance among transplant professionals is possible but requires investigation.

Other changes are required to reduce risk aversion. Transplant-specific guidelines that review decision-making barriers are required. These must provide evidence-based toolkits to support transplant professionals accepting organs and facilitation of risk communication. However, patient/public involvement is necessary to ensure communication is appropriately framed to aid understanding. Surgery must occur in adequately resourced and supported environments, with exact requirements varying between centers. This

includes optimizing numbers of surgeons, physicians, allied health professionals, operating theatres, intensive care facilities, inpatient and outpatient follow-up facilities (16). This is unlikely to be achieved without significant monetary investment so other strategies (e.g., collaborative networks, shared decision-making, etc.) must be investigated for efficacy with quality improvement studies, audit and governance. Recognition that early post-transplant complications are not necessarily attributable to poor decision-making at the time of organ offer is important, for medico-legal purposes and regulatory oversight. This includes financial reimbursement, which may be insufficient with less-than-ideal organs that can lead to more complications and/or hospitalizations but are still in the best interest for patients.

Shared decision-making is important. This can be between patients and their healthcare providers, ensuring patients are at the centre of the transplantation decision (30). However, it is also desirable among the clinical transplant team, transforming individual professional risk to collective departmental risk. Responsibility must be shared with all multi-disciplinary professionals involved in the full spectrum from procurement to implantation. With adequate counselling, all parties must fully embrace the possibility of risk to gain the opportunity of benefit. This requires multi-stakeholder consensus, including patients and professionals, on optimized decision-making under risk for wait-listed candidates.

Fundamentally, we must learn to become risk tolerant. For example, early deaths after transplantation are usually rigorously investigated at a local level, with national involvement if centers deviate from the median. However, early post-transplant deaths are far outweighed by deaths for wait-listed candidates while awaiting a graft which have been hitherto ignored. As recently stated, "*we perceive greater risk in acts of commission than in acts of omission: if a patient dies during or after transplantation, it's the doctor's responsibility; if the patient dies from organ failure while awaiting a transplant, we can blame the indifference of the Universe*." (31) Plus patient survival is not the only milestone to measure the success of transplantation. Quality of life benefits should also be considered in the decision-making of organ offers.

An adverse outcome is not necessarily an indicator that the decision to accept and/or transplant the organ was wrong. Indeed, I suggest any unit that has none is too risk averse and not transplanting enough. Paradoxically, higher surgical activity may lead to attenuation of adverse outcomes. Birkmeyer et al., in a study linking surgical skill and complication rates after bariatric surgery, observed technical skill was strongly correlated to procedural volume (32). Compared with the top quartile of skill, surgeons ranked in the bottom quartile experienced higher rates of reoperation, readmission within 30 days and return visits to the emergency department. Therefore, surgeons with low transplant activity will enter a Catch-22 situation; greater inclination for risk averse behavior that further reduces their procedural volume.

CONCLUSION

Risk aversion by transplant professionals is an understandable but unwelcome barrier for optimized organ acceptance and/or utilization. Despite significant advancements in behavioral economics studying decision-making with risk, little reciprocal work has been undertaken in transplantation. National efforts to increase organ acceptance/utilization are important, with scientific and technological breakthroughs potentially ushering in exciting future possibilities (33, 34). However, we cannot overlook the human component to organ acceptance and/or utilization. While external factors are important, some center-specific and others regulatory or medico-legal, individual cognitive biases remain important. A concerted effort to study decision-making under risk for transplant professionals, and targeted behavioral measures to shift risk aversion to risk tolerance when accepting organ offers, should be strongly encouraged.

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

AS is solely responsible for this manuscript.

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.

REFERENCES

1. NHS Blood and Transplant. Organ Donation and Transplantation 2030: Meeting the Need: A Ten-Year Vision for Organ Donation and Transplantation in the United Kingdom (2018). Available from: https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/23463/meeting-the-need-2030.pdf.
2. Vanholder R, Domínguez-Gil B, Busic M, Cortez-Pinto H, Craig JC, Jager KJ, et al. Organ Donation and Transplantation: a Multi-Stakeholder Call to Action. *Nat Rev Nephrol* (2021) 17(8):554–68. doi:10.1038/s41581-021-00425-3
3. White SL. Nudging the Organ Discard Problem. *Transplantation* (2017) 101(7):1518–9. doi:10.1097/tp.0000000000001718
4. Mohan S, Chiles MC, Patzer RE, Pastan SO, Husain SA, Carpenter DJ, et al. Factors Leading to the Discard of Deceased Donor Kidneys in the United States. *Kidney Int* (2018) 94(1):187–98. doi:10.1016/j.kint.2018.02.016
5. Stewart DE, Garcia VC, Rosendale JD, Klassen DK, Carrico BJ. Diagnosing the Decades-Long Rise in the Deceased Donor Kidney Discard Rate in the United States. *Transplantation* (2017) 101(3):575–87. doi:10.1097/tp.0000000000001539
6. Council of Europe. *Newsletter Transplant: International Figures on Donation and Transplantation*. Strasbourg, France: EQDM 2019. ISSN (2018). p. 2171–4118.

7. Aubert O, Reese PP, Audry B, Bouatou Y, Raynaud M, Viglietti D, et al. Disparities in Acceptance of Deceased Donor Kidneys between the United States and France and Estimated Effects of Increased US Acceptance. *JAMA Intern Med* (2019) 179(10):1365–74. doi:10.1001/jamainternmed.2019.2322
8. Organ Procurement and Transplantation Network. OPTN Board Adopts New Transplant Program Performance Metrics (2021). Available from: https://optn.transplant.hrsa.gov/news/optn-board-adopts-new-transplant-program-performance-metrics/.
9. Husain SA, King KL, Pastan S, Patzer RE, Cohen DJ, Radhakrishnan J, et al. Association between Declined Offers of Deceased Donor Kidney Allograft and Outcomes in Kidney Transplant Candidates. *JAMA Netw Open* (2019) 2(8):e1910312. doi:10.1001/jamanetworkopen.2019.10312
10. Choi AY, Mulvihill MS, Lee H-J, Zhao C, Kuchibhatla M, Schroder JN, et al. Transplant Center Variability in Organ Offer Acceptance and Mortality Among US Patients on the Heart Transplant Waitlist. *JAMA Cardiol* (2020) 5(6):660–8. doi:10.1001/jamacardio.2020.0659
11. Goldberg DS, French B, Lewis JD, Scott FI, Mamtani R, Gilroy R, et al. Liver Transplant center Variability in Accepting Organ Offers and its Impact on Patient Survival. *J Hepatol* (2016) 64(4):843–51. doi:10.1016/j.jhep.2015.11.015
12. Heilman RL, Green EP, Reddy KS, Moss A, Kaplan B. Potential Impact of Risk and Loss Aversion on the Process of Accepting Kidneys for Transplantation. *Transplantation* (2017) 101(7):1514–7. doi:10.1097/tp.0000000000001715
13. Kahneman D, Lovallo D, Sibony O. Before You Make that Big decision. *Harv Bus Rev* (2011) 89(6):50–60.
14. Cardinal H, Ballesteros Gallego F, Affdal A, Fortin MC. Canadian Transplant Nephrologists' Perspectives on the Decision-Making Process for Accepting or Refusing a Kidney from a Deceased Organ Donor. *Clin Transpl* (2020) 34(3):e13793. doi:10.1111/ctr.13793
15. Kocher MG, Pahlke J, Trautmann ST. Tempus Fugit: Time Pressure in Risky Decisions. *Management Sci* (2013) 59:2380–91. doi:10.1287/mnsc.2013.1711
16. Neuberger J, Callaghan C. Organ Utilization - the Next Hurdle in Transplantation? *Transpl Int* (2020) 33(12):1597–609. doi:10.1111/tri.13744
17. Dilaver NM, Gwilym BL, Preece R, Twine CP, Bosanquet DC. Systematic Review and Narrative Synthesis of Surgeons' Perception of Postoperative Outcomes and Risk. *BJS Open* (2020) 4(1):16–26. doi:10.1002/bjs5.50233
18. Tubbs EP, Elrod JAB, Flum DR. Risk Taking and Tolerance of Uncertainty: Implications for Surgeons. *J Surg Res* (2006) 131(1):1–6. doi:10.1016/j.jss.2005.06.010
19. Sacks GD, Dawes AJ, Ettner SL, Brook RH, Fox CR, Russell MM, et al. Impact of a Risk Calculator on Risk Perception and Surgical Decision Making. *Ann Surg* (2016) 264(6):889–95. doi:10.1097/sla.0000000000001750
20. Sacks GD, Dawes AJ, Ettner SL, Brook RH, Fox CR, Maggard-Gibbons M, et al. Surgeon Perception of Risk and Benefit in the Decision to Operate. *Ann Surg* (2016) 264(6):896–903. doi:10.1097/sla.0000000000001784
21. Contessa J, Suarez L, Kyriakides T, Nadzam G. The Influence of Surgeon Personality Factors on Risk Tolerance: a Pilot Study. *J Surg Education* (2013) 70(6):806–12. doi:10.1016/j.jsurg.2013.07.014
22. Bogacheva N, Kornilova T, Pavlova E. Relationships between Medical Doctors' Personality Traits and Their Professional Risk Perception. *Behav Sci (Basel)* (2019) 10(1):6. doi:10.3390/bs10010006
23. Yeung E. Evidence Based versus Defensive Medicine. *BMJ* (2018) 363:k4114. doi:10.1136/bmj.k4114
24. Calikoglu EO, Aras A. 'Defensive Medicine Among Different Surgical Disciplines: A Descriptive Cross-Sectional Study. *J Forensic Leg Med* (2020) 73:101970. doi:10.1016/j.jflm.2020.101970
25. Sharif A, Montgomery RA. Regulating the Risk-reward Trade-off in Transplantation. *Am J Transpl* (2020) 20(8):2282–3. doi:10.1111/ajt.15882
26. Bradshaw P. Good Communication Reduces Risk of a Complaint or Claim. *BMJ* (2019) 367:l6160. doi:10.1136/bmj.l6160
27. Sharif A. Improving Risk Counseling for Kidney Transplant Candidates Offered Deceased Donor Kidneys. *Kidney Int Rep* (2020) 5(12):2351–3. doi:10.1016/j.ekir.2020.09.016
28. Milkman KL, Chugh D, Bazerman MH. How Can Decision Making Be Improved? *Perspect Psychol Sci* (2009) 4(4):379–83. doi:10.1111/j.1745-6924.2009.01142.x
29. Michie S, Richardson M, Johnston M, Abraham C, Francis J, Hardeman W, et al. The Behavior Change Technique Taxonomy (V1) of 93 Hierarchically Clustered Techniques: Building an International Consensus for the Reporting of Behavior Change Interventions. *Ann Behav Med* (2013) 46:81–95. doi:10.1007/s12160-013-9486-6
30. Stiggelbout AM, Weijden TVd., Wit MPTD, Frosch D, Legare F, Montori VM, et al. Shared Decision Making: Really Putting Patients at the centre of Healthcare. *BMJ* (2012) 344:e256. doi:10.1136/bmj.e256
31. Montgomery RA. Getting Comfortable with Risk. *N Engl J Med* (2019) 381(17):1606–7. doi:10.1056/nejmp1906872
32. Birkmeyer JD, Finks JF, O'Reilly A, Oerline M, Carlin AM, Nunn AR, et al. Surgical Skill and Complication Rates after Bariatric Surgery. *N Engl J Med* (2013) 369(15):1434–42. doi:10.1056/nejmsa1300625
33. Weissenbacher A, Vrakas G, Nasralla D, Ceresa CDL. The Future of Organ Perfusion and Re-conditioning. *Transpl Int* (2019) 32(6):586–97. doi:10.1111/tri.13441
34. Elisseeff J, Badylak SF, Boeke JD. Immune and Genome Engineering as the Future of Transplantable Tissue. *N Engl J Med* (2021) 385(26):2451–62. doi:10.1056/nejmra1913421

ESOT | Transplant International

FORUM
published: 12 April 2022
doi: 10.3389/ti.2022.10038

Development of *Ex Situ* Normothermic Reperfusion as an Innovative Method to Assess Pancreases After Preservation

Arnau Panisello-Roselló[1,2]*, *Emma Folch-Puy*[1], *Joan Roselló-Catafau*[1] and *René Adam*[2]

[1]Experimental Pathology Department, Institute of Biomedical Research of Barcelona (IIBB), CSIC, Barcelona, Spain, [2]AP-HP Hôpital Paul Brousse, UR Chronothérapie, Cancers et Transplantation, Université Paris-Saclay, Paris, France

Keywords: pancreas, HOPE, normothermia, reperfusion, preservation solutions

A Forum discussing:

Development of *Ex Situ* Normothermic Reperfusion as an Innovative Method to Assess Pancreases After Preservation
by Ogbemudia AE, Hakim G, Dengu F, El-Gilani F, Dumbill R, Mulvey J, et al. (2021) Transpl Int 34(9):1630–42. doi:10.1111/tri.13990

Dear Editors,

We read with great interest the paper entitled "*Development of Ex Situ Normothermic Reperfusion as an Innovative Method to Assess Pancreases After Preservation*" (1). After analyzing the *ex situ* assessment of pancreases by normothermic reperfusion (NR), the authors suggested that HMPO2 may be better than SCS; they a further compared two different HMPO2 perfusates: Belzer-MPS and IGL-2.

We would like to point out some considerations concerning this perfusate comparison. Specifically, when the water content in pancreas grafts (as a surrogate for edema assessment) was measured in hypothermic conditions. Under those conditions, IGL2HMP pancreases showed a lower water content than the UWHMP group. These results are concomitant with the lower amylase and lipase levels, well known as injury markers for pancreas in static preservation, which has been validated recently as well in dynamic condition by Branchereau et al. (2). This higher injury prevention exerted by IGL2 would be associated with the water content gain, which in turn is regulated by the presence of oncotic agents such as PEG35 (in IGL2HMP) and HES (in UWHMP). One of the concerns related to pancreas preservation is the development of edema, which is generally regarded as undesirable. Thus, the lower water content in INGL2HMP indicates a better oncotic efficiency of PEG as compared to HES. Moreover, it is widely reported that HES acts as a red blood cell pro-aggregating agent (3), which is a major factor when considering a solution containing PEG to be more suitable for pancreas washout (4).

Recently, we have reported the benefits of that using PEG35 can be beneficial for preventing IRI damage (5) and can have an anti-inflammatory role in acute pancreatitis (6). This is especially relevant for pancreas IR pathophysiology, as a tendency to develop pancreatitis and vascular thrombosis after ischemia has been widely reported (2); notably, these are main causes of early patient morbidity and mortality after pancreas transplantation (7, 8). In addition, IGL2 (PEG35) is very suitable to better preserving luminal glycocalyx deterioration against the mechano-transduction events in hypothermic oxygenated perfusion (HOPE) due to inherent fluid dynamics, whereby the lower viscosity of IGL2 (9) as well as the vasodilatory action derived from NO generation by PEG35 may be relevant factors to be considered (4).

Forum

OPEN ACCESS

***Correspondence:**
Arnau Panisello-Roselló
arnau.panisello@iibb.csic.es

Received: *15 September 2021*
Accepted: *14 March 2022*
Published: *12 April 2022*

Citation:
Panisello-Roselló A, Folch-Puy E, Roselló-Catafau J and Adam R (2022) Development of Ex Situ Normothermic Reperfusion as an Innovative Method to Assess Pancreases After Preservation. Transpl Int 35:10038. doi: 10.3389/ti.2022.10038

We agree that future investigations are needed to confirm and expand relevant study, especially considering the number of animals used. We highlight the use of a PEG-based solution (IGL2) and its improvement of HOPE strategies, given that the favorable results reported in other solid organs could be extended to pancreas. This would be, for instance, the case of the protection of the mitochondria, as previously reported by us for liver measured as glutamate dehydrogenase (GLDH) (mitochondrial damage) (9, 10).

Especially given the lack of consensus regarding the optimal perfusion solutions and methods for pancreas preservation prior to transplantation, we are grateful for this important paper, as it opens up the dialogue about developing a new paradigm for pancreas preservation.

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

AP-R, EF-P, JR-C, and RA participated in the design, draft, revision, and approval of the work. The authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

FUNDING

This research was funded by Instituto de Salud Carlos III through FIS project PI 15/00110 co-funded by FEDER from Regional Development European Funds (European Union).

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. Ogbemudia AE, Hakim G, Dengu F, El-Gilani F, Dumbill R, Mulvey J, et al. Development of *Ex Situ* Normothermic Reperfusion as an Innovative Method to Assess Pancreases After Preservation. *Transpl Int* (2021) 34:1630–42. doi:10.1111/tri.13990
2. Prudhomme T, Renaudin K, Lo Faro ML, Cantarovich D, Kervella D, Minault D, et al. *Ex Situ* Hypothermic Perfusion of Nonhuman Primate Pancreas: A Feasibility Study. *Artif Organs* (2020) 44(7):736–43. doi:10.1111/aor.13655
3. Mosbah IB, Franco-Gou R, Abdennebi HB, Hernandez R, Escolar G, Saidane D, et al. Effects of Polyethylene Glycol and Hydroxyethyl Starch in University of Wisconsin Preservation Solution on Human Red Blood Cell Aggregation and Viscosity. *Transplant Proc* (2006) 38(5):1229–35. doi:10.1016/j.transproceed.2006.02.068
4. Zaouali MA, Bejaoui M, Calvo M, Folch-Puy E, Pantazi E, Pasut G, et al. Polyethylene Glycol Rinse Solution: an Effective Way to Prevent Ischemia-Reperfusion Injury. *Wjg* (2014) 20:16203. doi:10.3748/wjg.v20.i43.16203
5. Pasut G, Panisello A, Folch-Puy E, Lopez A, Castro-Benítez C, Calvo M, et al. Polyethylene Glycols: An Effective Strategy for Limiting Liver Ischemia Reperfusion Injury. *Wjg* (2016) 22(28):6501. doi:10.3748/wjg.v22.i28.6501
6. Ferrero-Andrés A, Panisello-Roselló A, Serafín A, Roselló-Catafau J, Folch-Puy E. Polyethylene Glycol 35 (PEG35) Protects against Inflammation in Experimental Acute Necrotizing Pancreatitis and Associated Lung Injury. *Ijms* (2020) 21:917. doi:10.3390/ijms21030917
7. Sakorafas GH, Tsiotos GG, Sarr MG. Ischemia/Reperfusion-Induced Pancreatitis. *Dig Surg* (2000) 17:3–14. doi:10.1159/000018793
8. Troppmann C. Complications after Pancreas Transplantation. *Curr Opin Organ Transplant* (2010) 15:112–8. doi:10.1097/mot.0b013e3283355349
9. Panisello Rosello A, Teixeira da Silva R, Castro C, G Bardallo R, Calvo M, Folch-Puy E, et al. Polyethylene Glycol 35 as a Perfusate Additive for Mitochondrial and Glycocalyx Protection in HOPE Liver Preservation. *Int J Mol Sci* (2020) 21(16):570. doi:10.3390/ijms21165703
10. Panisello-Rosello A, Roselló-Catafau J. HOPE (Hypothermic Oxygenated Perfusion) Strategies in the Era of Dynamic Liver Graft Preservation. *EBioMedicine* (2020) 61:103071. doi:10.1016/j.ebiom.2020.103071

ESOT | Transplant International

REVIEW
published: 21 April 2022
doi: 10.3389/ti.2022.10333

Patient Selection for Downstaging of Hepatocellular Carcinoma Prior to Liver Transplantation—Adjusting the Odds?

Daniel Seehofer[1], Henrik Petrowsky[2], Stefan Schneeberger[3], Eric Vibert[4], Jens Ricke[5], Gonzalo Sapisochin[6], Jean-Charles Nault[7,8] and Thomas Berg[9]*

[1]Department of Visceral, Transplant, Thoracic and Vascular Surgery, University Hospital, Leipzig, Germany, [2]Swiss HPB and Transplantation Center, Department of Surgery and Transplantation, University Hospital Zürich, Zurich, Switzerland, [3]Department of Visceral, Transplantation and Thoracic Surgery, Medical University of Innsbruck, Innsbruck, Austria, [4]Centre Hépato-Biliaire, Hôpital Paul Brousse, Villejuif, France, [5]Department of Radiology, LMU Munich, Munich, Germany, [6]Ajmera Transplant Program and HPB Surgical Oncology, Department of Surgery, Toronto General Hospital, University of Toronto, Toronto, ON, Canada, [7]Service d'Hépatologie, Hôpital Avicenne, Hôpitaux Universitaires Paris-Seine-Saint-Denis, Université Paris Nord, Paris, France, [8]INSERM UMR 1138 Functional Genomics of Solid Tumors Laboratory, Paris, France, [9]Division of Hepatology, Department of Medicine II, Leipzig University Medical Center, Leipzig, Germany

Background and Aims: Morphometric features such as the Milan criteria serve as standard criteria for liver transplantation (LT) in patients with hepatocellular carcinoma (HCC). Since it has been recognized that these criteria are too restrictive and do not adequately display the tumor biology, additional selection parameters are emerging.

Methods: Concise review of the current literature on patient selection for downstaging and LT for HCC outside the Milan criteria.

Results: The major task in patients outside the Milan criteria is the need for higher granularity with patient selection, since the benefit through LT is not uniform. The recent literature clearly shows that beneath tumor size and number, additional selection parameters are useful in the process of patient selection for and during downstaging. For initial patient selection, the alpha fetoprotein (AFP) level adds additional information to the size and number of HCC nodules concerning the chance of successful downstaging and LT. This effect is quantifiable using newer selection tools like the WE (West-Eastern) downstaging criteria or the Metroticket 2.0 criteria. Also an initial PET-scan and/or tumor biopsy can be helpful, especially in the high risk group of patients outside the University of California San Francisco (UCSF) criteria. After this entry selection, the clinical course during downstaging procedures concerning the tumor and the AFP response is of paramount importance and serves as an additional final selection tool.

OPEN ACCESS

Correspondence:
Daniel Seehofer
GFD_VTTG@medizin.uni-leipzig.de

Received: *03 January 2022*
Accepted: *22 February 2022*
Published: *21 April 2022*

Citation:
Seehofer D, Petrowsky H, Schneeberger S, Vibert E, Ricke J, Sapisochin G, Nault J-C and Berg T (2022) Patient Selection for Downstaging of Hepatocellular Carcinoma Prior to Liver Transplantation—Adjusting the Odds? Transpl Int 35:10333. doi: 10.3389/ti.2022.10333

Abbreviations: AFP, alpha fetoprotein; cPR, complete pathologic response; DS, downstaging; HCC, hepatocellular carcinoma; ITT, intention to treat analysis; LT, liver transplantation; MC, Milan criteria; MC-in, inside the Milan criteria; MC-out, outside the Milan criteria; mVI, microvascular invasion; PET, positron emission tomography; PR, partial response; SD, stable disease; TTV, total tumor volume; UCSF, University of California, San Francisco; UCSF-in, inside the UCSF criteria; UCSF-out, outside the UCSF criteria; WE-DS, West-Eastern downstaging criteria.

Conclusion: Selection criteria for liver transplantation in HCC patients are becoming more and more sophisticated, but are still imperfect. The implementation of molecular knowledge will hopefully support a more specific risk prediction for HCC patients in the future, but do not provide a profound basis for clinical decision-making at present.

Keywords: review, liver transplantation, hepatocellular carcinoma, downstaging, transarterial chemoembolization (TACE), drop-out, intention-to-treat

INTRODUCTION

Liver transplantation (LT) has become the mainstay of curative treatment for hepatocellular carcinoma (HCC) in cirrhosis, as it can provide the best long-term results (>5 years) in selected patients (1). The size and number of HCC nodules are suggestive for the risk of early tumor recurrence after LT according to the "Milan criteria" (MC) (**Table 1**) or the "Metroticket 2.0" criteria (2) assessments. Morphometric features have served as the main criteria for LT for many years, although from a tumor-biology viewpoint, they do not display the tumor biology. Thus, more specific selection parameters are warranted. With advances in the understanding of HCC biology, the MC appear too restrictive since a significant proportion of patients with HCC outside the MC (MC-out) are curable with LT. Recently, a prospective randomized trial confirmed that LT in selected MC-out patients markedly improved the 5-year survival from 31.2% to 77.5% (3). The major task in MC-out patients is the need for higher granularity with patient selection, since the benefit through LT is not uniform.

To overcome this issue, modifications or expansion of the MC should include parameters for estimating tumor biology and thus aid in patient selection for LT (**Table 1**). These parameters mainly include four categories: 1) serum biomarkers, 2) histological parameters, 3) tumor imaging, and 4) dynamic parameters during neoadjuvant measures. The following review focuses on LT candidate selection in patients initially presenting outside the MC. This includes a concise review of recently published clinical series in this field. Since many published studies are of a retrospective nature and of low quality according to the GRADE criteria (4), they do not include intention to treat (ITT) analysis and different pathways of patient selection. Hence, direct comparison of the reported results is difficult and a "meta-analysis" in the narrow sense was not considered useful for this review.

SETTING THE STAGE–PATHO-MOLECULAR CLASSIFICATION OF HEPATOCELLULAR CARCINOMA

Although direct investigation of tumor tissue is potentially the gold standard for HCC characterization, the investigation of biopsy material pre-LT has limitations. From the molecular and pathological characteristics, HCC is a heterogeneous tumor, both regarding the intra- and inter-tumor variability, and a major reason for the complexity of classifications; no hat fits all. Tumor development is a multistep process with malignant transformation of precursor lesions into early HCC, as described elsewhere in detail (5). During carcinogenesis and tumor progression various signaling pathways are frequently affected by recurrent somatic mutations. Despite the presence of around 50 proteins altering somatic mutations per tumor across all stages, only a few of these mutations are considered to be relevant drivers of carcinogenesis (two to six per tumor) (6). These mainly include genetic alterations in the following signaling pathways: 1) telomere maintenance, 2) Wnt/b-catenin, 3) P53/cell cycle regulation, 4) AKT/mTOR, 5) MAP kinase, 6) epigenetic modification, and 7) oxidative stress (5). Based on transcriptomic profiling, HCC subclassification interlinks dysregulation of signaling pathways with genetic alterations, histological subtypes, and prognosis underlying the molecular heterogeneity of HCC. This classification includes two major types, a proliferation and a non-proliferation type, with each encompassing different biological subclasses (**Table 2**). Despite these tremendous advances, biopsy-derived parameters are still underused in clinical pathways of LT and the use of genetic screening could hold important prognostic value.

One reason for the ongoing lack of specific histopathological parameters prior to LT might be the intratumor heterogeneity with trunk mutations present in all cells and other private mutations present in only parts of the tumor. This results in different grades of differentiation, even within the same lesion and leads to primary or secondary resistance to systemic treatments.

TABLE 1 | Morphometric and combined (Toronto) selection criteria for LT.

	Solitary HCC	Multifocal HCC
Milan criteria (MC)	≤5 cm	Maximal 3 nodules ≤3 cm
Up-to-seven criteria (UT7)	≤7 cm	Sum of maximum tumor diameter (cm) and number of tumors ≤7
UCSF criteria	≤6.5 cm	HCC: largest nodule ≤4.5 cm and sum of the diameter of all nodules ≤8 cm
Extended Toronto criteria (eTC)	No limit in size	No limits in size and number
	Only G1 und G2 tumors (obligatory biopsy)	Only G1 und G2 tumors (obligatory biopsy)
	No tumor-associated symptoms	No tumor-associated symptoms

TABLE 2 | Molecular subclassification of HCC.

	"Proliferation class" (50%of HCC)	"Non-proliferation class" (50%of HCC)
G1-G6 classification	G1-G3	G4-G6
Histological/clinical characteristics	Poor differentiation	Well/moderate differentiation
	High frequency of vascular invasion	Low frequency of vascular invasion
	High AFP levels	Low AFP levels
	Frequent HBV etiology (G1-G2)	Mainly HCV and alcohol
	G3: Macrotrabecular-massive histological subtypes (poor prognosis)	G4: Contain the steatohepatitic subtypes of HCC, inflammatory infiltrates, and CRP expression
Molecular features	Chromosomal instability and TP53 mutations	Chromosomal stability
	G1: Stem cell features, RPS6KA3 mutations	G4: Retain hepatocyte-like features, IL-6/JAK/STAT activation, and rare CTNNB1 and TP53 mutations
	G1-G2: AXIN1 mutations	G5 and G6: Wnt/b-catenin pathway activation due to CTNNB1 mutations
	G3: Dysregulation of cell cycle genes, FGF19 amplification, and TSC1/2 mutations	

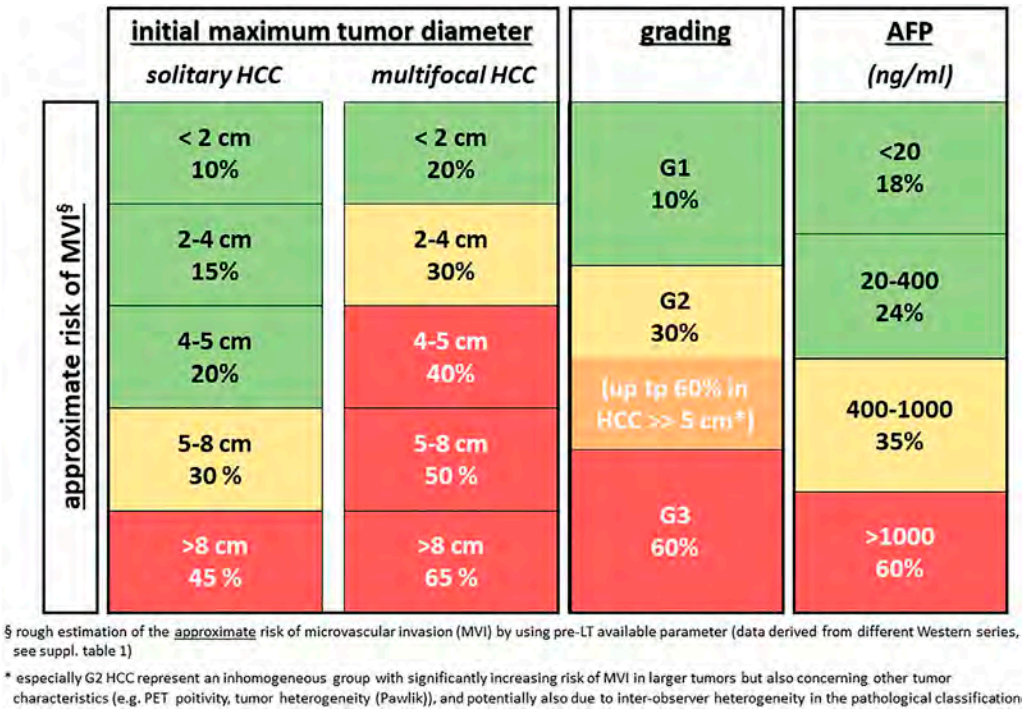


FIGURE 1 | Rough estimation of the risk of microvascular invasion (mVI) by using pre-LT available parameters (data derived from different Western series, see **Supplementary Table S1**). * especially G2 HCC represents an inhomogeneous group with significantly increasing risk of mVI in larger tumors but also concerning other tumor characteristics (e.g., PET positivity, tumor heterogeneity [Pawlik]), and potentially also due to inter-observer heterogeneity in the pathological classification.

However, it has been repeatedly shown that even basic tumor characteristics, like poor tumor grading (G3 = poorly differentiated HCC) (7) or aneuploidy (8) are important predictors of tumor recurrence. To note, HCC analyzed from liver explants gave a good prognostic tumor score according to the molecular prognosticator five-gene score and in HCC from G4 molecular subgroups, which included small well-differentiated HCC without microvascular invasion (mVI) developed on cirrhosis and expressing a transcriptomic program close to mature hepatocytes (9). However, these results should be read with caution since some of the most aggressive tumors may not be included due to drop out from the waiting list. Using whole-genome sequencing, a recent analysis has shown that in multifocal tumors, only 20%–40% are intrahepatic metastases arising from the same clone, whereas the remaining are based on *de novo* independent carcinogenesis of the diseased liver parenchyma at different sites (10). Therefore, the predictive potential of confined biopsy material, e.g., prior to LT, has natural limits where multifocal disease is frequently observed. In patients of (known) tumor heterogeneity, the worst grade determines the prognosis (11).

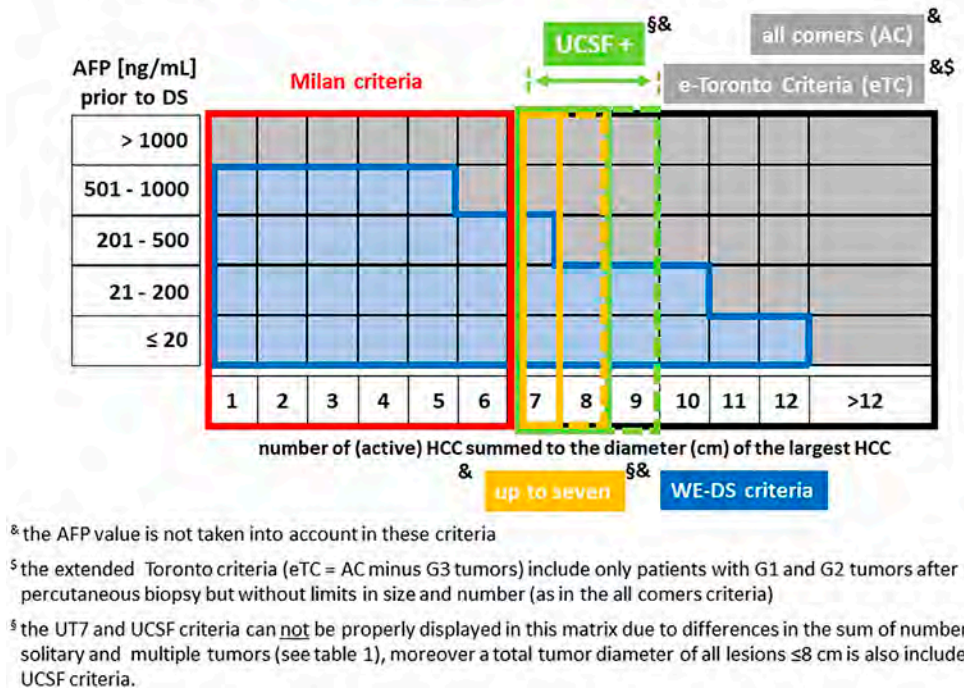


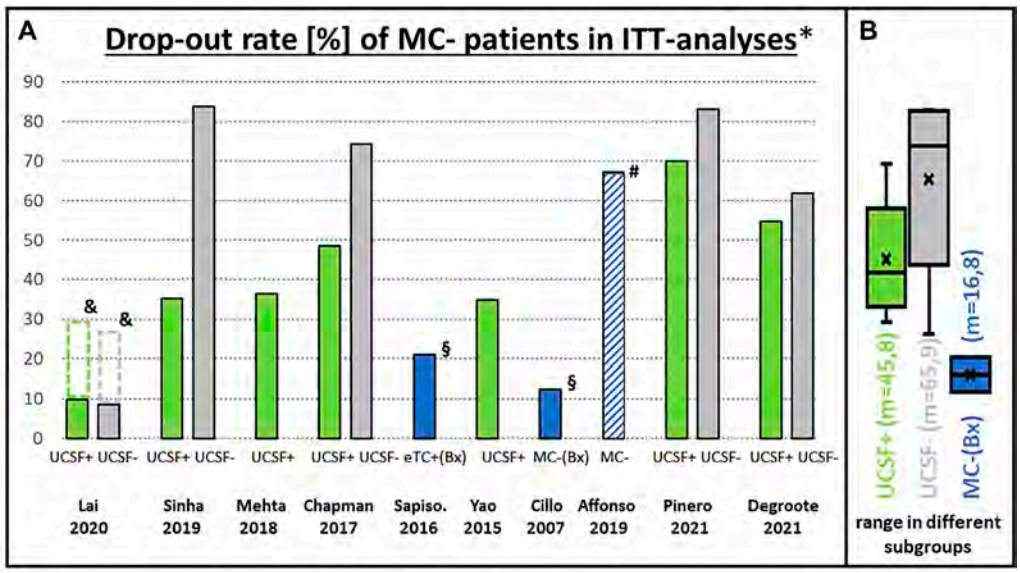
FIGURE 2 | Potential criteria for downstaging-approaches prior to LT. and the AFP value is not taken into account in these criteria. $ the extended Toronto criteria include only patients with G1 and G2 tumors after percutaneous biopsy but without limits in size and number (eTC = AC minus G3 tumors). § the UT7 and UCSF criteria cannot be properly displayed in this matrix due to differences in the sum of number in solitary and multiple tumors (see **Table 1**), moreover a total tumor diameter of all lesions ≤8 cm is also included in the UCSF criteria.

mVI, as one of the most relevant risk factors for HCC recurrence after LT, has been shown to be more predictive of tumor recurrence than, for example, standard staging criteria (12). While macrovascular invasion as a contraindication for LT can nowadays be diagnosed more precisely due to better imaging modalities (13), mVI can only be detected in surgical specimens and not by imaging or in biopsy material. Surrogate parameters like tumor size and number or serum alpha fetoprotein (AFP) levels are still in use for predicting the risk of mVI (**Figure 1**). Moreover, some new markers, such as serum and tissue PIVKA-II expression (14), combination of microRNA expression in HCC (15), or a 35-gene molecular tumor signature (16) have been proposed to predict mVI more precisely but these results still require external prospective validation. Overall, there is clearly a critical unmet need for reliable invasive or non-invasive preoperative detection of mVI and/or tumor biology taking into account biological diversity and intra- and inter-tumor heterogeneity.

STILL NOT OUTDATED: MORPHOMETRIC PARAMETERS—SHOULD THERE BE AN UPPER LIMIT OF SIZE AND NUMBER FOR DOWNSTAGING?

Since HCC size and number are easily accessible information by imaging, these parameters are traditionally used as a basis for further discussion and decision-making in many HCC-LT patient selection algorithms. As the risk for mVI and/or a G3 tumor corresponds with tumor size and number in unselected cohorts, these parameters ensure a relatively low drop-out rate during listing (17). While the morphometric selection criteria fulfill the idea of a great outcome per an ITT perspective, they are too unspecific as a (sole) surrogate for tumor biology. This shows the risk of withholding a life-saving procedure from a group of patients with large/multilocular but biologically favorable HCCs.

In patients outside the MC, a majority of Western transplant centers use some form of downstaging technique before LT. Center policies for including patients in downstaging protocols vary widely (**Figure 2**), resulting in diverse drop-out rates. In this context, it is important to emphasize that an "acceptable drop-out rate" remains ill-defined and may be difficult to specify from an ethical perspective. The justification for downstaging and an acceptable drop-out rate needs to be seen and determined in light of the efficacy of alternative treatment methods, organ availability, and a patient's attitude toward a concept that holds a limited chance of success. Patients outside the University of California San Francisco criteria (UCSF-out) have a significantly lower rate of successful downstaging, as described by Sinha et al. who reported that the success of downstaging decreases with increasing tumor burden. The proportion of successful downstaging after 12 months was 68% in patients with a sum of maximum diameter and tumor number ≤8 compared to 47% in



& dashed lines represent drop-out rate in Western centers in UT7+ and UT7- patients (no data on UCSF criteria in the western subgroup given), solid lines represent the drop out rate in the whole cohort with a high number of living donor transplantations in the eastern subgroup
§ pre-selection of AC-patients prior to DS using tumor biopsy (Bx) and exclusion of G3-tumors
# no seperate data für UCSF+ and UCSF- patients
m =mean (x)
* detailed data are given in suppl. table 2

FIGURE 3 | (A) Drop-out rate of patients outside the Milan criteria (MC) in the literature (only ITT analyses included): Percentage of patients not transplanted mainly because downstaging failed to reach the Milan criteria; **(B)** statistical summary of the different studies (box plot). & dashed lines represent drop-out rate in Western centers in UT7+ and UT7-patients (no data on UCSF criteria in the Western subgroup given). Solid lines represent the drop-out rate in the whole cohort for UCSF criteria with a high number of living donor transplantations in the Eastern subgroup. § pre-selection of AC patients prior to downstaging using tumor biopsy (Bx) and exclusion of G3 tumors. # no separate data for UCSF+ and UCSF- patients. $ the low drop-out rate of 27% in UT7-patients was observed only in one multicenter analysis (Lai, 2020) with a very short median waiting time of approximately 4 months * detailed data are given in **Supplementary Table S2**.

patients with a sum of 12, and 38% in patients with a sum of 14 tumors (18). However, no patient selection was performed in any of these trials at the entry of downstaging (i.e., true all-comers population).

The rationale for considering the tumor burden also relates to the reported drop-out rates of 54% and 77% after 1 and 2 years, respectively for UCSF-out patients compared to 25% and 35% in UCSF-in patients (18). The overall drop-out risk in completely unselected all-comers was 84% (62 out of 74 UCSF-out patients), which can be considered unreasonably high (**Figure 3**). Others have described a drop-out rate of 50% or more in unselected (no biopsy, no PET, no AFP limit) patients after entering a downstaging protocol (19). The drop-out risk clearly correlates with the tumor burden on presentation.

The UCSF criteria might be considered a reasonable upper limit for applying downstaging protocols on a solely morphometric basis. However, in the all-comers cohort, a proportion of patients can be cured by LT, although the likelihood is clearly lower (20,21). Considering this, it might be 'too restrictive' or more precisely 'too unspecific', if patient selection for downstaging is only based on tumor size and number. Moreover, the dichotomous nature of such criteria, which might also be subject to indistinctness in measurement technique, does not reflect the complex tumor biology of HCC. Other and more specific parameters to modulate the entry risk are therefore required, but relevant clinical data are only available for tumor grading, AFP levels, and PET-CT (see below).

IMPLICATIONS FOR TUMOR BIOPSY BEFORE INITIATION OF DOWNSTAGING

Considering the available data, the only biopsy-generated parameter with sufficient evidence for patient selection in LT is (poor) tumor differentiation. However, despite the progress in the patho-molecular classification of HCC, the exclusion of macrotrabecular-massive subtypes as a well-defined histological entity (22), which can be assessed on biopsy, should be deliberated. This subtype is associated with a poor prognosis after resection and ablation (23), but until now this has not been tested in a pre-transplantation setting. Since HCC is one of the rare tumors, where the final diagnosis can be made on the basis of non-invasive imaging, many centers do not perform a tumor biopsy prior to LT. This is, among others, based on the potential hazard of tumor cell dissemination. The risk of tumor seeding is undeniable, and cases of needle tract metastases have been described. However, the risk of (isolated) needle tract seeding and HCC recurrence after LT in two large cohorts was only 1.3% (24) and 1.9% (7) when using an adequate biopsy technique. Patients within the MC might not need a tumor

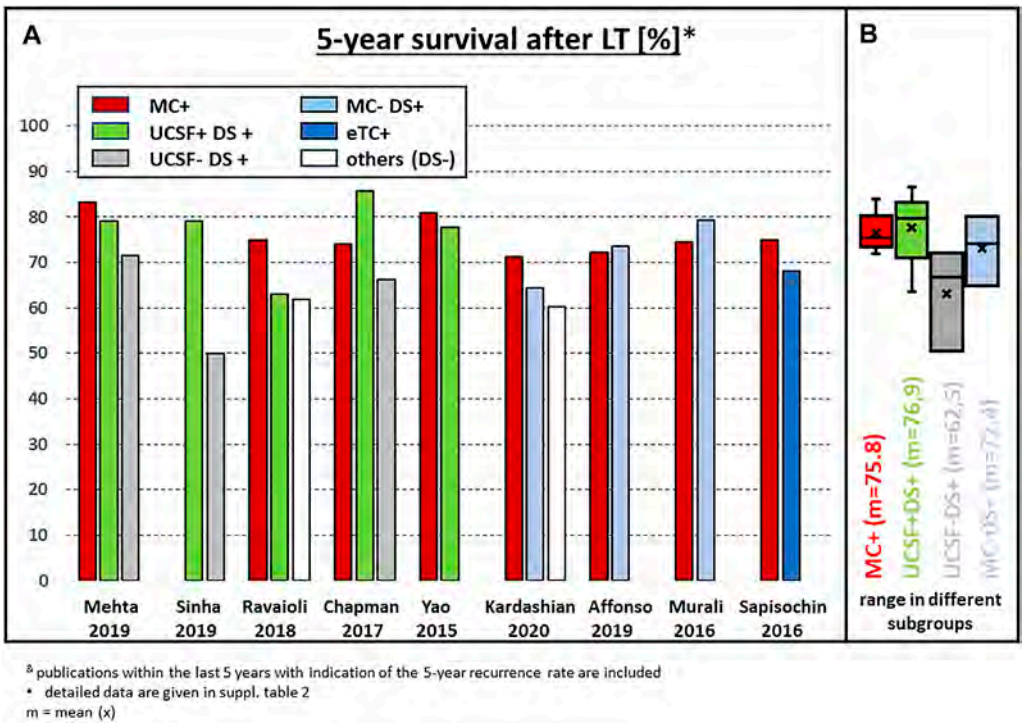


FIGURE 4 | (A) Actuarial 5-year survival after LT in recently published series of patients with subgroups outside the MC; **(B)** statistical summary of the different studies (box plot). & publications within the last 5 years with indication of the 5-year recurrence rate are included. * detailed data are given in **Supplementary Table S2**.

biopsy, due to the relatively low risk of G3 tumors, and the acceptable risk of mVI in those with small G3 tumors. In some studies, these patients have also shown that with relevant waiting time, the test of time or the dynamic response to bridging therapy ensures adequate patient selection, proven by the excellent long-term outcome without pre-LT biopsy (**Figures 4**, **5**). However, in patients within the MC, the very low risk has to be weighed against the potential benefit of tumor biopsy, with the seeding risk being negligible in MC-out patients.

Two single center experiences showed that upfront exclusion of G3 tumors in MC-out patients as an entry criterion for downstaging protocols might be a key factor to improve the survival results. Indeed, using this approach, Cillo et al. reported a 75% 5-year survival after LT irrespective of size and number (25). More recently, the updated Toronto experience confirmed these data, with 5- and 10-year actuarial patient survival rates of 68% and 50%, respectively in the MC-out group, which was slightly but not significantly different from the MC-in group (76% and 60%) (7) (**Figure 4**). However, pretransplant biopsy results might also be misleading due to tumor heterogeneity (26) and thereby produce a relevant number of false-positive or false-negative results. In the experience of Court et al., only 29% of G3 tumors were diagnosed correctly by pre-LT needle biopsy, while 17 out of 155 (11%) tumors with G1 or G2 differentiation in the final explant histology were classified as G3 by needle biopsy (27). In addition, the recurrence rate in this analysis did not correlate with the pre-LT biopsy, but with the grading in the final explant pathology.

Nevertheless, two prospective analyses have shown that a biopsy and eventually repeated biopsies are able to exclude a substantial proportion but not all G3 tumors. In the Toronto [7] and Padua cohorts (25), only 8% and 16% of MC-out patients were finally found to have a G3 tumor, respectively, even though the initial pre-LT biopsy did not show a G3 tumor. Not surprisingly, the few patients with G3 tumors had a 5-year disease-free survival of 47% compared to 82% in non-G3 patients (p = 0.008) (7). In comparison, the multicentric analysis by Mazzaferro et al. revealed a 27% incidence of G3 tumors in the MC-out subgroup (28). None of the available biopsy-driven series capture the "true ITT-population" including those patients with G3 tumors on biopsy, which were excluded from further downstaging. Therefore, this quota remains elusive.

But, conversely, a preoperative biopsy was 84%–92% effective in excluding G3 lesions. In addition, the incidence of mVI was only 26% in the MC-out patients and not statistically different from the MC-in group (25). Besides the excellent long-term results, the drop-out rate on an ITT basis was relatively low in both series with 21.4% and 12.5% in MC-out patients, indicating an effective reduction of the entry risk prior to downstaging (**Figure 3**). In contrast, series without any selection prior to downstaging revealed drop-out rates of more than 50% in the UCSF-out subgroup (**Figure 3**), relating to a drop-out reduction risk of approximately 50%–60% from the initial risk. The Padua cohort also showed that the risk of drop out increases with waiting time, leading to a 12.5% drop-out rate at 18 months and a 40% drop-out rate at 24 months (25). However, the 3- and

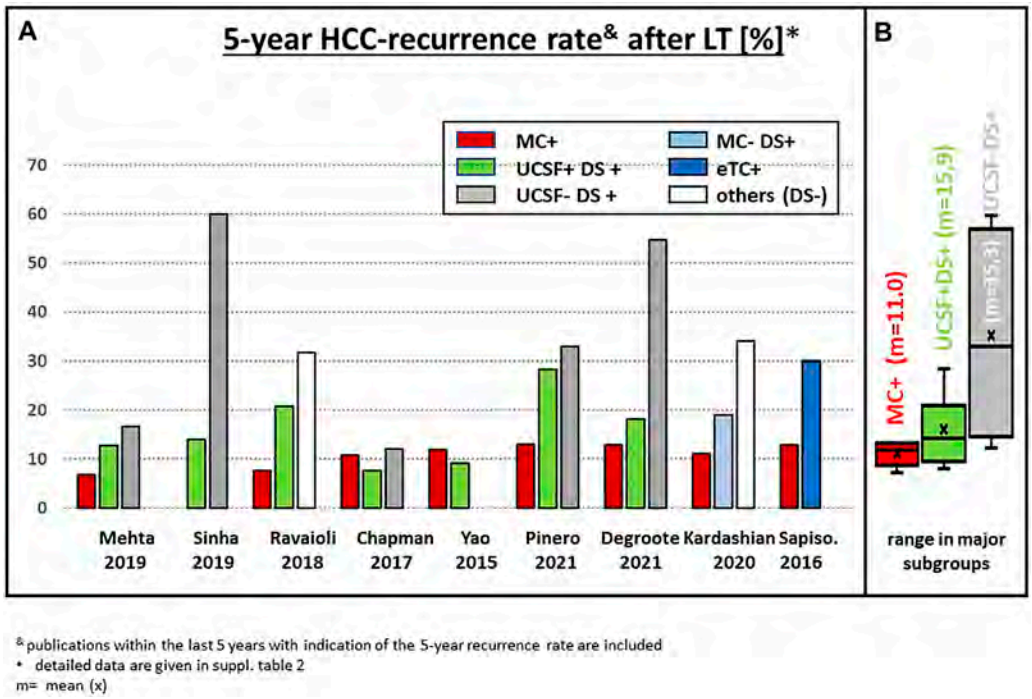


FIGURE 5 | (A) Actuarial 5-year HCC recurrence rate after LT in recently published series of patients with subgroups outside the MC; **(B)** statistical summary of the different studies (box plot). & publications within the last 5 years with indication of the 5-year recurrence rate are included. * detailed data are given in **Supplementary Table S2**.

5-year survival rates on an ITT basis were not significantly different between the UCSF-out (85% and 76%) and the UCSF-in (85 and 85%) groups, suggesting that in pre-selected cohorts, e.g., by exclusion of G3 tumors, the UCSF criteria do not seem to be an ideal discriminator.

In summary, excluding G3 tumors using tumor biopsies means the entry risk in all-comers populations can be reduced to a risk comparable to UCSF-in patients in terms of drop out. In addition, the percentage of mVI and (overlooked) G3 tumors might be reduced to a level comparable to MC-in patients. From the present data, exclusion of G3 tumors might be beneficial in UCSF-in patients, and particularly useful in UCSF-out patients prior to downstaging. However, controversy remains as to whether tumor biopsy is the ideal method, or if non-invasive parameters might be comparably adequate.

CAN THE ROLE OF THE INITIAL AFP LEVEL ACT AS A GATEKEEPER?

High AFP levels are known to be associated with tumor aggressiveness, poorly differentiated tumors, and mVI. Accordingly, it has been clearly shown that AFP provides prognostic information beyond tumor size and number (29). The prognostic value of an increasing AFP during waitlisting but also the AFP level at the time of LT has been shown in several analyses (see below). The establishment of the Hazard Associated with Liver Transplantation for HCC (HALT-HCC) score suggests that the addition of AFP levels facilitates the identification of patients with a poor prognosis within the MC and also of patients with a favorable prognosis outside the MC, using a cut-off HALT score of 17 (30). Consequently, AFP levels at LT have gained relevance for organ allocation. However, data on the initial AFP level and its relevance on patient selection for downstaging are less clear. Analyses of patients with the majority of patients undergoing living donor liver transplantation mainly without bridging or downstaging therapy also move towards inclusion of biological parameters, mainly the AFP value, e.g., in the Japanese "5-5-500 rule" (31). The impact of waiting time (i.e., living vs. deceased donor LT) on tumor recurrence outside the MC is also an interesting issue, but clearly beyond the scope of the present review.

On the one hand, AFP might help to identify patients with a high drop-out risk during downstaging, and on the other hand, an initial AFP <20 ng/ml might be predictive of a good response to downstaging therapies and a low recurrence rate after LT. About one-third of HCC patients inside or outside the MC present with normal AFP levels (<20 ng/ml) (32). It has been shown that in AFP-negative tumors the proportion of G3 tumors is significantly lower than in AFP-positive tumors (15% vs. 28%, $p < 0.001$). Accordingly, the rate of vascular invasion was significantly lower (20% vs. 31%, $p < 0.001$) and the percentage of pathological complete tumor necrosis was significantly higher (25 % vs. 16%, $p = 0.01$) in AFP-negative patients [32]. In a French cohort, when AFP levels were <100 ng/ml, only 2% of patients had a G3 tumor and only 20% of patients had mVI (plus 5% macroinvasion) (33).

Despite this, there still seems to be a subgroup of AFP-negative patients with impaired prognosis after LT. Additional initial selection criteria like AFP-DCP/PIVKA-II (34), PET-CT, or tumor biopsy might be of help with this specific subgroup but has yet to be fully investigated. In contrast, the response to downstaging as a predictive factor was also confirmed in AFP-negative tumors, as a tumor burden outside the MC at LT is a major risk factor for HCC recurrence (HR 10, 0.0; 3.7–33.3; $p < 0.001$). In the whole AFP-negative group, no recurrence was observed in the subgroup of patients with negative AFP and successful downstaging (32). Data generated from a US multicenter analysis unraveled that an AFP <20 ng/ml was a predictor of complete pathologic response (cPR) [63]. The rate of cPR was 26.6% in AFP-negative tumors, but only 19.5% in tumors with AFP >20 ng/ml at any time, but the majority of patients were MC-in. However, the percentage of AFP-negative tumors seems to be around 30% in MC-out and MC-in patients (32).

In AFP-producing tumors, it is increasingly clear that the predictive value of morphometrical parameters can be refined by simultaneous consideration of the AFP level. Likewise, an AFP level of >1,000 ng/ml at the time of LT has been validated as a poor prognostic factor in MC-out patients (33), as well as in MC-in patients, even though the incidence of AFP >1,000 ng/ml in MC-in patients was below 5% (29). This cut-off of 1,000 ng/ml or an increasing AFP prior to LT is generally accepted as a poor prognostic factor. In MC-out patients, the risk of tumor recurrence gradually increases with AFP values between 20 and 1,000 ng/ml at LT, thereby providing a risk stratification within defined criteria of tumor size and number. The additional value of the AFP level to improve patient selection in this context was first shown in the French AFP model, in which the upper limit of size and number could be increased from the MC criteria to ≤6 cm in cases of ≤3 nodules and to ≤3 cm in cases of ≥4 tumor nodules in patients with pre-LT AFP levels ≤100 ng/ml without a significantly increased risk of HCC recurrence (33). However, validating studies revealed a poor predictive value of this model, also pointing out the importance of the underlying liver disease (e.g., HCV vs. non-HCV) in different cohorts (35). In view of the fact that HCV is also displaced by NASH in the LT population (36), this might become extremely relevant, since many of the models are derived from cohorts with high numbers of patients with viral hepatitis. Other series have focused on an AFP limit of <400 ng/ml and found a low 5-year recurrence rate of 4.9% in patients with a total tumor diameter of <8 cm (37), or a 4-year recurrence rate of 9.4% in patients with a total tumor volume (TTV) of <115 cm^3 and AFP <400 ng/ml (38).

Lai et al. (39) raised the point that size and number alone are insufficient selection parameters and the AFP levels at first referral might overcome or at least reduce this problem. In a multicentric analysis of 3091 HCC patients at 12 centers, an ITT model was used for an upper limit of tumor burden for downstaging. A successful LT was defined as a 30% 5-year survival after LT and recalibrated to >13% 5-year survival rate after the time of first referral, otherwise LT was estimated to become an unrealistic goal. In this model, the upper limit of tumor burden at presentation revealed an inverse relation with the initial AFP level. Whereas in patients with an AFP level ≤20 ng/ml, an up to 12 sum of HCC number and diameter was acceptable, which decreased with increasing AFP to 10 (AFP 21–200 ng/ml), 7 (AFP 201–500 ng/ml), and 5 (AFP 501–1,000 ng/ml) (**Figure 6**). Using this West-Eastern downstaging criteria (WE-DS) the drop-out rate in Western patients (i.e., with low frequency of living donor liver transplantation [LDLT]) was below 15% and therefore not significantly different from the UCSF-in group. In contrast, 30.4% of patients outside the WE-DS criteria experienced drop out. When comparing the WE-DS criteria with the UCSF criteria, the WE-DS group included more patients than the UCSF-in group, and only 54% of the MC-out patients would have been considered for LT according to the UCSF criteria, but 79% according to the WE-DS criteria. Nevertheless, the WE-DS group revealed the same 5-year post-LT HCC-related death rate (14.4% vs. 15%). These data confirm that the (morphometric) UCSF criteria can be easily adjusted by including biological parameters. However, even in the WE-DS-out patients, only 38% of HCC-related deaths were observed within 5 years and 42% within 10 years after LT. In other words, based on these data, 3 out of 10 WE-DS-out patients will experience drop out prior to LT and a further 3 will develop HCC recurrence after LT, but 4 out of 10 WS-DS-out patients (ITT) are theoretically good candidates for LT, but the overall number would be relatively small (i.e., 2.5% in this series) [Error! Bookmark not defined.].

On the other end of the AFP scale, it could be shown that an AFP >1,000 ng/ml is a poor prognostic factor. A large multicenter analysis has even shown that cases with pre-LT AFP >1,000 ng/ml had no survival benefit after LT (40). However, this analysis did not consider the AFP at initial referral or the treatment response. Therefore, the situation of an initial AFP >1,000 ng/ml is still unclear. It was shown in low numbers that successful downstaging is possible in patients with AFP >1,000 ng/ml, but probably achievable only in less than 20% of patients (12.5% successful downstaging in (41) and 18.8% in (19)).

In summary, the AFP level may be used as a gatekeeper prior to downstaging, as well as prior to LT. Since all proposed models are based on adjustment of probabilities, decreasing upper limits of AFP levels should be considered with increasing tumor burden to maintain the rate of futile downstaging and/or LT approaches within accepted limits (42). However, at least for patients with a tumor burden outside the WE-DS criteria, additional parameters are advisable, especially since the selection of patients with a favorable prognosis on the basis of AFP levels means the percentage of patients with predicted poor prognosis is disproportionately increasing (**Figure 7**). Moreover, since the WE-DS model is based solely on parameters prior to downstaging, the dynamic response to downstaging is not captured. Therefore, this model might be a useful gatekeeper. However, in patients with high tumor burden, additional parameters might be useful together with dynamic re-evaluation during downstaging protocols.

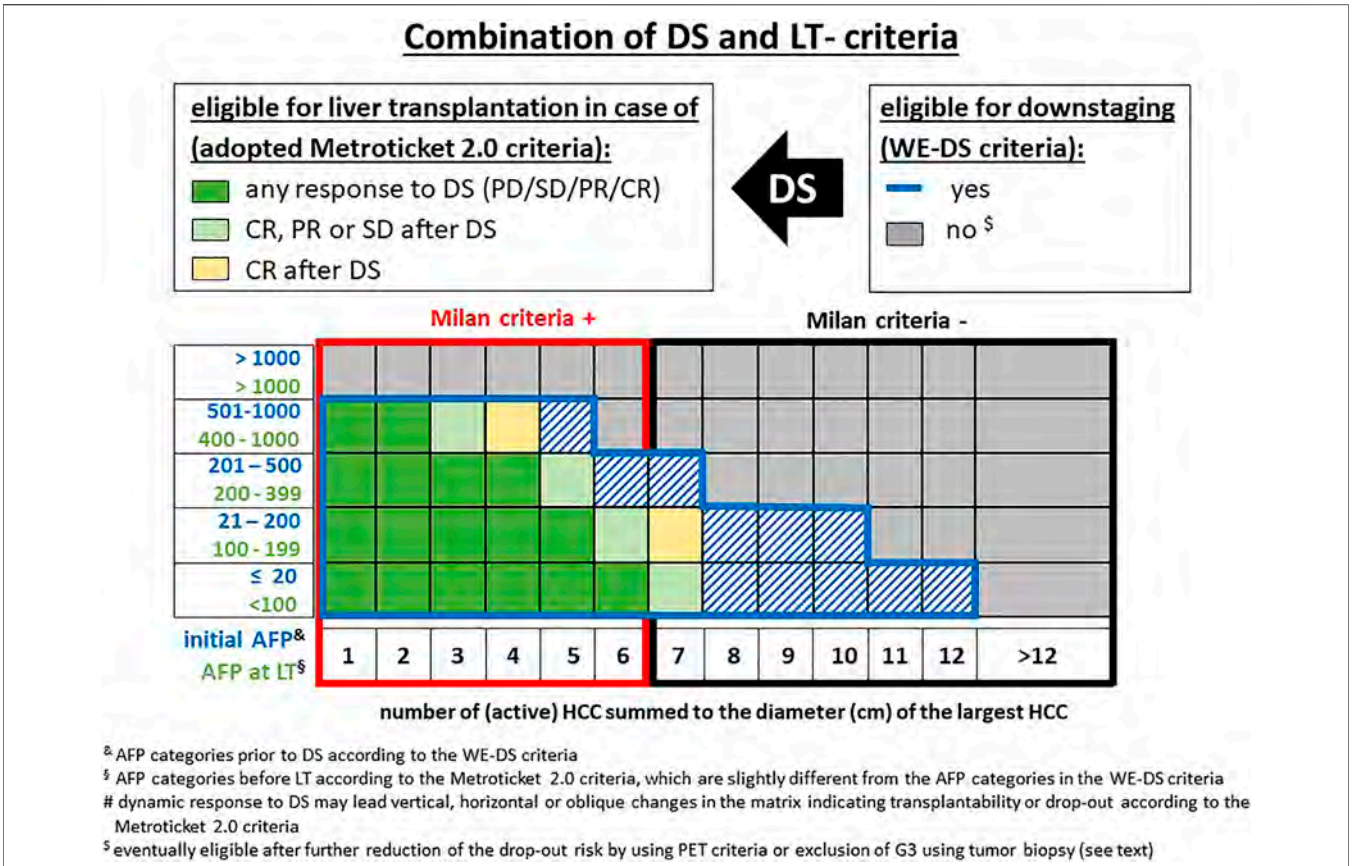


FIGURE 6 | Combination of published downstaging and LT criteria (DS: downstaging, PD: progressive disease, PR: partial response, CR: complete response). & AFP categories prior to DS according to the WE-DS criteria. § AFP categories before LT according to the Metroticket 2.0 criteria, which are slightly different from the AFP categories in the WE-DS criteria. $ eventually eligible after further reduction of the drop-out risk by using PET criteria or exclusion of G3 using tumor biopsy (see text).

BEYOND AFP–PET, HEPATOBILIARY MRI, AND OTHER BIOMARKERS

Whereas differentiated HCCs share a similar enzymatic activity with normal liver tissue, poorly differentiated tumors reveal a low glucose-6 phosphatase activity and high uptake of 18F-FDG. Therefore, it has been postulated that poorly differentiated tumors can be identified by means of PET positivity (PET+) (43). Therefore, PET holds the potential of being a non-invasive alternative to pre-LT tumor biopsy. Kornberg et al. showed that PET+ was the only independent predictor of tumor recurrence in patients outside the up-to-seven criteria (HR of 19.25). Independently of tumor size and number, the 5-year survival in PET-patients was 88.7% compared to 46.3% in PET+ patients ($p < 0.001$). Moreover, in the PET-patients, the percentage of mVI and G3 tumor was 12% and 9.3%, respectively, compared to 82.9% mVI and 34.1% G3 tumors in PET+ patients ($p < 0.001$). Thus, 14 of 21 poorly differentiated HCCs were PET positive (44). PET+ correlates with tumor burden, as 26% of MC-in patients were found to be PET+ compared to 47% in MC-out patients and inside the up-to-seven criteria and 48% in patients outside the up-to-seven criteria. Overall, these data confirm that morphometric criteria alone might not be ideal discriminators in MC-out patients. In a recent review, the accuracy of 18F-FDG-PET/CT for predicting mVI was 68%–88% and 55%–71% for poor tumor differentiation (43).

Data on the predictive potential of the combination of PET with AFP values are mainly derived from Asian cohorts, mainly using LDLT and often no consequent downstaging protocols. A multicentric experience from 16 Japanese LT centers showed that in multivariate analysis, exceeding MC, AFP ≥115 ng/ml, and PET+ status were independent risk factors for HCC recurrence (45). Of the 49 MC-out patients, 47% had PET+ scans and the 2-year recurrence rate in PET+ was significantly higher than in PET-patients (80.0% vs. 29.4%). A Korean analysis confirmed that PET in combination with the AFP value might be a better predictor of survival than each parameter alone. By using an AFP cut-off of 200 ng/ml they were able to define groups with a low (AFP <200 ng/ml and PET-), intermediate (PET+ or AFP >200 ng/ml), and high risk (PET+ and AFP >200 ng/ml) for tumor recurrence, leading to 5-year disease-free survival rates of 86.1%, 79.0%, and 18.5%, respectively (46).

Preliminary data on MRI criteria in transplanted patients show that the presence of satellite nodules and peri-tumoral hypo-intensity is associated with a 3-year tumor recurrence rate of 75.5% compared to 28.6% in cases of their absence ($p < 0.001$)

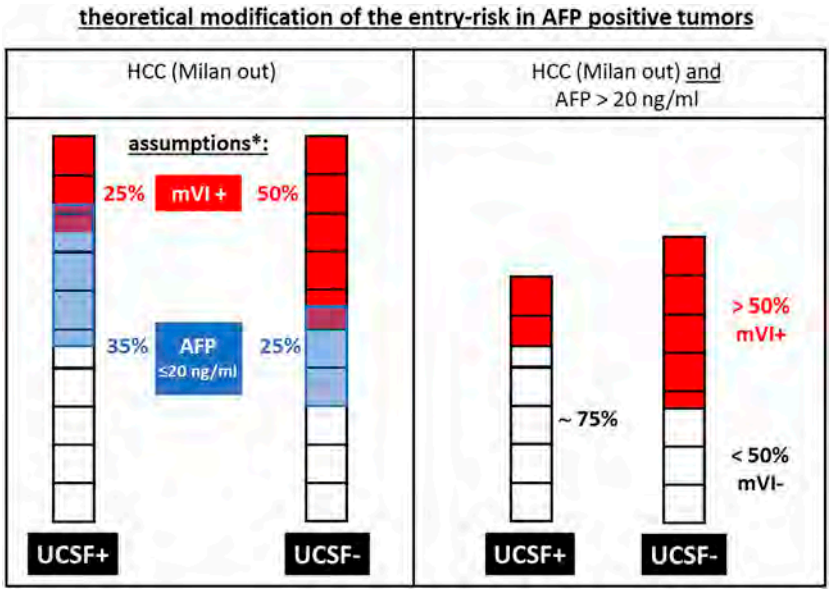


FIGURE 7 | Exemplary modification of the theoretical entry risk: in AFP-positive (i.e., > 20 ng/ml) tumors the risk of microvascular invasion (mVI+) is markedly increasing in UCSF- tumors compared to UCSF+ tumors, since the remaining patients represent a "negative selection" in a group with an already increased risk of mVI+. This increasing risk by increasing AFP levels is the theoretical basis for the limitation of morphometric selection criteria with increasing AFP levels in the WE-DS criteria as well as in the adopted Metroticket 2.0 criteria as shown in **Figure 6**. * the assumed values for mVI+ tumors and AFP levels >20 ng/ml are based on the average data from **Figure 1**, but are of a theoretical nature to expertly clarify the increasing risk by patient selection criteria, which are used in addition to morphometric criteria.

in 32 MC-out patients (13). Imaging features of HBI MRI predicting mVI have been investigated extensively, i.e., peri-tumoral arterial enhancement, irregular tumor margin, and peri-tumoral hypo-intensity on hepatobiliary phase. All parameters correlate well with the presence of mVI and therefore warrant further investigation for transplant candidate selection (47,48). Moreover, MRI might also be helpful in identifying macrotrabecular-massive HCC with high specificity (49). Hepatobiliary MRI adding criteria beyond wash-in and wash-out has proven higher sensitivity with comparable specificity for HCC depiction in cirrhotic patients (50). Diffusion weighted imaging is another promising MRI feature to predict HCC treatment outcome. A single center trial reported lower apparent diffusion coefficient values, which predicted early recurrence after LT (51). However, the heterogenous nature of HCC, especially in larger lesions, could potentially limit the value of the diffusion technique in more advanced patients outside MC. Other biomarkers like C-reactive protein, PIVKA-II (=DCP), and the neutrophil-lymphocyte ratio have been studied in LT candidates either alone or in combination with morphometric parameters. Relevant data are only available for DCP, which has been systemically evaluated prior to LDLT in Asian centers. In this context, a DCP cut-off between 300 and 450 mAU/ml was shown to indicate a five-fold increased risk for HCC recurrence after LT (52). Its value exceeding the use of AFP and the validation in Western series is currently lacking.

Besides the AFP value, PET+ seems to be at present the only non-invasive parameter with enough clinical evidence for inclusion in clinical pathways. In contrast to MC-in patients, where even PET+ patients seem to have a good prognosis, in MC-out patients the recurrence rate is considerably increased. Therefore, a PET scan might be an additional tool for patient selection in MC-out patients, potentially in combination with other markers, like the AFP value (for dynamic AFP response during downstaging see below).

BIOLOGY IS KING: VALUE OF DYNAMIC PARAMETERS DURING DOWNSTAGING

Prediction models, which are based on parameters available at first referral, allow only a gross a priori estimation of the HCC recurrence risk after LT. Additional dynamic parameters, such as the response to downstaging or a test of time without downstaging measures are not captured in such models. However, the response to therapy represents essential information for appropriate patient selection to further improve the predictive power, especially in MC-out patients.

Therefore, one possible approach to improve prediction could be that all potential LT candidates with HCC undergo upfront downstaging therapy irrespective of size and number. The final decision for or against LT would then be based on the treatment response (53). However, the chance of successful downstaging in (unselected) patients with a TTV >200 cm^3 is below 5% (1 out of 22) according to data by Murali et al. (19). Whether the reported low likelihood is acceptable remains to be defined by each center, otherwise some entry criteria for the downstaging approach should be considered as discussed above. Using the TTV

threshold of 200 cm^3, the study showed that downstaging was successful in 76% (52 out of 68) of patients outside the MC. But the maximum TTV of the UCSF criteria of 144 cm^3 indicates that the UCSF criteria are too unspecific (19). However, the TTV at initial presentation is ultimately only a random snapshot of tumor biology, reflecting a certain risk of aggressiveness, but not the individual risk. While poorly differentiated tumors (G3) outside MC might be excluded a priori by means of biopsy and/or PET scan, dynamic parameters (54) might add additional information in the remaining population, which still includes a mixture of low, intermediate, and high-risk patients. The combination of dynamic morphological and biological parameters represents not only the most concise approach of prognostic prediction prior to downstaging but is also important for patient selection during downstaging. Besides the dynamic radiological criteria (e.g., mRECIST), dynamic changes of AFP levels during the waiting time and/or downstaging procedures need be considered as dynamic changes of AFP levels are shown to be more relevant than static AFP values at initial referral [33]. Unquestionably, the progressive increase in AFP values (e.g., AFP slope >15 ng/ml (33,40)) during waiting time is associated with poor survival benefit after LT. On the other hand, a large SRTR study demonstrated that patients with AFP levels >400 ng/ml at time of listing who experienced an AFP decrease to <400 ng/ml during downstaging had a significantly improved 3-year ITT survival (81% vs. 48%) compared to those without AFP reduction (55). A simplified score has been calculated from three US centers based on tumor size and number plus AFP response on a gradual basis (200, 400, and 1,000 ng/ml). This NYCA score has been shown to provide an appropriate risk stratification [(56)].

Overall, radiological tumor progression and/or AFP progression during downstaging are associated with a significantly higher recurrence rate after LT in patients inside (57), as well as outside MC. According to available data, AFP level progression or radiologic progressive disease should therefore be considered as contraindication for LT, particularly in MC-out patients. The same applies to AFP levels >1,000 ng/ml. For AFP levels below 1,000 ng/ml, the overall risk is determined by the tumor burden. It is essential to restrict the maximum eligible tumor burden in increasing AFP categories to keep the risk of HCC recurrence within acceptable limits. This has been proven for the clinical scenarios of first patient referral, as well as for patients after downstaging and prior to LT (39) (**Figure 6**).

Along with AFP response, direct histological tumor response is also a known predictive factor, especially cPR after downstaging therapy as it is associated with a very low recurrence rate of 5.8% at 5 years irrespective of the initial tumor size (63). In these patients finally undergoing transplantation (i.e., in pre-selected patients), the percentage of cPR might be irrespective of the tumor burden. Mehta et al. reported cPR in 19% of patients inside MC, in 12% of UCSF-in patients, and in 19% of UCSF-out patients (58). Although these data were not collected on an ITT basis, the study underlines that tumor biology is only partially reflected by the MC. Because cPR is only definitively known after LT, it represents a difficult parameter for decision-making prior to LT. This is especially true since the radiological finding of "no vital tumor" was confirmed as cPR only in 46.5% of patients on explant pathology in a recent (2) and previous analyses (59–62).

However, advances in MRI technology might help to increase the prediction of cPR in the future [63]. Nevertheless, the radiological response to downstaging, defined by the mRECIST criteria, is a good predictor of the risk of recurrence after LT. This is underlined by the fact that radiological response to downstaging therapy was an essential parameter in order to refine the Metroticket 2.0 criteria (2). The combination of dynamic changes of tumor response to downstaging by radiological mRECIST classification and AFP levels might be able to better refine patient selection before and during downstaging procedures, especially since progressive disease during downstaging is a worse prognostic factor in patients outside MC. Furthermore, a recent analysis using a competing risk approach confirmed that non-response to neo-adjuvant therapies assessed by mRECIST increases the HCC recurrence rate after LT from <10% to >25% (64). Accordingly, the Metroticket 2.0 criteria have been modified for patients with progressive disease during downstaging, where tumor criteria have been reduced by 1–2 cm in all AFP categories (**Figure 6**) (2). Looking at patients who are still outside MC after downstaging (i.e., outside the "up to 6" criteria: 5 + 1 or 3 + 3), no patient with progressive disease should undergo LT according to this calculation. MC-out patients with partial response or stable disease can undergo LT when tumor burden is within the up-to-seven criteria and serum AFP is <100 ng/ml (2).

In summary, an available single marker of measuring the risk of tumor recurrence is still far away. Tumor biology has to be assessed by tumor burden, AFP levels, and response to therapy, including the use of PET and/or tumor biopsy in selected cases. However, the selection process might be refined in the future due to expanding knowledge of available prediction parameters. An overview of potential selection parameters for and during downstaging, as discussed above, is illustrated in **Figure 8**. These criteria might represent only rough approximations due to differences in underlying populations and available data, as well as differences in acceptable 5-year outcome parameters in various publications. Therefore, the refined Metroticket 2.0 criteria might currently reflect the most sophisticated endpoint of downstaging, whereas the entry criteria might be applied, as depicted in **Figures 6**, **8**.

FUTURE DIRECTIONS

Although selection criteria for LT in HCC patients are becoming more and more sophisticated, all established parameters are still "imperfect." In any group with a negative prognosis determined by the available criteria, there remains a small proportion of patients who will achieve long-term survival "against all odds." This has been shown for G3 tumors, PET-positive tumors, an AFP >1,000 ng/ml, and even for patients with macrovascular invasion (65). Until more specific biomarkers are available, in subgroups with predicted poor prognosis, only exceptional cases will achieve long-term survival. Therefore, LT might be considered later on after good response to initial locoregional therapy. However, in most centers, LT will not be

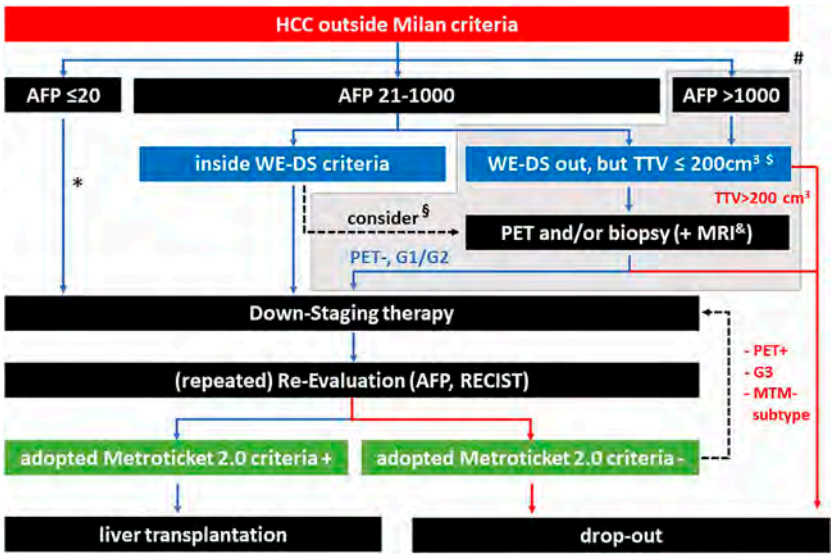


FIGURE 8 | Summary of potential selection criteria before and during downstaging. * according to the WE-DS criteria, the upper limit of sum and number would be 12, above this value a PET/biopsy might be considered. § depending on the tumor burden, also inside the WE-DS criteria, additional information concerning tumor biology might be useful. # patients outside the WE-DS criteria (grey background) have a significantly elevated risk of drop out during waiting time, however, this does not preclude selection of a low percentage of patients with good prognosis after LT (depending on individual center policies and organ availability). $ with a TTV <200 cm^3, the chance of successful downstaging is below 5% (Murali et al. [12]). & MRI techniques are evolving for the prediction of mVI and the macrotrabecular-massive (MTM) HCC subtype. Hepatobiliary MRI has proven higher sensitivity with comparable specificity for HCC depiction in cirrhotic patients.

considered ab initio in the high-risk groups, due to the very low rate of successful downstaging and LT.

Further approaches should explore personalized prediction and therapy approaches and implement molecular knowledge in clinical practice for patients with HCC listed for LT. For this, prospective evaluation is required and intra- and inter-tumor heterogeneity and the reproducibility of molecular analysis from tumor biopsy material must be taken into account. Newer selection parameters, which are currently under investigation include newer imaging methods, like fluorocholine PET (66), molecular markers derived from biopsy material, and increasing use of liquid biopsies (67). Future data on these parameters will hopefully support a more specific risk prediction in candidates for LT outside the conventional selection criteria.

AUTHOR CONTRIBUTIONS

DS, Conceptualization, Data curation, Formal analysis, Methodology, Visualization, Writing—original draft; HP, Conceptualization, Writing—original draft, Writing—review and editing; SS, Conceptualization, Formal analysis, Methodology, Writing—original draft, Writing—review and editing; EV , Conceptualization, Data curation, Writing—original draft, Writing—review and editing; JR, Conceptualization, Data curation, Writing—original draft, Writing—review and editing; GS, Conceptualization, Data curation, Writing—original draft, Writing review and editing; J-CN, Conceptualization, Data curation, Formal analysis, Visualization, Writing—original draft, Writing—review and editing; TB, Conceptualization, Data curation, Formal analysis, Methodology, Visualization, Writing—review and editing.

FUNDING

The authors acknowledge support from the German Research Foundation (DFG) and Universität Leipzig within the program of Open Access Publishing or Dieser Artikel Wurde Durch den Publikationsfonds der Universität Leipzig und das Programm Open Access Publizieren der DFG Gefördert.

CONFLICT OF INTEREST

DS has received honoraria or consultation fees from BTG, Johnson & Johnson, Gubert, Novartis, Olympus, and SIRTEX; and has participated in the following company's sponsored speaker's bureaus: Abbvie, Astellas, Bayer, BTG, Eisai, Johnson & Johnson, MSD/Merck, Novartis, and Olympus. GS has received research grants from Bayer and Roche, and consultancy fees from AstraZeneca and Roche. J-CN has received research grants from Bayer for INSERM UMR 1138. TB declares the following grants/research support: Abbvie, BMS, Gilead, MSD/Merck, Humedics, Intercept, Merz, Novartis, Sequana Medical. TB has received honoraria or consultation fees from Abbvie, Alexion, Bayer, Gilead, Eisai, Humedics, Intercept, Ipsen, Janssen, MSD/Merck, Novartis, Roche, Sequana Medical, SIRTEX, SOBI, and Shionogi; and has participated in the following company's sponsored speaker's bureaus: Abbvie, Alexion, Bayer, Gilead,

Eisai, Intercept, Ipsen, Janssen, MedUpdate GmbH, MSD/Merck, Novartis, Sequana Medica, and SIRTEX.

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.2022.10333/full#supplementary-material

REFERENCES

1. Yang A, Ju W, Yuan X, Han M, Wang X, Guo Z, et al. Comparison between Liver Resection and Liver Transplantation on Outcomes in Patients with Solitary Hepatocellular Carcinoma Meeting UNOS Criteria: a Population-Based Study of the SEER Database. *Oncotarget* (2017) 8(57):97428–38. doi:10.18632/oncotarget.22134
2. Cucchetti A, Serenari M, Sposito C, Di Sandro S, Mosconi C, Vicentin I, et al. Including mRECIST in the Metroticket 2.0 Criteria Improves Prediction of Hepatocellular Carcinoma-Related Death after Liver Transplant. *J Hepatol* (2020) 73(2):342–8. doi:10.1016/j.jhep.2020.03.018
3. Mazzaferro V, Citterio D, Bhoori S, Bongini M, Miceli R, De Carlis L, et al. Liver Transplantation in Hepatocellular Carcinoma after Tumour Downstaging (XXL): a Randomised, Controlled, Phase 2b/3 Trial. *Lancet Oncol* (2020) 21(7):947–56. doi:10.1016/S1470-2045(20)30224-2
4. Al-Ameri AAM, Wei X, Wen X, Wei Q, Guo H, Zheng S, et al. Systematic Review: Risk Prediction Models for Recurrence of Hepatocellular Carcinoma after Liver Transplantation. *Transpl Int* (2020) 33:697–712. doi:10.1111/tri.13585
5. Calderaro J, Couchy G, Imbeaud S, Amaddeo G, Letouzé E, Blanc J-F, et al. Histological Subtypes of Hepatocellular Carcinoma Are Related to Gene Mutations and Molecular Tumour Classification. *J Hepatol* (2017) 67(4):727–38. doi:10.1016/j.jhep.2017.05.014
6. Dhanasekaran R, Nault J-C, Roberts LR, Zucman-Rossi J. Genomic Medicine and Implications for Hepatocellular Carcinoma Prevention and Therapy. *Gastroenterology* (2019) 156(2):492–509. doi:10.1053/j.gastro.2018.11.001
7. Sapisochin G, Goldaracena N, Laurence JM, Dib M, Barbas A, Ghanekar A, et al. The Extended Toronto Criteria for Liver Transplantation in Patients with Hepatocellular Carcinoma: A Prospective Validation Study. *Hepatology* (2016) 64(6):2077–88. doi:10.1002/hep.28643
8. Andreou A, Gül S, Pascher A, Schöning W, Al-Abadi H, Bahra M, et al. Patient and Tumour Biology Predict Survival beyond the Milan Criteria in Liver Transplantation for Hepatocellular Carcinoma. *Hpb* (2015) 17(2):168–75. doi:10.1111/hpb.12345
9. Nault JC, Martin Y, Caruso S, Hirsch TZ, Bayard Q, Calderaro J, et al. Clinical Impact of Genomic Diversity from Early to Advanced Hepatocellular Carcinoma. *Hepatology* (2020) 71(1):164–82. doi:10.1002/hep.30811
10. Furuta M, Ueno M, Fujimoto A, Hayami S, Yasukawa S, Kojima F, et al. Whole Genome Sequencing Discriminates Hepatocellular Carcinoma with Intrahepatic Metastasis from Multi-Centric Tumors. *J Hepatol* (2017) 66:363–73. doi:10.1016/j.jhep.2016.09.021
11. Han DH, Choi GH, Kim KS, Choi JS, Park YN, Kim SU, et al. Prognostic Significance of the Worst Grade in Hepatocellular Carcinoma with Heterogeneous Histologic Grades of Differentiation. *J Gastroenterol Hepatol* (2013) 28:1384–90. doi:10.1111/jgh.12200
12. Erstad DJ, Tanabe KK. Prognostic and Therapeutic Implications of Microvascular Invasion in Hepatocellular Carcinoma. *Ann Surg Oncol* (2019) 26(5):1474–93. doi:10.1245/s10434-019-07227-9
13. Kim AY, Sinn DH, Jeong WK, Kim YK, Kang TW, Ha SY, et al. Hepatobiliary MRI as Novel Selection Criteria in Liver Transplantation for Hepatocellular Carcinoma. *J Hepatol* (2018) 68(6):1144–52. doi:10.1016/j.jhep.2018.01.024
14. Poté N, Cauchy F, Albuquerque M, Voitot H, Belghiti J, Castera L, et al. Performance of PIVKA-II for Early Hepatocellular Carcinoma Diagnosis and Prediction of Microvascular Invasion. *J Hepatol* (2015) 62(4):848–54. doi:10.1016/j.jhep.2014.11.005
15. Lin Z, Cai Y-J, Chen R-C, Chen B-C, Zhao L, Xu S-H, et al. A microRNA Expression Profile for Vascular Invasion Can Predict Overall Survival in Hepatocellular Carcinoma. *Clinica Chim Acta* (2017) 469:171–9. doi:10.1016/j.cca.2017.03.026
16. Mínguez B, Hoshida Y, Villanueva A, Toffanin S, Cabellos L, Thung S, et al. Gene-expression Signature of Vascular Invasion in Hepatocellular Carcinoma. *J Hepatol* (2011) 55(6):1325–31. doi:10.1016/j.jhep.2011.02.034
17. Pawlik TM, Delman KA, Vauthey J-N, Nagorney DM, Ng IO-L, Ikai I, et al. Tumor Size Predicts Vascular Invasion and Histologic Grade: Implications for Selection of Surgical Treatment for Hepatocellular Carcinoma. *Liver Transpl* (2005) 11:1086–92. doi:10.1002/lt.20472
18. Sinha J, Mehta N, Dodge JL, Poltavskiy E, Roberts J, Yao F. Are There Upper Limits in Tumor Burden for Down-Staging of Hepatocellular Carcinoma to Liver Transplant? Analysis of the All-Comers Protocol. *Hepatology* (2019) 70(4):1185–96. doi:10.1002/hep.30570
19. Murali AR, Romero-Marrero C, Miller C, Aucejo F, Levitin A, Gill A, et al. Predictors of Successful Downstaging of Hepatocellular Carcinoma outside Milan Criteria. *Transplantation* (2016) 100:2391–7. doi:10.1097/tp.0000000000001402
20. Degroote H, Piñero F, Costentin C, Notarpaolo A, Boin IF, Boudjema K, et al. International Study on the Outcome of Locoregional Therapy for Liver Transplant in Hepatocellular Carcinoma beyond Milan Criteria. *JHEP Rep* (2021) 3:100331. doi:10.1016/j.jhepr.2021.100331
21. Mazzotta AD, Pascale A, Cano L, Rosmorduc O, Allard MA, Sa Cunha A, et al. Number of Hepatocellular Carcinoma Nodules in Patients Listed for Liver Transplantation within Alpha-fetoprotein Score: a New Prognostic Risk Factor. *Transpl Int* (2021) 34:954–63. doi:10.1111/tri.13858
22. Calderaro J, Meunier L, Nguyen CT, Boubaya M, Caruso S, Luciani A, et al. ESM1 as a Marker of Macrotrabecular-Massive Hepatocellular Carcinoma. *Clin Cancer Res* (2019) 25(19):5859–65. doi:10.1158/1078-0432.CCR-19-0859
23. Ziol M, Poté N, Amaddeo G, Laurent A, Nault J-C, Oberti F, et al. Macrotrabecular-massive Hepatocellular Carcinoma: A Distinctive Histological Subtype with Clinical Relevance. *Hepatology* (2018) 68(1):103–12. doi:10.1002/hep.29762
24. Fuks D, Cauchy F, Fusco G, Paradis V, Durand F, Belghiti J. Preoperative Tumour Biopsy Does Not Affect the Oncologic Course of Patients with Transplantable HCC. *J Hepatol* (2014) 61:589–93. doi:10.1016/j.jhep.2014.04.046
25. Cillo U, Vitale A, Grigoletto F, Gringeri E, D'Amico F, Valmasoni M, et al. Intention-to-treat Analysis of Liver Transplantation in Selected, Aggressively Treated HCC Patients Exceeding the Milan Criteria. *Am J Transpl* (2007) 7(4):972–81. doi:10.1111/j.1600-6143.2006.01719.x
26. Pawlik TM, Gleisner AL, Anders RA, Assumpcao L, Maley W, Choti MA. Preoperative Assessment of Hepatocellular Carcinoma Tumor Grade Using Needle Biopsy. *Ann Surg* (2007) 245:435–42. doi:10.1097/01.sla.0000250420.73854.ad
27. Court CM, Harlander-Locke MP, Markovic D, French SW, Naini BV, Lu DS, et al. Determination of Hepatocellular Carcinoma Grade by Needle Biopsy Is Unreliable for Liver Transplant Candidate Selection. *Liver Transpl* (2017) 23(9):1123–32. doi:10.1002/lt.24811
28. Mazzaferro V, Llovet JM, Miceli R, Bhoori S, Schiavo M, Mariani L, et al. Predicting Survival after Liver Transplantation in Patients with Hepatocellular Carcinoma beyond the Milan Criteria: a Retrospective, Exploratory Analysis. *Lancet Oncol* (2009) 10(1):35–43. doi:10.1016/S1470-2045(08)70284-5
29. Hameed B, Mehta N, Sapisochin G, Roberts JP, Yao FY. Alpha-fetoprotein Level > 1000 ng/mL as an Exclusion Criterion for Liver Transplantation in

Patients with Hepatocellular Carcinoma Meeting the Milan Criteria. *Liver Transpl* (2014) 20(8):945–51. doi:10.1002/lt.23904

30. Sasaki K, Firl DJ, Hashimoto K, Fujiki M, Diago-Uso T, Quintini C, et al. Development and Validation of the HALT-HCC Score to Predict Mortality in Liver Transplant Recipients with Hepatocellular Carcinoma: a Retrospective Cohort Analysis. *Lancet Gastroenterol Hepatol* (2017) 2(8):595–603. doi:10.1016/S2468-1253(17)30106-1
31. Shimamura T, Akamatsu N, Fujiyoshi M, Kawaguchi A, Morita S, Kawasaki S, et al. Expanded Living-donor Liver Transplantation Criteria for Patients with Hepatocellular Carcinoma Based on the Japanese Nationwide Survey: the 5-5-500 Rule - a Retrospective Study. *Transpl Int* (2019) 32:356–68. doi:10.1111/tri.13391
32. Agopian VG, Harlander-Locke MP, Markovic D, Zarrinpar A, Kaldas FM, Cheng EY, et al. Evaluation of Patients with Hepatocellular Carcinomas that Do Not Produce α-Fetoprotein. *JAMA Surg* (2017) 152(1):55–64. doi:10.1001/jamasurg.2016.3310
33. Duvoux C, Roudot–Thoraval F, Decaens T, Pessione F, Badran H, Piardi T, et al. Liver Transplantation for Hepatocellular Carcinoma: A Model Including α-Fetoprotein Improves the Performance of Milan Criteria. *Gastroenterology* (2012) 143(4):986–94. doi:10.1053/j.gastro.2012.05.052
34. Hu B, Tian X, Sun J, Meng X. Evaluation of Individual and Combined Applications of Serum Biomarkers for Diagnosis of Hepatocellular Carcinoma: a Meta-Analysis. *Ijms* (2013) 14:23559–80. doi:10.3390/ijms141223559
35. Mourad M, Lebossé F, Merle P, Levrero M, Antonini T, Lesurtel M, et al. External Validation of the French Alpha-fetoprotein Model for Hepatocellular Carcinoma Liver Transplantation in a Recent Unicentric Cohort - a Retrospective Study. *Transpl Int* (2021) 34:535–45. doi:10.1111/tri.13819
36. Ivanics T, Shwaartz C, Claasen MPAW, Patel MS, Yoon P, Raschzok N, et al. Trends in Indications and Outcomes of Liver Transplantation in Canada: A Multicenter Retrospective Study. *Transpl Int* (2021) 34:1444–54. doi:10.1111/tri.13903
37. Lai Q, Avolio AW, Manzia TM, Sorge R, Agnes S, Tisone G, et al. Combination of Biological and Morphological Parameters for the Selection of Patients with Hepatocellular Carcinoma Waiting for Liver Transplantation. *Clin Transpl* (2012) 26:E125–E131. doi:10.1111/j.1399-0012.2011.01572.x
38. Toso C, Meeberg G, Hernandez-Alejandro R, Dufour JF, Marotta P, Majno P, et al. Total Tumor Volume and Alpha-fetoprotein for Selection of Transplant Candidates with Hepatocellular Carcinoma: A Prospective Validation. *Hepatology* (2015) 62:158–65. doi:10.1002/hep.27787
39. Lai Q, Vitale A, Halazun K, Iesari S, Viveiros A, Bhangui P, et al. Identification of an Upper Limit of Tumor Burden for Downstaging in Candidates with Hepatocellular Cancer Waiting for Liver Transplantation: A West-East Collaborative Effort. *Cancers* (20202020) 12(2):452. doi:10.3390/cancers12020452
40. Lai Q, Vitale A, Iesari S. Intention-to-treat Survival Benefit of Liver Transplantation in Patients with Hepatocellular Cancer. *Hepatology* (2017) 66(6):1910–9. doi:10.1002/hep.29342
41. Yao FY, Mehta N, Flemming J, Dodge J, Hameed B, Fix O, et al. Downstaging of Hepatocellular Cancer before Liver Transplant: Long-Term Outcome Compared to Tumors within Milan Criteria. *Hepatology* (2015) 61(6):1968–77. doi:10.1002/hep.27752
42. Toso C, Meeberg G, Andres A, Shore C, Saunders C, Bigam DL, et al. Downstaging Prior to Liver Transplantation for Hepatocellular Carcinoma: Advisable but at the price of an Increased Risk of Cancer Recurrence - a Retrospective Study. *Transpl Int* (2019) 32:163–72. doi:10.1111/tri.13337
43. Lee SD, Kim SH. Role of Positron Emission Tomography/computed Tomography in Living Donor Liver Transplantation for Hepatocellular Carcinoma. *Hepatobiliary Surg Nutr* (2016) 5:408–14. doi:10.21037/hbsn.2016.08.01
44. Kornberg A, Witt U, Schernhammer M, Kornberg J, Ceyhan GO, Mueller K, et al. Combining 18F-FDG Positron Emission Tomography with Up-To-Seven Criteria for Selecting Suitable Liver Transplant Patients with Advanced Hepatocellular Carcinoma. *Sci Rep* (2017) 7(1):14176. doi:10.1038/s41598-017-14430-9
45. Takada Y, Kaido T, Shirabe K, Nagano H, Egawa H, Sugawara Y, et al. Significance of Preoperative Fluorodeoxyglucose-Positron Emission Tomography in Prediction of Tumor Recurrence after Liver Transplantation for Hepatocellular Carcinoma Patients: a Japanese Multicenter Study. *J Hepatobiliary Pancreat Sci* (2017) 24(1):49–57. doi:10.1002/jhbp.412
46. Hong SK, Lee K-W, Kim H-S, Yoon KC, Yi N-J, Suh K-S. Living Donor Liver Transplantation for Hepatocellular Carcinoma in Seoul National University. *Hepatobiliary Surg Nutr* (2016) 5(6):453–60. doi:10.21037/hbsn.2016.08.07
47. Kim KA, Kim M-J, Jeon HM, Kim KS, Choi J-S, Ahn SH, et al. Prediction of Microvascular Invasion of Hepatocellular Carcinoma: Usefulness of Peritumoral Hypointensity Seen on Gadoxetate Disodium-Enhanced Hepatobiliary Phase Images. *J Magn Reson Imaging* (2012). 35(3):629–34. doi:10.1002/jmri.22876
48. Lee S, Kim SH, Lee JE, Sinn DH, Park CK. Preoperative Gadoxetic Acid-Enhanced MRI for Predicting Microvascular Invasion in Patients with Single Hepatocellular Carcinoma. *J Hepatol* (2017) 67(3):526–34. doi:10.1016/j.jhep.2017.04.024
49. Mulé S, Galletto Pregliasco A, Tenenhaus A, Kharrat R, Amaddeo G, Baranes L, et al. Multiphase Liver MRI for Identifying the Macrotrabecular-Massive Subtype of Hepatocellular Carcinoma. *Radiology* (2020) 295(3):562–71. doi:10.1148/radiol.2020192230
50. Ricke J, Steffen IG, Bargellini I, Berg T, Bilbao Jaureguizar JIJI, Gebauer B, et al. Gadoxetic Acid-Based Hepatobiliary MRI in Hepatocellular Carcinoma. *JHEP Rep* (2020) 2(6):100173. doi:10.1016/j.jhepr.2020.100173
51. Chuang Y-H, Ou H-Y, Yu C-Y, Chen C-L, Weng C-C, Tsang LL-C, et al. Diffusion-weighted Imaging for Identifying Patients at High Risk of Tumor Recurrence Following Liver Transplantation. *Cancer Imaging* (2019) 19(1):74. doi:10.1186/s40644-019-0264-y
52. Lai Q, Iesari S, Levi Sandri GB, Lerut J. Des-gamma-carboxy Prothrombin in Hepatocellular Cancer Patients Waiting for Liver Transplant: a Systematic Review and Meta-Analysis. *Int J Biol Markers* (2017) 32(4):e370–e374. doi:10.5301/ijbm.5000276
53. Mazzaferro V. Squaring the circle of Selection and Allocation in Liver Transplantation for HCC: An Adaptive Approach. *Hepatology* (2016) 63(5):1707–17. doi:10.1002/hep.28420
54. Clavien P-A, Lesurtel M, Bossuyt PM, Gores GJ, Langer B, Perrier A. Recommendations for Liver Transplantation for Hepatocellular Carcinoma: an International Consensus Conference Report. *Lancet Oncol* (2012) 13:e11–e22. doi:10.1016/s1470-2045(11)70175-9
55. Merani S, Majno P, Kneteman NM, Berney T, Morel P, Mentha G, et al. The Impact of Waiting List Alpha-Fetoprotein Changes on the Outcome of Liver Transplant for Hepatocellular Carcinoma. *J Hepatol* (2011) 55:814–9. doi:10.1016/j.jhep.2010.12.040
56. Halazun KJ, Tabrizian P, Najjar M, Florman S, Schwartz M, Michelassi F, et al. Is it Time to Abandon the Milan Criteria? *Ann Surg* (2018) 268(4):690–9. doi:10.1097/SLA.0000000000002964
57. Lai Q, Avolio AW, Graziadei I, Otto G, Rossi M, Tisone G, et al. Alpha-fetoprotein and Modified Response Evaluation Criteria in Solid Tumors Progression after Locoregional Therapy as Predictors of Hepatocellular Cancer Recurrence and Death after Transplantation. *Liver Transpl* (2013) 19:1108–18. doi:10.1002/lt.23706
58. Mehta N, Dodge JL, Grab JD, Yao FY. National Experience on Down-Staging of Hepatocellular Carcinoma before Liver Transplant: Influence of Tumor Burden, Alpha-Fetoprotein, and Wait Time. *Hepatology* (2020) 71(3):943–54. doi:10.1002/hep.30879
59. Otto G, Herber S, Heise M, Lohse AW, Mönch C, Bittinger F, et al. Response to Transarterial Chemoembolization as a Biological Selection Criterion for Liver Transplantation in Hepatocellular Carcinoma. *Liver Transpl* (2006) 12(8):1260–7. doi:10.1002/lt.20837
60. Seehofer D, Nebrig M, Denecke T, Kroencke T, Weichert W, Stockmann M, et al. Impact of Neoadjuvant Transarterial Chemoembolization on Tumor Recurrence and Patient Survival after Liver Transplantation for Hepatocellular Carcinoma: a Retrospective Analysis. *Clin Transpl* (2012) 26(5):764–74. doi:10.1111/j.1399-0012.2012.01609.x

61. Agopian VG, Morshedi MM, McWilliams J, Harlander-Locke MP, Markovic D, Zarrinpar A, et al. Complete Pathologic Response to Pretransplant Locoregional Therapy for Hepatocellular Carcinoma Defines Cancer Cure after Liver Transplantation. *Ann Surg* (2015) 262(3):536–45. doi:10.1097/SLA.0000000000001384
62. DiNorcia J, Florman SS, Haydel B, Tabrizian P, Ruiz RM, Klintmalm GB, et al. Pathologic Response to Pretransplant Locoregional Therapy Is Predictive of Patient Outcome after Liver Transplantation for Hepatocellular Carcinoma. *Ann Surg* (2020) 271(4):616–24. doi:10.1097/SLA.0000000000003253
63. Gordic S, Corcuera-Solano I, Stueck A, Besa C, Argiriadi P, Guniganti P, et al. Evaluation of HCC Response to Locoregional Therapy: Validation of MRI-Based Response Criteria versus Explant Pathology. *J Hepatol* (2017) 67: 1213–21. doi:10.1016/j.jhep.2017.07.030
64. Lee DD, Samoylova M, Mehta N, Musto KR, Roberts JP, Yao FY, et al. The mRECIST Classification Provides Insight into Tumor Biology for Patients with Hepatocellular Carcinoma Awaiting Liver Transplantation. *Liver Transpl* (2019) 25(2):228–41. doi:10.1002/lt.25333
65. Assalino M, Terraz S, Grat M, Lai Q, Vachharajani N, Gringeri E, et al. Liver Transplantation for Hepatocellular Carcinoma after Successful Treatment of Macrovascular Invasion - a Multi-center Retrospective Cohort Study. *Transpl Int* (2020) 33(5):567–75. doi:10.1111/tri.13586
66. Wallace MC, Sek K, Francis RJ, Samuelson S, Ferguson J, Tibballs J, et al. Baseline and Post-treatment 18F-Fluorocholine PET/CT Predicts Outcomes in Hepatocellular Carcinoma Following Locoregional Therapy. *Dig Dis Sci* (2020) 65(2):647–57. doi:10.1007/s10620-019-05781-6
67. Felden J, Villanueva A. Role of Molecular Biomarkers in Liver Transplantation for Hepatocellular Carcinoma. *Liver Transpl* (2020) 26(6):823–31. doi:10.1002/lt.25731

ESOT | Transplant International

ORIGINAL RESEARCH
published: 11 April 2022
doi: 10.3389/ti.2022.10297

Modeling the Potential of Treg-Based Therapies for Transplant Rejection: Effect of Dose, Timing, and Accumulation Site

Maya M. Lapp[1], Guang Lin[2], Alexander Komin[3], Leah Andrews[4], Mei Knudson[5], Lauren Mossman[4], Giorgio Raimondi[3] and Julia C. Arciero[6]**

[1]Department of Mathematics, The College of Wooster, Wooster, OH, United States, [2]Department of Mathematics, Purdue University, West Lafayette, IN, United States, [3]Department of Plastic and Reconstructive Surgery, Johns Hopkins School of Medicine, Baltimore, MD, United States, [4]Department of Mathematics, St. Olaf College, Northfield, MN, United States, [5]Department of Mathematics, Carleton College, Northfield, MN, United States, [6]Department of Mathematical Sciences, Indiana University-Purdue University of Indianapolis, Indianapolis, IN, United States

Introduction: The adoptive transfer of regulatory T cells (Tregs) has emerged as a method to promote graft tolerance. Clinical trials have demonstrated the safety of adoptive transfer and are now assessing their therapeutic efficacy. Strategies that generate large numbers of antigen specific Tregs are even more efficacious. However, the combinations of factors that influence the outcome of adoptive transfer are too numerous to be tested experimentally. Here, mathematical modeling is used to predict the most impactful treatment scenarios.

Methods: We adapted our mathematical model of murine heart transplant rejection to simulate Treg adoptive transfer and to correlate therapeutic efficacy with Treg dose and timing, frequency of administration, and distribution of injected cells.

Results: The model predicts that Tregs directly accumulating to the graft are more protective than Tregs localizing to draining lymph nodes. Inhibiting antigen-presenting cell maturation and effector functions at the graft site was more effective at modulating rejection than inhibition of T cell activation in lymphoid tissues. These complex dynamics define non-intuitive relationships between graft survival and timing and frequency of adoptive transfer.

Conclusion: This work provides the framework for better understanding the impact of Treg adoptive transfer and will guide experimental design to improve interventions.

Keywords: regulatory T cells, rejection, heart transplant, mathematical model, adoptive transfer, immune response

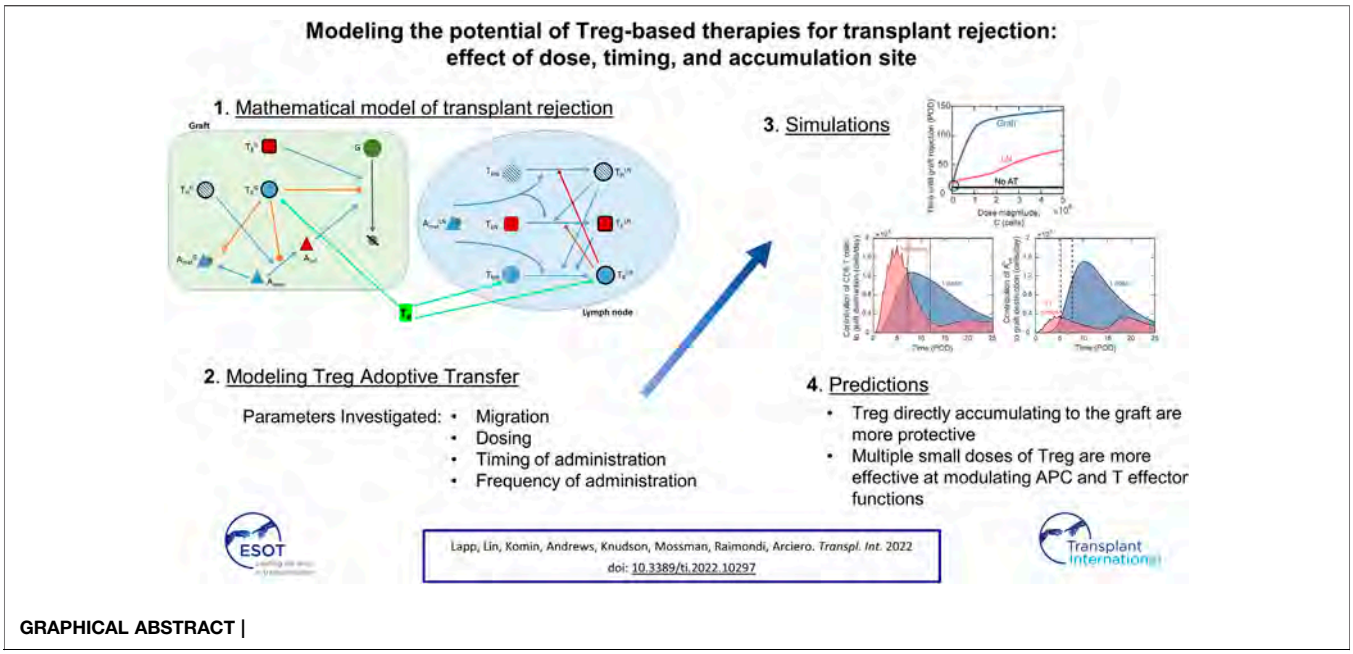
OPEN ACCESS

Correspondence:
Giorgio Raimondi
g.raimondi@jhmi.edu
Julia C. Arciero
jarciero@iupui.edu

Received: *10 December 2021*
Accepted: *17 March 2022*
Published: *11 April 2022*

Citation:
Lapp MM, Lin G, Komin A, Andrews L, Knudson M, Mossman L, Raimondi G and Arciero JC (2022) Modeling the Potential of Treg-Based Therapies for Transplant Rejection: Effect of Dose, Timing, and Accumulation Site. Transpl Int 35:10297. doi: 10.3389/ti.2022.10297

Abbreviations: APC, antigen-presenting cell; AT, adoptive transfer; CAR, chimeric antigen receptors; DC, dendritic cell; IL-2, interleukin 2; LN, lymph node; ODE, ordinary differential equation; POD, post-operative day; Tregs, regulatory T cells.



GRAPHICAL ABSTRACT |

INTRODUCTION

Following transplantation, lifelong immunosuppression is required to prevent allograft rejection (1). Unfortunately, because of drug-associated complications (1–3) and the unfeasibility of complete immunosuppression, 5 years survival rates for patients undergoing solid organ transplantation range anywhere from 40–70%, depending on the organ transplant type (4–6). Thus, there is an urgent need to develop alternative treatment strategies to promote graft tolerance and improve the quality of life for transplant recipients.

One such promising strategy is the adoptive transfer of regulatory T cells (Tregs). Tregs are lymphocytes that suppress the activity of other immune cells and are critical for maintaining peripheral tolerance and preventing autoimmune pathologies (7). The idea is that adoptive transfer of large numbers of Tregs can suppress transplant rejection and promote establishment of tolerance to the transplanted organ. Studies in pre-clinical animal models have shown feasibility and efficacy of polyclonal Treg infusion (8–13). Results from the completed clinical trials provide evidence that delivery of expanded polyclonal Treg is safe (14–16) and possibly effective (17). Excitingly, preclinical studies suggest that the therapeutic effect can be improved by using alloantigen-specific Tregs (18–20). To achieve this, Tregs can be modified to express Chimeric Antigen Receptors (CAR) or transgenic T cell receptors, which endow large-scale production of Tregs with the desired antigen specificity (10, 21, 22). In animal transplant models, CAR-Tregs have been shown to reach the grafts (19, 23, 24) and to control skin graft rejection to a greater extent than polyclonal Tregs (19, 24). CAR-Treg application and safety is currently being investigated in the first clinical trial in Europe (STEADFAST study, Sangamo Therapeutics, Inc.).

Despite these promising outcomes, much remains unknown about the consequences and efficacy of Treg adoptive transfer and what conditions maximize their therapeutic effect. It is difficult to compare experimental outcomes between studies due to differences in model organisms, transplanted organs, dose magnitude, dose timing, and Treg population quality (affected by *in vitro* expansion). Moreover, disparities in the results of some Phase I clinical trials highlight this lack of understanding of their optimal use (13, 25, 26). Further clinical trials will continue to improve our comprehension of adoptive transfer efficacy. However, it takes years to evaluate the long-term effects of this treatment, and development of methods to facilitate a more rapid understanding of the impact and optimization of treatment regimen would be invaluable.

Mathematical and computational models have been widely used in conjunction with experimental methods in cancer and virology to understand immune system dynamics and aid in the design of effective immunotherapies (27–29), but their application in transplantation is lacking. Some theoretical models for solid organ transplant rejection have been proposed (30–35), several of which focused on the impact of immunosuppression only (31, 33–35) and used simplified representations of immune components. We established one of the first theoretical models to describe the immune system and transplant dynamics that give rise to transplant rejection, and ours is currently the only transplantation model using differential equations to track Tregs independently from other T cell populations (30). Both our work and that of De Gaetano et al. incorporated experimental methods in developing transplant rejection models (30, 31). However, no mathematical model to date provides a robust mechanism for analysis of Treg adoptive transfer on the immune response to transplantation.

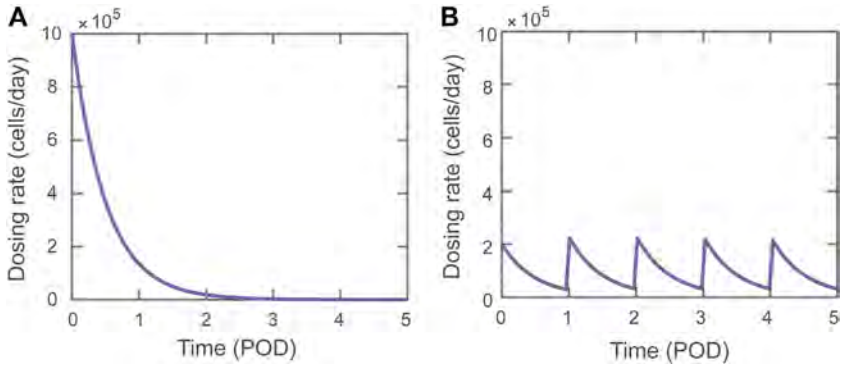


FIGURE 1 | Visual representations of Dosing function, $D(t)$, delivering a total of $C = 5 \times 10^5$ Tregs. **(A)** A single dose with dosing rate $D_0 = 10^6$ cells/day. **(B)** Five doses delivered on POD0-4 with dosing rate $D_0 = \frac{10^6}{5}$ cells/day.

In the present study, we adapted our model (30) to include new equations and terms to simulate the impact of alloantigen-specific Treg adoptive transfer on graft survival (**Supplementary Figure S1**). We updated the model equations for antigen-presenting cells (APCs) to match their behavior observed *in vivo* (36). We also performed parameter estimation on the updated model using a non-dominated sorting-based multi-objective evolutionary algorithm (37), called NSGA-II (Non-dominated Sorting Genetic Algorithm II). Although adoptive transfer is not sufficient to prevent graft rejection independently of immunosuppression (10), in this study we focus exclusively on adoptive transfer treatments to elucidate their direct effect on transplant rejection without the complicating and possibly confounding effects of concomitant immunosuppression. Thus, using the updated model, this study aimed at: 1) identifying optimal conditions for Treg delivery, specifically the activation status and tissue distribution, magnitude of dose, timing of delivery, and frequency of dosing, 2) analyzing immune dynamics to explain the effects of adoptive transfer on graft survival, and 3) suggesting future avenues for experimental studies into adoptive transfer treatment.

Our model simulations and analysis identified that timely inhibition of dendritic cell (DC) maturation in the graft and prolonged modulation of cytotoxic CD8 T cells and inflammatory macrophage activity in the graft by Treg were significantly more impactful than inhibiting the activation of alloreactive T cells in draining lymphoid tissues. Use of the model allowed us to identify a non-intuitive correlation between Treg dosing and administration frequency that delineates more effective interventions. Overall, our model enables the rapid simulation of a vast number of conditions that would be prohibitive to cover experimentally. It also provides the framework for future modeling efforts that will assess combinatorial treatments involving both Treg adoptive transfer and immunoregulatory agents.

MATERIALS AND METHODS

This study does not involve any new animal or human studies, and thus it was exempt from IRB approval.

Model Description

We expanded our previously developed model describing the dynamics of murine heart transplant rejection (30) to examine the impact of adoptive transfer of Tregs on graft survival. The original model consists of 13 nonlinear first order ordinary differential equations (ODEs) tracking the following populations in a representative lymph node compartment (LN) and graft compartment (G): CD4 T cells (T_H^{LN} and T_H^G), CD8 T cells (T_E^{LN} and T_E^G), regulatory T cells (T_R^{LN} and T_R^G), immature DCs (A_{imm}), mature DCs (A_{mat}^{LN} and A_{mat}^G), inflammatory macrophages (A_{inf}), pro- and anti-inflammatory cytokines (C_p and C_a), and graft cells (G). An additional ODE is introduced to track naïve Tregs (T_{RN}^{LN}). All model equations and parameters appear in the Supplemental Digital Content, and all model variables and their initial values are provided in **Supplementary Table S1**. A complete list of model assumptions is provided in (30). While this model is parametrized for mouse heart transplantation, the predicted interactions between the immune response and transplanted organ would be similar for other transplants.

In this study, the model equations tracking the immature and mature DCs are adapted to include more realistic representations of experimental observations (36). Specifically, the decay rate of immature DCs is assumed to depend on the remaining graft mass (**Supplementary Eq. S10**, second term). In addition, DC maturation is assumed to occur in the presence of pro-inflammatory cytokines or CD4 T cells (**Supplementary Eq. S10**, third term; **Supplementary Eq. S11**, first term). For verification of the modified model and updated predictions for host immune dynamics without adoptive transfer treatment, see the Supplemental Digital Content (**Supplementary Figures S2, S3**).

A dosing function, $D(t)$, for the adoptive transfer of Tregs is defined in **Eq. 1** (also in **Supplementary Eq. S12**). Parameters f_G, f_{LN}, and f_N correspond to the fraction of the Treg dose that enters the graft as activated Tregs (**Eq. 2**), the lymph node as activated Tregs (**Eq. 3**), and the lymph node as naïve Tregs (**Eq. 4**), respectively. For simulations involving adoptive transfer, $f_G + f_{LN} + f_N = 1$. These three parameters are set to zero when adoptive transfer is not simulated. The dosing function

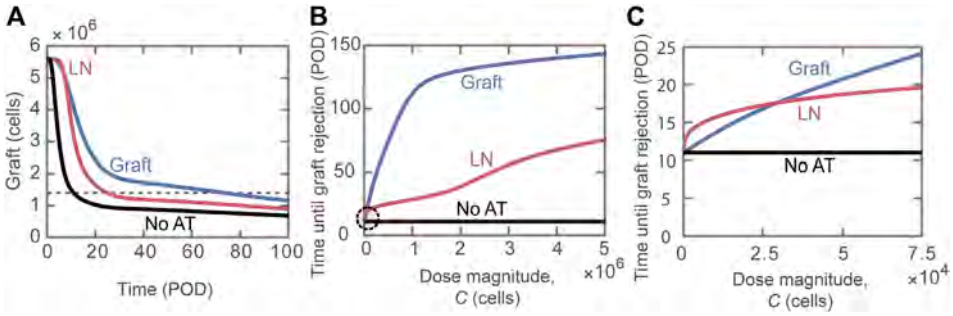


FIGURE 2 | Impact of distribution of adoptively transferred Tregs. **(A)** Number of graft cells shown over time for three different cases: activated Tregs administered to the graft (blue), activated Tregs administered to the lymph node (red), and no Tregs administered (black). Tregs are administered on POD0 with dose magnitude $C = 5 \times 10^5$ cells. The horizontal dashed line indicates a 75% reduction in initial graft size. The intersections of the curves with this dashed line give the model predicted values of graft rejection time. **(B)** Model predicted rejection time as Treg dose magnitude (C) is varied. The different curves depict predictions for the delivery of activated Tregs to the graft (blue) and activated Tregs to the lymph node (red). The black line marks POD11, which is when rejection occurs without adoptive transfer. **(C)** Circled region in panel B is magnified to examine the effect of small dose magnitudes on graft rejection.

is composed of one or multiple exponentially decaying functions of time, where D_0 indicates the dosing rate, n indicates the total number of doses delivered, t_i indicates the post-operative day (POD) of delivery of the ith Treg injection, and β indicates the decay rate. A decay rate of $\beta = 2\ \text{day}^{-1}$ is used to simulate the relatively fast absorption of Tregs during adoptive transfer. We assume around 50% of injected cells are lost or distribute in non-draining lymphoid tissues (which are excluded from the model), and we define the dose magnitude C to be the total number of injected cells that localize to the modeled lymph node and graft compartments. Thus, for the majority of this study, we simulate adoptive transfer with $C = 5 \times 10^5$ Tregs. The dose magnitude is the area under the curve of our dosing function, which is given by $C = \int_0^\infty (D_0 \sum_{i=1}^n d_i(t))\, dt = \frac{nD_0}{\beta}$ cells. A single dose with $D_0 = 10^6$ cells/day or n doses with $D_0 = \frac{10^6}{n}$ cells/day both administer a total of $C = 5 \times 10^5$ Tregs. **Figure 1** shows the dosing rate over time for a single dose administered on POD0 with $D_0 = 10^6$ cells/day (**Figure 1A**) and 5 doses administered on POD0, 1, 2, 3, and 4 with $D_0 = \frac{10^6}{5}$ cells/day (**Figure 1B**). The dose magnitude C is varied during model analysis.

$$D(t) = D_0 \Sigma_{i=1}^n d(t),\ where\ d_i(t) = \begin{cases} 0, & t < t_i \\ e^{-\beta(t-t_i)}, & t \geq t_i \end{cases} \quad (1)$$

$$\frac{dT_R^G}{dt} = k e_R T_R^{LN} - \mu_R T_R^G + \frac{r_{RG} T_R^G \left(T_E^G + T_H^G\right)}{\alpha_6 + T_R^G} + f_G D(t) \quad (2)$$

$$\frac{dT_R^{LN}}{dt} = \frac{a_R T_{RN}^{LN} A_{mat}^{LN}}{\gamma_2 + A_{mat}^{LN}} - \mu_R T_R^{LN} + \frac{r_R T_R^{LN} \left(T_E^{LN} + T_H^{LN}\right)}{\alpha_2 + T_R^{LN}} - e_R T_R^{LN} + f_{LN} D(t) \quad (3)$$

$$\frac{dT_{RN}^{LN}}{dt} = \mu_{RN} \left(T_0 - T_{RN}\right) + f_N D(t) \quad (4)$$

Model Simulations

The activation state, accumulation site, magnitude, timing, and frequency of adoptive transfer are varied in the model to simulate the impact of each of these factors on the immune response to the graft. Parameters f_G, f_{LN}, and f_N are varied to determine the impact of activation state (i.e., naïve or activated) and accumulation site of Tregs. The effects of dose magnitude are assessed by varying C. The timing and frequency of Treg doses are evaluated by varying the start day of the dose (t_0) and the number of doses, n. If multiple doses are administered, it is assumed that the doses are given at 1-day intervals. Following our previous work, graft rejection is defined as a 75% reduction in the original number of graft cells (30). The time at which the model predicts graft rejection is used in this study to identify optimal dosing strategies for the adoptive transfer of Tregs.

Parameter Estimation

To calibrate the model parameters in the updated model (see parameters shaded in **Supplementary Table S2**), we employed a non-dominated sorting-based multi-objective evolutionary algorithm (37), called NSGA-II (Non-dominated Sorting Genetic Algorithm II), which can greatly improve the efficiency in constrained multi-objective optimization tasks. NSGA-II is a popular non-domination based genetic algorithm for multi-objective optimization and parameter estimation. NSGA-II improves elitism and there is no need to choose sharing parameters a priori.

RESULTS

Impact of Treg Accumulation Site

To analyze the effect of Treg adoptive transfer using our model, we first evaluated the impact of the activation status of the injected cells on graft survival. It is well recognized that pre-activated Treg (even after resting) are an order of magnitude more suppressive than naïve Tregs (38, 39). For this analysis, we simulated the equivalent of delivering $C = 5 \times 10^5$ Tregs on POD0. As expected, accumulation of naïve Tregs into the lymph node was not as effective as the adoptive transfer of pre-activated Tregs (Supplemental Digital Content, **Supplementary Figure S4**). This confirmed the benefit of using *ex-vivo* expanded Tregs, which is a necessary step in

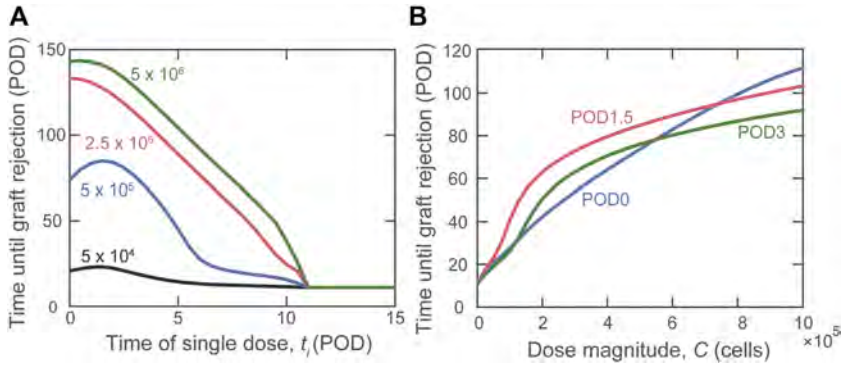


FIGURE 3 | Impact of the timing of Treg administration on graft rejection. **(A)** Predicted graft rejection time as the day of dose administration (t_i) is varied given four different dose magnitudes: $C = 5 \times 10^4$ (black), $C = 5 \times 10^5$ (blue), $C = 2.5 \times 10^6$ (red), and $C = 5 \times 10^6$ (green) cells. **(B)** Predicted graft rejection time as dose magnitude (C) is varied for Tregs administered on POD0 (blue), POD1.5 (red), and POD3 (green).

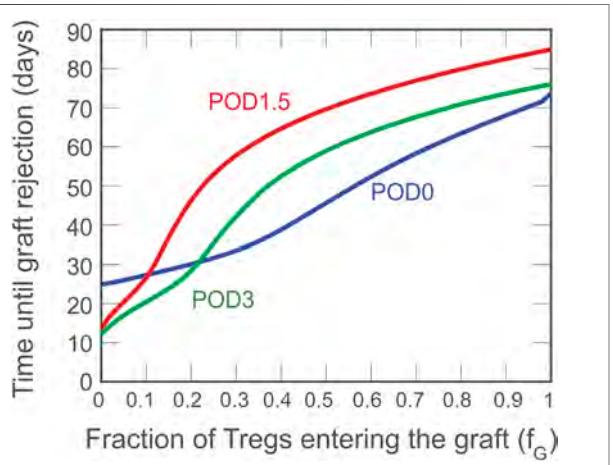


FIGURE 4 | Impact on rejection time of the fraction of activated Tregs that enters the graft. A dose of $C = 5 \times 10^5$ cells is administered on POD0 (blue), POD1.5 (red), and POD3 (green) and the impact on graft survival is projected in relation to the distribution of the injected Treg between graft and lymphoid compartments.

most clinical preparations of antigen-specific cells that renders homogeneous populations of pre-activated Treg. For the remainder of the study, we simulated the use of pre-activated Tregs.

Next, we focused on the question: does the tissue distribution of Tregs between the lymphoid compartment and the graft post-adoptive transfer impact transplant survival? **Figure 2A** depicts the change in the graft mass over time for the scenarios where the transferred Tregs exclusively accumulate in the lymphoid versus graft compartments, as well as for the case of no adoptive transfer (black curve). There is a substantial benefit with the accumulation of Tregs in the graft in comparison to lymphoid tissues, with estimated rejection on POD74 vs. POD25, respectively. The model can simulate a wide range of Tregs dose magnitudes; for almost all ranges, accumulation of Tregs directly to the graft (**Figure 2B**, blue curve) is more effective at extending survival than localization to the lymph node compartment (**Figure 2B**, red curve). For very small doses ($C < 2.9 \times 10^4$ cells), delivering activated Tregs to the lymph node is predicted to be more effective than delivering to the graft (**Figure 2C**); though, it improves graft survival by no more than 2.4 days.

Impact of Single Dose Timing

Having shown the benefit of graft localization, we studied the impact of the timing of a single injection of varying amounts of Tregs. **Figure 3** shows a non-monotonic relationship between rejection time and day of Treg administration, observed for some Treg dose magnitudes (**Figure 3A**). Specifically, when using $C = 5 \times 10^5$ cells (blue curve), we observed that delaying Treg administration to POD1.5 yields optimal graft survival time. The non-monotonic behavior between dose timing and graft survival is highlighted for physiological dose magnitudes ($C < 10^6$ cells) in **Figure 3B**. Once the dose magnitude is increased above $C = 7.5 \times 10^5$ cells, a monotonic relationship is re-established in which graft survival time decreases with the delay of Treg administration.

Although a theoretical model can assume that all Tregs administered to an individual accumulate exclusively in the graft or lymphoid tissues, physiological *in vivo* constraints and Treg properties dictate a variable partitioning between the two compartments. Thus, in **Figure 4**, we show the simulated impact of varying the fraction of Tregs that enter the graft (f_g) between 0 and 1; the remaining fraction ($1–f_g$) is assumed to enter the draining lymphoid tissue compartment. As indicated by the red curve, the model predicts that if more than 10% of the injected Tregs locate to the graft, delaying injection until POD1.5 is the most beneficial strategy to prolong the time to graft rejection.

Impact of Multiple Doses

The model allows us to compare the protective effect of a single injection of $C = 5 \times 10^5$ Tregs versus splitting that total number of cells among multiple daily injections (an approach that can also represent sustaining the presence of a smaller amount of Treg over time). As depicted in **Figure 5**, distributing a fixed dose prolongs graft survival. The relationship between the number of consecutive doses and graft survival was not monotonic, with

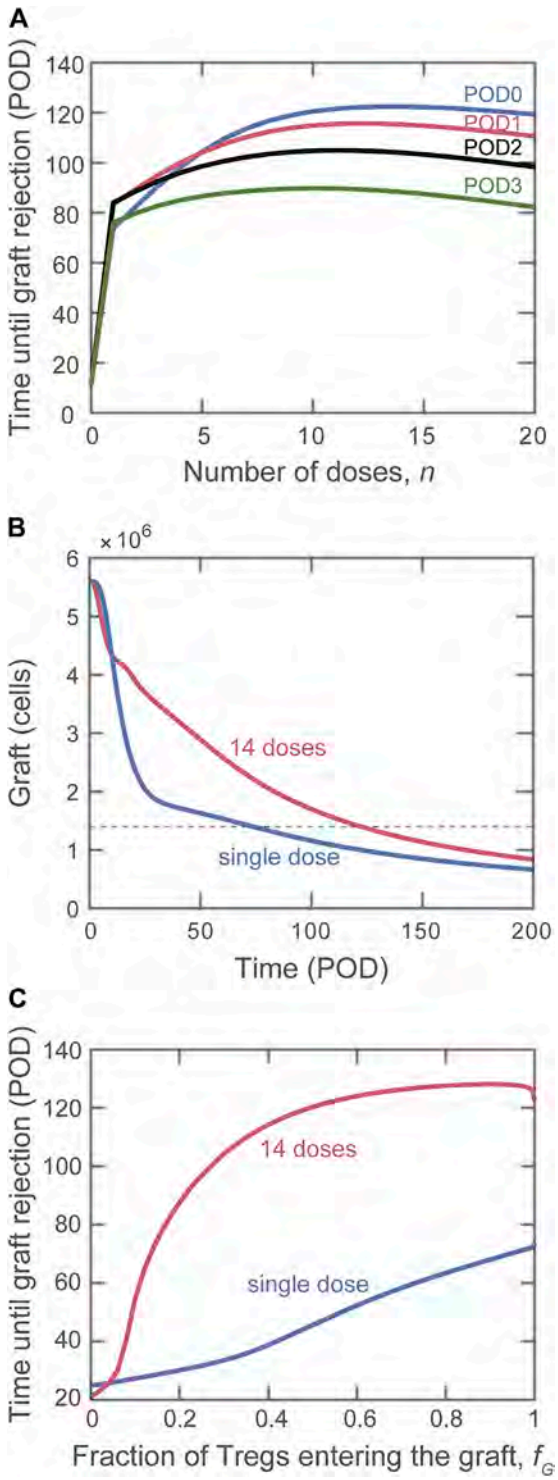


FIGURE 5 | Impact on graft rejection of splitting the same total number of Treg into multiple doses. **(A)** Plot of the simulated rejection time as a function of the indicated number of doses administered starting on POD0 (blue), POD1 (red), POD2 (black), and POD3 (green). The total dose magnitude for each simulation is $C = 5 \times 10^5$ cells. **(B)** Number of graft cells as a function of time for a single dose (blue curve) and for the optimal case of multiple doses (14 daily doses of Tregs beginning on POD0, red curve). **(C)** Comparison of rejection time for single (blue curve) and multiple (red curve) dose administration if the fraction of Tregs that reach the graft is varied.

graft survival peaking around 10–15 equivalent doses, depending on the first day of injection (**Figure 5A**). Significantly, when enough doses are administered, delaying the start of adoptive transfer treatment is no longer beneficial. The model predicts that administering $C = 5 \times 10^5$ cells divided evenly among 14 daily doses beginning on POD0 is the most effective treatment, extending graft survival until POD115. As shown in **Figure 5B** (graft mass over time), it is noteworthy that in addition to extending transplant survival, multiple doses of Treg provide better protection of the graft (i.e., slower rate of mass decline). **Figure 5C** shows that the impact of multiple doses compared with a single dose is apparent even if only a small fraction of Tregs (>5%) enters the graft.

Impact of Treg Adoptive Transfer on Host Immune Dynamics

Differently from bioinformatic approaches (e.g., machine learning), mathematical modeling enables the analysis of the dynamics of host immune cells that determine the simulated outcome. This valuable property can be used to gain insight into why certain adoptive transfer treatments are more effective than others. We investigated in detail the difference in effects between using a single injection of graft-infiltrating Tregs versus splitting the total number over multiple injections. **Figure 6** depicts the predicted dynamics of host immune cells in the graft with single dose adoptive transfer (blue) and 14 dose adoptive transfer (red). For a similar investigation into the impact of site of accumulation and timing of single injections, please see the Supplemental Digital Content (**Supplementary Figures S5, S6**).

Delivering a large single dose of Tregs minimizes the maturation of graft infiltrating DCs (**Figure 6B**) and therefore limits the activation and graft accumulation of cytotoxic CD8 T cells (**Figure 6C**) and delays the activation of macrophages (**Figure 6D**). Therefore, delivering a substantial portion of Tregs soon after transplantation is effective at limiting the early inflammatory response. Nevertheless, the regulation of the destructive capacity of those cells is not sustained with a single administration.

When the injected cells are split among 14 doses, elevated Treg levels are maintained during the surge of cytotoxic T cells and inflammatory macrophages (**Figures 6A,C,D**, red). In contrast, for single dose adoptive transfer, Tregs in the graft decay before the surge of these graft destructive subsets (**Figures 6A,C,D**, blue). The lack of Treg-mediated control in the last scenario allows each cytotoxic T cell and inflammatory macrophage to cause greater graft damage despite a lower accumulation level. This phenomenon is highlighted in the boxed regions in **Figures 6C–F**. We note that **Figures 6E,F** show the value of each term on the right-hand side of the differential equation for graft cells that contributes to graft destruction (**Supplementary Eq. S9**). Therefore, higher values of the curve in panel E or F indicate a greater contribution to graft destruction. For example, from POD7.2 to POD11.9, although more cytotoxic T cells are predicted to be present with the 14-dose regimen (**Figure 6C**; the 14-dose curve is above the 1-dose curve in the dotted boxed region), their effector functions are regulated and less graft

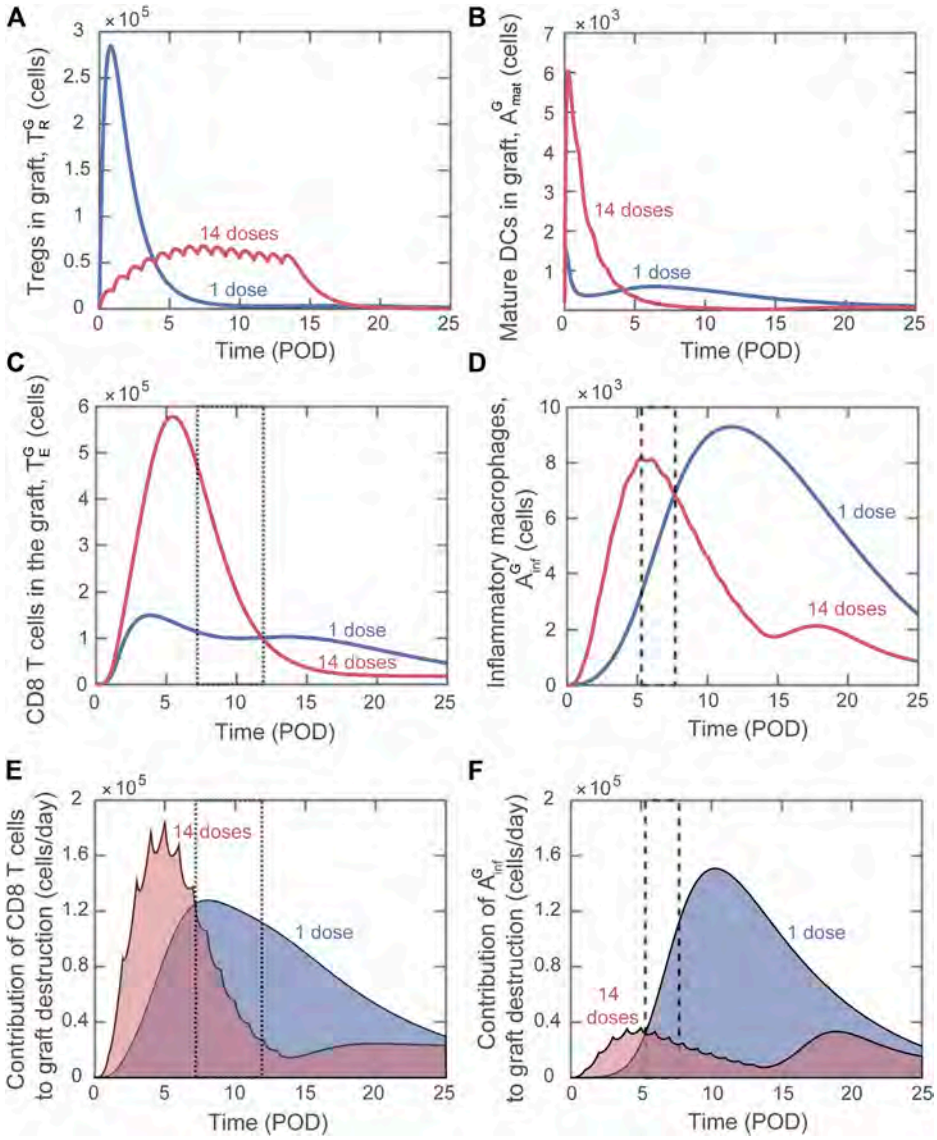


FIGURE 6 | Model predicted alterations of immune dynamics induced by $C = 5 \times 10^5$ Tregs delivered as a single dose (blue) or distributed across 14 doses (red). **(A)** Number of Tregs in the graft, T_R^G. **(B)** Number of mature DCs in the graft, A_{mat}^G. **(C)** Number of cytotoxic CD8 T cells in the graft, T_E^G. **(D)** Number of inflammatory macrophages in the graft, A_{inf}^G. **(E)** Rate of graft destruction caused by cytotoxic CD8 T cells. **(F)** Rate of destruction caused by inflammatory macrophages. Dashed boxes [in **(C–F)**] highlight timeframes when indicated destructive cell quantity is higher for the 14 dose treatment but the resulting rate of destruction is lower than for the single dose treatment.

destruction occurs (**Figure 6E**; the 1-dose curve is above the 14-dose curve). Similarly, from POD5.3 to POD7.7, although more inflammatory macrophages are predicted with the 14-dose regimen (**Figure 6D**), less graft destruction is mediated by this subset (**Figure 6F**).

Overall, analyzing the population dynamics for various Treg dose frequencies indicates that, to optimize adoptive transfer, cell delivery must 1) maintain elevated Treg levels during the surge of cytotoxic T cells and inflammatory macrophages in the graft and 2) deliver enough Tregs early after transplantation to inhibit DC maturation and therefore limit T cell activation. These same principles hold true when examining dose timing and can explain why administering a single dose on POD1.5 is more effective than delivering a single dose on POD0 (see Supplemental Digital Content, **Supplementary Figure S6**). When examining site of Treg accumulation, minimizing DC maturation is again critical to understand the benefit of delivering Tregs to the graft rather than the lymph node (see Supplemental Digital Content, **Supplementary Figure S5**).

DISCUSSION

In this study, we expanded our previous mathematical model of murine heart transplant rejection to incorporate the impact

of Treg adoptive transfer. Importantly, and complementary to bioinformatic approaches (40–43), we took advantage of the power of mathematical modeling to enable analysis of the immune dynamics underlying the results of simulations and thereby offer rationales for the therapeutic optimizations proposed. With the advent of feasible and reliable approaches of genetic engineering to generate high numbers of antigen-specific Treg for adoptive transfer (10), our data suggest important optimizations that would maximize the therapeutic efficacy of these cells.

Impact of Treg Accumulation Site

Our model predicts that Tregs accumulating to the transplant are more therapeutically effective than Tregs distributing to the lymph node (**Figure 2**). Importantly, analysis of immune dynamics provides the rationale behind such a different outcome: inhibition of DC maturation in the graft is more effective than direct inhibition of T cell activation in the draining lymphoid tissue at minimizing the number of CD8 and CD4 T cells that reach the graft (Supplemental Digital Content, **Supplementary Figure S5**). Interestingly, experimental reports in rodent models have suggested that an immediate accumulation of Treg in the graft promotes longer survival (44, 45). Overall, our analysis suggests that identifying methods to increase the fraction of Tregs that translocate early on to the graft will maximize the impact of adoptive transfer. It is noteworthy that the conditions of *ex-vivo* Treg expansion impart specific migratory capacity to the cells and these differences impact the therapeutic result obtained (45–49). Moreover, the breadth of genetic engineering that has become possible for clinical products (e.g., CRISPR/Cas9 based modifications) could provide useful tools to impart the ideal behavior in adoptively transferred Tregs.

Impact of Single Dose Timing

When administering a single dose of Tregs, our simulations suggest that delaying cell delivery is more effective than administering cells on the day of transplantation (**Figure 3B**). The justification for this unexpected result is similar to that given for splitting Treg into multiple doses. Slightly delaying administration allows Tregs to directly inhibit the destructive functions of cytotoxic T cells and inflammatory macrophages while ensuring a timely reduction of DC activation and, therefore, T cell activation (Supplemental Digital Content, **Supplementary Figure S5**). Obviously, this scenario is specific to the unique conditions of administering Tregs without any additional manipulation of the recipient (like immunosuppression, see below), but it highlights the impact of examining cellular interactions at the system level.

Impact of Multiple Doses

The model predicts that maintaining a prolonged influx of Tregs is the most effective method of lengthening graft survival. Delivering multiple, smaller doses of Tregs preserves the graft longer than delivering the same total number of Tregs in a single bolus. This regimen allows for elevated levels of Tregs to remain in the graft during the surge of graft-destructive cells (and control them), while also compromising on an "early enough" limitation of DC maturation. Although the clinical implementation of multiple daily dosing of Treg is unrealistic, this result highlights the important point of sustaining the survival and function of transferred Tregs. This is a contentious issue with the reported negative effect that many immunosuppressive drugs have on the homeostasis and function of Treg (50, 51). There is growing interest in devising approaches to sustain the persistence and function of Tregs. The use of IL-2/anti-IL2 complexes, IL-2 muteins, as well as the genetic engineering of Tregs to respond to "orthogonal" IL-2 are all examples of active investigations to promote Tregs persistence (10, 20, 52). In parallel, the utilization of biomaterials to promote the sustained accumulation of Tregs in proximity to the transplant represent a very promising strategy (53). Our model results provide the rationale to strongly support these ongoing efforts.

Limitations, Parallelisms, and Future Work

While the processes of sensitivity analysis and parameter estimation performed in this study (based on experimental data) have improved the accuracy of several model parameters, some parameters remain uncertain. For example, the persistence and proliferation of Tregs in the graft are unquantified variables that have a profound impact on the dynamics of graft infiltrating immune cells. Similarly, the relationship between number of Tregs injected and the number of cells that reach the graft is not quantified; the factors that influence such a relationship are poorly defined, and thus, further experimentation is needed (54).

While ours is the only ODE transplantation model to date that tracks Tregs independently of other T cells, several immunological mathematical models have recently been developed to elucidate the role of Tregs on self-tolerance and to identify key Treg interactions with other immune populations (55–58). The assumptions from these models may be used to improve our existing model to better replicate Treg behavior. In particular, given the importance of IL-2 to Treg survival, proliferation, and function, explicitly tracking IL-2 concentrations as in (57) would strengthen our current model and allow further investigation into IL-2 therapies as a method of extending Treg survival.

Although there is very limited quantitative information, other pre-clinical transplant models show important concordance with some of our model simulations. In a mouse model of pancreatic islet transplantation (45), Zhang et al. compared the i.v. infusion of Treg with the co-transplantation at the site of grafting. The significantly longer survival obtained in the latter case agrees with our model-suggested principle of a higher therapeutic impact when Treg can rapidly and directly modulate graft immune populations. Unfortunately, their report did not present different doses or timing of Treg administration. A similar scenario emerges from reports using Treg in mouse skin transplant models. The use of CAR-Treg (59), cell lines

of Treg (60), or polyclonal Treg (61) injected on the day of the transplant promotes only a modest increase in transplant survival, a result that correlates with simulation of a fixed number of Treg that probably mostly home to the lymphoid compartment. In all cases, the combination with so called "adjunct therapies" (ranging from thymectomy and T cell depletion to irradiation and bone marrow co-transplantation) is demonstrated as necessary to achieve lasting therapeutic effects. Currently, we can only draw qualitative comparisons to these experimental models since theoretical model parameters would need to be adapted to each different transplant model.

Overall, our modified model is beneficial in identifying methods to maximize the benefits of Treg adoptive transfer and in generating hypotheses on the key immune dynamics that govern its outcome and that can be tested experimentally. However, as demonstrated in this study and by experimental evidence to date, Treg adoptive transfer alone is insufficient to prevent transplant rejection. These results highlight the need to understand what additional perturbations to the system would better support or enhance the protective function of Tregs. Our mathematical model provides the framework into which treatments like immunosuppression (existing or hypothetical) can be included and used to dissect their very complex effects. Combined with the promising technological advances in both the investigation and manipulation of cells, there is tangible optimism toward the ultimate goal of optimizing therapeutic strategies for transplantation.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

JA and GR conceived the idea of the study; all authors contributed to the rationalization of immunological concepts into equations; JA, ML, LA, LM, and MK created and optimized the model code; GL and LA performed model optimization; GR and JA defined immunological assumptions; JA, ML, GR, and AK wrote the manuscript.

FUNDING

This work was supported by United States Army Medical Research Acquisition Activity (USMRAA) grant W81XWH-19-1-0352, and internal support from the Department of Plastic and Reconstructive Surgery (both to GR). This work was also supported by NSF DMS-1559745, NSF DMS-1654019, and NIH 1R01EY030851 (to JA).

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 thank Olga Dorabiala, Ross Walgren, Julia Shapiro, Hannah Schmidt, and Anirudh Arun for invaluable support with literature search and conceptualization of immune interactions.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10297/full#supplementary-material

REFERENCES

1. Allison TL. Immunosuppressive Therapy in Transplantation. *Nurs Clin North America* (2016) 51(1):107–20. doi:10.1016/j.cnur.2015.10.008
2. Min DI, Monaco AP. Complications Associated with Immunosuppressive Therapy and Their Management. *Pharmacotherapy* (1991) 11(5):119S–125S. doi:10.1002/j.1875-9114.1991.tb02641.x
3. de Fijter JW, Holdaas H, Øyen O, Sanders J-S, Sundar S, Bemelman FJ, et al. Early Conversion from Calcineurin Inhibitor- to Everolimus-Based Therapy Following Kidney Transplantation: Results of the Randomized ELEVATE Trial. *Am J Transpl* (2017) 17(7):1853–67. doi:10.1111/ajt.14186
4. Shyu S, Dew MA, Pilewski JM, DeVito Dabbs AJ, Zaldonis DB, Studer SM, et al. Five-year Outcomes with Alemtuzumab Induction after Lung Transplantation. *J Heart Lung Transplant* (2011) 30(7):743–54. doi:10.1016/j.healun.2011.01.714
5. Grant D, Abu-Elmagd K, Mazariegos G, Vianna R, Langnas A, Mangus R, et al. Intestinal Transplant Registry Report: Global Activity and Trends. *Am J Transpl* (2015) 15(1):210–9. doi:10.1111/ajt.12979
6. Hellemans R, Hazzan M, Durand D, Mourad G, Lang P, Kessler M, et al. Daclizumab versus Rabbit Antithymocyte Globulin in High-Risk Renal Transplants: Five-Year Follow-Up of a Randomized Study. *Am J Transpl* (2015) 15(7):1923–32. doi:10.1111/ajt.13191
7. Vignali DAA, Collison LW, Workman CJ. How Regulatory T Cells Work. *Nat Rev Immunol* (2008) 8(7):523–32. doi:10.1038/nri2343
8. Safinia N, Leech J, Hernandez-Fuentes M, Lechler R, Lombardi G. Promoting Transplantation Tolerance; Adoptive Regulatory T Cell Therapy. *Clin Exp Immunol* (2013) 172(2):158–68. doi:10.1111/cei.12052
9. Tang Q, Bluestone JA. Regulatory T-Cell Therapy in Transplantation: Moving to the Clinic. *Cold Spring Harb Perspect Med* (2013) 3(11):a015552. doi:10.1101/cshperspect.a015552
10. Ferreira LMR, Muller YD, Bluestone JA, Tang Q. Next-generation Regulatory T Cell Therapy. *Nat Rev Drug Discov* (2019) 18(10):749–69. doi:10.1038/s41573-019-0041-4
11. MacDonald KN, Piret JM, Levings MK. Methods to Manufacture Regulatory T Cells for Cell Therapy. *Clin Exp Immunol* (2019) 197(1):52–63. doi:10.1111/cei.13297
12. Issa F, Strober S, Leventhal JR, Kawai T, Kaufman DB, Levitsky J, et al. The Fourth International Workshop on Clinical Transplant Tolerance. *Am J Transpl* (2021) 21(1):21–31. doi:10.1111/ajt.16139
13. Sawitzki B, Harden PN, Reinke P, Moreau A, Hutchinson JA, Game DS, et al. Regulatory Cell Therapy in Kidney Transplantation (The ONE Study): a Harmonised Design and Analysis of Seven Non-randomised, Single-Arm, Phase 1/2A Trials. *The Lancet* (2020) 395(10237):1627–39. doi:10.1016/s0140-6736(20)30167-7

14. Trzonkowski P, Bacchetta R, Battaglia M, Berglund D, Bohnenkamp HR, ten Brinke A, et al. Hurdles in Therapy with Regulatory T Cells. *Sci Transl Med* (2015) 7(304):304ps18. doi:10.1126/scitranslmed.aaa7721
15. Brunstein CG, Miller JS, Cao Q, McKenna DH, Hippen KL, Curtsinger J, et al. Infusion of *Ex Vivo* Expanded T Regulatory Cells in Adults Transplanted with Umbilical Cord Blood: Safety Profile and Detection Kinetics. *Blood* (2011) 117(3):1061–70. doi:10.1182/blood-2010-07-293795
16. Bluestone JA, Buckner JH, Fitch M, Gitelman SE, Gupta S, Hellerstein MK, et al. Type 1 Diabetes Immunotherapy Using Polyclonal Regulatory T Cells. *Sci Transl Med* (2015) 7(315):315ra189. doi:10.1126/scitranslmed.aad4134
17. Brunstein CG, Miller JS, McKenna DH, Hippen KL, DeFor TE, Sumstad D, et al. Umbilical Cord Blood-Derived T Regulatory Cells to Prevent GVHD: Kinetics, Toxicity Profile, and Clinical Effect. *Blood* (2016) 127(8):1044–51. doi:10.1182/blood-2015-06-653667
18. Tang Q, Vincenti F. Transplant Trials with Tregs: Perils and Promises. *J Clin Invest* (2017) 127(7):2505–12. doi:10.1172/jci90598
19. Boardman DA, Philippeos C, Fruhwirth GO, Ibrahim MAA, Hannen RF, Cooper D, et al. Expression of a Chimeric Antigen Receptor Specific for Donor HLA Class I Enhances the Potency of Human Regulatory T Cells in Preventing Human Skin Transplant Rejection. *Am J Transpl* (2017) 17(4):931–43. doi:10.1111/ajt.14185
20. Romano M, Fanelli G, Albany CJ, Giganti G, Lombardi G. Past, Present, and Future of Regulatory T Cell Therapy in Transplantation and Autoimmunity. *Front Immunol* (2019) 10:43. doi:10.3389/fimmu.2019.00043
21. Raffin C, Vo LT, Bluestone JA. Treg Cell-Based Therapies: Challenges and Perspectives. *Nat Rev Immunol* (2020) 20(3):158–72. doi:10.1038/s41577-019-0232-6
22. Boardman DA, Levings MK. Cancer Immunotherapies Repurposed for Use in Autoimmunity. *Nat Biomed Eng* (2019) 3(4):259–63. doi:10.1038/s41551-019-0359-6
23. Dawson NA, Lamarche C, Hoeppli RE, Bergqvist P, Fung VC, McIver E, et al. Systematic Testing and Specificity Mapping of Alloantigen-specific Chimeric Antigen Receptors in Regulatory T Cells. *JCI Insight* (2019) 4(6):e123672. doi:10.1172/jci.insight.123672
24. MacDonald KG, Hoeppli RE, Huang Q, Gillies J, Luciani DS, Orban PC, et al. Alloantigen-specific Regulatory T Cells Generated with a Chimeric Antigen Receptor. *J Clin Invest* (2016) 126(4):1413–24. doi:10.1172/jci82771
25. Atif M, Conti F, Gorochov G, Oo YH, Miyara M. Regulatory T Cells in Solid Organ Transplantation. *Clin Transl Immunol* (2020) 9(2):e01099. doi:10.1002/cti2.1099
26. Koyama I, Bashuda H, Uchida K, Seino K-i., Habu S, Nakajima I, et al. A Clinical Trial with Adoptive Transfer of Ex Vivo-induced, Donor-specific Immune-Regulatory Cells in Kidney Transplantation-A Second Report. *Transplantation* (2020) 104(11):2415–23. doi:10.1097/tp.0000000000003149
27. Altrock PM, Liu LL, Michor F. The Mathematics of Cancer: Integrating Quantitative Models. *Nat Rev Cancer* (2015) 15(12):730–45. doi:10.1038/nrc4029
28. Culshaw RV. Review of HIV Models: The Role of the Natural Immune Response and Implications for Treatment. *J Biol Syst* (2004) 12(02):123–35. doi:10.1142/s0218339004001099
29. Kirschner D, Pienaar E, Marino S, Linderman JJ. A Review of Computational and Mathematical Modeling Contributions to Our Understanding of *Mycobacterium tuberculosis* Within-Host Infection and Treatment. *Curr Opin Syst Biol* (2017) 3:170–85. doi:10.1016/j.coisb.2017.05.014
30. Arciero JC, Maturo A, Arun A, Oh BC, Brandacher G, Raimondi G. Combining Theoretical and Experimental Techniques to Study Murine Heart Transplant Rejection. *Front Immunol* (2016) 7:448. doi:10.3389/fimmu.2016.00448
31. Gaetano A, Matone A, Agnes A, Palumbo P, Ria F, Magalini S. Modeling Rejection Immunity. *Theor Biol Med Model* (2012) 9:18. doi:10.1186/1742-4682-9-18
32. Day JD, Metes DM, Vodovotz Y. Mathematical Modeling of Early Cellular Innate and Adaptive Immune Responses to Ischemia/Reperfusion Injury and Solid Organ Allotransplantation. *Front Immunol* (2015) 6:484. doi:10.3389/fimmu.2015.00484
33. Banks HT, Hu S, Jang T, Kwon H-D. Modelling and Optimal Control of Immune Response of Renal Transplant Recipients. *J Biol Dyn* (2012) 6:539–67. doi:10.1080/17513758.2012.655328
34. An G. Introduction of a Framework for Dynamic Knowledge Representation of the Control Structure of Transplant Immunology: Employing the Power of Abstraction with a Solid Organ Transplant Agent-Based Model. *Front Immunol* (2015) 6:561. doi:10.3389/fimmu.2015.00561
35. Akinwumi SM. *Modeling the Kinetics of EBV in Primary Carriers and Transplant Recipients*. Edmonton, AB: University of Alberta (2017). doi:10.7939/R3HH6CK80
36. Oberbarnscheidt MH, Zeng Q, Li Q, Dai H, Williams AL, Shlomchik WD, et al. Non-self Recognition by Monocytes Initiates Allograft Rejection. *J Clin Invest* (2014) 124(8):3579–89. doi:10.1172/jci74370
37. Deb K, Pratap A, Agarwal S, Meyarivan T. A Fast and Elitist Multiobjective Genetic Algorithm: NSGA-II. *IEEE Trans Evol Computat* (2002) 6(2):182–97. doi:10.1109/4235.996017
38. Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T Cells and Immune Tolerance. *Cell* (2008) 133(5):775–87. doi:10.1016/j.cell.2008.05.009
39. Wood KJ, Bushell A, Hester J. Regulatory Immune Cells in Transplantation. *Nat Rev Immunol* (2012) 12(6):417–30. doi:10.1038/nri3227
40. Bontha SV, Maluf DG, Mueller TF, Mas VR. Systems Biology in Kidney Transplantation: The Application of Multi-Omics to a Complex Model. *Am J Transpl* (2017) 17(1):11–21. doi:10.1111/ajt.13881
41. Mastoridis S, Martínez-Llordella M, Sanchez-Fueyo A. Emergent Transcriptomic Technologies and Their Role in the Discovery of Biomarkers of Liver Transplant Tolerance. *Front Immunol* (2015) 6:304. doi:10.3389/fimmu.2015.00304
42. Stegall MD, Borrows R. Computational Biology: Modeling Chronic Renal Allograft Injury. *Front Immunol* (2015) 6:385. doi:10.3389/fimmu.2015.00385
43. Yang JYC, Sarwal MM. Transplant Genetics and Genomics. *Nat Rev Genet* (2017) 18(5):309–26. doi:10.1038/nrg.2017.12
44. Issa F, Hester J, Milward K, Wood KJ. Homing of Regulatory T Cells to Human Skin Is Important for the Prevention of Alloimmune-Mediated Pathology in an *In Vivo* Cellular Therapy Model. *PLoS One* (2012) 7(12):e53331. doi:10.1371/journal.pone.0053331
45. Zhang N, Schröppel B, Lal G, Jakubzick C, Mao X, Chen D, et al. Regulatory T Cells Sequentially Migrate from Inflamed Tissues to Draining Lymph Nodes to Suppress the Alloimmune Response. *Immunity* (2009) 30(3):458–69. doi:10.1016/j.immuni.2008.12.022
46. Campbell DJ. Control of Regulatory T Cell Migration, Function, and Homeostasis. *J.Immunol.* (2015) 195(6):2507–13. doi:10.4049/jimmunol.1500801
47. Issa F, Robb RJ, Wood KJ. The where and when of T Cell Regulation in Transplantation. *Trends Immunol* (2013) 34(3):107–13. doi:10.1016/j.it.2012.11.003
48. Jhunjhunwala S, Chen LC, Nichols EE, Thomson AW, Raimondi G, Little SR. All-trans Retinoic Acid and Rapamycin Synergize with Transforming Growth Factor-B1 to Induce Regulatory T Cells but Confer Different Migratory Capacities. *J Leukoc Biol* (2013) 94(5):981–9. doi:10.1189/jlb.0312167
49. MacDonald KG, Orban PC, Levings MK. T Regulatory Cell Therapy in Transplantation. *Curr Opin Organ Transplant* (2012) 17(4):343–8. doi:10.1097/mot.0b013e328355aaaf
50. Camirand G, Riella LV. Treg-Centric View of Immunosuppressive Drugs in Transplantation: A Balancing Act. *Am J Transpl* (2017) 17(3):601–10. doi:10.1111/ajt.14029
51. Furukawa A, Wisel SA, Tang Q. Impact of Immune-Modulatory Drugs on Regulatory T Cell. *Transplantation* (2016) 100(11):2288–300. doi:10.1097/tp.0000000000001379
52. Eskandari SK, Sulkaj I, Melo MB, Li N, Allos H, Alhaddad JB, et al. Regulatory T Cells Engineered with TCR Signaling-Responsive IL-2 Nanogels Suppress Alloimmunity in Sites of Antigen Encounter. *Sci Transl Med* (2020) 12(569):eaaw4744. doi:10.1126/scitranslmed.aaw4744
53. Fisher JD, Zhang W, Balmert SC, Aral AM, Acharya AP, Kulahci Y, et al. *In Situ* recruitment of Regulatory T Cells Promotes Donor-specific Tolerance in Vascularized Composite Allotransplantation. *Sci Adv* (2020) 6(11):eaax8429. doi:10.1126/sciadv.aax8429
54. Brown LV, Gaffney EA, Ager A, Wagg J, Coles MC. Quantifying the Limits of CAR T-Cell Delivery in Mice and Men. *J R Soc Interf* (2021) 18(176):20201013. doi:10.1098/rsif.2020.1013
55. Alexander HK, Wahl LM. Self-tolerance and Autoimmunity in a Regulatory T Cell Model. *Bull Math Biol* (2011) 73(1):33–71. doi:10.1007/s11538-010-9519-2

56. Fouchet D, Regoes R. A Population Dynamics Analysis of the Interaction between Adaptive Regulatory T Cells and Antigen Presenting Cells. *Plos One* (2008) 3(5):e2306. doi:10.1371/journal.pone.0002306
57. Garcia-Martinez K, Leon K. Modeling the Role of IL2 in the Interplay between CD4+ Helper and Regulatory T Cells: Studying the Impact of IL2 Modulation Therapies. *Int Immunol* (2012) 24(7):427–46. doi:10.1093/intimm/dxr120
58. León K, Lage A, Carneiro J. Tolerance and Immunity in a Mathematical Model of T-Cell Mediated Suppression. *J Theor Biol* (2003) 225(1):107–26. doi:10.1016/s0022-5193(03)00226-1
59. Sicard A, Lamarche C, Speck M, Wong M, Rosado-Sánchez I, Blois M, et al. Donor-specific Chimeric Antigen Receptor Tregs Limit Rejection in Naive but Not Sensitized Allograft Recipients. *Am J Transpl* (2020) 20(6):1562–73. doi:10.1111/ajt.15787
60. Golshayan D, Jiang S, Tsang J, Garin MI, Mottet C, Lechler RI. In Vitro-expanded Donor Alloantigen-specific CD4+CD25+ Regulatory T Cells Promote Experimental Transplantation Tolerance. *Blood* (2007) 109(2):827–35. doi:10.1182/blood-2006-05-025460
61. Joffre O, Santolaria T, Calise D, Saati TA, Hudrisier D, Romagnoli P, et al. Prevention of Acute and Chronic Allograft Rejection with CD4+CD25+Foxp3+ Regulatory T Lymphocytes. *Nat Med* (2008) 14(1):88–92. doi:10.1038/nm1688

ESOT | Transplant International

ORIGINAL RESEARCH
published: 13 April 2022
doi: 10.3389/ti.2022.10057

Single Donor Infusion of S-Nitroso-Human-Serum-Albumin Attenuates Cardiac Isograft Fibrosis and Preserves Myocardial Micro-RNA-126-3p in a Murine Heterotopic Heart Transplant Model

Anne-Kristin Schaefer[1,2], Attila Kiss[1], André Oszwald[3,4], Felix Nagel[1], Eylem Acar[1], Arezu Aliabadi-Zuckermann[2], Matthias Hackl[5], Andreas Zuckermann[2], Renate Kain[3,4], Andrzej Jakubowski[6,7], Peter Ferdinandy[8], Seth Hallström[9] and Bruno K. Podesser[1]**

[1]Ludwig Boltzmann Institute for Cardiovascular Research, Center for Biomedical Research, Medical University of Vienna, Vienna, Austria, [2]Department of Cardiac Surgery, Medical University of Vienna, Vienna, Austria, [3]Department of Pathology, Medical University of Vienna, Vienna, Austria, [4]Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria, [5]TamiRNA GmbH, Vienna, Austria, [6]Department of Pharmacology, Jagiellonian University Medical College, Kraków, Poland, [7]Department of Anesthesiology and Intensive Care, Małopolska Orthopedic and Rehabilitation Hospital, Kraków, Poland, [8]Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, Semmelweis University, Budapest, Hungary, [9]Division of Physiological Chemistry, Otto Loewi Research Center, Medical University of Graz, Graz, Austria

Objectives: Cold ischemia and subsequent reperfusion injury are non-immunologic cornerstones in the development of graft injury after heart transplantation. The nitric oxide donor S-nitroso-human-serum-albumin (S-NO-HSA) is known to attenuate myocardial ischemia-reperfusion (I/R)-injury. We assessed whether donor preservation with S-NO-HSA affects isograft injury and myocardial expression of GATA2 as well as miR-126-3p, which are considered protective against vascular and endothelial injury.

Methods: Donor C57BL/6 mice received intravenous (0.1 µmol/kg/h) S-NO-HSA ($n = 12$), or 0.9% saline (control, $n = 11$) for 20 min. Donor hearts were stored in cold histidine-tryptophan-α-ketoglutarate-N solution for 12 h and underwent heterotopic, isogenic transplantation, except 5 hearts of each group, which were analysed immediately after preservation. Fibrosis was quantified and expression of GATA2 and miR-126-3p assessed by RT-qPCR after 60 days or immediately after preservation.

Results: Fibrosis was significantly reduced in the S-NO-HSA group (6.47% ± 1.76 vs. 11.52% ± 2.16; $p = 0.0023$; 12 h-S-NO-HSA-hHTX vs. 12 h-control-hHTX). Expression of miR-126-3p was downregulated in all hearts after ischemia compared to native myocardium, but the effect was significantly attenuated when donors received S-NO-

OPEN ACCESS

Correspondence:
Bruno K. Podesser
bruno.podesser@meduniwien.ac.at
Seth Hallström
seth.hallstroem@medunigraz.at

Received: *24 September 2021*
Accepted: *17 March 2022*
Published: *13 April 2022*

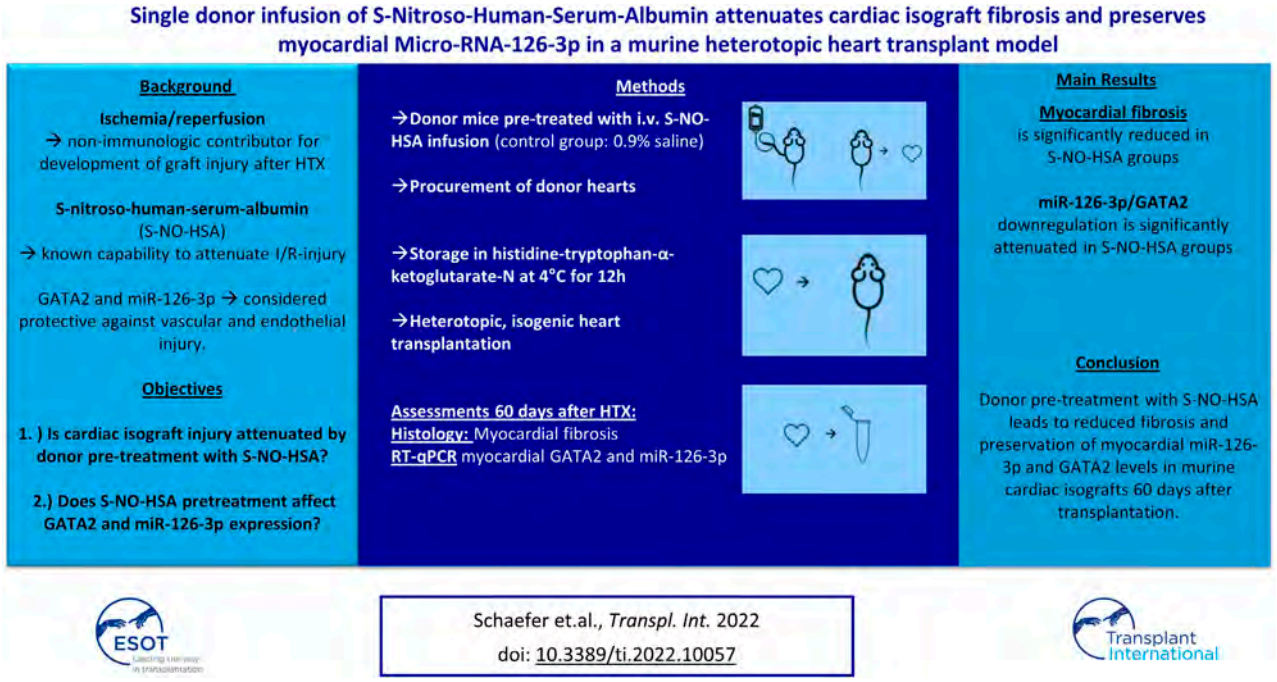
Citation:
Schaefer A-K Kiss A, Oszwald A, Nagel F, Acar E, Aliabadi-Zuckermann A, Hackl M, Zuckermann A, Kain R, Jakubowski A, Ferdinandy P, Hallström S and Podesser BK (2022) Single Donor Infusion of S-Nitroso-Human-Serum-Albumin Attenuates Cardiac Isograft Fibrosis and Preserves Myocardial Micro-RNA-126-3p in a Murine Heterotopic Heart Transplant Model. Transpl Int 35:10057. doi: 10.3389/ti.2022.10057

Abbreviations: CAI, Chronic allograft injury; CAV, cardiac allograft vasculopathy; eNOS, endothelial nitric oxide synthase; HTK-N, histidine-tryptophan-α-ketoglutarate-N; hHTX, heterotopic heart transplantation; HTX, heart transplantation; I/R, ischemia/reperfusion; IVC, inferior vena cava; miR, microRNA; NO, nitric oxide; RT-qPCR, real time quantitative polymerase chain reaction; S-NO-HSA, S-nitroso-human-serum-albumin.

HSA (1 ± 0.27 vs. 0.33 ± 0.31; $p = 0.0187$; 12 h-S-NO-HSA-hHTX vs. 12 h-control-hHTX; normalized expression to U6 snRNA).

Conclusion: Donor pre-treatment with S-NO-HSA lead to reduced fibrosis and preservation of myocardial miR-126-3p and GATA2 levels in murine cardiac isografts 60 days after transplantation.

Keywords: heart transplantation, graft preservation, cardiac isograft injury, cardiac graft fibrosis, experimental transplantation



GRAPHICAL ABSTRACT |

INTRODUCTION

Chronic allograft injury (CAI), consisting of vasculopathy and interstitial fibrosis, affects approximately 50% of patients after 10 years and limits long-term survival following heart transplantation (1). There is substantial evidence that endothelial injury during organ procurement and preservation, caused by ischemia and subsequent reperfusion, results in endothelial dysfunction. The latter is a non-immunologic contributor to pathogenesis and progression of CAI (2–4). Besides endothelial dysfunction, the progression of interstitial and perivascular fibrosis consecutively leads to impaired diastolic and systolic graft function, thus preservation of endothelial and vascular function is certainly a clinically desirable goal.

Recent studies have demonstrated that supplementation of nitric oxide (NO), or increased expression of endothelial NO-synthase (eNOS) protects against both IR-injury and fibrosis (5, 6). We have proposed the concept of donor- and recipient management using the NO-donor S-nitroso-human-serum-albumin (S-NO-HSA) (7), a high-molecular-weight S-nitrosothiol with a high S-nitrosograde and exact equimolar nitrosation (8).

NO release by S-NO-HSA can downregulate eNOS activity by feedback inhibition (9), and thereby prevent eNOS uncoupling and subsequent superoxide and peroxynitrite formation caused by eNOS uncoupling during I/R. Supporting evidence for this concept comes from previous small-and large animal preclinical studies, where addition of S-NO-HSA to the preservation solution has shown to enhance hemodynamic and metabolic recovery after cardioplegic arrest in the isolated rabbit heart after 6 h of hypothermic, cardioplegic arrest (5), and intravenous infusion of S-NO-HSA at a dose of 0.1 µmol/kg/h reduced ischemia/reperfusion injury in the pig heart after unprotected warm ischemia (7, 10). Whether S-NO-HSA provides similar protective effects beyond acute functional and metabolic improvements in the setting of heart transplantation (HTX) has not yet been investigated.

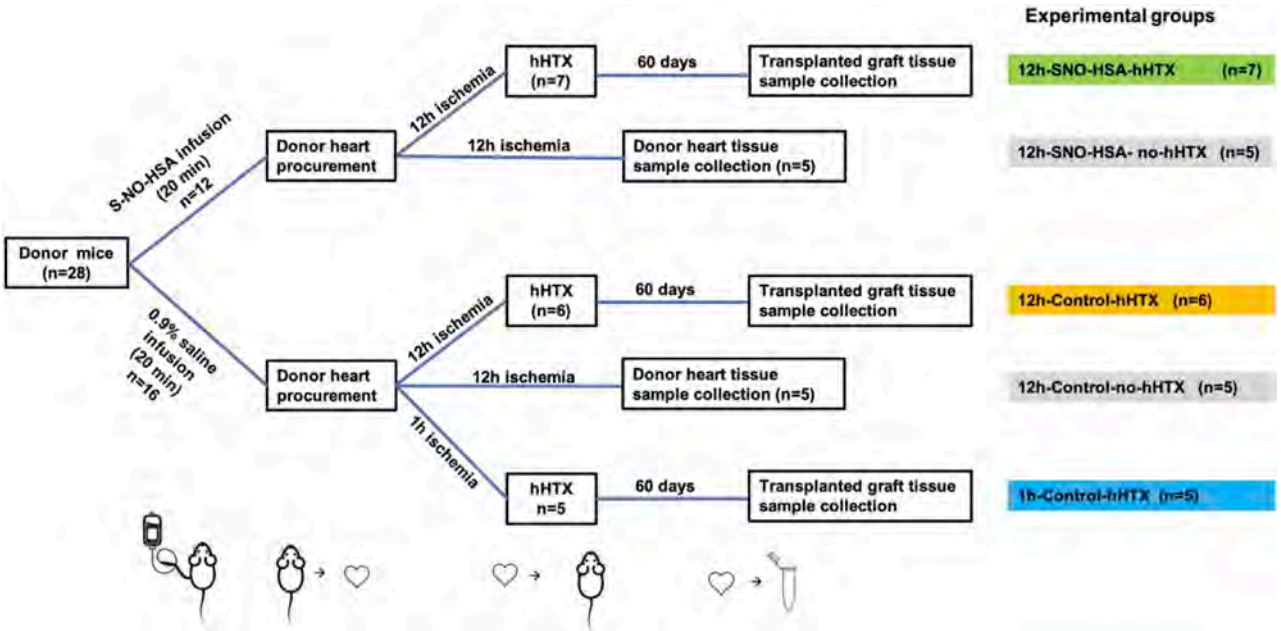


FIGURE 1 | Experimental setup. Palpation score, myocardial fibrosis, GATA2 and miR-126-3p were assessed in 3 groups 60 days after heterotopic transplantation (hHTX). HHTX took place either after 12 h of cold ischemia with donor preparation using SNO-HSA (12 h-SNO-HSA-hHTX), donor preparation with normal saline (12 h-control-hHTX), or after 1 h of cold ischemia (1 h-control-hHTX). Additionally, GATA2 and miR-126-3p expression was assessed in 3 non-transplanted groups directly after the cold ischemia period after 12 h with donor preparation using SNO-HSA (12 h-SNO-HSA-no-hHTX) or normal saline (12 h-control-no-hHTX). GATA2 and miR-126-3p expression was analyzed in some grafts without cold ischemia (no ischemia) as an additional control group. An overview of experimental groups is depicted in **Table 1**.

TABLE 1 | Overview of experimental groups.

Experimental group	Total (n)	Histology (n)	miRNA analysis (n)
Transplanted groups			
12 h-SNO-HSA-hHTX	7	7	5
12 h-control-hHTX	6	6	6
1 h-control-hHTX	5	5	5
Non-transplanted groups			
12 h-SNO-HSA-no-hHTX	5	—	5
12 h-control-no-hHTX	5	—	5
no-ischemia	19	—	19

Endothelial cell function and eNOS expression in different organs, including the heart, is highly regulated on epigenetic levels, particularly by the GATA2 transcription factor (11). In general, GATA2 also activates the expression of miR-126, the most abundant microRNA in endothelial cells (12). Recent clinical studies demonstrated the diagnostic relevance of miR-126 in association with the presence of CAI in HTX recipients (13, 14). Nevertheless, little is known about 1) the spatial-temporal expression of both GATA2 and miR-126 in transplanted hearts; 2) the effect of S-NO-HSA on their expression levels.

The aim of the present study was to investigate whether donor pretreatment with S-NO-HSA attenuates long-term development of graft fibrosis, and to characterize the expression of GATA2 and miR-126-3p with and without S-NO-HSA pretreatment in a mouse model of isogenic, heterotopic HTX after prolonged cold ischemia and reperfusion.

MATERIALS AND METHODS

Experimental Animals

Male C57BL/6 mice aged 8–9 weeks (Department for Laboratory Animal Science and Genetics, Himberg, Austria) were used in this study. The experimental protocol was approved by the regional Ethics Committee for Laboratory Animal Experiments at the Medical University of Vienna and the Federal Ministry Republic of Austria, Education, Science and Research (authorization protocol number GZ 66.009/0158-WF/V/3b/2015) and conforms with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

S-NO-HSA Preparation

HSA was processed as previously described (10, 15). S-NO-HSA preparation is depicted in detail in **Supplementary Appendix S1**. S-NO-HSA was dissolved in 0.9% saline solution and continuously infused *via* a catheter in the femoral vein for 20 min (0.1 µmol S-NO-HSA/kg/h) prior to donor heart procurement.

Experimental Groups

In order to clarify the impact of S-NO-HSA on graft preservation and miR-126-3p and GATA2 expression, the experimental setup depicted in **Figure 1** was used.

Hearts without ischemia (n = 19; no ischemia) served as additional controls for the expression analysis of GATA2 and miR-126-3p assessed by RT-qPCR. The experimental groups are summarized in **Table 1**.

Donor Heart Procurement

Donor mice were anesthetized by intraperitoneal injection of the mixture of xylazine (5 mg/kg) and ketamine (100 mg/kg), followed by catheterization of the femoral vein and intravenous infusion of S-NO-HSA (0.1 μmol/kg/h) dissolved in 0.9% saline solution, or 0.9% saline solution only (control groups) for 20 min, followed by thoracotomy and administration of 1 ml of HTK-N solution (4°C, Dr. Franz Köhler Chemie GmbH, Bensheim, Germany) supplemented with 100 units of heparin *via* the inferior vena cava to arrest the heart. The ascending aorta and pulmonary trunk were divided. After ligation of the superior venae cavae, and en block ligation of the pulmonary veins, the graft was excised, flushed with heparinized HTK-N solution, and stored in HTK-N solution at 4°C for either 1 h or 12 h.

Heterotopic Abdominal Heart Transplantation

Recipient surgeries were conducted as described previously (16, 17). Analgesia was provided by subcutaneous injection of buprenorphine (0.1 mg/kg bodyweight) and anaesthesia maintained with inhaled isoflurane. Briefly, after laparotomy and dissection of the infrarenal aorta and IVC, the abdominal aorta and IVC were cross-clamped infrarenally and directly proximal to the iliac bifurcation. After longitudinal aortotomy and venotomy, the donor's acending aorta was anastomosed to the recipient's abdominal aorta and the donor's pulmonary trunk to the recipient's IVC using running 10-0 nylon sutures. The duration of warm ischemia during the implantation process was standardized to 30 min.

Assessment of Functional Graft Status

Graft viability was assessed and rated by transabdominal palpation using a score from 0 (no palpable contraction) to 5 (strong beat and adequate heart rate) before sample collection 60 days after transplantation as described previously (16).

Myocardial Tissue Sample Collection

Sixty days after transplantation, recipient mice were anaesthetized with the mixture of ketamine and xylazine (0.1 ml/10 g bodyweight), and anaesthesia was confirmed by hind foot and tail pinch. The transplanted heart was excised and transversally cut at mid-papillary level. The base of the hearts was frozen in liquid nitrogen and stored at −80°C, and the apex fixed in 7.5% formaldehyde for histopathology analysis.

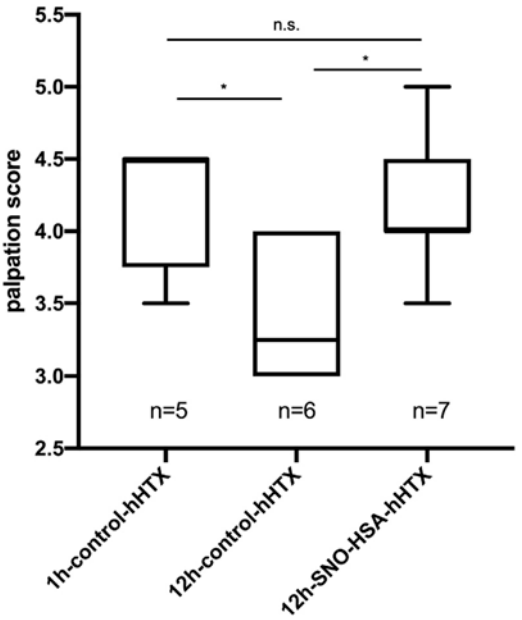


FIGURE 2 | Palpation score of control and S-NO-HSA pretreatment groups. Graft viability was assessed and rated by transabdominal palpation using a score from 0 (no palpable contraction) to 5 (strong beat and adequate heart rate) prior sample collection at day 60 after transplantation (hHTX) in the 3 transplanted groups. *$p < 0.05$; n.s. = non-significant.

Assessment of miR-126-3p and GATA2 Expression

Assessment of miR-126-3p and GATA2 expression are described in **Supplementary Appendix S1**.

Histological Analysis

Histological analysis is described in detail in **Supplementary Appendix S1**. For quantification of interstitial fibrosis, sections of 4 μm were cut and stained with Sirius red. The areas of total and positively stained tissue within a region were quantified using CellProfiler (18).

Human Cardiac Fibroblast Experiments

Human ventricular cardiac fibroblasts (Lonza, Basel, Switzerland) were cultured in fibroblast basal medium supplemented with 0.1% insulin, 0.1% fibroblast growth factor, 0.1% GA-1000, and 10% FBS (all Lonza, Basel, Switzerland) as described previously (19). Cultures were washed once with DPBS (Thermo Fisher Scientific, CA, United States) when indicated, and split at a confluency level of 70%. Cells were treated for 24 h follows: 1) No treatment—control; 2) 20 ng/ml TGF-β (Abcam, Cambridge, United Kingdom); 3) 25 μmol/L HSA; 4) 25 μmol/L S-NO-HSA; 5) 20 ng/ml TGF-β + 25 μmol/L HSA and 6) 20 ng/ml TGF-β + 25 μmol/L S-NO-HSA. Total RNA was extracted, and expression of target genes (**Supplementary Table S5**) were assessed by RT-qPCR (**Supplementary Appendix S1**).

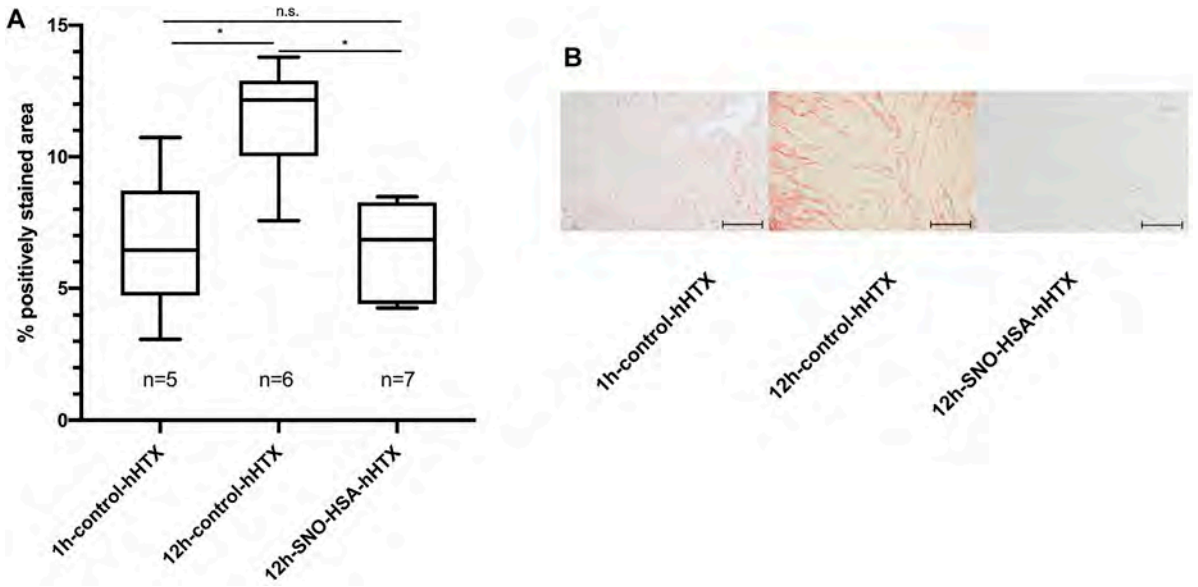


FIGURE 3 | Panel **(A)**: Extent of myocardial interstitial fibrosis 60 days after hHTX. Control (1 h-control-hHTX, 12 h-control-hHTX) and S-NO-HSA pretreated (12 h-SNO-HSA-hHTX) groups. Extent of fibrosis in isografts 60 days after hHTX At least two regions of myocardium each of the interventricular septum (IVS) and the right ventricle (RV) were selected for quantification. The areas of total and positively stained tissue within a region were quantified using CellProfiler (18). *$p < 0.05$; n.s. = non-significant Panel **(B)**: Representative images of sirius red stain of transplanted myocardium 60 days after hHTX. Sections of 4 µm were cut and stained with Sirius red for quantification of interstitial fibrosis at day 60 after transplantation (hHTX). Images were acquired using an upright microscope using a ×40 objective and a CCD-camera with a ×0.63 adapter (Axio Imager. M2 and Axiocam 512 color, Carl Zeiss, Aalen, Germany). Scale bar = 100 µm.

Statistical Analysis

Data are presented as mean ± standard deviation. Testing for normality was performed using the Kolmogorov-Smirnov-test. One-way ANOVA with Tukey HSD post-hoc test was used for multiple comparisons between the groups. Two-tailed $p < 0.05$ was considered statistically significant. Spearman correlation was used to assess correlation of miR-126-3p and GATA2 expression. Analysis was performed using Prism 8 software for macOS (GraphPad Inc., San Diego, CA, United States).

RESULTS

Baseline Characteristics and Functional Graft Assessment

Baseline characteristics of experimental animals are shown in **Supplementary Table S1**.

Palpation score 60 days after transplantation was significantly higher in grafts transplanted after 1 h ischemia and 12 h ischemia when donors received S-NO-HSA compared to the 12 h control-group. (4.2 ± 0.45 vs. 3.42 ± 0.49; $p = 0.041$; 1 h-control-hHTX vs. 12 h-control-hHTX and 4.21 ± 0.49 vs. 3.42 ± 0.49; $p = 0.023$ 12 h-S-NO-HSA-hHTX vs. 12 h-control-hHTX). Palpation score is depicted in **Figure 2**.

Myocardial Interstitial Fibrosis

In hearts transplanted after prolonged cold ischemia (12 h), fibrosis was significantly reduced 60 days after transplantation when donors were pretreated with S-NO-HSA (6.47% ± 1.76 vs.

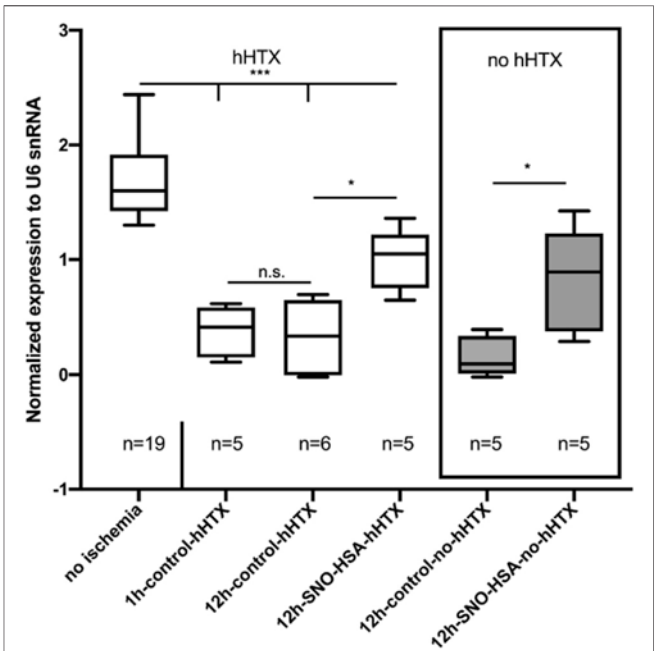


FIGURE 4 | Myocardial miR-126-3p expression levels in control (1 h-control-hHTX, 12 h-control-hHTX, 12 h-control-no-hHTX) and S-NO-HSA pretreated (12 h-SNO-HSA-hHTX, 12 h-SNO-HSA-no-hHTX) groups. Box plots from myocardium not subjected to ischemia (no ischemia), from non-transplanted reference hearts with 12 h ischemia (12 h-control-no-hHTX) and S-NO-HSA pretreated non-transplanted hearts (12 h-SNO-HSA-no-hHTX). ***$p < 0.001$; *$p < 0.05$; n.s = .non-significant.

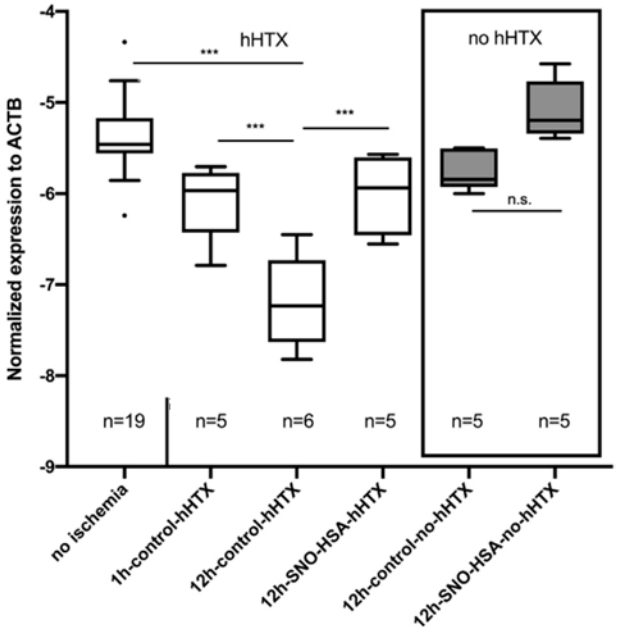


FIGURE 5 | Myocardial GATA2 expression levels in control and S-NO-HSA pretreatment groups. Box plots from myocardium not subjected to ischemia (no ischemia), from non-transplanted reference hearts with 12 h ischemia (12 h-Control-no-hHTX) and S-NO-HSA pretreated non-transplanted hearts (12 h-SNO-HSA-no-hHTX). Assessment of GATA2 expression levels from the myocardial biopsies are described in detail in **Supplementary Appendix S1**. ***$p < 0.001$; *$p < 0.05$; n.s. = non-significant.

11.52% ± 2.16; p = 0.0023; 12 h-S-NO-HSA-hHTX vs. 12 h-control-hHTX). The extent of myocardial interstitial fibrosis is depicted in **Figure 3A**, and representative images of each group are shown in **Figure 3B**. Regarding duration of ischemia, the extent of fibrosis in hearts transplanted after prolonged (12 h) ischemia was significantly higher than the extent of fibrosis in the second control group transplanted after 1 h of ischemia (11.52% ± 2.16 vs. 6.66% ± 2.72; p = 0.006; 12 h-control-hHTX vs. 1 h-control-hHTX) at 60 days after transplantation. Fibrosis in donors pretreated with S-NO-HSA and prolonged (12 h) ischemia was not significantly different to the reference group transplanted after 1 h of ischemia at 60 days after transplantation (6.47% ± 1.76 vs. 6.66% ± 2.72; p = 0.99; 12 h-S-NO-HSA-hHTX vs. 1 h-control-hHTX).

MiR-126-3p Expression in the Myocardium

Figure 4 depicts the expression of miR-126-3p in myocardial tissue. When compared to myocardium not subjected to ischemia (no ischemia), miR-126-3p was significantly reduced in all grafts (transplanted and non-transplanted) subjected to ischemia. However, transplanted grafts from donors pretreated with S-NO-HSA showed a significantly increased miR-126-3p expression compared to control groups without S-NO-HSA-pretreatment (transplanted groups: 1 ± 0.27 vs. 0.33 ± 0.31; p = 0.0187; 12 h-SNOHSA-hHTX vs. 12 h-control-hHTX; normalized expression to U6 snRNA).

In the groups analyzed directly after the ischemic period (12 h) without subsequent transplantation, expression of miR-126-3p was significantly higher in the group with S-NO-HSA pretreated donors when compared to grafts procured without prior S-NO-HSA-administration. (0.82 ± 0.46 vs. 0.16 ± 0.17; p = 0.029; 12 h-SNOHSA-no-hHTX vs. 12 h-control-no-hHTX; normalized expression to U6 snRNA).

There was no significant difference in miR-126-3p expression levels between the control groups transplanted after 12 h vs. only 1 h of ischemia (0.33 ± 0.31 vs. 0.38 ± 0.22; p = 0.99; 12 h-control-hHTX vs. 1 h-control-hHTX; normalized expression to U6 snRNA).

Myocardial GATA2 Expression

Myocardial GATA2 expression is depicted in **Figure 5**. Sixty days after hHTX, GATA2 expression was significantly downregulated in grafts subjected to 12 h of ischemia, but this effect was reversed when donors had received S-NO-HSA (−6.00 ± 0.45 vs. −7.188 ± 0.5; 12 h-S-NO-HSA-hHTX vs. 12 h-control-hHTX: p = 0.0008; normalized expression to ACTB).

Grafts that were subjected to ischemia but not transplanted showed no significant difference in GATA2 expression levels compared to control hearts without ischemia. In the non-transplanted groups, there was also no significant difference in GATA2-expression depending on whether donors received S-NO-HSA (−5.1 ± 0.33 vs. −5.74 ± 0.22; p = 0.134; 12 h-SNO-HSA-no-hHTX vs. 12 h-control-no-hHTX; normalized expression to ACTB).

A positive correlation was found between GATA2 and miR-126-3p expression levels of all samples [r = 0.496, p = 0.0006; **Supplemental Figure S1** (**Supplementary Appendix S1**)].

Role of Nitric Oxide on the Expression of Markers for Fibrosis in Human Cardiac Fibroblasts

To further evaluate the role of an intact eNOS (endothelium) and its effect on fibrosis we utilized S-NO-HSA as a tool. S-NO-HSA at a concentration of 25 μmol/L releases NO in a physiological range of approximately 150 nmol/L in cell culture medium or physiological saline (20). The potential anti-fibrotic effect of intact eNOS (intact endothelium) was studied in human cardiac fibroblasts, which were cultivated and treated with TGF-β in order to stimulate fibroblast to myofibroblast transition. As appropriate control to 25 μmol/L S-NO-HSA 25 μmol/L HSA was used. In direct comparison NO released *via* S-NO-HSA significantly decreased α-SMA mRNA levels (**Figure 6A**, panel a; p = 0.0006) and transforming growth factor-β (TGF-β) type II receptors (TGFBR2) expression levels (**Figure 6B**; p = 0.0139) in TGF-β stimulated fibroblasts. In addition, perostin levels (another marker of activated fibroblast) was reduced with S-NO- HSA but did not reach significance compared to HSA (**Figure 6C**). Both HSA and S-NO-HSA reduced collagen I expression levels in TGF-β stimulated fibroblasts (revealing no specific NO effect; **Figure 6D**).

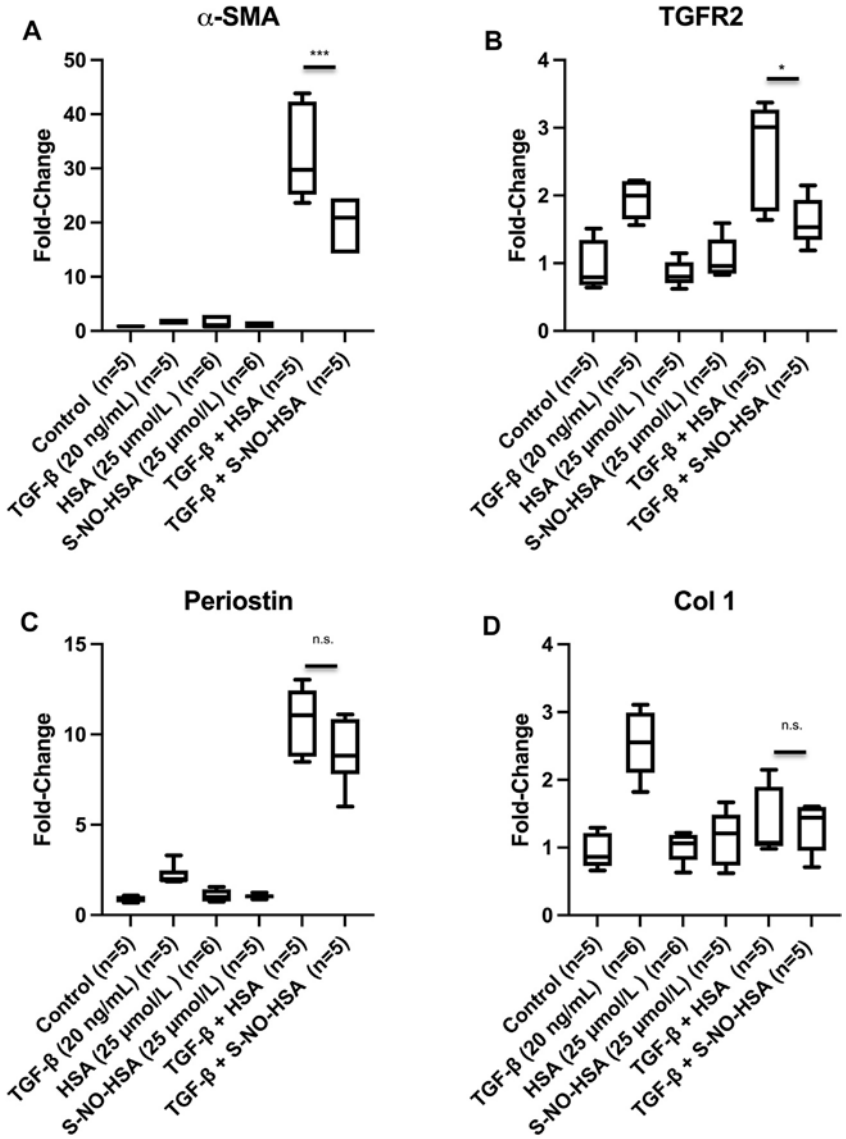


FIGURE 6 | Expression levels of α-smooth muscle actin [αSMA, panel **(A)**], transforming growth factor-β type II receptors [TGFBR2, panel **(B)**], perostin **(C)** and collagen I **(D)** in transforming growth factor (TGF-β) stimulated human ventricular cardiac fibroblasts. Cells were treated for 24 h as follows: No treatment—control; TGFβ (20 ng/ml); HSA (25 µmol/L); S-NO-HSA (25 µmol/L); TGF-β (20 ng/ml) + HSA (25 µmol/L) and TGFβ (20 ng/ml) + S-NO-HSA (25 µmol/L). Total RNA was extracted, and expression of target genes (**Supplementary Table S5**) were assessed by RT-qPCR. mean ± SD (n = 6 per treatment); ***p < 0.001; *p < 0.05; n.s. = non-significant.

However, it has to be mentioned that in TGF-β stimulated fibroblasts both HSA and S-NO-HSA further increased α SMA levels (HSA: 18-fold) and perostin expression levels (HSA: 4.9-fold) in TGF-β stimulated fibroblasts. It is known that HSA can enhance mRNA expression levels as we observed in these two cases (21).

DISCUSSION

Optimizing preservation methods is crucial, as improving cold storage enables increasing the donor pool by long-distance procurements and acceptance of marginal donors. Previous studies have demonstrated the superior cardioprotective effect of HTK-N (22), an effect that can even be augmented by the addition of the nitric oxide donor S-NO-HSA (8). However, these studies have focused on acute functional parameters, and little is known about long-term effects on the myocardium after transplantation.

In the present study, donor pre-treatment with intravenous S-NO-HSA prior to graft procurement significantly reduced the long-term development of interstitial fibrosis in heterotopically transplanted murine cardiac isografts. This effect was accompanied by preservation of myocardial GATA2 and miR-

126-3p expression. Whilst depletion of miR-126-3p was present in all grafts subjected to cold ischemia, this effect was significantly attenuated by donor pre-treatment with S-NO-HSA.

Our data suggests that miR-126-3p downregulation seems to be related to the ischemic period per se, since downregulation was also observed in non-transplanted grafts after the ischemic period. MiR-126-3p downregulation seems also to be less dependent on the duration of ischemic period, since no significant difference in miR-126-3p expression between transplanted grafts after 1 h and 12 h of ischemia was observed.

In contrast to miR-126-3p levels, GATA2 expression was markedly reduced only in transplanted grafts, and this effect was reversed in the S-NO-HSA group, suggesting preserved endothelial cell function. Preservation of myocardial miR-126-3p levels by S-NO-HSA administration is an important novel finding and suggests that dysregualation of miR-126-3p in the myocardium is primarily caused by ischemia. In further consequence, depletion of miR-126-3p may play a causative role in the development of cardiac fibrosis.

Mechanistically, there is evidence that miR-126-3p has pro-angiogenic properties by degradation of negative regulators in the vascular endothelial growth factor pathway, phosphoinositol-3 kinase regulatory subunit 2 (PI3KR2) and sprouty related protein 1 (SPRED1), thereby maintaining the integrity of blood vessels (23). In line with our results, previous studies demonstrated that depletion of miR-126-3p is associated with impaired cardiac and vascular function (14). Yang et al. found that overexpression of miR-126-3p protected human cardiac microvascular endothelial cells against hypoxia/reoxygenation injury via a mechanism activating the PI3K/Akt/eNOS signaling pathway (24). Accordingly, we found that cold ischemia is accompanied by a marked decline of miR-126-3p in transplanted hearts. Furthermore, it has been shown that miR-126-3p does not only affect endothelial cells, but also initiates cardioprotection against ischemia-reperfusion-injury in cardiomyocytes (25).

Dysregulated circulating miRs are potential biomarkers for cardiovascular diseases: (13, 26). A recent clinical study has shown that *circulating* miR-126-3p was upregulated in patients with CAV compared to transplanted patients without CAV (13). In contrast, downregulation of *tissue* miR-126-3p has been described in a very recent study in myocardial biopsies of transplant recipients with allograft vasculopathy, which is in line with our findings (14). Nevertheless, further preclinical and clinical studies are warranted to clarify the role and spatial-temporal expression pattern of miR-126-3p in HTX.

A recent pioneering study by Hartmann et al. demonstrated that GATA2 regulates miR-126-3p in endothelial cells (12). In line with this finding, we found a correlation between mir-126-3p expression and GATA2 levels. In addition, we observed a decline of GATA2 expression in transplanted grafts. These effects were partially counteracted by donor pretreatment with S-NO-HSA. In our study, we did not investigate the mode of action how S-NO-HSA modifies GATA2, however it is tempting to speculate that preserved endothelial cell viability may lead to maintainance of GATA2 levels and subsequent functional improvement.

An ischemia duration-dependent increase in myocardial fibrosis in transplanted cardiac isografts 60 days after transplantation was detected. The extent of fibrosis was significantly attenuated when donors were pretreated with S-NO-HSA before procurement.

NO deficiency due to its consumption by superoxide (O_2^-), produced in high concentrations during ischemia and reperfusion are known to play an important role in the pathophysiology of I/R injury (15). The mechanism by which S-NO-HSA as an exogenous NO-donor can protect the dysfunction of the endothelium and prevent excessive O_2^- formation is based on prevention of eNOS uncoupling (10, 15). The uncoupled eNOS can intermittently produce both NO and superoxide (27, 28). It is of note that the slow and long-lasting release of NO by S-NO-HSA compared to small molecular weight S-nitroso thiols is a special feature of the applied drug. Mean arterial blood pressure is not affected at a dose of 0.1 µmol/kg/h of S-NO-HSA (29). Recently, this difference in kinetics of NO release by S-NO-HSA has also been demonstrated intracellularly by live-cell imaging of nitric oxide dynamics with novel FP-based probes (20).

In the present study, administration of S-NO-HSA as pretreatment to the donor may increase/preserve NO bioavailibility due to prevention of eNOS uncoupling during the cold ischemia and reperfusion period, leading to a decrease in oxidative/nitroxidative stress induced by O_2^- and peroxynitrite ($ONOO^-$) formation, and maintainance of endothalial cell integrity and function during the period of I/R (10). In the pretreatment phase, NO provided by S-NO-HSA may downregulate eNOS activity through feedback inhibition and thereby preserve its function (prevent eNOS uncoupling) (30). Therefore, in this setting, beneficial effects can be explained by two mechanisms: on the one hand, pretreatment with S-NO-HSA downregulates eNOS prior to ischemia and reperfusion by supplementing NO and thereby preserving and stabilizing its function during the prolonged ischemic phase, leading to sufficient NO production after transplantation (reperfusion), on the other hand, the improved NO production has further an indirect/direct positive inotropic and lusitropic effect on the myocardial cell and thereby preserves cardiac function (10). S-NO-HSA administration for only 20 min prior to donor heart procurement seems to minimize/prevent eNOS uncoupling during the 12 h storage in HTK-N solution at 4°C and subsequent transplantation. Interestingly, this pretreatment with the NO-donor is sufficient to reduce fibrosis after 60 days of transplantation. NO has also been reported to act as an antifibrotic effector in animal models of experimental fibrosis and a loss of NO bioavailability in eNOS knock-out mice resulted in increased fibrosis (6, 31, 32). These data are in line with our observed results and emphasize the importance of NO bioavailability in the prevention of fibrosis (32).

As S-NO-HSA prevents eNOS uncoupling in our transplant model and in further consequence reduces fibrosis, we utilized S-NO-HSA as NO donor to simulate a preserved endothelium in experiments with human fibroblasts and TGF-ß stimulation. NO provided by S-NO-HSA at physiologically relevant concentration significantly decreased α-smooth muscle actin (α-SMA) levels as well as TGFRII expression levels in TGF-β stimulated fibroblasts when compared to HSA (**Figures 6A,B**). Park et al. have recently

demonstrated that NO (*via* nitrite) significantly decreased α-SMA expression in TGF-ß stimulated fibroblasts thereby attenuating myofibroblast differentiation of human keratocytes (33). In addition, inhibiting the production of NO causes endothelial cells to produce factors that promote the expression in fibroblasts of α-SMA and collagen type I (34). In our model we could not attribute the reduction of expression of collagen type I to NO as both HSA and S-NO-HSA showed a reduction (**Figure 6D**). It has also been shown that increased levels of TGFRII from matrix-producing interstitial cells such as fibroblast are sufficient to increase the severity of fibrosis (35). The expression levels of periostin (a marker of activated fibroblasts) (36) was also reduced with S-NO-HSA compared to HSA but did not reach a level of significance (**Figure 6C**). Taking together the data reveals the importance of NO (and intact eNOS) in the prevention of fibrosis. The data with the NO donor S-NO-HSA on expression of markers for fibrosis in TGF-β stimulated human cardiac fibroblasts are in line with our finding that hearts transplanted after prolonged cold ischemia showed significantly reduced fibrosis 60 days after transplantation when donors were pretreated with S-NO-HSA.

Limitations

The animal model used in the present study is not able to fully mimic the clinical scenario of cardiac transplantation. After heterotopic transplantation, the graft is perfused and beating, but the left ventricle is unloaded, leading to graft atrophy and thrombus formation in the left ventricular cavity over time. However, quantification of fibrosis is possible in the right ventricular myocardium and the interventricular septum.

Our study did not aim to investigate the influence of immunologic responses and we therefore did not choose an allograft model. In the isogenic transplantation model applied, we did not expect nor observe graft vasculopathy.

However, the control role of miR-126-3p on the progression of neointima formation and vascular smooth muscle cell proliferation has been demonstrated in previous studies (37), suggesting that the depletion of miR-126-3p in transplanted hearts demonstrated in our study may be an indicator for CAI. This let us hypothesize that preservation of miR-126-expression may also inhibit CAV pathogenesis. Further studies are also required to assess other variables with, e.g., PET-MRI which will enable to establish correlations with graft viability and functional analysis of the transplanted hearts.

CONCLUSION

Intravenous administration of S-NO-HSA to the donor prior to organ procurement significantly attenuated myocardial interstitial fibrosis, and lead to preservation of both GATA2 and miR-126-3p expression in cardiac isografts. These results indicate that the signaling pathways involving GATA2 and miR-126-3p participate in the pathogenesis of CAI, and targeting miR-126-3p might represent a potential novel therapeutic approach to limit ischaemia-mediated cardiac and vascular dysfunction in heart transplant recipients. S-NO-HSA may represent a useful therapeutic adjunct to pre-transplant graft preservation, which is clinically easily applicable without requiring a direct intervention on the organ recipient.

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 Ethics committee of the Center of Biomedical research and federal ministry republic of austria, education, science and research.

AUTHOR CONTRIBUTIONS

A-KS: Conduction of the experiments, writing of manuscript draft. AK: Supervision, resources. AO: Histopathology analyses. FN: experimental assistance, review and editing of manuscript draft. EA: human cardiac fibroblast experiments AA-Z: supervision, review and editing of manuscript draft. MH: microRNA analyses. AZ: supervision, conceptualization, review and editing of manuscript draft. RK: histopathology analyses, supervision. AJ: conceptualization, review and editing of manuscript draft. PF: conceptualization, review and editing of manuscript draft. SH: conceptualization, resources, review and editing of manuscript draft. BP: conceptualization, supervision, resources, review and editing of manuscript draft.

FUNDING

This study was funded by the Ludwig Boltzmann Institute for Cardiovascular Research (No. REM 2017-2020, BP), Vienna, Austria. The authors have no other financial conflicts of interest or disclosures.

CONFLICT OF INTEREST

MH was employed by the company TamiRNA GmbH.

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 Susanna Skalicky (TAmiRNA) for her technical assistance in the project.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10057/full#supplementary-material

REFERENCES

1. Yusen RD, Edwards LB, Kucheryavaya AY, Benden C, Dipchand AI, Goldfarb SB, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirty-Second Official Adult Lung and Heart-Lung Transplantation Report-2015; Focus Theme: Early Graft Failure. *J Heart Lung Transplant* (2015) 34(10):1264–77. doi:10.1016/j.healun.2015.08.014
2. Davis SF, Yeung AC, Meredith IT, Charbonneau F, Ganz P, Selwyn AP, et al. Early Endothelial Dysfunction Predicts the Development of Transplant Coronary Artery Disease at 1 Year Posttransplant. *Circulation* (1996) 93(3): 457–62. doi:10.1161/01.cir.93.3.457
3. Lee JH, Okada K, Khush K, Kobayashi Y, Sinha S, Luikart H, et al. Coronary Endothelial Dysfunction and the Index of Microcirculatory Resistance as a Marker of Subsequent Development of Cardiac Allograft Vasculopathy. *Circulation* (2017) 135(11):1093–5. doi:10.1161/circulationaha.116.025268
4. Bedi DS, Riella LV, Tullius SG, Chandraker A. Animal Models of Chronic Allograft Injury: Contributions and Limitations to Understanding the Mechanism of Long-Term Graft Dysfunction. *Transplantation* (2010) 90(9):935–44. doi:10.1097/tp.0b013e3181efcfbc
5. Wilck N, Markó L, Balogh A, Kräker K, Herse F, Bartolomaeus H, et al. Nitric Oxide-Sensitive Guanylyl Cyclase Stimulation Improves Experimental Heart Failure with Preserved Ejection Fraction. *JCI Insight* (2018) 3(4):e96006. doi:10.1172/jci.insight.96006
6. Chung MP, Monick MM, Hamzeh NY, Butler NS, Powers LS, Hunninghake GW. Role of Repeated Lung Injury and Genetic Background in Bleomycin-Induced Fibrosis. *Am J Respir Cell Mol Biol* (2003) 29(3 Pt 1):375–80. doi:10.1165/rcmb.2003-0029OC
7. Semsroth S, Fellner B, Trescher K, Bernecker OY, Kalinowski L, Gasser H, et al. S-nitroso Human Serum Albumin Attenuates Ischemia/reperfusion Injury after Cardioplegic Arrest in Isolated Rabbit Hearts. *J Heart Lung Transplant* (2005) 24(12):2226–34. doi:10.1016/j.healun.2005.08.004
8. Trescher K, Dzilic E, Kreibich M, Gasser H, Aumayr K, Kerjaschki D, et al. The Nitric Oxide Donor, S-Nitroso Human Serum Albumin, as an Adjunct to HTK-N Cardioplegia Improves protection during Cardioplegic Arrest after Myocardial Infarction in Rats. *Interact Cardiovasc Thorac Surg* (2015) 20(3): 387–94. doi:10.1093/icvts/ivu383
9. Griscavage JM, Hobbs AJ, Ignarro LJ. Negative Modulation of Nitric Oxide Synthase by Nitric Oxide and Nitroso Compounds. *Adv Pharmacol* (1995) 34: 215–34. doi:10.1016/s1054-3589(08)61088-1
10. Hallström S, Franz M, Gasser H, Vodrazka M, Semsroth S, Losert UM, et al. S-nitroso Human Serum Albumin Reduces Ischaemia/reperfusion Injury in the Pig Heart after Unprotected Warm Ischaemia. *Cardiovasc Res* (2008) 77(3):506–14. doi:10.1093/cvr/cvm052
11. De Val S, Black BL. Transcriptional Control of Endothelial Cell Development. *Developmental Cell* (2009) 16(2):180–95. doi:10.1016/j.devcel.2009.01.014
12. Hartmann D, Fiedler J, Sonnenschein K, Just A, Pfanne A, Zimmer K, et al. MicroRNA-Based Therapy of GATA2-Deficient Vascular Disease. *Circulation* (2016) 134(24):1973–90. doi:10.1161/circulationaha.116.022478
13. Singh N, Heggermont W, Fieuws S, Vanhaecke J, Van Cleemput J, De Geest B. Endothelium-enriched microRNAs as Diagnostic Biomarkers for Cardiac Allograft Vasculopathy. *J Heart Lung Transplant* (2015) 34(11):1376–84. doi:10.1016/j.healun.2015.06.008
14. Heggermont WA, Delrue L, Dierickx K, Dierckx R, Verstreken S, Goethals M, et al. Low MicroRNA-126 Levels in Right Ventricular Endomyocardial Biopsies Coincide with Cardiac Allograft Vasculopathy in Heart Transplant Patients. *Transpl Direct* (2020) 6(5):e549. doi:10.1097/txd.0000000000000995
15. Hallström S, Gasser H, Neumayer C, Fügl A, Nanobashvili J, Jakubowski A, et al. S-nitroso Human Serum Albumin Treatment Reduces Ischemia/reperfusion Injury in Skeletal Muscle via Nitric Oxide Release. *Circulation* (2002) 105(25):3032–8. doi:10.1161/01.cir.0000018745.11739.9b
16. Hasegawa T, Visovatti SH, Hyman MC, Hayasaki T, Pinsky DJ. Heterotopic Vascularized Murine Cardiac Transplantation to Study Graft Arteriopathy. *Nat Protoc* (2007) 2(3):471–80. doi:10.1038/nprot.2007.48
17. Liu F, Kang SM. Heterotopic Heart Transplantation in Mice. *JoVE* (2007) 6: 238. doi:10.3791/238
18. Carpenter AE, Jones TR, Lamprecht MR, Clarke C, Kang I, Friman O, et al. CellProfiler: Image Analysis Software for Identifying and Quantifying Cell Phenotypes. *Genome Biol* (2006) 7(10):R100. doi:10.1186/gb-2006-7-10-r100
19. Perera-Gonzalez M, Kiss A, Kaiser P, Holzweber M, Nagel F, Watzinger S, et al. The Role of Tenascin C in Cardiac Reverse Remodeling Following Banding-Debanding of the Ascending Aorta. *Int J Mol Sci* (2021) 22(4):2023. doi:10.3390/ijms22042023
20. Eroglu E, Rost R, Bischof H, Blass S, Schreilechner A, Gottschalk B, et al. Application of Genetically Encoded Fluorescent Nitric Oxide (NO●) Probes, the geNOps, for Real-Time Imaging of NO● Signals in Single Cells. *J Vis Exp* (2017) 121. doi:10.3791/55486
21. Higai K, Satake M, Nishioka H, Azuma Y, Matsumoto K. Glycated Human Serum Albumin Enhances Macrophage Inflammatory Protein-1β mRNA Expression through Protein Kinase C-δ and NADPH Oxidase in Macrophage-like Differentiated U937 Cells. *Biochim Biophys Acta (Bba) - Gen Subjects* (2008) 1780(2):307–14. doi:10.1016/j.bbagen.2007.11.010
22. Trescher K, Hasun M, Baumgartner A, Dietl W, Wolfsberger M, Hallström S, et al. New HTK-N46b Cardioplegia Provides superior protection during Ischemia/reperfusion in Failing Hearts. *J Cardiovasc Surg (Torino)* (2013) 54(3):413–21.
23. Fish JE, Santoro MM, Morton SU, Yu S, Yeh R-F, Wythe JD, et al. miR-126 Regulates Angiogenic Signaling and Vascular Integrity. *Developmental Cell* (2008) 15(2):272–84. doi:10.1016/j.devcel.2008.07.008
24. Yang H-H, Chen Y, Gao C-Y, Cui Z-T, Yao J-M. Protective Effects of MicroRNA-126 on Human Cardiac Microvascular Endothelial Cells against Hypoxia/Reoxygenation-Induced Injury and Inflammatory Response by Activating PI3K/Akt/eNOS Signaling Pathway. *Cell Physiol Biochem* (2017) 42(2):506–18. doi:10.1159/000477597
25. Shi H, Chen L, Wang H, Zhu S, Dong C, Webster KA, et al. Synergistic Induction of miR-126 by Hypoxia and HDAC Inhibitors in Cardiac Myocytes. *Biochem Biophysical Res Commun* (2013) 430(2):827–32. doi:10.1016/j.bbrc.2012.11.061
26. Neumann A, Napp LC, Kleeberger JA, Benecke N, Pfanne A, Haverich A, et al. MicroRNA 628-5p as a Novel Biomarker for Cardiac Allograft Vasculopathy. *Transplantation* (2017) 101(1):e26–e33. doi:10.1097/tp.0000000000001477
27. Huk I, Nanobashvili J, Neumayer C, Punz A, Mueller M, Afkhampour K, et al. l -Arginine Treatment Alters the Kinetics of Nitric Oxide and Superoxide Release and Reduces Ischemia/Reperfusion Injury in Skeletal Muscle. *Circulation* (1997) 96(2):667–75. doi:10.1161/01.cir.96.2.667
28. Pou S, Pou WS, Bredt DS, Snyder SH, Rosen GM. Generation of Superoxide by Purified Brain Nitric Oxide Synthase. *J Biol Chem* (1992) 267(34):24173–6. doi:10.1016/s0021-9258(18)35745-4
29. Jakubowski A, Maksimovich N, Olszanecki R, Gebska A, Gasser H, Podesser BK, et al. S-nitroso Human Serum Albumin Given after LPS challenge Reduces Acute Lung Injury and Prolongs Survival in a Rat Model of Endotoxemia. *Naunyn-schmied Arch Pharmacol* (2009) 379(3):281–90. doi:10.1007/s00210-008-0351-2
30. Ignarro LJ, Napoli C. Novel Features of Nitric Oxide, Endothelial Nitric Oxide Synthase, and Atherosclerosis. *Curr Diab Rep* (2005) 5(1):17–23. doi:10.1007/s11892-005-0062-8
31. Yoshimura S, Nishimura Y, Nishiuma T, Yamashita T, Kobayashi K, Yokoyama M. Overexpression of Nitric Oxide Synthase by the Endothelium Attenuates Bleomycin-Induced Lung Fibrosis and Impairs MMP-9/TIMP-1 Balance. *Respirology* (2006) 11(5):546–56. doi:10.1111/j.1440-1843.2006.00894.x
32. Dooley A, Bruckdorfer KR, Abraham DJ. Modulation of Fibrosis in Systemic Sclerosis by Nitric Oxide and Antioxidants. *Cardiol Res Pract* (2012) 2012: 521958. doi:10.1155/2012/521958

33. Park J-H, Kim M, Yim B, Park CY. Nitric Oxide Attenuated Transforming Growth Factor-β Induced Myofibroblast Differentiation of Human Keratocytes. *Sci Rep* (2021) 11(1):8183. doi:10.1038/s41598-021-87791-x
34. Sun YBY, Qu X, Li X, Nikolic-Paterson DJ, Li J. Endothelial Dysfunction Exacerbates Renal Interstitial Fibrosis through Enhancing Fibroblast Smad3 Linker Phosphorylation in the Mouse Obstructed Kidney. *PLoS One* (2013) 8(12):e84063. doi:10.1371/journal.pone.0084063
35. Khalil H, Kanisicak O, Prasad V, Correll RN, Fu X, Schips T, et al. Fibroblast-specific TGF-β-Smad2/3 Signaling Underlies Cardiac Fibrosis. *J Clin Invest* (2017) 127(10):3770–83. doi:10.1172/jci94753
36. Kashima TG, Nishiyama T, Shimazu K, Shimazaki M, Kii I, Grigoriadis AE, et al. Periostin, a Novel Marker of Intramembranous Ossification, Is Expressed in Fibrous Dysplasia and in C-Fos-Overexpressing Bone Lesions. *Hum Pathol* (2009) 40(2):226–37. doi:10.1016/j.humpath.2008.07.008
37. Jansen F, Stumpf T, Proebsting S, Franklin BS, Wenzel D, Pfeifer P, et al. Intercellular Transfer of miR-126-3p by Endothelial Microparticles Reduces Vascular Smooth Muscle Cell Proliferation and Limits Neointima Formation by Inhibiting LRP6. *J Mol Cell Cardiol* (2017) 104:43–52. doi:10.1016/j.yjmcc.2016.12.005

ESOT | Transplant International

ORIGINAL RESEARCH
published: 21 April 2022
doi: 10.3389/ti.2022.10204

Immune Response to Third Dose BNT162b2 COVID-19 Vaccine Among Kidney Transplant Recipients—A Prospective Study

Dafna Yahav[1,2†], Ruth Rahamimov[2,3,4†], Tiki Mashraki[3,4], Naomi Ben-Dor[2,3], Tali Steinmetz[2,3], Timna Agur[2,3], Boris Zingerman[2,3], Michal Herman-Edelstein[2,3], Shelly Lichtenberg[2,3], Haim Ben-Zvi[2,5], Erez Bar-Haim[6], Hila Cohen[6], Shahar Rotem[6], Uri Elia[6], Ili Margalit[1,2] and Benaya Rozen Zvi[2,3]*

[1]Infectious Diseases Unit, Rabin Medical Center, Petah-Tikva, Israel, [2]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel, [3]Rabin Medical Center, Department of Nephrology and Hypertension, Petah-Tikva, Israel, [4]Department of Transplantation, Rabin Medical Center, Petah-Tikva, Israel, [5]Clinical Microbiology Laboratory, Rabin Medical Center, Beilinson Hospital, Petah-Tikva, Israel, [6]Department of Biochemistry and Molecular Genetics, Israel Institute for Biological Research, Ness-Ziona, Israel

Immune response to two SARS-CoV-2 mRNA vaccine doses among kidney transplant recipients (KTRs) is limited. We aimed to evaluate humoral and cellular response to a third BNT162b2 dose. In this prospective study, 190 KTRs were evaluated before and ~3 weeks after the third vaccine dose. The primary outcomes were anti-spike antibody level >4160 AU/ml (neutralization-associated cutoff) and any seropositivity. Univariate and multivariate analyses were conducted to identify variables associated with antibody response. T-cell response was evaluated in a subset of participants. Results were compared to a control group of 56 healthcare workers. Among KTRs, we found a seropositivity rate of 70% (133/190) after the third dose (37%, 70/190, after the second vaccine dose); and 27% (52/190) achieved levels above 4160 AU/ml after the third dose, compared to 93% of controls. Variables associated with antibody response included higher antibody levels after the second dose (odds ratio [OR] 30.8 per log AU/ml, 95% confidence interval [CI]11–86.4, $p < 0.001$); and discontinuation of antimetabolite prior to vaccination (OR 9.1,95% CI 1.8–46.5, $p = 0.008$). T-cell response was demonstrated in 13% (7/53). In conclusion, third dose BNT162b2 improved immune response among KTRs, however 30% still remained seronegative. Pre-vaccination temporary immunosuppression reduction improved antibody response.

Keywords: kidney transplant recipients, COVID-19 vaccine, immunosuppression reduction, antibody response, cellular response

OPEN ACCESS

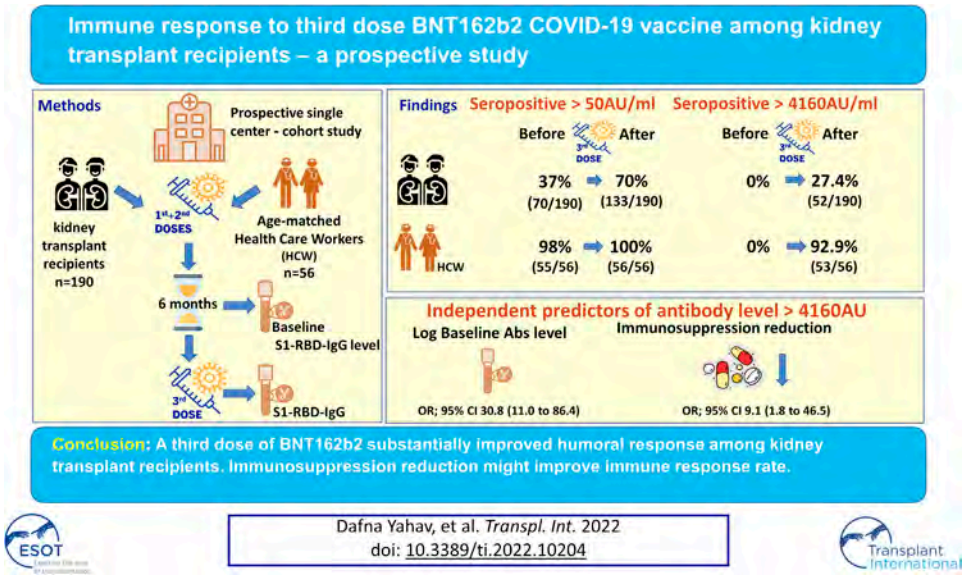
***Correspondence:**
Dafna Yahav
dafna.yahav@gmail.com

†These authors have contributed equally to this work

Received: *09 November 2021*
Accepted: *08 March 2022*
Published: *21 April 2022*

Citation:
Yahav D, Rahamimov R, Mashraki T, Ben-Dor N, Steinmetz T, Agur T, Zingerman B, Herman-Edelstein M, Lichtenberg S, Ben-Zvi H, Bar-Haim E, Cohen H, Rotem S, Elia U, Margalit I and Zvi BR (2022) Immune Response to Third Dose BNT162b2 COVID-19 Vaccine Among Kidney Transplant Recipients—A Prospective Study. Transpl Int 35:10204. doi: 10.3389/ti.2022.10204

Abbreviations: CNI, Calcineurin inhibitor; IFNg, interferon-gamma; KTRs, Kidney transplant recipients; SOT, Solid organ transplant.



GRAPHICAL ABSTRACT |

INTRODUCTION

Kidney transplant recipients (KTRs) are at increased risk for severe disease and death from COVID-19, and hence are prioritized for vaccination (1). Several studies evaluating the immune response following a two-dose mRNA vaccine schedule among KTRs demonstrated diminished humoral and cellular response (2–4). Seroconversion rates among KTRs receiving two doses of mRNA vaccine in these studies ranged from 36–54% compared to 100% in healthy controls (2–4). Similarly, T-cell response rates of 30–54% were demonstrated among KTRs, compared to over 95% among healthy controls (4,5). In addition, clinical cases of severe COVID-19, including fatal cases, were reported among fully (two-dose) vaccinated KTRs (6,7). The third mRNA vaccine dose has been recommended for severely immunocompromised patients since April 2021 in France, as well as by the European Medicines Agency (EMA) since October 2021 (1,8). Several previous studies evaluated the effectiveness and safety of a third mRNA vaccine dose among solid organ transplant (SOT) recipients (9–14), with three studies including solely KTRs (9,13,14). Humoral response among SOT recipients was demonstrated in 32–55% of those seronegative after two vaccine doses, without serious adverse events. Cellular response and predictors of negative immune response were partially evaluated (9–14). Immune response to two-dose mRNA vaccines varied between SOT types in previous studies, with KTRs being more responsive than lung transplant recipients, but less responsive than heart and liver transplant recipients (15–17).

In the current study, we aimed to evaluate humoral and cellular response specifically among KTRs ~3 weeks after a third dose of BNT162b2 vaccine dose in Israel. We also aimed to identify variables associated with positive antibody response.

Patients and Methods

This is a prospective comparative study conducted in continuation with our previous study, evaluating the effectiveness and safety of a two-dose schedule of BNT162b2 vaccine among KTRs (2). Participants (N = 190) in the current study were consenting KTRs, who participated in the previous study, received a third BNT162b2 vaccine dose (according to the Israeli Ministry of Health recommendation for the entire population, at least 5 months after the second dose), and had antibody levels collected before and after the third dose. These were compared with 56 healthy controls. Vaccines were administered between July 12, 2021 and August 29, 2021 and patients were followed for up to 9 weeks. Participants were scheduled for a study visit ~3 weeks after the third vaccine dose to collect blood for anti-spike antibody levels and cellular response (See below). Follow up for acute kidney rejection episodes was performed by collecting creatinine levels at the time of antibody levels collection, and requesting that participants report any unusual symptoms. The study was approved by the local ethics committee of the Rabin Medical Center. We collected demographics and data concerning the immunosuppressive medication regimen. Blood samples for anti-spike SARS-CoV-2 antibodies were tested using the SARS-CoV-2 IgG II Quant (Abbott©) assay. A test was considered positive when IgG was ≥50 AU/ml (18). Calcineurin inhibitor (CNI) blood levels (tacrolimus or cyclosporine) and creatinine values were also obtained on study visit. Renal function was calculated using the chronic kidney disease epidemiology collaboration (CKD-EPI) equation. T-cell response was measured for 55 randomly selected participants using the SARS-CoV-2 interferon-gamma

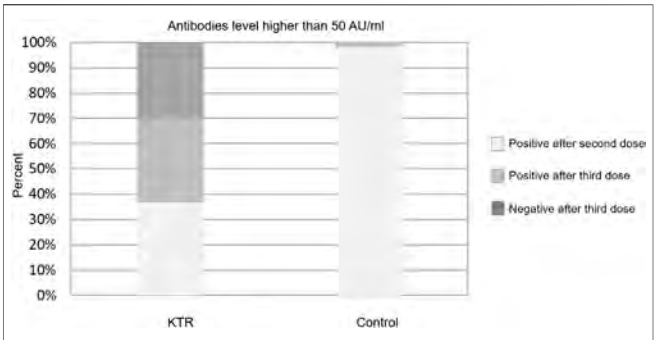


FIGURE 1 | Antibody response rates following second and third dose among kidney transplant recipients and controls–cut-off at 50 AU/ml.

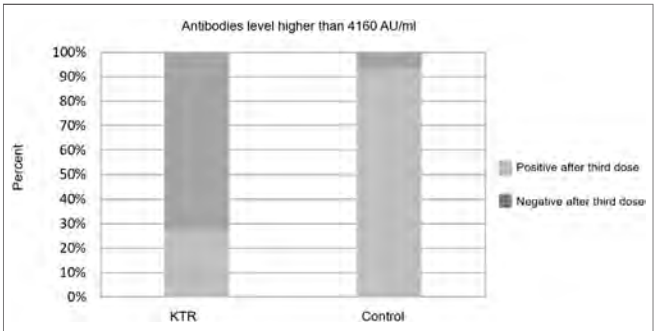


FIGURE 2 | Antibody response rates following second and third dose among kidney transplant recipients and controls–cut-off at 4160 AU/ml.

(IFNg) release assay (EUROIMMUN, Lübeck, Germany) with strict adherence to the manufacturer's instructions. In this quantitative assay, whole blood was stimulated for 24 h with spike antigen and a control with no antigen. Secreted IFNg in response to stimulation was measured by ELISA (DuoSet, R&D Systems, Minneapolis, MN) and results were presented as the difference between IFNg levels in response to spike versus background response to no antigen control. The results were measured in pg/ml, and a test was considered positive when the difference was >10 pg/ml (weak positive 10–50, positive >50 pg/ml) (19,20).

The primary outcomes were 1) proportion of antibody response above 4160 AU/ml, a threshold that was previously shown to correspond with a 95% probability of viral neutralization (21,22); and 2) any seropositivity (>50 AU/ml) among previously seronegative participants. Secondary outcomes included log transformed antibody levels as a continuous variable, T-cell response, and acute rejection.

Statistical Analysis

Categorical variables were presented as numbers (percentages), and continuous variables as median (interquartile range, IQR) or mean (SD), according to their distribution. The former was compared using the Chi-square test or Fisher's exact test and the latter using *t*-test or Mann Whitney test, as appropriate.

Univariate and multivariate logistic regression models were used for the evaluation of variables associated with response (of both >4160 AU/ml, and >50 AU/ml). All variables were considered for inclusion into the multivariate analysis after testing for collinearity using a forward stepwise regression model with a p value below 0.05 used for inclusion. Linear regression analyses were performed to explore factors associated with higher log transformed antibody titer among KTRs.

Results were compared with a control group of 56 healthcare workers aged 60–75 years that were immunized with a third BNT162b2 dose during the same period. A General linear model (GLM) was used for comparison of log transformed Ab level between the KTR and control groups with age, gender, creatinine value, body mass index (BMI), and diabetes as covariates using a fixed effect model. Estimated marginal mean (EMM) adjusted for the above variables was calculated to evaluate the adjusted difference of log Ab level with 95% confidence interval. Analyses were performed using IBM SPSS statistics, version 27.

RESULTS

Of the 308 KTRs in the original cohort (2), 190 (61%) had a baseline anti-spike antibody test collected before the third vaccine dose, and were included in the current study. (See flow chart of patients' selection in **Supplementary Figure S1**). Mean age was 59 years (SD 12), and 32% were females (61/190). Median time from third vaccination to antibody test collection was 29 days (IQR 20–33).

Antibody Response Among KTRs Group

Overall, 133 (70.0%) KTRs had a positive antibody response (>50 AU/ml) after the third vaccine dose, compared with 70 (36.8%) after the second dose ($p < 0.001$). Sixty three of 120 KTRs (52.5%) were seronegative after second dose but turned seropositive after the third dose (**Figure 1**). Using a cutoff of 4160 AU/ml, 52 (27.4%) KTRs achieved this antibody level after third dose compared with 52 (92.9%) of the control group ($p < 0.001$). None of the study participants (KTRs or controls) achieved antibody levels >4160 AU/ml after the second vaccine dose (**Figure 2**).

Characteristics of the study population are presented in **Table 1**, stratified by antibody response >4160 AU/ml. Twenty-seven KTRs (14.2%) had their immunosuppression reduced permanently or temporarily prior to the third vaccine dose. Among 70% (19/27) of them, antimetabolites were discontinued, usually temporarily; for the other eight KTRs, dose was reduced (reasons for discontinuation and regimens, see **Supplementary Table S1**).

Variables Associated With Antibody Response

Univariate analysis for variables associated with antibody response over 4160 AU/ml demonstrated that lower antibody level after the second vaccine dose, older age, lower estimated

TABLE 1 | Characteristics of 190 KTRs, stratified by antibody response >4160 AU/ml.

Variable	All (N = 190)	Response (N = 52, 27%)[a]	No Response (N = 138, 72%)[a]	*p*-value
Age (years) (mean, SD)	59.03 (12.35%)	54.58 (11.86)	60.71 (12.16)	0.002
Female gender (No., percentage)	61 (32.11%)	21 (40.38%)	40 (28.99%)	0.133
Time from transplantation (years) (mean, SD)	7.48 (7.98)	6.62 (6.41)	7.80 (8.49)	0.363
Living donor (No., percentage)	147 (77.37%)	47 (90.38%)	100 (72.46%)	0.008
eGFR (per ml/min/1.73m^2) (mean, SD)	61.13 (21.48)	70.01 (20.93)	57.78 (20.78)	0.001
Diabetes mellitus (No., percentage)	37 (19.47%)	5 (9.62%)	32 (23.19%)	0.035
Baseline log antibody level (mean, SD)	1.31326 (0.905)	2.34099 (0.515)	0.925999 (0.692)	<0.001
Time from second vaccine dose (days) (mean, SD)	163.38 (1841.01%)	160.96 (2,354.15%)	164.3178 (1,600.00%)	0.275
Immunosuppression reduction (yes) (No., percentage)	27 (14.21%)	9 (17.31%)	18 (13.04%)	0.425
BMI (per kg/m^2) (mean, SD)	27.22 (4.43)	27.30 (4.12)	27.19 (4.56)	0.877
High antimetabolite dose[b] (No., percentage)	120 (63.16%)	32 (61.54%)	88 (63.77%)	0.776
High tacrolimus level[c] (No., percentage)	110 (57.89%)	25 (48.08%)	85 (61.59%)	0.092
mTOR inhibitor (No., percentage)	17 (8.95%)	5 (9.62%)	12 (8.70%)	0.843
Treatment with ATG (No., percentage)	8 (4.21%)	1 (1.92%)	7 (5.07%)	0.335
Cyclosporine use (No., percentage)	30 (15.79%)	10 (19.23%)	20 (14.49%)	0.425

eGFR, estimated glomerular filtration; mTOR, mammalian target of rapamycin; ATG, anti thymocyte globulin.
[a]*Response for this analysis was considered if antibody level increased beyond 4160 AU/ml.*
[b]*High antimetabolite dose ≥720 mg per day.*
[c]*High tacrolimus level >7 mg/ml.*

TABLE 2 | Univariate and multivariate analyses for variables associated with antibody response >4160 AU/ml among 190 KTRs

Variable	Univariate			Multivariate		
	OR	95% CI for OR	p	OR	95% CI for OR	p
Age (per year)	0.961	0.936–0.987	0.003	—	—	—
Female gender	1.660	0.854–3.227	0.135	—	—	—
Time from transplantation (years)	0.980	0.939–1.023	0.362	—	—	—
Living donor	3.572	1.321–9.659	0.012	—	—	—
eGFR (per ml/min/1.73m^2)	1.029	1.012–1.046	0.001	—	—	—
Diabetes mellitus	0.352	0.129–0.961	0.042	—	—	—
Baseline log antibody level	22.976	9.018–58.540	<0.001	30.78	10.97–86.36	<0.001
Time from second vaccine dose (days)	0.990	0.972–1.008	0.274	—	—	—
Immunosuppression reduction	1.405	0.608–3.244	0.426	9.06	1.76–46.48	0.008
BMI (per kg/m^2)	1.006	0.936–1.081	0.876	—	—	—
High antimetabolite dose[a]	0.909	0.471–1.755	0.776	—	—	—
High tacrolimus level[b]	0.577	0.303–1.098	0.094	—	—	—
mTOR inhibitor	1.117	0.373–3.341	0.843	—	—	—
Treatment with ATG	0.367	0.044–3.057	0.354	—	—	—
Cyclosporine use	0.542	0.149–1.970	0.352	—	—	—

OR, odds ratio; eGFR, estimated glomerular filtration rate; BMI, body mass index; mTOR, mammalian target of rapamycin; ATG, anti thymocyte globulin.
[a]*High antimetabolite dose ≥720 mg per day.*
[b]*High tacrolimus level >7 mg/ml.*

glomerular filtration rate (eGFR), presence of diabetes mellitus, and transplant from none-living donor were associated with no response (**Table 1**). Multivariate analysis, introducing immunosuppression reduction into the model, only demonstrated antibody levels after the second vaccine dose and immunosuppression reduction was significantly associated with antibody response (odds ratio [OR] 30.78, 95% confidence interval [CI] 10.97–86.36, $p < 0.001$; and OR 9.06, 95% CI 1.76–46.48, $p = 0.008$, respectively) (**Table 2**). Performing the same analysis to predict any antibody response (>50 AU/ml) for the 120 nonresponding patients, only baseline antibody level and treatment with cyclosporine (instead of tacrolimus) were demonstrated as significant. (See **Supplementary Table S2**).

Antibody Response in KTRs Versus Controls

Comparison of the KTR cohort's baseline characteristics and outcomes versus the healthcare workers control group is detailed in **Table 3**. Among the control group, 100% were seropositive (>50 AU/ml) after the third dose, while 98% were positive after the second dose. In this group, none of the participants had antibody level >4160 AU/ml prior to the third dose, while this

TABLE 3 | comparison of the 190 KTRs and 56 controls included in the study.

Variable Name	All		KTR (190)		Control (56)		p
Age (years) (Mean, SD)	61.36	11.940	59.03	12.355	69.27	5.303	<0.001
Female gender (No., percentage)	88	35.77%	61	32.11%	27	48.21%	0.027
Diabetes mellitus (No., percentage)	44	17.89%	37	19.47%	7	12.50%	0.231
BMI (kg/m^2) (mean, SD)	27.0828	4.229	27.22	4.431	26.60	3.400	0.35
Serum creatinine (mg/dl) (mean, SD)	1.25	0.733	1.36	0.790	0.86	0.207	<0.001
Time to booster dose[a] (mean, SD)	172.17	23.222	163.38	18.410	201.85	8.468	<0.001
Bassline antibody level (AU/ml) (median, IQR)	52.75	3.68–343	13.80	2.6–111.55	514.35	259.68–857.8	<0.001
Antibody levels after third dose (AU/ml) (median, IQR)	1881.45	59.48–13,299.2	622.40	19.35–5,474.4	23,800.15	13,343–41,511.75	<0.001
Baseline log antibody level (AU/ml) (mean, SD)	1.62	0.99	1.31	0.90	2.65	0.40	<0.001
Log antibody level after third dose (mean, SD)	2.92	1.432	2.51	1.365	4.31	0.417	<0.001
Adjusted log antibody level after third dose[b] (median, IQR)	—	—	2.32	3.7–4.63	4.17	2.07–2.56	<0.001
Antibody level above 50 AU/ml (No., percentage)	189	76.8%	133	70.0%	56	100.0%	<0.001
Antibody level above 4160 AU/ml (No., percentage)	104	42.3%	52	27.4%	52	92.9%	<0.001

IQR, interquartile range.

[a]*Time between the second and third vaccine dose in days.*

[b]*Estimated marginal mean with 95% CI, adjusted for age, gender; BMI, serum creatinine and diabetes mellitus.*

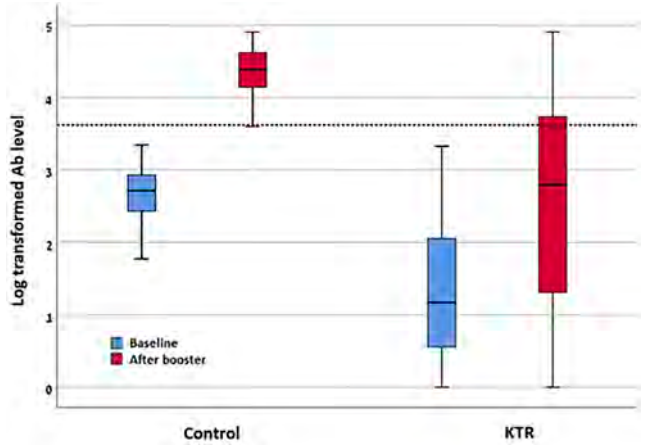


FIGURE 3 | Log transformed antibody levels before and after third vaccine dose among KTR and control. KTR, kidney transplant recipient; "Baseline"—levels as tested after second vaccine dose. "After booster"—after third vaccine dose.

level was achieved in 93% (52/56) after the third dose. Regarding antibody levels achieved, among 190 KTRs, median anti-spike antibody level was significantly increased from 13.8 (IQR 2.6–111.55) AU/ml before, to 514.35 (IQR 19.35–5,474.4) AU/ml after the third dose ($p < 0.001$). Similarly, log transformed antibody level was increased from 1.3 ± 0.9 AU/ml to 2.51 ± 1.37 AU/ml ($p < 0.001$). In comparison, among 56 control group participants, antibody level was increased from 514 (IQR 259.68–857.8) AU/ml before, to 23,800.15 (IQR 259.68–857.8) AU/ml after the third dose. Log antibody level increased from 2.65 ± 0.4 to 4.31 ± 0.42. After adjustment for age, gender, BMI, diabetes mellitus status and creatinine level, the adjusted mean difference of the log transformed antibody level between the control and KTR groups was 1.98 (95% CI 1.57–2.39) AU/ml, reflecting significantly increased antibody levels among the control group. Antibody levels before and after the third vaccine dose are presented in **Figure 3**.

Variables Associated With Higher Titer Antibody Response

When the log transformed antibody level was evaluated as it continued to be variable, the factors that were significantly associated with higher log antibody level were baseline antibody levels before the third vaccine dose, immunosuppression reduction, non-diabetic status, treatment with cyclosporine, and treatment with TOR inhibitors (instead of antimetabolites) (See **Supplementary Table S3**).

T-Cell Response

T-cell response, tested in 55 randomly selected KTRs, of whom two were excluded from the analysis because of very high negative control response; of the remaining 53 participants, seven (13.2%) patients had a positive response. This randomly selected subgroup of patients did not differ in baseline characteristics compared to the study population. (**Supplementary Table S4** for comparison). Forty patients (75.5%) of the 53 were seropositive after the third vaccine dose.

During the follow up of median 61 days (IQR 56–63), none of the KTRs developed acute graft rejection.

DISCUSSION

In this study including 190 KTRs, we found 70% seropositivity rates in response to a three dose BNT162b2 regimen, increasing from 37% after two doses. Twenty seven percent (52/190) achieved antibody response over 4160 AU/ml, associated with neutralization. T-cell response rate among KTRs was low, with 7/53 (13%) presenting adequate anti spike T-cell response. Variables associated with antibody response among KTRs included antibody levels after the second vaccine dose and immunosuppression reduction. The antibody response rate documented in our study is in accordance with recent studies reporting improved humoral response 28 days following a third mRNA dose in SOT recipients (9–14). Three of them including

solely KTRs: Massa et al. reported antibody response in 32% of 61 seronegative KTRs, improving seropositivity rates from 44% to 62% after three BNT162b2 doses (13). Benotmane et al. reported 49% response among 159 previously seronegative KTRs after three doses of the mRNA-1273 vaccine (9). The relatively increased response rates in the latter study may represent a higher humoral immunogenicity with mRNA-1273 vaccine compared to BNT162b2, previously demonstrated in healthy people (23). Schrezenmeier et al. reported a 36% response rate among 25 previously seronegative KTRs, following either the third homologous BNT162b2 dose or heterologous ChAdOx1 (14). In SOT recipients in general, Kamar et al. reported a 44% response rate among 59 seronegative SOTs, most of them KTRs, with seropositivity rates increasing from 40% to 66% of 101 SOT recipients vaccinated with BNT162b2 (10). Finally, Hall et al. randomized 120 SOT recipients (29 KTRs, 25 kidney-pancreas), 10% seropositive at baseline, to either mRNA-1273 or placebo. Fifty five percent of 60 patients in the vaccine arm were seropositive after the third dose, compared with 18% in the placebo group (11). Magnitude of response was also demonstrated to increase after third dose, similar to our study (9,24).

Surprisingly, using the cutoff of 4160 AU/ml, the control group in our study was seronegative prior to the third dose. The mean age of participants in the control group was 69 years, and they were on average 200 days after the second vaccine. Waning of vaccine response has been demonstrated, mainly in older adults, which may explain the relatively low antibody titer (25). In addition, the cutoff used as surrogate for neutralization in our study was taken from previous studies. Additional studies may be needed to validate this cutoff as a surrogate for neutralization.

We found in our cohort 13% (7/53) of patients with adequate T-cell response, evaluated by SARS-CoV-2 spike-specific IFNg secretion. These rates are lower than the 30–60% cellular response rates reported after second vaccine dose among KTRs, measured by IFNg secreting cells frequency, which is of higher sensitivity than IFNg secretion assay (4). A significant increase in IFNg secreting cells was demonstrated in studies evaluating SOT recipients before and after the third vaccine dose (11,13,14). Percentage of responders was not reported in these studies, and assays differed, limiting our ability to compare them to our results. In a small study in cancer patients, no improvement in T-cell response was observed after the BNT162b2 third dose (26). Schrezenmeier et al. reported that KTRs with a humoral response to the third vaccine dose had significantly higher portions of antigen-reactive T cells than those without a humoral response (14).

Various predictors of antibody response to the third mRNA dose were reported from previous studies in SOT recipients. Among KTRs, use of antimetabolite, low lymphocyte count, and previous negative antibody response to the second vaccine dose predicted negative response (9). In SOT recipients in general, older age, higher degree of immunosuppression, and a lower estimated glomerular filtration rate were associated with no antibody response (10). Previous studies assessing immune response to second mRNA vaccine dose in KTRs demonstrated mycophenolate including regimen as strongly associated with low seroconversion rates (2,3). In the study by Schrezenmeier et al. only three individuals achieved high positive antibodies, one of them was the only person in the study without mycophenolate mofetil at the time of vaccination (14).

These findings may explain our results, showing immunosuppression reduction prior to vaccination to be associated with positive antibody response.

Following the third vaccine dose and although immunosuppression was discontinued for 14.2% of the cohort, no acute rejection episodes were found in our cohort. Previous studies demonstrated no serious adverse events and no acute rejection episodes among SOT recipients who received a third mRNA vaccine dose (9–11,24). In addition, previous studies have demonstrated no graft or patient survival impairment following temporary discontinuation of mycophenolate mofetil in KTRs treated with triple immunosuppression, as in our study (27,28).

Our study has several limitations. The sample size was limited with a small number of events included in the multivariable analysis. Cellular response was tested only after the third vaccine dose, with no previous testing after second dose for comparison. It was also assessed only in a limited subset of patients due to technical limitations. In addition, neutralizing antibodies were not directly assessed. Nevertheless, we used a cut-off of 4160 AU/ml of anti-spike antibodies as a surrogate, as previously described (21,22). Hall et al. demonstrated significant median percent virus neutralization (71%) with a third dose versus placebo (13%); and Massa et al. also demonstrated a significant increase in serum neutralizing activity between the second and third doses (11,13). The significance of higher antibody response as reflecting protection against infection and severe disease is still debated, though data are accumulating to support an association. A recent study from Israel demonstrated among healthcare workers both higher antibody levels and recused risk for infection after third versus second BNT162b2 dose. Greater incidence of infection was demonstrated among those with lower antibody levels (29). Immunosuppression reduction was not planned for the study and was initiated by either patients or treating physicians, due to reasons related or unrelated to the vaccine. This strategy should be tested in clinical trials, designated for this question (30).

Though our results and others present improved humoral immunity following three vaccine doses, still, at least 30% of KTRs remain seronegative, potentially susceptible to SARS-CoV-2 infection. These results support the administration of a third vaccine dose to KTRs; however, additional strategies should be discussed. These may include immunosuppression reduction prior to vaccination (30). Considering that anti-metabolites are consistently reported as associated with seronegative response, transient discontinuation of anti-metabolites prior to and shortly after the vaccine dose may be considered, monitoring meticulously for acute rejection (14). An alternative approach could be heterologous vaccination, i.e., using a non-mRNA vaccine for the third dose. Such a “mix and match” approach was recently supported by the EMA for the general population, stating that there are currently no data to support heterologous boosting among immunocompromised individuals (31). Two studies in KTRs used heterologous third dose boosting, with

approximately a third to half of the recipients developing humoral response (12,14). This approach should be further tested in this population. An additional approach could be a fourth mRNA vaccine dose. Three studies evaluating a fourth vaccine dose among KTRs have been published so far, demonstrating improved immunogenicity, though with lower response rates among those still seronegative after the third dose (32–34). Otherwise, consideration of post-exposure or even pre-exposure prophylaxis could be an alternative to vaccination, using monoclonal antibodies or antiviral drugs (35–38). In addition, safety results should be verified in larger, longer term follow-up studies, including follow-up on rejection risk (39).

In summary, third dose BNT162b2 improves immune response over two doses in KTRs; however a significant percentage of these patients still remain seronegative and at risk for SARS-CoV-2 infection. Temporary withdrawal of the antimetabolite should be considered before the administration of the vaccine dose, taking into account individual risk for rejection. This strategy and others for improved immunization should be tested in clinical studies.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available upon reasonable request to the authors.

ETHICS STATEMENT

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

AUTHOR CONTRIBUTIONS

DY, BRZ, RR, IM, BZ, and TA conceived the idea and wrote the protocol for the study. TM, NB-D, TS, TA, BZ, MH-E and RR collected the data and blood samples. HB-Z, MH-E, EB-H, HC, SR, and UE performed the laboratory analysis. BRZ, DY, RR, and IM performed the statistical analysis and wrote the manuscript. All authors reviewed the manuscript and approved it for submission.

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 wish to thank Michal Nachtomy-Disatnik and Ehud Ben-Dor for their contribution to this study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10204/full#supplementary-material

REFERENCES

1. American Society of Transplantation. *COVID-19: Vaccine FAQ Sheet [Internet]*. United States: American Society of Transplantation (2020). Available from: https://www.myast.org/covid-19-vaccine-faq-sheet (cited Sep 26, 2021).
2. Rozen-Zvi B, Yahav D, Agur T, Zingerman B, Ben-Zvi H, Atamna A, et al. Antibody Response to mRNA SARS-CoV-2 Vaccine Among Kidney Transplant Recipients - Prospective Cohort Study. *Clin Microbiol Infect* (2021) 27:1173.e1–1173.e4. doi:10.1016/j.cmi.2021.04.028
3. Boyarsky BJ, Werbel WA, Avery RK, Tobian AAR, Massie AB, Segev DL, et al. Antibody Response to 2-Dose SARS-CoV-2 mRNA Vaccine Series in Solid Organ Transplant Recipients. *JAMA* (2021) 325(21):2204–6. doi:10.1001/jama.2021.7489
4. Crespo M, Barrilado-Jackson A, Padilla E, Eguía J, Echeverria-Esnal D, Cao H, et al. Negative Immune Responses to Two-Dose mRNA COVID-19 Vaccines in Renal Allograft Recipients Assessed with Simple Antibody and Interferon Gamma Release Assay Cellular Monitoring. *Am J Transpl* (2022) 22:786–800. doi:10.1111/ajt.16854
5. Stumpf J, Siepmann T, Lindner T, Karger C, Schwöbel J, Anders L, et al. Humoral and Cellular Immunity to SARS-CoV-2 Vaccination in Renal Transplant versus Dialysis Patients: A Prospective, Multicenter Observational Study Using mRNA-1273 or BNT162b2 mRNA Vaccine. *The Lancet Reg Health - Europe* (2021) 9:100178. doi:10.1016/j.lanepe.2021.100178
6. Caillard S, Chavarot N, Bertrand D, Kamar N, Thaunat O, Moal V, et al. Occurrence of Severe COVID-19 in Vaccinated Transplant Patients. *Kidney Int* (2021) 100(2):477–9. doi:10.1016/j.kint.2021.05.011
7. Tau N, Yahav D, Schneider S, Rozen-Zvi B, Abu Sneineh M, Rahamimov R. Severe Consequences of COVID-19 Infection Among Vaccinated Kidney Transplant Recipients. *Am J Transpl* (2021) 21(8):2910–2. doi:10.1111/ajt.16700
8. Hrabovszki G. *Comirnaty and Spikevax: EMA Recommendations on Extra Doses Boosters [Internet]*. Amsterdam, Netherlands: European Medicines Agency (2021). Available from: https://www.ema.europa.eu/en/news/comirnaty-spikevax-ema-recommendations-extra-doses-boosters (cited Jan 12, 2022).
9. Benotmane I, Gautier G, Perrin P, Olagne J, Cognard N, Fafi-Kremer S, et al. Antibody Response after a Third Dose of the mRNA-1273 SARS-CoV-2 Vaccine in Kidney Transplant Recipients with Minimal Serologic Response to 2 Doses. *JAMA* (2021) 326(11):1063–5. doi:10.1001/jama.2021.12339
10. Kamar N, Abravanel F, Marion O, Couat C, Izopet J, Del Bello A. Three Doses of an mRNA Covid-19 Vaccine in Solid-Organ Transplant Recipients. *N Engl J Med* (2021) 385(7):661–2. doi:10.1056/nejmc2108861
11. Hall VG, Ferreira VH, Ku T, Ierullo M, Majchrzak-Kita B, Chaparro C, et al. Randomized Trial of a Third Dose of mRNA-1273 Vaccine in Transplant Recipients. *N Engl J Med* (2021) 385(13):1244–6. doi:10.1056/nejmc2111462
12. Werbel WA, Boyarsky BJ, Ou MT, Massie AB, Tobian AAR, Garonzik-Wang JM, et al. Safety and Immunogenicity of a Third Dose of SARS-CoV-2 Vaccine in Solid Organ Transplant Recipients: A Case Series. *Ann Intern Med* (2021) 174(9):1330–2. doi:10.7326/l21-0282

13. Massa F, Cremoni M, Gérard A, Grabsi H, Rogier L, Blois M, et al. Safety and Cross-Variant Immunogenicity of a Three-Dose COVID-19 mRNA Vaccine Regimen in Kidney Transplant Recipients. *EBioMedicine* (2021) 73:103679. doi:10.1016/j.ebiom.2021.103679
14. Schrezenmeier E, Rincon-Arevalo H, Stefanski A-L, Potekhin A, Staub-Hohenbleicher H, Choi M, et al. B and T Cell Responses after a Third Dose of SARS-CoV-2 Vaccine in Kidney Transplant Recipients. *J Am Soc Nephrol* (2021) 32(12):3027–33. doi:10.1681/asn.2021070966
15. Shostak Y, Shafran N, Heching M, Rosengarten D, Shtraichman O, Shitenberg D, et al. Early Humoral Response Among Lung Transplant Recipients Vaccinated with BNT162b2 Vaccine. *Lancet Respir Med* (2021) 9(6): e52–e53. doi:10.1016/s2213-2600(21)00184-3
16. Itzhaki Ben Zadok O, Shaul AA, Ben-Avraham B, Yaari V, Ben Zvi H, Shostak Y, et al. Immunogenicity of the BNT162b2 mRNA Vaccine in Heart Transplant Recipients - a Prospective Cohort Study. *Eur J Heart Fail* (2021) 23(9):1555–9. doi:10.1002/ejhf.2199
17. Rabinowich L, Grupper A, Baruch R, Ben-Yehoyada M, Halperin T, Turner D, et al. Low Immunogenicity to SARS-CoV-2 Vaccination Among Liver Transplant Recipients. *J Hepatol* (2021) 75(2):435–8. doi:10.1016/j.jhep.2021.04.020
18. Abbott laboratories. SARS-CoV-2 IgG II Quant. 2020 [Internet] (2021). Available from: https://www.corelaboratory.abbott/int/en/offerings/segments/infectious-disease/sars-cov-2-.
19. Huzly D, Panning M, Smely F, Enders M, Komp J, Steinmann D. Validation and Performance Evaluation of a Novel Interferon-γ Release Assay for the Detection of SARS-CoV-2 Specific T-Cell Response [Internet] (2021). Available from: https://www.medrxiv.org/content/10.1101/2021.07.17.21260316v1.
20. Malipiero G, Moratto A, Infantino M, D'Agaro P, Piscianz E, Manfredi M, et al. Assessment of Humoral and Cellular Immunity Induced by the BNT162b2 SARS-CoV-2 Vaccine in Healthcare Workers, Elderly People, and Immunosuppressed Patients with Autoimmune Disease. *Immunol Res* (2021) 69(6):576–83. doi:10.1007/s12026-021-09226-z
21. Ebinger JE, Fert-Bober J, Printsev I, Wu M, Sun N, Prostko JC, et al. Antibody Responses to the BNT162b2 mRNA Vaccine in Individuals Previously Infected with SARS-CoV-2. *Nat Med* (2021) 27(6):981–4. doi:10.1038/s41591-021-01325-6
22. Redjoul R, Le Bouter A, Beckerich F, Fourati S, Maury S. Antibody Response after Second BNT162b2 Dose in Allogeneic HSCT Recipients. *The Lancet* (2021) 398(10297):298–9. doi:10.1016/s0140-6736(21)01594-4
23. Steensels D, Pierlet N, Penders J, Mesotten D, Heylen L. Comparison of SARS-CoV-2 Antibody Response Following Vaccination with BNT162b2 and mRNA-1273. *JAMA* (2021) 7(8):1133–40. doi:10.1001/jama.2021.15125
24. Massa F, Cremoni M, Gerard A, Grabsi H, Rogier L, Blois M, et al. *Safety and Cross-Variant Immunogenicity of a Three-Dose COVID-19 mRNA Vaccine Regimen in Kidney Transplant Recipients [Internet]*. Report No.: ID 3890865. Rochester, NY: Social Science Research Network (2021). Available from: https://papers.ssrn.com/abstract=3890865 (cited Sep 26, 2021).
25. Naaber P, Tserel L, Kangro K, Sepp E, Jürjenson V, Adamson A, et al. Dynamics of Antibody Response to BNT162b2 Vaccine after Six Months: a Longitudinal Prospective Study. *The Lancet Reg Health – Europe* (2021) 10: 100208. doi:10.1016/j.lanepe.2021.100208
26. Shroff RT, Chalasani P, Wei R, Pennington D, Quirk G, Schoenle MV, et al. Immune Responses to Two and Three Doses of the BNT162b2 mRNA Vaccine in Adults with Solid Tumors. *Nat Med* (2021) 27(11):2002–11. doi:10.1038/s41591-021-01542-z
27. Pascual J, van Hooff JP, Salmela K, Lang P, Rigotti P, Budde K. Three-year Observational Follow-Up of a Multicenter, Randomized Trial on Tacrolimus-Based Therapy with Withdrawal of Steroids or Mycophenolate Mofetil after Renal Transplant. *Transplantation* (2006) 82(1):55–61. doi:10.1097/01.tp.0000225806.80890.5e
28. Yahav D, Sulimani O, Green H, Margalit I, Ben-Zvi H, Mor E, et al. Immunosuppression Reduction in Kidney Transplant Recipients during Bacterial Infection-A Retrospective Study. *Clin Transpl* (2019) 33(10): e13707. doi:10.1111/ctr.13707
29. Spitzer A, Angel Y, Marudi O, Zeltser D, Saiag E, Goldshmidt H, et al. Association of a Third Dose of BNT162b2 Vaccine with Incidence of SARS-CoV-2 Infection Among Health Care Workers in Israel. *Jama* (2012) 327:341. doi:10.1001/jama.2021.23641
30. Furlow B. Immunocompromised Patients in the USA and UK Should Receive Third Dose of COVID-19 Vaccine. *Lancet Rheumatol* (2021) 3:e756. doi:10.1016/S2665-9913(21)00313-1
31. Berbece C. *EMA and ECDC Recommendations on Heterologous Vaccination Courses against COVID-19: 'mix-And-Match' Approach Can Be Used for Both Initial Boosters*. Amsterdam, Netherlands: European Medicines Agency (2021). Available from: https://www.ema.europa.eu/en/news/ema-ecdc-recommendations-heterologous-vaccination-courses-against-covid-19-mix-match-approach-can-be (cited Jan 12, 2022).
32. Caillard S, Thaunat O, Benotmane I, Masset C, Blancho G. Antibody Response to a Fourth Messenger RNA COVID-19 Vaccine Dose in Kidney Transplant Recipients: A Case Series. *Ann Intern Med* (2022) 175(3):455–6. doi:10.7326/l21-0598
33. Alejo JL, Mitchell J, Chiang TP-Y, Abedon AT, Boyarsky BJ, Avery RK, et al. Antibody Response to a Fourth Dose of a SARS-CoV-2 Vaccine in Solid Organ Transplant Recipients: A Case Series. *Transplantation* (2021) 105(12): e280–e281. doi:10.1097/tp.0000000000003934
34. Kamar N, Abravanel F, Marion O, Romieu-Mourez R, Couat C, Del Bello A, et al. Assessment of 4 Doses of SARS-CoV-2 Messenger RNA-Based Vaccine in Recipients of a Solid Organ Transplant. *JAMA Netw Open* (2021) 4(11): e2136030. doi:10.1001/jamanetworkopen.2021.36030
35. AstraZeneca. AZD7442 PROVENT Phase III Prophylaxis Trial Met Primary Endpoint in Preventing COVID-19 [Internet] (2021). Available from: https://www.astrazeneca.com/media-centre/press-releases/2021/azd7442-prophylaxis-trial-met-primary-endpoint.html (cited Sep 26, 2021).
36. Gupta A, Gonzalez-Rojas Y, Juarez E, Crespo Casal M, Moya J, Falci DR, et al. Early Treatment for Covid-19 with SARS-CoV-2 Neutralizing Antibody Sotrovimab. *N Engl J Med* (2021) 385(21):1941–50. doi:10.1056/nejmoa2107934
37. Pfizer. Pfizer's Novel COVID-19 Oral Antiviral Treatment Candidate Reduced Risk of Hospitalization or Death by 89% in Interim Analysis of Phase 2/3 EPIC-HR Study | Pfizer [Internet] (2021). Available from: https://www.pfizer.com/news/press-release/press-release-detail/pfizers-novel-covid-19-oral-antiviral-treatment-candidate (cited Jan 12, 2022).
38. Jayk Bernal A, Gomes da Silva MM, Musungaie DB, Kovalchuk E, Gonzalez A, Delos Reyes V, et al. Molnupiravir for Oral Treatment of Covid-19 in Nonhospitalized Patients. *New Engl J Med* (2021) 386(6):509–20.
39. Williams WW, Ingelfinger JR. Third Time's a Charm - Covid-19 Vaccine Hope for Solid-Organ Transplant Recipients. *N Engl J Med* (2021) 385(13):1233–4. doi:10.1056/nejme2112866

ESOT | Transplant International

ORIGINAL RESEARCH
published: 05 April 2022
doi: 10.3389/ti.2022.10352

Posttransplantation Diabetes Mellitus Among Solid Organ Recipients in a Danish Cohort

Quenia Dos Santos[1], Mads Hornum[2,3], Cynthia Terrones-Campos[1], Cornelia Geisler Crone[1], Neval Ete Wareham[1], Andreas Soeborg[1], Allan Rasmussen[4], Finn Gustafsson[3,5], Michael Perch[3,5], Soeren Schwartz Soerensen[2], Jens Lundgren[1], Bo Feldt-Rasmussen[2] and Joanne Reekie[1]*

[1]Centre of Excellence for Health, Immunity and Infections (CHIP), Rigshospitalet, University of Copenhagen, Copenhagen, Denmark, [2]Department of Nephrology, Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark, [3]Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, [4]Department of Surgical Gastroenterology, Rigshospitalet, Copenhagen, Denmark, [5]Department of Cardiology, Rigshospitalet, Copenhagen, Denmark

Post-transplant diabetes mellitus (PTDM) is associated with a higher risk of adverse outcomes. We aimed to describe the proportion of patients with diabetes prior to solid organ transplantation (SOT) and post-transplant diabetes mellitus (PTDM) in three time periods (early-likely PTDM: 0–45 days; 46–365 days and >365 days) post-transplant and to estimate possible risk factors associated with PTDM in each time-period. Additionally, we compared the risk of death and causes of death in patients with diabetes prior to transplant, PTDM, and non-diabetes patients. A total of 959 SOT recipients (heart, lung, liver, and kidney) transplanted at University Hospital of Copenhagen between 2010 and 2015 were included. The highest PTDM incidence was observed at 46–365 days after transplant in all SOT recipients. Age and the Charlson Comorbidity Index (CCI Score) in all time periods were the two most important risk factors for PTDM. Compared to non-diabetes patients, SOT recipients with pre-transplant diabetes and PTDM patients had a higher risk of all-cause mortality death (aHR: 1.77, 95% CI: 1.16–2.69 and aHR: 1.89, 95% CI: 1.17–3.06 respectively). Pre-transplant diabetes and PTDM patients had a higher risk of death due to cardiovascular diseases and cancer, respectively, when compared to non-diabetes patients.

Keywords: diabetes mellitus, mortality, transplant, post-transplant diabetes mellitus, solid organ transplant recipient

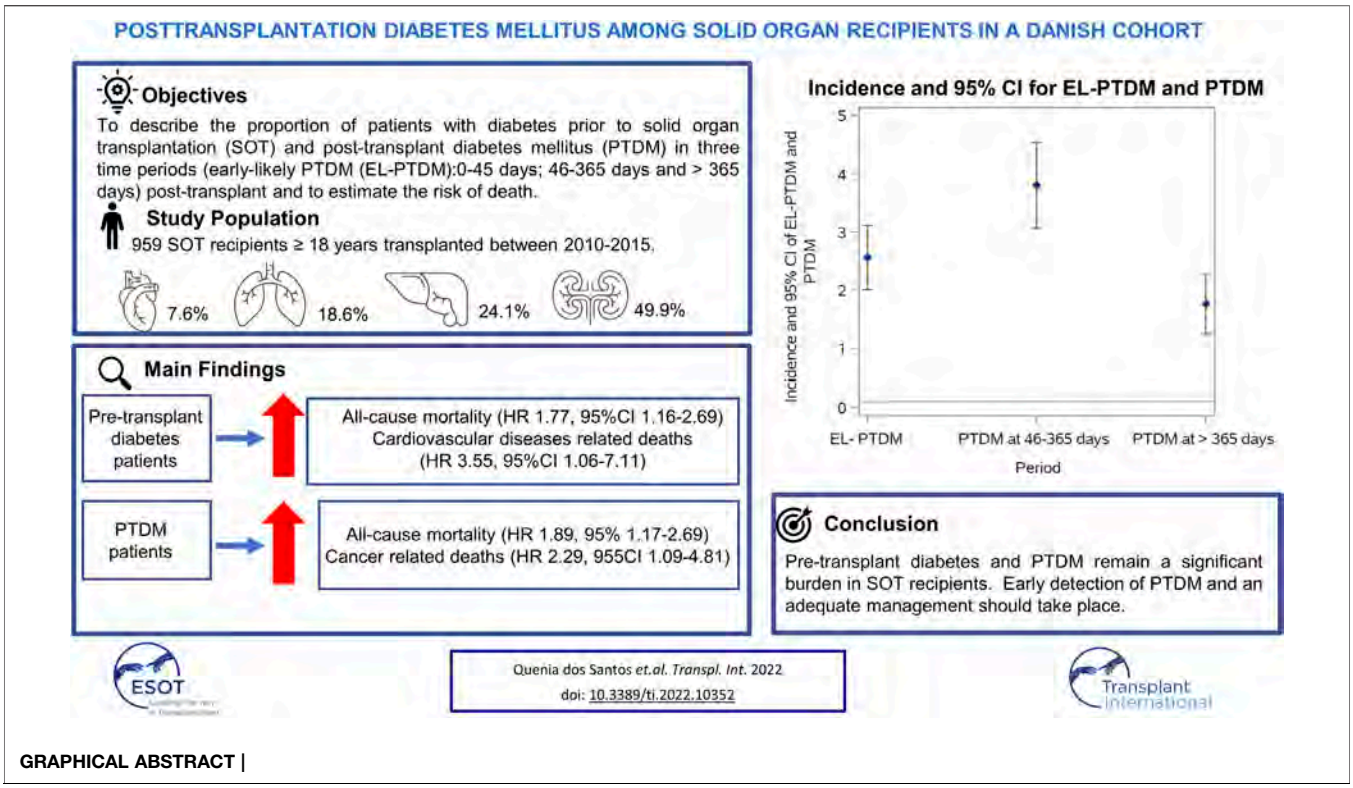
OPEN ACCESS

Correspondence:
Quenia Dos Santos
quenia.dos.santos.riedel@regionh.dk

Received: *11 January 2022*
Accepted: *15 March 2022*
Published: *05 April 2022*

Citation:
Dos Santos Q, Hornum M, Terrones-Campos C, Crone CG, Wareham NE, Soeborg A, Rasmussen A, Gustafsson F, Perch M, Soerensen SS, Lundgren J, Feldt-Rasmussen B and Reekie J (2022) Posttransplantation Diabetes Mellitus Among Solid Organ Recipients in a Danish Cohort. Transpl Int 35:10352. doi: 10.3389/ti.2022.10352

Abbreviations: ATC, anatomical therapeutic chemical; BMI, body mass index; CCI, Charlson comorbidity index; CI, confidence interval; CLASS, classification of death causes after transplantation; DPDD, Danish Prescription Database Data; EPM, electronic prescription medication; ICD, International Classification of Diseases; IR, incidence rate; IRR, incidence rate ratio; LPR, National Patient Registry; MATCH, Management of post-transplant infections in collaborating Hospitals; PERSIMUNE, Personalized Medicine for Infectious Complications in Immune Deficiency; PTDM, post-transplant diabetes mellitus; SDB, Sundhedsdatabanken; PYFU, Person Year Follow-up; SOT, solid organ transplant.



GRAPHICAL ABSTRACT |

INTRODUCTION

In 2014, the term post-transplantation diabetes mellitus (PTDM) was adopted to refer to newly diagnosed diabetes mellitus in the post transplantation period, irrespective of diagnostic timing or whether diabetes was present but undetected prior to transplantation or not (1). PTDM has been associated with greater mortality and a higher prevalence of infections in Solid Organ Transplant (SOT) recipients (2, 3). PTDM has also been associated with premature or more frequent cardiovascular events among kidney and lung recipients (4, 5) and an increased risk of adverse outcomes in heart recipients, such as cardiovascular morbidity and increased mortality (6–8).

The prevalence of PTDM in the first year after SOT varies by transplanted organ, with previous studies reporting PTDM in 10–20% of patients who have undergone a kidney transplantation (3) and in 20–40% of patients who received other solid organs (9). In addition to transplant type, factors thought to affect the incidence of PTDM include age, body mass index (BMI), race/ethnicity, and the immunosuppression regimen (9). The use of calcineurin inhibitors, especially tacrolimus (10), has been reported to increase the risk of developing PTDM because it can lead to insulin hyposecretion (11–13). Corticosteroids are used as maintenance immunosuppression as well as treatment of rejection and the relationship between this medication and hyperglycemia is well established (2). Therefore, awareness of PTDM risk factors and PTDM management are of importance for post-transplantation care (14).

The International Consensus Meeting on Post Transplant Diabetes Mellitus identified two critical time periods for assessing PTDM (46–365 days and >365 days after transplantation) (1). The consensus meeting also highlighted that due to transient post transplantation hyperglycemia a formal diagnosis of PTDM should not be made in the early time period of 0–45 days post-transplant.

Thus, the aim of this study was to estimate the percentage of SOT recipients with diabetes prior to transplantation and to determine the incidence of and risk factors associated with both early-likely PTDM (EL-PTDM) diagnosed in the 0–45 days post-transplant and PTDM in diagnosed in the two time-periods, 46–365 days and >365 days after transplantation. The inclusion of the EL-PTDM category, was to provide additional information on the transient nature of this period and to determine whether increased monitoring of potential early-likely PTDM patients could be beneficial. In addition, the risk of all cause and cause-specific mortality post-transplant was also assessed and compared in pre-transplant diabetes, those developing PTDM and non-diabetes patients.

MATERIALS AND METHODS

Study Design and Participants

The study cohort included all patients aged ≥18 years who underwent a SOT (heart, liver, lung and kidney) at Rigshospitalet, University Hospital of Copenhagen, a large tertiary transplant center, between January 2010 and

TABLE 1 | Characteristics of non-diabetes and diabetes patients at baseline.

Characteristics at baseline	Non-diabetes baseline (*n* = 625)	Pre-transplant diabetes (*n* = 334)	*p*-Value
Type of Transplant-N (%)			
Kidney	288 (60.1)	191 (39.9)	0.001
Liver	185 (80.1)	46 (19.9)	
Lung	120 (68.2)	56 (31.8)	
Heart	32 (43.8)	41 (56.2)	
Sex-N (%)			
Male	366 (63.2)	213 (36.8)	0.11
Female	259 (68.2)	121 (31.8)	
BMI categories-N (%)			
BMI < 25.0	244 (64.9)	132 (35.1)	0.006
BMI ≥ 25.0	191 (58.6)	135 (41.4)	
Missing	190 (72.0)	67 (28.0)	
Age in years (Median & IQR)	48.9 (39.6–55.0)	52.8 (44.7–60.2)	0.002
CCI in points (Median & IQR)	2.0 (2.0–3.0)	2.0 (2.0–3.0)	0.99

December 2015. All SOT patients were prospectively enrolled in the Management of post-Transplant infections in Collaborating Hospitals (MATCH) cohort (15).

For patients with more than one transplantation, only data related to the first transplant after 2010 was assessed**.** Since pancreas transplantation has been demonstrated to accomplish restoration of long-term glucose homeostasis (16, 17), pancreas recipients were excluded.

Data Sources

Clinical characteristics, sociodemographic and biochemical data were extracted from the MATCH database stored at the Centre of Excellence for Personalized Medicine for Infectious Complications in Immune Deficiency (PERSIMUNE) data warehouse. The data warehouse includes both regional and nationwide data collected prospectively as part of routine care.

Data on prescribed medications including insulin and oral anti-diabetic medication were extracted from the Electronic Prescription Medication (EPM), a database with hospital prescriptions from 2006 to 2016, and the Danish Prescription Database Data (DPDD), a database with outpatient prescriptions from 2004 onwards. Due to a change in systems there was a gap in data from EPM from May 2011 to December 2011. Individual patient data on specific immunosuppressive therapies was not available however, detailed information on the immunosuppressive schemes per transplant type can be found in a previous published article (18) and as a **Supplementary Material S1**.

Data on admissions and diagnosis were retrieved from the National Patient Registry (LPR) (19) and Sundhedsdatabanken (SDB). LPR was established in 1977 and has national data up to 2016 while SDB has data for patients in the capital region of Denmark from 2008 to 2019. For death, we used data from the Danish Civil Registration System on mortality (20).

In this study we used data on underlying cause of death. Underlying cause of death was defined as the disease or comorbidity leading to the death or directly causing the event classified as the immediate cause of death.

The specific underlying cause of death was obtained in accordance with a modified version of the validated Classification of Death Causes after Transplantation (CLASS) method (21) which includes completion of a Case Record Form for deceased patients and a review and adjudication process involving experts within the field of transplantation (21). The underlying cause of death was selected from 15 pre-defined transplant specific and non-specific categories of death causes, including 267 specific conditions (22). Specific recorded underlying causes of death were further grouped in seven wider categories in order to perform statistical analyses, including deaths due to cardiac or vascular disease, graft failure, graft rejection, infections, cancer, unknown causes and other organ specific diseases (aside from those specified above) and non-organ specific causes. Non-organ specific causes include hemorrhage, alcohol abuse, suicide and other causes.

Definition of Diabetes

Diabetes was assessed at four time periods, 1) Pre-transplant diabetes, which was defined as a diagnosis of diabetes at any timepoint prior to transplantation. 2) "Early-likely PTDM" (EL-PTDM) assessed in the period 0–45 days post-transplant to help estimate the transient nature of this period and understand whether similar risk factors were identified for EL-PTDM and PTDM. PTDM was then assessed at two time periods post-transplantation according to the periods defined by the International Consensus Meeting on Post Transplant Diabetes Mellitus: 3) 46–365 days, and 4) >365 days post-transplant (1).

Patients were defined as having developed diabetes if they fulfilled at least one of the following criteria during the time period of interest (for all time periods, except before transplant):

- A Hemoglobin A1C test ≥6.5 mmol/L or (1);
- A prescription of antidiabetic medication from either EPM or DPDD (Use of insulin-ATC code A10A, or use of oral antidiabetic medication-ATC code A10B) (23);
- A diagnosis of diabetes (ICD-10 codes: E10, E11, E13) (24).

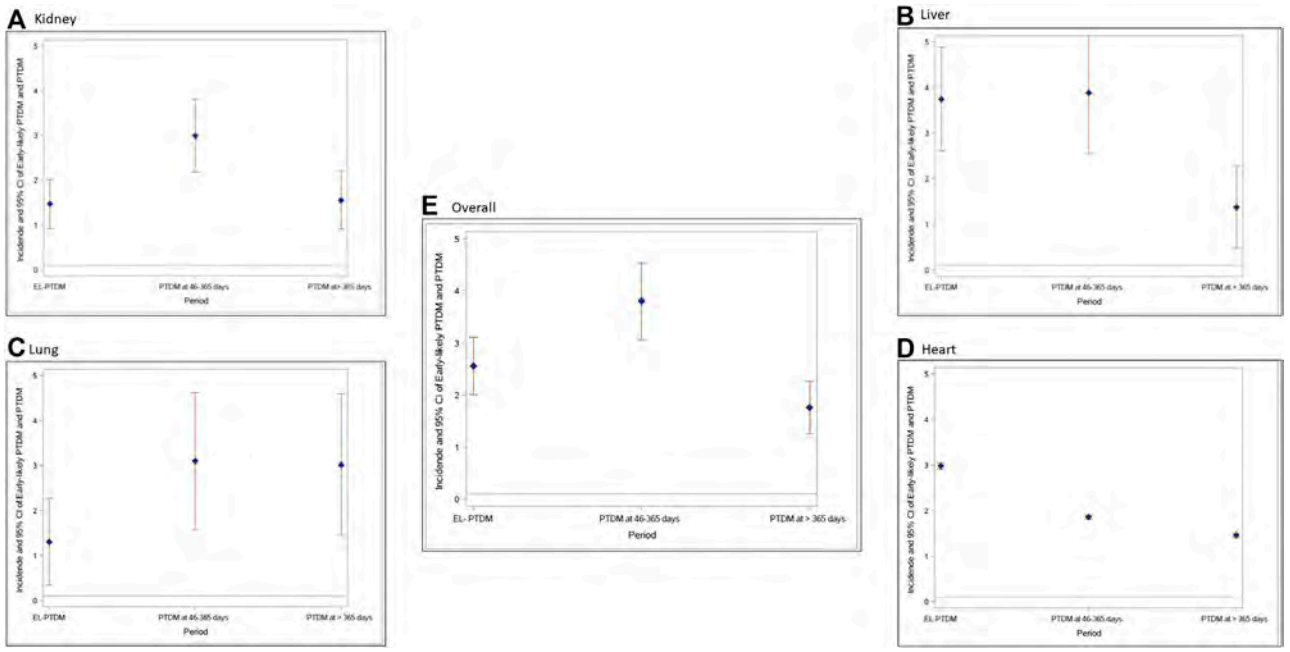


FIGURE 1 | Incidence rate per 100 person-days follow up and 95% Confidence interval of EL-PTDM and PTDM overall and per transplant type at each time period. **(A)** Kidney; **(B)** Liver, **(C)** Lung; **(D)** Heart; **(E)** Overall.

TABLE 2 | Univariable risk factors for the development of EL-PTDM and PTDM in each time period.

	EL-PTDM		**46–365 days after transplant**		**>365 days after transplant**	
	***n* = 625**		***n* = 611**		***n* = 579**	
Variables	OR with 95% CI	*p*-Value	OR with 95% CI	*p*-Value	OR with 95% CI	*p*-Value
Sex						
Male	1 (ref)		1 (ref)		1 (ref)	
Female	0.82 (0.51–1.31)	0.41	0.89 (0.62–1.28)	0.55	1.01 (0.69–1.49)	0.92
Transplant type						
Kidney	1 (ref)		1 (ref)		1 (ref)	
Liver	2.43 (1.46–4.04)	0.006	1.76 (1.18–2.64)	0.005	1.37 (0.88–2.11)	0.15
Lung	0.67 (0.31–1.45)	0.31	0.66 (0.39–1.13)	0.13	0.91 (0.52–1.57)	0.73
Heart	1.91 (0.73–5.01)	0.18	1.01 (0.42–2.33)	0.99	0.81 (0.32–2.08)	0.67
BMI categories						
BMI < 25	1 (ref)		1 (ref)		1 (ref)	
BMI ≥ 25	2.42 (1.42–4.19)	0.001	1.59 (1.05–2.43)	0.02	2.16 (1.38–3.37)	0.007
BMI-missing	1.32 (0.72–2.41)	0.35	0.99 (0.64–1.55)	0.98	1.06 (0.64–1.74)	0.81
Age (each 10 years)	1.43 (1.18–1.74)	0.002	1.39 (1.20–1.62)	0.001	1.44 (1.22–1.69)	0.001
CCI score (per point higher)	1.37 (1.15–1.63)	0.003	1.22 (1.06–1.42)	0.006	1.23 (1.05–1.44)	0.009

Patients were classified as having pre-transplant diabetes if they met the above criteria prior to transplantation with the exception of insulin treatment used during hospitalization (from EPM database). Due to high incidence of corticoid-induced hyperglycemia in patients listed for transplantation, patients meeting the definition based only on a record of insulin treatment during hospital admission were not classed as having pre-transplant diabetes.

Patients classified with pre-transplant diabetes remained classified as having diabetes in the entire post-transplantation period. Patients who were not classified as having pre-transplant diabetes could be classified as developing EL-PTDM or PTDM if they met the diabetes definition in the time-period of interest post-transplant. Patients diagnosed with EL-PTDM or PTDM in one period could subsequently return to non-diabetes status in the following time-period if they did not meet the diabetic definition in the new time-period.

During the first 15 days post-transplantation prescription for antidiabetic medication were not included in the definition. A large number of transplant recipients have glucose intolerance and hyperglycemia in the first few weeks post-transplant, detectable in approximately 90% of kidney allograft recipients

TABLE 3 | Number of deaths and incidence rate (95% CI) of death per 100 PYFU and univariable and multivariable Cox models for death per diabetes group.

	N of deaths	Incidence Rate (95% CI)	Univariable[a]		Multivariable[b]		Multivariable[c]	
			HR (95%CI)	*p*-Value	HR (95%CI)	*p*-Value	HR (95%CI)	*p*-Value
Non-diabetes	71	3.14 (2.41–3.87)	1	-	1	-	1	-
Pre-transplant diabetics	64	4.09 (3.09–5.09)	1.32 (0.93–1.88)	0.11	1.35 (0.93–1.98)	0.11	1.77 (1.16–2.69)	0.007
PTDM	39	4.84 (3.32–6.36)	1.46 (1.03–2.07)	0.03	1.53 (0.99–2.39)	0.05	1.89 (1.17–3.06)	0.008

[a]*Univariable model: adjusted by diabetes groups.*
[b]*Multivariable model: adjusted for sex, age (per 10 years), BMI, diabetes groups and CCI.*
[c]*Multivariable model: adjusted for sex, age (per 10 years), BMI, diabetes groups; CCI, and transplant type.*

TABLE 4 | Number of deaths per cause of death and incidence rate (95% CI) of deaths per 100 PYFU and univariable and multivariable Cox models for death causes per diabetes group.

Causes of death	Diabetes group	N of deaths	Incidence rate (95% CI)	Univariable[a]	Multivariable[b]	HR (95% CI)	*p*-Value
				HR (95% CI)	*p*-Value		
Graft rejection	Non-diabetes	19.0	0.84 (0.46; 1.22)	1	-	1	-
	Pre-transplant diabetes	10.0	0.64 (0.24; 1.03)	0.77 (0.35; 1.65)	0.50	1.53 (0.55; 4.21)	0.40
	PTDM	9.0	1.12 (0.39; 1.85)	1.33 (0.59; 2.99)	2.72	1.50 (0.89; 8.24)	0.08
Infections	Non-diabetes	12.0	0.53 (0.23; 0.83)	1	-	1	-
	Pre-transplant diabetes	9.0	0.57 (0.20; 0.95)	1.08 (0.45; 2.60)	0.85	1.85 (0.74; 4.63)	0.18
	PTDM	6.0	0.74 (0.15; 1.34)	1.40 (0.51; 3.78)	0.50	2.06 (0.67; 6.33)	0.20
Cardiovascular diseases	Non-diabetes	5.0	0.22 (0.03; 0.41)	1	-	1	-
	Pre-transplant diabetes	13.0	0.83 (0.38; 1.28)	3.89 (1.38; 10.95)	0.01	3.55 (1.06; 11.74)	0.03
	PTDM	3.0	0.37 (0.21; 0.79)	1.73 (0.41; 7.28)	0.44	1.57 (0.34; 7.11)	0.55
Cancer	Non-diabetes	14.0	0.62 (0.29; 0.94)	1	-	1	-
	Pre-transplant diabetes	17.0	1.09 (0.57; 1.60)	1.78 (0.87; 3.63)	0.11	1.83 (0.86; 3.93)	0.11
	PTDM	13.0	1.61 (0.74; 2.49)	2.62 (1.22; 5.62)	0.01	2.29 (1.09; 4.81)	0.02

[a]*Univariable model: adjusted by diabetes groups.*
[b]*Multivariable model: adjusted for sex, age (per 10 years), BMI, diabetes groups; CCI, and transplant type.*

in the early few weeks after transplant (25, 26). Thus prescription of insulin or oral antidiabetics immediately following transplant and while the patient is hospitalized is high (2), but not an indication of EL-PTDM.

Approvals

All procedures performed in this study were in accordance with the ethical standards of the 1975 Helsinki Declaration. All relevant approvals were obtained from the Danish National Data Protection Agency (2012-58-0004, RH-2015-67, with I-Suite number: 03787) according to national legislations on retrospective studies. The study was approved by the MATCH steering committee. This work was supported by Danish National Research Foundation (Grant number 126).

Statistical Analyses

Patient characteristics at transplant were described and compared for those with and without pre-transplant diabetes. Continuous variables, were analyzed using the Wilcoxon test (nonparametric data) and categorical variables, using the χ^2 test.

The prevalence of diabetes before transplant and incidence of EL-PTDM (0–45 days) and PTDM (46–365 days after transplant and >365 days after transplant) in each of the time periods was calculated among patients alive at the beginning of the time.

Univariable logistic regression analysis was used to evaluate risk factors for developing EL-PTDM and PTDM. Factors that were significant in the univariable analyses (p-value < 0.1) were included in the multivariable model. Models were developed separately for each of the three post-transplant time periods. Potential risk factors were selected according to the literature and availability in our dataset (27–30). They included sex; age at transplant; type of transplant; BMI ≥ 25 kg/m^2 at transplant and the Charlson Comorbidity Index (CCI) (31).

For the CCI (31), two dimensions related to diabetes (presence of diabetes mellitus with and without chronic complications) were excluded from calculation of the index to avoid collinearity issues with our outcome. Therefore 15 dimensions of this index were used.

Survival analysis was used to compare the risk of death in non-diabetes patients to those with pre-transplant diabetes, and those who developed PTDM (>45 days post-transplant). All patients were included in the analysis from day 46 post-transplant (thus individuals who only met the diabetes definition in the EL-PTDM period (0–45 days) were included in the non-diabetes group). Patients were followed until the date of death, a new transplant date, or the end of follow-up, whichever occurred first. For this analysis, the end of the follow-up was set to 31.12.2019 (the last date that cause of death information was available). Diabetes was treated as a time-updated variable, with all patients initially classified as either non-diabetes or pre-transplant diabetes. Patients in the non-diabetes category contributed person-time to that group until such a time as they met our definition for

PTDM. They then contributed person-time to the PTDM group from the first date they met our definition for the remainder of the follow-up. Cox proportional hazard models were used to compare the risk of all-cause and cause specific death in the three groups after adjusting for other factors.

As a sensitivity analysis the analysis was re-run using Fine and Grey methodology with deaths not related to the specific cause of interest treated as a competing risk. Categories of causes of death were only assessed if there were more than 20 deaths with that cause.

All data analyses were performed using SAS Studio.

RESULTS

A total of 959 SOT recipients were included in this study. Two patients with a kidney-pancreas transplant were excluded. The most common transplant type was kidney (479, 50.0%), followed by liver (231, 24.0%), lung (176, 18.0%) and heart (73, 8.0%). Pre-transplant diabetes was observed in 334 (34.8%–95% CI: 31.8–37.9) SOT recipients, with 78.0% meeting our definition in the year prior to transplantation. Of those 334 patients with pre-transplant diabetes, 33.5% (112 patients) met all three diabetes criteria; 7 (2.0%) had a medication prescription and a hemoglobin A1C ≥ 6.5 mmol/L only; 25 (7.5%) had a hemoglobin A1C ≥ 6.5 mmol/L and a diagnosis code only; 30 (9.0%) had a diagnosis code and a medication prescription only; 133 patients (39.9%) with one hemoglobin A1C ≥ 6.5 mmol/L, 23 (6.9%) with a diagnosis code, 4 (1.2%) with a medication prescription.

Table 1 shows the patient characteristics by pre-transplant diabetes status. There was a higher percentage of patients with BMI < 25 among non-diabetes compared to pre-transplant diabetes (64.9%, 95% CI: 59.8–69.7 vs. 35.1%, 95% CI: 30.2–40.1). A higher percentage of pre-transplant diabetes was observed among heart (56.2–95% CI: 44.0–67.7) and kidney transplants (39.9–95% CI: 35.4–44.4) compared to lung (31.8–95% CI: 25.0–39.2) and liver (19.9–95% CI: 14.9–25.6) ($p = 0.001$). The median age at transplant was also higher in those with pre-transplant diabetes compared to non-diabetes: 52.8 years (95% CI: 44.7–60.2) vs. 48.9 years (95% CI: 39.6–55.0).

The number and percentage of non-diabetes, pre-transplant diabetes and PTDM overall and per transplant type at each time period is found in **Supplementary Material S2**. The highest incidence of PTDM was observed at 46–365 days after transplant (IR of 3.80, 95% CI: 3.07–4.53) per 100 PYFU vs. IR of 2.56, 95% CI: 2.01–3.11 for EL-PTDM and IR of 1.76, 95% CI: 1.25–2.26 at >365 days after transplant (**Figure 1**). Among the 625 SOT recipients with no pre-transplant diabetes, 83 (13.3%) fulfilled the diagnosis criteria for EL- PTDM in the first 45 days post-transplant. Between day 46 and day 365 post-transplant, 171 patients (28.0%), out of 611 patients under follow-up at day 46, met our criteria for PTDM; 104 were new PTDM and 67 had also been diagnosed in the previous time period and 16 PTDM detected in the previous period reverted to non-diabetes. In the late period (>365 days) 143 patients out of 579 still under follow-up after 1 year had PTDM (24.7%) of whom 47 met the criteria for the first time and 96 were already diagnosed as PTDM in one of the previous periods and 62 patients diagnosed with PTDM in the previous period reverted to non-diabetes. The number of patients and the distribution of EL-PTDM and PTDM diagnostic criteria can be found in the **Supplementary Material S3**.

Risk factors associated with the development of EL-PTDM and PTDM in univariable analysis are shown in **Table 2**. For the multivariable analyses, older age and a higher CCI score in all time periods remained significantly associated with an increased likelihood of EL-PTDM and PTDM. For the EL-PTDM patients, the adjusted odds ratio (aOR) for age and CCI were (aOR: 1.44 per 10 years older, 95% CI: 1.18–1.75, $p = 0.0003$ and aOR: 1.39, 95% CI: 1.17–1.65, $p = 0.0002$ respectively). At 46–365 days, the estimates for age and CCI score were (aOR: 1.40 per 10 years older, 95% CI: 1.20–1.62, $p = 0.0001$ and aOR: 1.23, 95% CI: 1.06–1.42, $p = 0.005$) and in the later period (aOR: 1.44, 95% CI: 1.22–1.69, $p = 0.0001$ and aOR: 1.23, 95% CI: 1.04–1.44, $p = 0.01$ respectively).

A total of 174 patients died during 4,636 person years follow-up (PYFU) (**Table 3**) (IR 3.75, 95% CI: 3.20–4.31) per 100 PYFU. PTDM patients were found to have a higher risk of death when compared to non-diabetes patients in the univariable analysis (HR: 1.46, 95% CI: 1.03–2.07). This increased risk remained for PTDM and became significant for pre-transplant diabetes patients after adjusting for sex, age (per 10 years), BMI, diabetes groups, CCI and transplant type (aHR of 1.89, 95% CI: 1.17–3.06 and aHR of 1.77, 95% CI: 1.16–2.69 respectively, **Table 3**).

Table 4 shows the distribution of cause of deaths per diabetes group. In the univariable analysis, when compared to nondiabetes patients, a higher risk of death due to cardiovascular diseases was found and remained after adjustment for other risk factors (aHR 3.55, 95% CI 1.06–11.74). A higher rate of deaths due to cancer was observed in PTDM patients in both univariable and multivariable models (HR of 2.62, 95% CI: 1.22–5.62, $p = 0.01$ and aHR of 2.29, 95% CI: 1.09–4.81, $p = 0.02$, respectively). No other significant differences were found for the remaining causes of death among non-diabetes, pre-transplant diabetes and PTDM.

DISCUSSION

In this study of 959 SOT recipients, over one third fulfilled our diagnosis criteria for diabetes prior to transplantation. The highest proportion was among heart recipients where slightly over half of the patients met our criteria for pre-transplant diabetes. The proportion of non-diabetes patients at transplantation diagnosed with PTDM was also high, with the highest incidence rates observed at 46–365 days post-transplantation (IR of 3.80, 95% CI: 3.07–4.53). Older age and a higher CCI score (at all time periods) were associated with an increased risk of PTDM and similar risk factors were identified for EL-PTDM. A higher incidence rate of all-cause mortality was observed among individuals with diabetes prior to transplantation and PTDM patients. Pre-transplant diabetes and PTDM patients were found to have a higher risk of death due to cardiovascular disease and cancer in both univariable and multivariable analysis.

The characteristics of our patients with pre-transplant diabetes (**Table 1**) were consistent with previous studies (4, 32–34). We also observed a similar proportion with pre-transplant diabetes (34.8–95% CI: 31.8–37.9), where previous studies have estimated the prevalence to range from 17.5 to 38.0% (4, 32–34). These studies used a variety of different criteria to assess diabetes. Some included oral glucose tolerance test or fasting plasma glucose (35, 36), variables not available in this study or only the combinations of two components (such as two or more positive random glucose or a hemoglobin A1C ≥6.5 prior to transplant) (32), while other studies have relied on self-report diabetes status (34, 37). However, a recent study, using a criteria similar to ours, found high sensitivity (93%) and specificity (98%) when comparing their criteria against patient self-report diabetes status (37), and found the combined criteria better than using diagnosis or medication alone.

The formal diagnosis of PTDM is recommended when the patients are stable on their likely maintenance immunosuppression and in the absence of acute infections (1). In addition, most studies report the percentage of SOT recipients with PTDM at time periods equal or greater than 1 year after transplant. It is well known that an excess in blood glycemia can occur for myriad reasons post-transplantation (immunosuppressive therapy, infections, and other critical conditions), and thus it is important to exclude transient post transplantation hyperglycemia from PTDM diagnosis. Previous studies have reported hyperglycemia in approximately 90% of kidney allograft recipients during the first weeks post-transplant (25, 26). Consequently, in the immediate post-transplant setting, insulin therapy or prescription of a medication for diabetes is generally required to manage postoperative hyperglycemia, especially given the requirement for high-dose immunosuppressants in this setting (2). We split the present analysis into two PTDM time periods (46–365 days and >365 days), but we also reported patients that fulfilled the diabetes criteria in the first 45 days after transplant (EL-PTDM) to increase awareness of the number of transplant recipients that can potentially develop PTDM in the future. Furthermore, it is important to emphasize that the first weeks after transplant are critical periods and efforts should be made in a tentative effort to stabilize the patient. Of a total of 83 patients diagnosed with EL-PTDM in the first 45 days after transplant, 67 (80.7%) remained PTDM in the subsequent period.

The highest incidence of overall PTDM and overall diabetes was detected at 46–365 days. This is in line with the literature, that recommends that a diagnosis of PTDM is generally reserved for the outpatient setting, when the recipient had been discharged from the hospital, is stable, and in the absence of acute infections (1, 2).

The percentage of PTDM at 1 year post-transplant found in the literature ranged from 12 to 45% in liver recipients (32); 4–25% in kidney transplant recipients (34); 4–40% in heart transplant recipients (29, 38); and 5–45% in lung transplant recipients (39-41). This again is in line with our results reported for days 46–365 (liver recipients: 38.1%, heart recipients: 25.8% kidney: 25.7% and lung: 18.8%) (**Supplementary Material S2**).

The most important risk factors for the development of EL-PTDM and PTDM in this study were age and CCI score (in all time periods), which have been identified previously (27–30, 42). Some immunosuppressive regimens have also been associated with an increased risk of PTDM. This could not be investigated by our study due to limitations in our medication data and the lack of reliable information on the medication dosages.

Pre-transplant diabetes and PTDM patients were found to have a higher all-cause mortality rate and in cause-specific analysis patients with pre-transplant diabetes had a higher risk of death due to cardiovascular diseases when compared to non-diabetes. This is in line with previous studies (2, 3, 27, 30, 43, 44), that also used similar covariates in their analyses (age, gender, BMI, among others). An important study (2) found that PTDM may only reduce short-term survival after liver transplant, while the impact of PTDM on survival after lung transplant is unclear and PTDM after heart transplantation does not affect survival. In our study, PTDM patients, had a higher risk of death due to cancer in the univariable analysis and in the multivariable analysis. This was also observed in a previous study (44) while some other studies did not support this finding (4, 45). It is well known that diabetes mellitus has been widely associated with the increase the risk of malignancy due to the postulated mechanisms including stimulation of insulin-like growth factor-axis and increased cytokines production (46), but it is still uncertain whether the same association can be extrapolated to PTDM patients (44). For the remaining causes of death, no differences were found when comparing pre-transplant diabetes and PTDM to non-diabetes patients.

The limitations of this study should be highlighted. As mentioned previously (25, 26), increases in the glycemia levels are expected in the period right after transplant (25, 26), and it is not common for a patient to receive a diagnostic code for diabetes at 0–45 days after transplant. Additionally, our criteria to define PTDM does not include blood glucose levels as the available data did not discriminate between fasting and non-fasting glucose tests. Thus, it is possible, that the incidence of EL-PTDM could be underestimated particularly in the 0–45 days period. Further, patients with chronic kidney disease may have a lower hemoglobin because of erythropoietin deficit, especially right after transplant. However, the number of EL-PTDM patients diagnosed only based on HbA1C in this time period is low (27.8%) (**Supplementary Material S3**). One additional limitation is the lack of information about the immunosuppressive medication as previously mentioned. Furthermore, for some cause of deaths the number of events was very low, therefore their results must be interpreted cautiously. Lastly, as data on medication was available only until the December 31, 2016, incidence of PTDM could be underestimated from 2017 until 2019, since for this period it relied on hemoglobin A1c and diabetes diagnosis codes only.

The strengths of this study are to present the PTDM frequency in different time periods and to include different types of solid organs recipients (kidney, liver, lung and heart) as well as to report the number of EL-PTDM patients. An additional strength is that this is the first study that assess post-transplant death between non-diabetes, pre-transplant diabetics and PTDM since most of the published studies combine PTDM and pre-transplant

diabetes together or exclude pre-transplant diabetes and present the outcomes only for non-diabetes and PTDM.

In conclusion, this study found that a high proportion of SOT recipients have diabetes prior to transplantation, and that PTDM incidence was highest at 46–365 days after transplant in all transplant recipients. Compared to non-diabetes, pre-transplant diabetics and PTDM patients had a higher mortality rate after transplant. In relation to causes of death, pre-transplant diabetes and PTDM patients had a higher risk of death due to cardiovascular diseases and cancer, respectively, when compared to non-diabetes patients. Pre-transplant diabetes and PTDM remain a significant burden in the SOT population and an early detection of PTDM and an adequate management and treatment of both pre-transplant diabetes and PTDM should take place. For those patients, it is advisable to follow current general practice guidelines for blood glucose goals for both inpatients (47) and outpatients (48). Closer monitoring and frequent (49) follow-up are of the utmost importance to prevent or minimize adverse outcomes in those patients.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request. Requests to access the datasets should be directed to persimune.rigshospitalet@regionh.dk.

ETHICS STATEMENT

All procedures performed in this study were in accordance with the ethical standards of the 1975 Helsinki Declaration. All relevant approvals were obtained from the Danish National Data Protection Agency (2012-58-0004, RH- 2015-67, with I-Suite number: 03787) according to national legislations on retrospective studies. The study was approved by the MATCH steering committee. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

QDS designed the study, performed the data analysis, and drafted and edited the manuscript. CT-C, MH, JR, and BF-R designed the study, assisted in data analysis, and edited the manuscript. CC, NW, and AS assisted in data analysis, and edited the manuscript. AR, FG, MP, SS, and JL assisted in data collection and edited the manuscript.

FUNDING

This work was supported by Danish National Research Foundation (Grant number 126).

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.2022.10352/full#supplementary-material

REFERENCES

1. Sharif A, Hecking M, de Vries APJ, Porrini E, Hornum M, Rasoul-Rockenschaub S, et al. Proceedings from an International Consensus Meeting on Posttransplantation Diabetes Mellitus: Recommendations and Future Directions. *Am J Transpl* (2014) 14(9):1992–2000. doi:10.1111/ajt.12850
2. Shivaswamy V, Boerner B, Larsen J. Post-transplant Diabetes Mellitus: Causes, Treatment, and Impact on Outcomes. *Endocr Rev* (2016) 37(1):37–61. doi:10.1210/er.2015-1084
3. Kasiske BL, Snyder JJ, Gilbertson D, Matas AJ. Diabetes Mellitus after Kidney Transplantation in the United States. *Am J Transpl* (2003) 3(2):178–85. doi:10.1034/j.1600-6143.2003.00010.x
4. Eide IA, Halden TAS, Hartmann A, Åsberg A, Dahle DO, Reisaeter AV, et al. Mortality Risk in post-transplantation Diabetes Mellitus Based on Glucose and HbA1c Diagnostic Criteria. *Transpl Int* (2016) 29(5):568–78. doi:10.1111/tri.12757
5. Seoane-Pillado MT, Pita-Fernández S, Valdés-Cañedo F, Seijo-Bestilleiro R, Pértega-Díaz S, Fernández-Rivera C, et al. Incidence of Cardiovascular Events and Associated Risk Factors in Kidney Transplant Patients: a Competing Risks Survival Analysis. *BMC Cardiovasc Disord* (2017) 17(1):72. doi:10.1186/s12872-017-0505-6
6. Cho MS, Choi H-I, Kim I-O, Jung S-H, Yun T-J, Lee J-W, et al. The Clinical Course and Outcomes of post-transplantation Diabetes Mellitus after Heart Transplantation. *J Korean Med Sci* (2012) 27(12):1460–7. doi:10.3346/jkms.2012.27.12.1460
7. Kim HJ, Jung S-H, Kim J-J, Yun T-J, Kim JB, Choo SJ, et al. New-Onset Diabetes Mellitus after Heart Transplantation- Incidence, Risk Factors and Impact on Clinical Outcome. *Circ J* (2017) 81(6):806–14. doi:10.1253/circj.cj-16-0963
8. Martínez-Dolz L, Almenar L, Martínez-Ortiz L, Arnau MA, Chamorro C, Moro J, et al. Predictive Factors for Development of Diabetes Mellitus post-heart Transplant. *Transpl Proc* (2005) 37(9):4064–6. doi:10.1016/j.transproceed.2005.09.161
9. Jenssen T, Hartmann A. Post-transplant Diabetes Mellitus in Patients with Solid Organ Transplants. *Nat Rev Endocrinol* (2019) 15(3):172–88. doi:10.1038/s41574-018-0137-7
10. Heisel O, Heisel R, Balshaw R, Keown P. New Onset Diabetes Mellitus in Patients Receiving Calcineurin Inhibitors: a Systematic Review and Meta-Analysis. *Am J Transpl* (2004) 4(4):583–95. doi:10.1046/j.1600-6143.2003.00372.x
11. Santos L, Rodrigo E, Piñera C, Quintella E, Ruiz JC, Fernández-Fresnedo G, et al. New-onset Diabetes after Transplantation: Drug-Related Risk Factors. *Transplant Proc* (2012) 44(9):2585–7. doi:10.1016/j.transproceed.2012.09.053
12. Shah T, Kasravi A, Huang E, Hayashi R, Young B, Cho YW, et al. Risk Factors for Development of New-Onset Diabetes Mellitus after Kidney Transplantation. *Transplantation* (2006) 82(12):1673–6. doi:10.1097/01.tp.0000250756.66348.9a
13. Yates CJ, Fourlanos S, Hjelmesaeth J, Colman PG, Cohney SJ. New-onset Diabetes after Kidney Transplantation-Changes and Challenges. *Am J Transpl* (2012) 12(4):820–8. doi:10.1111/j.1600-6143.2011.03855.x

14. Weng LC, Chiang YJ, Lin MH, Hsieh CY, Lin SC, Wei TY, et al. Association between Use of FK506 and Prevalence of post-transplantation Diabetes Mellitus in Kidney Transplant Patients. *Transplant Proc* (2014) 46(2): 529–31. doi:10.1016/j.transproceed.2013.11.141
15. Lodding IP, Sengeløv H, da Cunha-Bang C, Iversen M, Rasmussen A, Gustafsson F, et al. Clinical Application of Variation in Replication Kinetics during Episodes of Post-transplant Cytomegalovirus Infections. *EBioMedicine* (2015) 2(7):699–705. doi:10.1016/j.ebiom.2015.05.003
16. Morel P, Goetz FC, Moudry-Munns K, Freier E, Sutherland DE. Long-term Glucose Control in Patients with Pancreatic Transplants. *Ann Intern Med* (1991) 115(9):694–9. doi:10.7326/0003-4819-115-9-694
17. Robertson RP, Sutherland DE, Lanz KJ. Normoglycemia and Preserved Insulin Secretory reserve in Diabetic Patients 10-18 Years after Pancreas Transplantation. *Diabetes* (1999) 48(9):1737–40. doi:10.2337/diabetes.48.9.1737
18. Ekenberg C, da Cunha-Bang C, Lodding IP, Sørensen SS, Sengeløv H, Perch M, et al. Evaluation of an Electronic, Patient-Focused Management System Aimed at Preventing Cytomegalovirus Disease Following Solid Organ Transplantation. *Transpl Infect Dis* (2020) 22(2):e13252. doi:10.1111/tid.13252
19. Lynge E, Sandegaard JL, Rebolj M. The Danish National Patient Register. *Scand J Public Health* (2011) 39(7_Suppl. l):30–3. doi:10.1177/1403494811401482
20. Pedersen CB. The Danish Civil Registration System. *Scand J Public Health* (2011) 39(7 Suppl. l):22–5. doi:10.1177/1403494810387965
21. Wareham NE, Da Cunha-Bang C, Borges ÁH, Ekenberg C, Gerstoft J, Gustafsson F, et al. Classification of Death Causes after Transplantation (CLASS). *Medicine (Baltimore)* (2018) 97(29):e11564. doi:10.1097/md.0000000000011564
22. Rigshospitalet UoC. *Cause of Death List: Rigshospitalet*. Copenhagen, Denmark: University of Copenhagen, Centre of Excellence for Health, Immunity and Infections (CHIP). Available from: https://chip.dk/Portals/0/files/MATCH/CLASS_Cause%20of%20Death%20List.pdf?ver=2018-10-01-095315-583×tamp=1538380416865 (Accessed December 16, 2021).
23. Methodology WHOCC. *Drugs Used in Diabetes*. Norway: Norwegian Institute of Public Health. Available from: https://www.whocc.no/atc_ddd_index/?code=a10 (Accessed November 15, 2021).
24. ICD10Data. Diabetes Mellitus E08-E13 (2022). Available from: ICD10Data.com.
25. Chakkera HA, Weil EJ, Castro J, Heilman RL, Reddy KS, Mazur MJ, et al. Hyperglycemia during the Immediate Period after Kidney Transplantation. *Cjasn* (2009) 4(4):853–9. doi:10.2215/cjn.05471008
26. Hecking M, Haidinger M, Döller D, Werzowa J, Tura A, Zhang J, et al. Early Basal Insulin Therapy Decreases New-Onset Diabetes after Renal Transplantation. *Jasn* (2012) 23(4):739–49. doi:10.1681/asn.2011080835
27. Cheng CY, Chen CH, Wu MF, Wu MJ, Chen JP, Liu YM, et al. Risk Factors in and Long-Term Survival of Patients with Post-Transplantation Diabetes Mellitus: A Retrospective Cohort Study. *Int J Environ Res Public Health* (2020) 17(12), 4581. Available from: http://europepmc.org/abstract/MED/32630562. https://europepmc.org/articles/PMC7345656. https://europepmc.org/articles/PMC7345656?pdf=render. doi:10.3390/ijerph17124581
28. Demirci MS, Toz H, Yılmaz F, Ertilav M, Asci G, Ozkahya M, et al. Risk Factors and Consequences of post-transplant Diabetes Mellitus. *Clin Transpl* (2010) 24(5):E170–E177. doi:10.1111/j.1399-0012.2010.01247.x
29. Pham PT, Sidhu HS, Pham PM, Pham PC Diabetes Mellitus after Solid Organ Transplantation. In: KR Feingold, B Anawalt, A Boyce, et al. editors. *Endotext [Internet]*. South Dartmouth (MA): MDText.com, Inc. (2019). Available from: https://www.ncbi.nlm.nih.gov/books/NBK378977/ (Accessed November 28, 2021).
30. Roccaro GA, Goldberg DS, Hwang W-T, Judy R, Thomasson A, Kimmel SE, et al. Sustained Posttransplantation Diabetes Is Associated with Long-Term Major Cardiovascular Events Following Liver Transplantation. *Am J Transpl* (2018) 18(1):207–15. doi:10.1111/ajt.14401
31. Quan H, Sundararajan V, Halfon P, Fong A, Burnand B, Luthi J-C, et al. Coding Algorithms for Defining Comorbidities in ICD-9-CM and ICD-10 Administrative Data. *Med Care* (2005) 43(11):1130–9. doi:10.1097/01.mlr.0000182534.19832.83
32. Lieber SR, Lee RA, Jiang Y, Reuter C, Watkins R, Szempruch K, et al. The Impact of post-transplant Diabetes Mellitus on Liver Transplant Outcomes. *Clin Transpl* (2019) 33(6):e13554. doi:10.1111/ctr.13554
33. Hjelmesaeth J, Hartmann A, Leivestad T, Holdaas H, Sagedal S, Olstad M, et al. The Impact of Early-Diagnosed New-Onset post-transplantation Diabetes Mellitus on Survival and Major Cardiac Events. *Kidney Int* (2006) 69(3): 588–95. doi:10.1038/sj.ki.5000116
34. Munshi VN, Saghafian S, Cook CB, Werner KT, Chakkera HA. Comparison of post-transplantation Diabetes Mellitus Incidence and Risk Factors between Kidney and Liver Transplantation Patients. *PLoS One* (2020) 15(1):e0226873. doi:10.1371/journal.pone.0226873
35. Hornum M, Jørgensen KA, Hansen JM, Nielsen FT, Christensen KB, Mathiesen ER, et al. New-onset Diabetes Mellitus after Kidney Transplantation in Denmark. *Cjasn* (2010) 5(4):709–16. doi:10.2215/cjn.05360709
36. Valderhaug TG, Jenssen T, Hartmann A, Midtvedt K, Holdaas H, Reisæter AV, et al. Fasting Plasma Glucose and Glycosylated Hemoglobin in the Screening for Diabetes Mellitus after Renal Transplantation. *Transplantation* (2009) 88(3):429–34. doi:10.1097/tp.0b013e3181af1f53
37. Miller DR, Safford MM, Pogach LM. Who Has Diabetes? Best Estimates of Diabetes Prevalence in the Department of Veterans Affairs Based on Computerized Patient Data. *Diabetes Care* (2004) 27(Suppl. 2):B10–21. doi:10.2337/diacare.27.suppl_2.b10
38. Davidson J, Wilkinson A, Dantal J, Dotta F, Haller H, Hernández D, et al. New-onset Diabetes after Transplantation: 2003 International Consensus Guidelines. Proceedings of an International Expert Panel Meeting. Barcelona, Spain, 19 February 2003. *Transplantation* (2003) 75(10):SS3–24. doi:10.1097/01.TP.0000069952.49242.3E
39. Ye X, Kuo H-T, Sampaio MS, Jiang Y, Bunnapradist S. Risk Factors for Development of New-Onset Diabetes Mellitus after Transplant in Adult Lung Transplant Recipients. *Clin Transpl* (2011) 25(6):885–91. doi:10.1111/j.1399-0012.2010.01383.x
40. Sharif A, Cohney S. Post-transplantation Diabetes-State of the Art. *Lancet Diabetes Endocrinol* (2016) 4(4):337–49. doi:10.1016/s2213-8587(15)00387-3
41. Lane JT, Dagogo-Jack S. Approach to the Patient with New-Onset Diabetes after Transplant (NODAT). *J Clin Endocrinol Metab* (2011) 96(11):3289–97. doi:10.1210/jc.2011-0657
42. Mizrahi N, Braun M, Ben Gal T, Rosengarten D, Kramer MR, Grossman A. Post-transplant Diabetes Mellitus: Incidence, Predicting Factors and Outcomes. *Endocrine* (2020) 69(2):303–9. doi:10.1007/s12020-020-02339-9
43. Cosio FG, Pesavento TE, Kim S, Osei K, Henry M, Ferguson RM. Patient Survival after Renal Transplantation: IV. Impact of post-transplant Diabetes. *Kidney Int* (2002) 62(4):1440–6. doi:10.1111/j.1523-1755.2002.kid582.x
44. Yeh H, Lin C, Li Y-R, Yen C-L, Lee C-C, Chen J-S, et al. Temporal Trends of Incident Diabetes Mellitus and Subsequent Outcomes in Patients Receiving Kidney Transplantation: a National Cohort Study in Taiwan. *Diabetol Metab Syndr* (2020) 12(1):34. doi:10.1186/s13098-020-00541-3
45. Lim WH, Wong G, Pilmore HL, McDonald SP, Chadban SJ. Long-term Outcomes of Kidney Transplantation in People with Type 2 Diabetes: a Population Cohort Study. *Lancet Diabetes Endocrinol* (2017) 5(1):26–33. doi:10.1016/s2213-8587(16)30317-5
46. Pandey A, Forte V, Abdallah M, Alickaj A, Mahmud S, Asad S, et al. Diabetes Mellitus and the Risk of Cancer. *Minerva Endocrinol* (2011) 36(3):187–209.
47. American Diabetes Association. Diabetes Care in the Hospital, Nursing home, and Skilled Nursing Facility. *Diabetes Care* (2015) 38:S80–5. doi:10.2337/dc15-S016
48. Kasiske BL, Zeier MG, Chapman JR, Craig JC, Ekberg H, Garvey CA, et al. KDIGO Clinical Practice Guideline for the Care of Kidney Transplant Recipients: a Summary. *Kidney Int* (2010) 77(4):299–311. doi:10.1038/ki.2009.377
49. Centre of Excellence for Health. Immunity and Infections (CHIP) (2021). Available from: https://chip.dk/Portals/0/files/MATCH/CLASS_Cause%20of%20Death%20List.pdf?ver=2018-10-01-095315-583&timestamp=1538380416865.

ESOT | Transplant International

ORIGINAL RESEARCH
published: 19 April 2022
doi: 10.3389/ti.2022.10113

Oral Glucose Tolerance Test for the Screening of Glucose Intolerance Long Term Post-Heart Transplantation

Stefan Roest[1,2], *Marleen M. Goedendorp-Sluimer*[1,2], *Julia J. Köbben*[3], *Alina A. Constantinescu*[1,2], *Yannick J. H. J. Taverne*[2,4], *Felix Zijlstra*[1], *Adrienne A. M. Zandbergen*[3] *and Olivier C. Manintveld*[1,2]*

[1]*Department of Cardiology, Thorax Center, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands,* [2]*Erasmus MC Transplant Institute, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands,* [3]*Department of Internal Medicine, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands,* [4]*Department of Cardiothoracic Surgery, Thorax Center, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands*

Post-transplant diabetes mellitus (PTDM) is a frequent complication post-heart transplantation (HT), however long-term prevalence studies are missing. The aim of this study was to determine the prevalence and determinants of PTDM as well as prediabetes long-term post-HT using oral glucose tolerance tests (OGTT). Also, the additional value of OGTT compared to fasting glucose and glycated hemoglobin (HbA1c) was investigated. All patients > 1 year post-HT seen at the outpatient clinic between August 2018 and April 2021 were screened with an OGTT. Patients with known diabetes, an active infection/rejection/malignancy or patients unwilling or unable to undergo OGTT were excluded. In total, 263 patients were screened, 108 were excluded. The included 155 patients had a median age of 54.3 [42.2–64.3] years, and 63 (41%) were female. Median time since HT was 8.5 [4.8–14.5] years. Overall, 51 (33%) had a normal range, 85 (55%) had a prediabetes range and 19 (12%) had a PTDM range test. OGTT identified prediabetes and PTDM in more patients (18% and 50%, respectively), than fasting glucose levels and HbA1c. Age at HT (OR 1.03 (1.00–1.06), p = 0.044) was a significant determinant of an abnormal OGTT. Prediabetes as well as PTDM are frequently seen long-term post-HT. OGTT is the preferred screening method.

Keywords: heart transplantation, comorbidity, oral glucose tolerance test, prediabetes, post-transplant diabetes mellitus

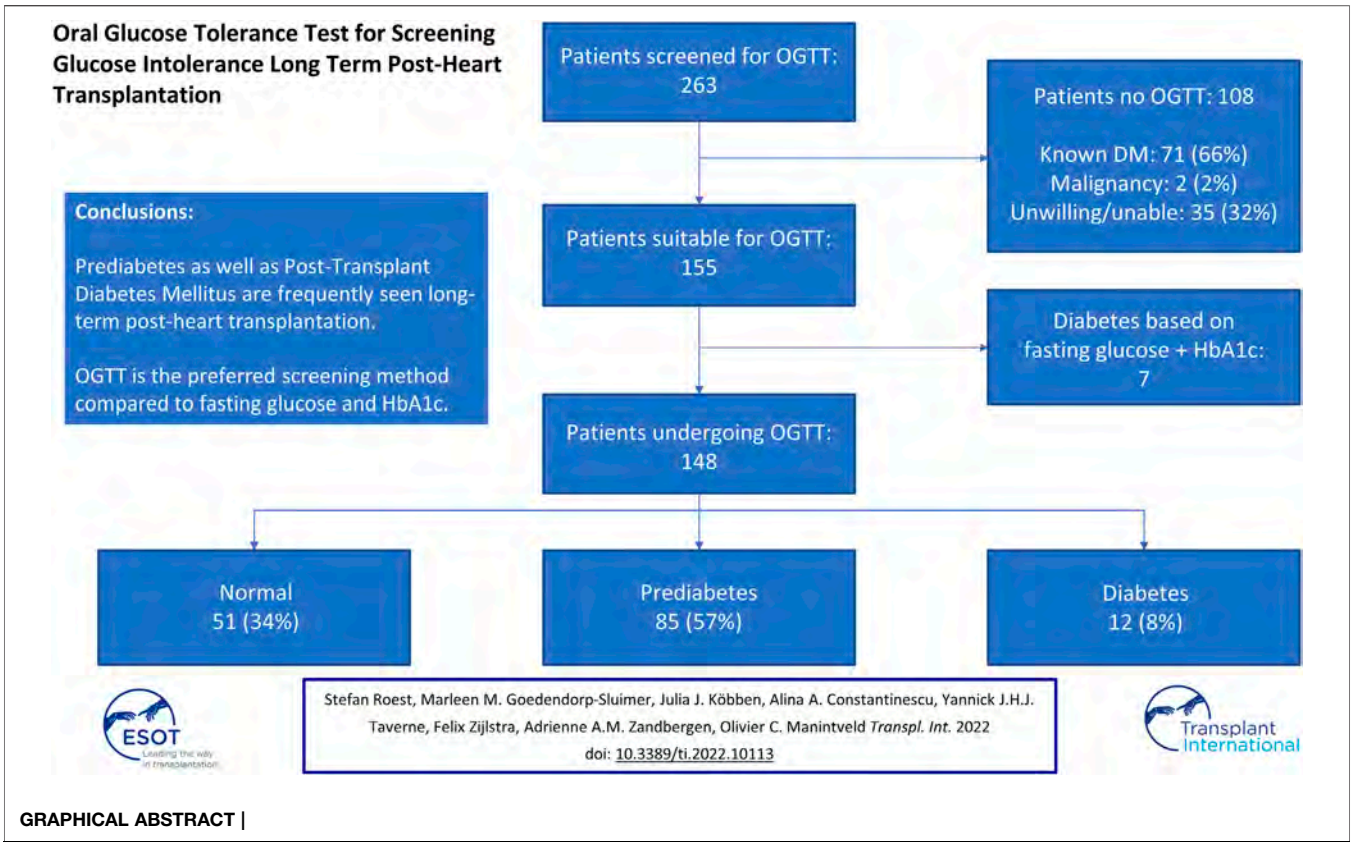
OPEN ACCESS

Correspondence:
Olivier C. Manintveld
o.manintveld@erasmusmc.nl

Received: *14 October 2021*
Accepted: *08 March 2022*
Published: *19 April 2022*

Citation:
Roest S, Goedendorp-Sluimer MM, Köbben JJ, Constantinescu AA, Taverne YJHJ, Zijlstra F, Zandbergen AAM and Manintveld OC (2022) Oral Glucose Tolerance Test for the Screening of Glucose Intolerance Long Term Post-Heart Transplantation. Transpl Int 35:10113. doi: 10.3389/ti.2022.10113

Abbreviations: ADA, American Diabetes Association; BMI, body mass index; CAV, cardiac allograft vasculopathy; CMV, cytomegalovirus; HbA1c, glycated hemoglobin; HT, heart transplantation; ISHLT, International Society for Heart and Lung Transplantation; LVAD, left ventricular assist device; OGTT, oral glucose tolerance test; OR, odds ratio; PTDM, post-transplantation diabetes mellitus.



GRAPHICAL ABSTRACT |

INTRODUCTION

Type 2 diabetes mellitus is an increasing problem worldwide, leading to reduced life expectancy and increased risk for cardiovascular complications (1–3). Prediabetes is an intermediate metabolic state between normal glucose tolerance and diabetes mellitus, with a growing prevalence worldwide as well (over 45% when aged 65 years or more) (4, 5). Patients with prediabetes have an increased risk (of up to 70%) of developing type 2 diabetes mellitus; both leading to an increased risk of cardiovascular morbidity and mortality, in the general population as well as in patients with manifest atherosclerotic disease (6–9).

In solid-organ transplant recipients, the incidences of post-transplant diabetes mellitus (PTDM) are high, varying between 10 and 40% depending on the transplanted organ and definitions used (10–15, 17). Risk factors for the development of PTDM include general cardiovascular risk factors (such as body mass index, age and family history) as well as transplant-related causes (such as immunosuppressive regimen, viral infections) (10). The incidence of PTDM 5 years post-heart transplantation (HT) reported by the registry of the International Society for Heart and Lung Transplantation (ISHLT) is 34% (16). Unfortunately, information after 5 years is lacking in this registry. Currently, no other studies have been performed determining the prevalence of PTDM after 5 years in HT recipients.

Recently, data from the ISHLT registry showed that PTDM was associated with an increased risk for severe renal dysfunction, retransplantation and death (17). This warrants an early recognition of the disease in order to modify risk factors to decrease the risk for these worse outcomes. Moreover, in HT recipients, only one small study has been published that demonstrated no difference in outcomes between patients with prediabetes and diabetes in the year pre-HT (18). Studies on prevalence and outcomes of prediabetes post-HT are missing.

Previous studies in the general population have shown the added value of the oral glucose tolerance test (OGTT) to fasting glucose levels and glycated hemoglobin (HbA1c) for identifying patients with (pre)diabetes, with a significant number of patients being missed without the OGTT (19, 20). Therefore, in transplant patients, adding an OGTT is preferred in patients who are stable on immunosuppressive regimen to make a diagnosis of (pre)diabetes according to the American Diabetes Association (ADA) guidelines (4). In the guidelines of the ISHLT it is advised to periodically screen for PTDM after HT by fasting glucose levels or OGTT and HbA1c levels (21). However, studies on the added value of an OGTT compared to fasting glucose and HbA1c in HT recipients are missing.

In the current study, we investigated the prevalence of prediabetes and diabetes mellitus long-term post-HT and determinants for an abnormal OGTT long-term post-HT. Additionally, the added value of an OGTT compared to a fasting glucose and HbA1c is investigated.

TABLE 1 | Baseline characteristics all screened patients and divided into groups (patient who underwent oral glucose tolerance test versus those who did not).

Parameters	Whole Cohort	Patient Not Undergoing OGTT	Patients Undergoing OGTT	p-value
Number of patients	263	108	155	
Female	96 (37)	33 (31)	63 (41)	0.10
Ethnicity				0.25
Caucasian	227 (86)	89 (82)	138 (89)	
Black	8 (3)	5 (5)	3 (2)	
Other	28 (11)	14 (13)	14 (9)	
Age at HT (years)	47.1 [32.5–55.0]	48.7 [38.1–56.8]	46.2 [26.1–53.6]	0.047
Age at OGTT (years)			54.3 [42.2–64.3]	
Time HT—OGTT (years)			8.5 [4.8–14.5]	
Etiology heart failure				0.001
Ischemic CMP	70 (27)	40 (37)	30 (19)	
Non-ischemic CMP	193 (73)	68 (63)	125 (81)	
LVAD pre-HT	45 (17)	11 (10)	34 (22)	0.01
BMI at HT	23.1 ± 4.5	24.0 ± 4.9	22.4 ± 4.1	0.006
BMI at OGTT			25.8 [23.7–27.7]	
Prednisolone use 1 year post-HT	236 (90)	102 (94)	134 (87)	0.03
Medication at OGTT				
Tacrolimus			140 (90)	
Ciclosporin			15 (10)	
Mycophenolate mofetil			65 (42)	
Everolimus			27 (17)	
Prednisolone			93 (60)	
Prednisolone dosage (mg)			5.0 [5.0–7.5]	
Glycemic status				
Transient hyperglycemia post-HT	115 (44)	31 (29)	84 (54)	<0.001
Reversed PTDM post-HT	37 (14)	7 (6)	30 (19)	0.003
Known DM at OGTT	71 (27)	71 (66)	0 (0)	<0.001
DM pre-HT	22 (8)	22 (20)	0 (0)	
DM post-HT	49 (19)	49 (45)	0 (0)	
Rejections[a]	1 [0–2]	1 [0–2]	1 [0–2]	0.007
CMV infection	53 (20)	19 (18)	34 (22)	0.39

[a]*Number of rejections treated with methylprednisolone.*

Baseline characteristics of all patients and those who underwent oral glucose tolerance test (including patients in whom the diagnosis was determined based on fasting glucose and HbA1c). Continuous variables are demonstrated with mean ± standard deviation when normally distributed and median with [25th—75th percentile] when not normally distributed. Categorical variables are demonstrated with numbers and (%).

Abbreviations: BMI, body mass index; CMP, cardiomyopathy; CMV, cytomegalovirus; DM, diabetes mellitus; HT, heart transplantation; LVAD, left ventricular assist device; OGTT, oral glucose tolerance test.

PATIENTS AND METHODS

Study Population

In this cross-sectional study, all adult HT patients who were more than 1 year post-HT that were seen at our outpatient clinic between August 2018 and April 2021 were screened to undergo an OGTT. Patients with known diabetes, an active infection/rejection treatment, patients who were treated for a malignancy and patients unwilling or unable to undergo OGTT were excluded. Information on immunosuppressive regimen has been published before (22). This study was conducted according to the Declaration of Helsinki and was approved by the Erasmus MC Ethics committee (MEC-2017-421).

Oral Glucose Tolerance Test

The OGTT was performed according to the guidelines of de American Diabetes Association (ADA) (4). A fasting glucose level and HbA1c were measured at the outpatient clinic after which 75 g of glucose in a 200 ml solution was administered. After 2 hours a second glucose measurement was performed. Glucose measurements were performed using a finger prick (Accu-Check® Inform II, Roche) while HbA1c was measured in normal blood draws. In case a patient was unable to undergo an OGTT at the outpatient clinic due to the travel distance to the hospital, the OGTT was performed by the patient's general practitioner with strict instructions.

Definitions

Transient hyperglycemia was defined as a patient needing insulin because of hyperglycemia in the first days post-operatively up until 45 days, based on the consensus document published by Sharif et al. (23). Patients still needing glucose-lowering drugs (oral or subcutaneous) after 45 days post-HT or developing PTDM during follow-up but who became normoglycemic without any glucose-lowering drugs during follow-up were labeled as recovered PTDM, as described in earlier studies (11). Cytomegalovirus (CMV) infection was defined as a patient with symptoms that were related to CMV replication.

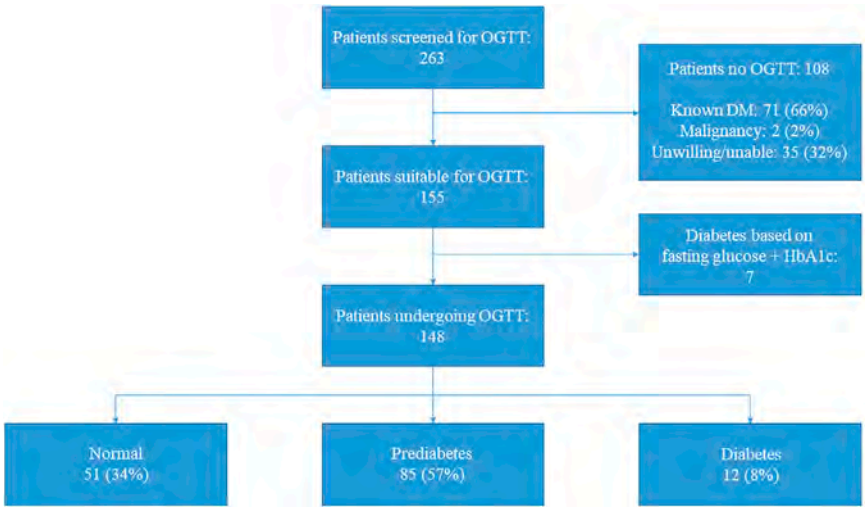


FIGURE 1 | Results screening and oral glucose tolerance test. Results of screening and oral glucose tolerance test for all patients (*n* = 263). Of the 35 patients unwilling/unable to undergo oral glucose tolerance test, six (17%) died before a test could be performed. Abbreviations: DM, diabetes mellitus; HbA1c, glycated hemoglobin; OGTT, oral glucose tolerance test.

TABLE 2 | Baseline characteristics based on oral glucose tolerance test result.

		Results OGTT		
Parameters	**Normal Range**	**Prediabetes**	**PTDM**	**p-value**
Number of patients	51	85	19	
Female	26 (51)	30 (35)	7 (37)	0.18
Ethnicity				0.28
Caucasian	47 (92)	73 (86)	18 (95)	
Black	2 (4)	1 (1)	0 (0)	
Other	2 (4)	11 (13)	1 (5)	
Age at HT (years)	38.7 [22.2–52.1]	49.3 [33.9–55.1]	45.6 [25.4–50.8]	0.08
Age at OGTT (years)	49.7 [30.4–56.7]	57.7 [46.9–66.1]	54.8 [42.0–65.4]	0.019
Time HT—OGTT (years)	8.4 [3.5–11.8]	8.5 [5.1–15.0]	11.5 [5.2–19.1]	0.13
Etiology heart failure				0.23
Ischemic CMP	6 (12)	19 (22)	5 (26)	
Non-ischemic CMP	45 (88)	66 (78)	14 (74)	
LVAD pre-HT	12 (24)	18 (21)	3 (16)	0.78
BMI at HT	22.3 ± 4.7	22.4 ± 3.6	22.9 ± 4.4	0.85
BMI at OGTT	25.1 [23.3–27.1]	25.9 [24.1–27.6]	26.7 [23.0–30.6]	0.56
Prednisolone use 1 year post-HT	42 (82)	75 (88)	17 (89)	0.74
Medication at OGTT				
Tacrolimus	49 (96)	75 (88)	16 (84)	0.21
Ciclosporin	2 (4)	10 (12)	3 (16)	0.21
Mycophenolate mofetil	23 (45)	35 (41)	7 (37)	0.81
Everolimus	6 (12)	19 (22)	2 (11)	0.20
Prednisolone	27 (53)	50 (59)	16 (84)	0.056
Prednisolone dosage (mg)	5 [5–7.5]	5 [5–7.5]	7.5 [5–10]	0.20
Glycemic status				
Transient hyperglycemia post-HT	32 (63)	46 (54)	6 (32)	0.054
Reversed PTDM	6 (12)	17 (20)	7 (37)	0.07
Rejections[a]	1 [0–1]	1 [0–2]	1 [0–2]	0.18
CMV infection	10 (20)	20 (24)	4 (21)	0.86

[a]*Number of rejections treated with methylprednisolone.*

Baseline characteristics of all patients and those who underwent oral glucose tolerance test (including patients in whom the diagnosis was determined based on fasting glucose and HbA1c). Continuous variables are demonstrated with mean ± standard deviation when normally distributed and median with [25th–75th percentile] when not normally distributed. Categorical variables are demonstrated with numbers and (%).

Abbreviations: BMI, body mass index; CMP, cardiomyopathy; CMV, cytomegalovirus; DM, diabetes mellitus; HT, heart transplantation; LVAD, left ventricular assist device; OGTT, oral glucose tolerance test; PTDM, post-transplant diabetes mellitus.

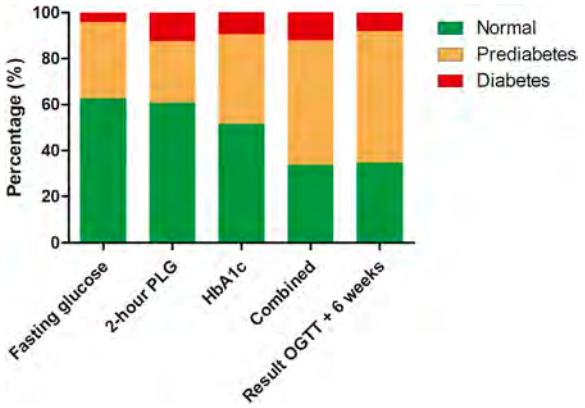


FIGURE 2 | Results of oral glucose tolerance test specified by detection method, combination of all the results and with confirmation test after 6 weeks. Abbreviations: HbA1c, glycated hemoglobin; OGTT, oral glucose tolerance test; PLG, postload glucose.

The definitions for the results of the OGTTs were according to the ADA guidelines (4), which are demonstrated in **Supplementary Table S1**. When a patient had a test result in the diabetic range, a confirmation test was repeated after 6 weeks. When a patient had both an increased HbA1c level within the diabetes range as well as an increased fasting glucose within the diabetes range at the outpatient clinic, diabetes was diagnosed.

Statistical Analysis

Normality of distribution was tested using the Shapiro-Wilks test. Continuous variables were expressed with a mean ± standard deviation (SD) when normally distributed and compared with a student t-test or one-way ANOVA depending on the number of groups. If the data were not normally distributed a median was presented with the 25th—75th percentile (Interquartile range, IQR) and compared using a Mann-Whitney or Kruskal Wallis test. Categorical variables were demonstrated as numbers with percentages (%) and compared with a Chi square or Fisher's exact test where appropriate. To determine risk factors for an abnormal test result from OGTT (PTDM and prediabetes range tests) a binary logistic regression analysis was performed. First, an univariable analysis was performed including the sex of the recipient and the recipient age at the time of the HT. In order to not overfit the analysis, a propensity score was created which included variables that have been linked to PTDM in the literature. These variables included: ethnicity, time between heart transplantation and OGTT, heart failure etiology, transient hyperglycemia, resolved PTDM, number of rejections (patients with more than 3 rejections included into one group), cytomegalovirus disease, body mass index at time of OGTT, tacrolimus use at time of OGTT, and prednisolone use at time of OGTT. In a multivariable analysis, age, recipient sex and the propensity score were included. Additionally, an ordinal regression analysis was performed. In this analysis, age and a propensity score including all previously mentioned variables and recipient gender were included. A p-value < 0.05 was considered as statistically significant. The data were analyzed with IBM SPSS statistics 25 (IBM Corp., New Orchard Road, Amonk, NY10504, United States).

TABLE 3 | Logistic regression analysis investigating determinants of an abnormal oral glucose tolerance test result (prediabetes or diabetes range test).

	Abnormal OGTT Result		
	OR (95% CI)		
	Univariable	**Model 1**	**Model 2**
Age recipient at HT	1.02 (1.00–1.04)	1.02 (1.00–1.04)	1.03 (1.00–1.06)
P-value	0.036	0.07	0.044
Female recipient	0.53 (0.27–1.05)	0.59 (0.29–1.18)	0.60 (0.28–1.31)
P-value	0.07	0.14	0.20

Univariable analysis of recipient sex and recipient age at heart transplantation individually in the model.
Model 1: Model including sex and age at the time of the heart transplantation of the recipient.
Model 2: Model including sex and age adjusted for the propensity score which included the following parameters: ethnicity, time between heart transplantation and CT scan, heart failure etiology, transient hyperglycemia, resolved PTDM, number of rejections, cytomegalovirus disease, body mass index at time of OGTT, tacrolimus use at time of OGTT, and prednisolone use at time of OGTT. *Patients with 3 or more rejections were combined into one group due to the small number of patients.*
Abbreviations: CI, confidence interval; HT, heart transplantation; OGTT, oral glucose tolerance test; OR, odds ratio.

TABLE 4 | Ordinal regression analysis investigating determinants of the oral glucose tolerance test results.

	Abnormal OGTT result	
	OR (95% CI)	
	Model 1	**Model 2**
Age recipient at HT	1.02 (1.00–1.04)	1.03 (1.00–1.05)
P-value	0.023	0.044

Model 1: unadjusted
Model 2: Model including age adjusted for the propensity score which included the following parameters: recipient sex, ethnicity, time between heart transplantation and CT scan, heart failure etiology, transient hyperglycemia, resolved PTDM, number of rejections, cytomegalovirus disease, body mass index at time of OGTT, tacrolimus use at time of OGTT, and prednisolone use at time of OGTT.*
**Patients with 3 or more rejections were combined into one group due to the small number of patients. Abbreviations: CI, confidence interval; HT, heart transplantation; OGTT, oral glucose tolerance test; OR, odds ratio.*

RESULTS

Study Population

In total, 263 patients were screened at the outpatient clinic of whom 96 (37%) were female. The median age at HT was 47.1 [32.5–55.0] years old. The etiology of heart failure pre-HT was ischemic cardiomyopathy in 27% of patients; 17% had a left ventricular assist device pre-HT. During the admission directly after HT, 115 (44%) developed transient hyperglycemia. Reversed PTDM was seen in 37 (14%) patients, while 71 (27%) patients had known diabetes mellitus. Other baseline characteristics are demonstrated in **Table 1**. Of the 263 patients, 108 were excluded for OGTT after screening (**Figure 1**) based on the following exclusion criteria: known diabetes mellitus in 71 (66%) patients (of whom 20% developed

diabetes pre-HT and 45% post-HT), 35 (32%) patients who were unable or unwilling to undergo OGTT (of whom six died before an OGTT was performed), and two (2%) patients underwent treatment for an active malignancy.

Oral Glucose Tolerance Test

In 148 out of 155 patients an OGTT was performed since in seven patients PTDM could be diagnosed based on solely the fasting glucose level in combination with the HbA1c (**Figure 1**). Baseline characteristics are demonstrated in **Table 1**. Forty-one percent were female and the most common ethnicity was Caucasian (89%). The median age at the time of the OGTT was 54.3 [42.2–64.3] years old and patients were 8.5 [4.8–14.5] years post-HT. Median BMI was 25.8 [23.7–27.7] kg/m^2 and transient hyperglycemia and reversed PTDM were seen in 54% and 19%, respectively.

When including the results after 6 weeks to confirm (when needed) the diagnosis of PTDM, based on OGTT 51 (34%) of patients had a normal glucose tolerance, 85 (57%) had a prediabetes range test and 12 (8%) had a PTDM range test. Together with the 7 patients in whom the diagnosis was confirmed using fasting glucose and HbA1c, 51/155 (33%) had a normal range test, 85/155 (55%) had a prediabetes range test and 19/155 (12%) of patients had a diabetes range test during the study period. The baseline characteristics of the patients undergoing OGTT (n = 155) stratified by OGTT result are demonstrated in **Table 2**.

In total, 40 patients (27%) were within 5 years post-HT and 108 (73%) were more than 5 years post-HT. When the results of the OGTTs were stratified according to the time post-transplant, no significant differences were seen in the results (p = 0.33) as is demonstrated in **Supplementary Table S2**.

Additional Value of Oral Glucose Tolerance Test

For the patients who underwent an OGTT, the results of each component of the OGTT (fasting glucose, 2 h postload glucose, and HbA1c) are demonstrated in **Figure 2**. Based on the OGTT data, 123 (83%) of the patients with (a combination of) only a fasting serum glucose test and an HbA1c measurement would have been be correctly classified. Of the remaining 25 (17%) patients who were not correctly classified, 14 (56%) had prediabetes and 11 (44%) diabetes. When including the results of the repeated OGTT after 6 weeks (confirming the PTDM diagnosis), 127 (86%) would have been classified correctly, while 21 (14%) would not. Of the patients who were not correctly classified, 15 (71%) had prediabetes and 6 (29%) PTDM. When looking at all patients with prediabetes range results, 15 (18%) of patients would have been missed without an OGTT. In diabetes range patients this would have been the case in 6 (50%) of the cases.

Determinants of Abnormal Oral Glucose Tolerance Test

In order to define determinants for an abnormal OGTT, patients with a prediabetes and PTDM range test were combined. Univariable analysis demonstrated that age at HT (OR 1.02 (1.00–1.04), p = 0.036) was a significant determinant of an abnormal test, while recipient gender was not (OR 0.53 (0.27–1.05), p = 0.07) (**Table 3**). When both recipient age, recipient gender and the propensity score were included in one model, age at HT was still a significant determinant (OR 1.03 (1.00–1.06), p = 0.044). In ordinal regression analysis, this association between recipient age at HT and OGTT outcome was confirmed (OR 1.03 (1.00–1.05), p = 0.044) (**Table 4**).

DISCUSSION

This study shows that impaired glucose metabolism is highly prevalent in patients long term post-HT. Based on OGTT, 104 of 155 patients without known PTDM (67%) had an abnormal test of whom 85 (55%) had a prediabetes range test, while 19 (12%) had a PTDM range test. When stratified by time since HT (≤ or > 5 years), there was no difference in the test results (p = 0.33). An OGTT demonstrated 18% more prediabetes and 50% more PTDM compared to a fasting glucose level and HbA1c only. Age at HT was significantly associated with the result of the OGTT.

This is the first study investigating the long-term prevalence of prediabetes and diabetes in HT recipients. In the registry from the ISHLT, diabetes mellitus is monitored up until 5 years post-HT, where diabetes status is reported in accordance with the clinical diagnostic guidance in place at the reporting transplant center (16). The incidence of diabetes mellitus at 5 years in the registry between 1995 and 2017 was 33.8%, which is higher than what was seen in our population before we performed the OGTT (27%) (16). Even though our patients were regularly seen at the outpatient clinic, 12% of patients had a PTDM range test when using the OGTT. Overall, this increased the total number of patients with PTDM from 27% to 34%, a relative increase of 26%. This demonstrates that a high percentage of patients have unnoticed PTDM despite regular blood test for abnormal glucose metabolism (mostly by assessment of non-fasting glucose levels). This is emphasized by our analysis on the added value of the OGTT compared to a fasting glucose and HbA1c. By performing an OGTT, prediabetes and PTDM were diagnosed in 18% and 50% more patients, respectively. To our knowledge, this is the first study demonstrating this significant advantage in heart transplantation recipients. Ussif et al. investigated kidney transplant recipients who underwent OGTT 1 year post-transplant (24). In this study, an OGTT only identified two patients who would have been missed compared to a combination of fasting glucose and HbA1c (24). This could be due to the difference in the timing of the OGTT, or a significant change in metabolism (given the other comorbidities that patients develop over time) in heart transplant recipients (16). However, our study illustrates that regular testing of the glycemic status through OGTT is warranted in (heart) transplant patients as is recommended by the American Diabetes Association (4).

In our study, a total of 55% of patients had a prediabetes range test. It is essential to monitor the glucose metabolism closely in this patient population because of their high cardiovascular risk profile and highly prevalent risk factors such as hypertension,

dyslipidemia, renal dysfunction but also the chronic use of steroids (25). When these patients also have PTDM, the cardiovascular risk significantly further increases (17). Whether this is also applicable in HT patients with prediabetes needs to be further investigated. Studies in non-transplant patients have demonstrated that individuals with prediabetes have a significantly increased risk to develop diabetes mellitus as well as cardiovascular morbidity and mortality (6, 7). Moreover, in a recent study in kidney transplant recipients, patients with prediabetes had similar risks for cardiovascular mortality as patients with PTDM (26). This demonstrates that patients with prediabetes should be monitored carefully and diagnosed early. As impaired glucose metabolism is a modifiable risk factor, the question remains whether an intervention will result in the reduction of cardiovascular events. Unfortunately, currently, no studies have been performed in HT recipients. Only one study showed that patients with prediabetes pre-HT had no increased risk of mortality after a median of 50 months post-HT (18). This study only used HbA1c levels to determine whether a patient had prediabetes, which could underestimate the true number of patients with prediabetes. Overall, the question remains whether patients who develop prediabetes post-HT have an accelerated progression of cardiac allograft vasculopathy (CAV) and/or an increased risk of micro- or macrovascular complications and mortality.

Age at HT was a significant determinant for an abnormal OGTT. Unfortunately, we were not able to include other determinants of PTDM in the analysis due to the relatively small study population, such as immunosuppressive regimen (prednisolone, tacrolimus), rejections, and CMV infection (10, 17). However, in univariable analysis, these factors were not associated with worse outcomes. There are several reasons for this. First of all, in patients who are more than 1 year post-HT, the significance of risk factors such as rejections probably become less important in the development of PTDM, especially since most rejections occur in the first year post-HT (16) and steroid use is tapered after the first year as is seen in our study. In univariable analysis, prednisolone use during OGTT was not associated with the OGTT outcome. Even though prednisolone is a risk factor for PTDM (10), one study in kidney transplant recipients demonstrated that patients who use prednisolone in a daily dosage of 5 mg or lower had no improvement in insulin sensitivity (assessed with glucose clamp technique) (27). This was confirmed by a Cochrane review including kidney transplant recipients which demonstrated that withdrawal of steroids did not decrease the risk on PTDM (28). Moreover, withdrawal of prednisolone increased the risk for acute rejection (28). In our opinion, prednisolone withdrawal should only be performed in patients who have a low risk for the development of an acute rejection episode and for other reasons than PTDM prevention. In other patients, steroids could be used with a daily dosage of 5 mg in order to reduce PTDM risk as much as possible. When patients do develop diabetes, the newer drugs to treat diabetes are of special interest since these drugs have cardio-renal-metabolic effects (i.e., sodium-glucose cotransporter-2 (SGLT2) inhibitors or glucagon-like peptide 1 (GLP1) analogues) (29,30).

Our study has several limitations. First of all, this is a single-center study which comes with all its limitations such as generalizability. In our study population, around 10% of patients were unable or unwilling to undergo OGTT which could increase the risk for selection bias. Ultimately, this could mean that the numbers in our study underestimate the frequency of prediabetes and PTDM long-term post-HT. Furthermore, most patients in our population were Caucasian which makes it difficult to extrapolate our study results to populations consisting of other ethnicities.

In conclusion, our study demonstrated that both prediabetes and PTDM are frequently observed in patients not known with PTDM long-term post-HT. Age at HT was a determinant for an abnormal OGTT. OGTT is the preferred test to screen for prediabetes and PTDM since it identifies significantly more patients than (fasting) glucose and HbA1c levels alone. Future studies are needed to investigate the impact of prediabetes and PTDM diagnosed long-term post-HT on transplant-related outcomes as well as future cardiovascular complications in this high-risk population. Furthermore, studies are needed to investigate the effects of glucose-lowering interventions (with lifestyle and/or medication) on progression of prediabetes to PTDM and prevention of (cardiovascular) complications.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available due to privacy reasons. Reasonable requests to access the datasets should be directed to o.manintveld@erasmusmc.nl.

ETHICS STATEMENT

This study involving human participants were reviewed and approved by Erasmus MC Ethics committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SR participated in conception and design of the research, in the acquisition of the data, the statistical analysis, drafting the manuscript and critical revision of the final version of the manuscript. MG-S participated in conception and design of the research, in the acquisition of the data, drafting the manuscript and critical revision of the final version of the manuscript. JK participated in conception and design of the research, drafting the manuscript and critical revision of the final version of the manuscript. AC participated in the

acquisition of the data, drafting the manuscript and critical revision of the final version of the manuscript. YT participated in drafting the manuscript and critical revision of the final version of the manuscript. FZ supervision, drafting the manuscript and critical revision of the final version of the manuscript AZ participated in conception and design of the research, supervision, drafting the manuscript and critical revision of the final version of the manuscript. OM participated in conception and design of the research, in the acquisition of the data, supervision, drafting the manuscript and critical revision of the final version of the manuscript.

CONFLICT OF INTEREST

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

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10113/full#supplementary-material

REFERENCES

1. Zheng Y, Ley SH, Hu FB. Global Aetiology and Epidemiology of Type 2 Diabetes Mellitus and its Complications. *Nat Rev Endocrinol* (2018) 14:88–98. doi:10.1038/nrendo.2017.151
2. Collaboration NCDRF. Worldwide Trends in Diabetes since 1980: a Pooled Analysis of 751 Population-Based Studies with 4.4 Million Participants. *Lancet* (2016) 387:1513–30. doi:10.1016/S0140-6736(16)00618-8
3. Global Burden of Metabolic Risk Factors for Chronic Diseases C. Cardiovascular Disease, Chronic Kidney Disease, and Diabetes Mortality burden of Cardiometabolic Risk Factors from 1980 to 2010: a Comparative Risk Assessment. *Lancet Diabetes Endocrinol* (2014) 2:634–47. doi:10.1016/S2213-8587(14)70102-0
4. American Diabetes A. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2019. *Diabetes Care* (2019) 42:S13–S28. doi:10.2337/dc19-S002
5. Menke A, Casagrande S, Geiss L, Cowie CC. Prevalence of and Trends in Diabetes Among Adults in the United States, 1988-2012. *Jama* (2015) 314:1021–9. doi:10.1001/jama.2015.10029
6. Cai X, Zhang Y, Li M, Wu JH, Mai L, Li J, et al. Association between Prediabetes and Risk of All Cause Mortality and Cardiovascular Disease: Updated Meta-Analysis. *Bmj* (2020) 370:m2297. doi:10.1136/bmj.m2297
7. Ford ES, Zhao G, Li C. Pre-Diabetes and the Risk for Cardiovascular Disease. *J Am Coll Cardiol* (2010) 55:1310–7. doi:10.1016/j.jacc.2009.10.060
8. Buysschaert M, Bergman M. Definition of Prediabetes. *Med Clin North America* (2011) 95:289–97. doi:10.1016/j.mcna.2010.11.002
9. Milman S, Crandall JP, Mechanisms of Vascular Complications in Prediabetes. *Med Clin North America* (2011) 95:309–25. doi:10.1016/j.mcna.2010.11.004
10. 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
11. Cho MS, Choi H-I, Kim I-O, Jung S-H, Yun T-J, Lee J-W, et al. The Clinical Course and Outcomes of post-transplantation Diabetes Mellitus after Heart Transplantation. *J Korean Med Sci* (2012) 27:1460–7. doi:10.3346/jkms.2012.27.12.1460
12. Kim HJ, Jung S-H, Kim J-J, Yun T-J, Kim JB, Choo SJ, et al. New-Onset Diabetes Mellitus after Heart Transplantation — Incidence, Risk Factors and Impact on Clinical Outcome —. *Circ J* (2017) 81:806–14. doi:10.1253/circj.cj-16-0963
13. Nieuwenhuis MG, Kirkels JH. Predictability and Other Aspects of post-transplant Diabetes Mellitus in Heart Transplant Recipients. *J Heart Lung Transplant* (2001) 20:703–8. doi:10.1016/s1053-2498(01)00257-1
14. Hackman KL, Snell GI, Bach LA. Prevalence and Predictors of Diabetes after Lung Transplantation: a Prospective, Longitudinal Study. *Diabetes Care* (2014) 37:2919–25. doi:10.2337/dc14-0663
15. Stepanova M, Henry L, Garg R, Kalwaney S, Saab S, Younossi Z. Risk of De Novo post-transplant Type 2 Diabetes in Patients Undergoing Liver Transplant for Non-alcoholic Steatohepatitis. *BMC Gastroenterol* (2015) 15:175. doi:10.1186/s12876-015-0407-y
16. Khush KK, Cherikh WS, Chambers DC, Harhay MO, Hayes D, Hsich E, et al. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: Thirty-Sixth Adult Heart Transplantation Report - 2019; Focus Theme: Donor and Recipient Size Match. *J Heart Lung Transplant* (2019) 38:1056–66. doi:10.1016/j.healun.2019.08.004
17. Vest AR, Cherikh WS, Noreen SM, Stehlik J, Khush KK. New-onset Diabetes Mellitus after Adult Heart Transplantation and the Risk of Renal Dysfunction or Mortality. *Transplantation* (2022) 106:178–87. doi:10.1097/tp.0000000000003647
18. Chamarthi B, Zafrir B, Plutzky J, Givertz MM. Impact of Pre-diabetes on Heart Transplant Outcomes in Patients with Advanced Heart Failure. *J Heart Lung Transplant* (2014) 33:215–7. doi:10.1016/j.healun.2013.10.018
19. Fonville S, Zandbergen AAM, Vermeer SE, Dippel DWJ, Koudstaal PJ, den Hertog HM. Prevalence of Prediabetes and Newly Diagnosed Diabetes in Patients with a Transient Ischemic Attack or Stroke. *Cerebrovasc Dis* (2013) 36:283–9. doi:10.1159/000353677
20. Ferrannini G, De Bacquer D, De Backer G, Kotseva K, Mellbin L, Wood D, et al. Screening for Glucose Perturbations and Risk Factor Management in Dysglycemic Patients with Coronary Artery Disease-A Persistent Challenge in Need of Substantial Improvement: A Report from ESC EORP EUROASPIRE V. *Diabetes Care* (2020) 43:726–33. doi:10.2337/dc19-2165
21. Costanzo MR, Dipchand A, Starling R, Anderson A, Chan M, Desai S, et al. The International Society of Heart and Lung Transplantation Guidelines for the Care of Heart Transplant Recipients. *J Heart Lung Transpl* (2010) 29:914–56. doi:10.1016/j.healun.2010.05.034
22. Zijlstra LE, Constantinescu AA, Manintveld O, Birim O, Hesselink DA, van Thiel R, et al. Improved Long-Term Survival in Dutch Heart Transplant Patients Despite Increasing Donor Age: the Rotterdam Experience. *Transpl Int* (2015) 28:962–71. doi:10.1111/tri.12503
23. Sharif A, Hecking M, de Vries APJ, Porrini E, Hornum M, Rasoul-Rockenschaub S, et al. Proceedings from an International Consensus Meeting on Posttransplantation Diabetes Mellitus: Recommendations and Future Directions. *Am J Transpl* (2014) 14:1992–2000. doi:10.1111/ajt.12850
24. Ussif AM, Åsberg A, Halden TAS, Nordheim E, Hartmann A, Jenssen T. Validation of Diagnostic Utility of Fasting Plasma Glucose and HbA1c in Stable Renal Transplant Recipients One Year after Transplantation. *BMC Nephrol* (2019) 20:12. doi:10.1186/s12882-018-1171-3
25. Lund LH, Edwards LB, Dipchand AI, Goldfarb S, Kucheryavaya AY, Levvey BJ, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirty-Third Adult Heart Transplantation Report-2016; Focus Theme: Primary Diagnostic Indications for Transplant. *J Heart Lung Transplant* (2016) 35:1158–69. doi:10.1016/j.healun.2016.08.017
26. Porrini E, Díaz JM, Moreso F, Lauzurrica R, Ibernon M, Torres IS, et al. Prediabetes Is a Risk Factor for Cardiovascular Disease Following Renal Transplantation. *Kidney Int* (2019) 96:1374–80. doi:10.1016/j.kint.2019.06.026

27. Midtvedt K, Hjelmesaeth J, Hartmann A. Insulin Resistance after Renal Transplantation: the Effect of Steroid Dose Reduction and Withdrawal. *J Am Soc Nephrol* (2004) 15:3233–9. doi:10.1097/01.asn.0000145435.80005.1e
28. Haller MC, Royuela A, Nagler EV, Pascual J, Webster AC. Steroid Avoidance or Withdrawal for Kidney Transplant Recipients. *Cochrane Database Syst Rev* (2016) CD005632. doi:10.1002/14651858.CD005632.pub3
29. Zelniker TA, Braunwald E. Mechanisms of Cardiorenal Effects of Sodium-Glucose Cotransporter 2 Inhibitors. *J Am Coll Cardiol* (2020) 75:422–34. doi:10.1016/j.jacc.2019.11.031
30. Cornell S. A Review of GLP-1 Receptor Agonists in Type 2 Diabetes: A Focus on the Mechanism of Action of Once-Weekly Agents. *J Clin Pharm Ther* (2020) 45(Suppl. 1):17–27. doi:10.1111/jcpt.13230

ESOT | Transplant International

ORIGINAL RESEARCH
published: 04 April 2022
doi: 10.3389/ti.2022.10172

Trends in Donation After Circulatory Death in Lung Transplantation in the United States: Impact Of Era

*Christopher M. Bobba[1,2], Bryan A. Whitson[2], Matthew C. Henn[2], Nahush A. Mokadam[2], Brian C. Keller[3], Justin Rosenheck[3] and Asvin M. Ganapathi[2]**

[1]Division of Thoracic and Cardiovascular Surgery, University of Florida Health, Gainesville, FL, United States, [2]Division of Cardiac Surgery, Department of Surgery, The Ohio State University Wexner Medical Center, Columbus, OH, United States, [3]Division of Pulmonary, Critical Care and Sleep Medicine, Department of Internal Medicine, The Ohio State University Wexner Medical Center, Columbus, OH, United States

Background: Use of lungs donated after circulatory death (DCD) has expanded, but changes in donor/recipient characteristics and comparison to brain dead donors (DBD) has not been studied. We examined the evolution of the use of DCD lungs for transplantation and compare outcomes to DBD lungs.

Methods: The SRTR database was used to construct three 5-year intervals. Perioperative variables and survival were compared by era and for DCD vs. DBD. Geographic variation was estimated using recipient permanent address.

Results: 728 DCD and 27,205 DBD lung transplants were identified. DCD volume increased from Era 1 (n = 73) to Era 3 (n = 528), representing 1.1% and 4.2% of lung transplants. Proportionally more DCD recipients were in ICU or on ECMO pre-transplant, and had shorter waitlist times. DCD donors were older, had lower PaO2/FiO2 ratios compared to DBD, more likely to be bilateral, had longer ischemic time, length of stay, post-op dialysis, and increased use of lung perfusion. There was no difference in overall survival. Geographically, use was heterogeneous.

Conclusion: DCD utilization is low but increasing. Despite increasing ischemic time and transplantation into sicker patients, survival is similar, which supports further DCD use in lung transplantation. DCD lung transplantation presents an opportunity to continue to expand the donor pool.

Keywords: lung transplantation, organ procurment, organ donation, donation after cardiac death, donation after brain death

OPEN ACCESS

***Correspondence:**
Asvin M. Ganapathi
asvin.ganapathi@osumc.edu

Received: *01 November 2021*
Accepted: *03 March 2022*
Published: *04 April 2022*

Citation:
Bobba CM, Whitson BA, Henn MC, Mokadam NA, Keller BC, Rosenheck J and Ganapathi AM (2022) Trends in Donation After Circulatory Death in Lung Transplantation in the United States: Impact Of Era. Transpl Int 35:10172. doi: 10.3389/ti.2022.10172

Abbreviations: ANOVA, analysis of variance; DBD, donation after brain death; DCD, donation after circulatory death; EVLP, ex vivo lung perfusion; ISHLT, international society for heart and lung transplantation; LAS, lung allocation score; PF, PaO2/FiO2; PGD, primary graft dysfunction; PMP, per million in population; OPO, organ procurement organization; TRR, transplant recipient registration form; UNOS/OPTN, united network for organ sharing/organ procurement and transplant network.

INTRODUCTION

Lung transplantation remains the gold standard therapy for end stage lung disease, however a shortage of viable organs remains (1, 2). Use of lungs from donors after circulatory death (DCD) has been instrumental in increasing organ supply. DCD use has increased to 4.8% of all lung transplants in 2018, and expansion in their use is an avenue for continued growth in available organs (3). Evidence demonstrating equivalent outcomes to donation after brain death (DBD) organs has led to increased utilization with similar mortality, primary graft dysfunction (PGD) and acute rejection rates at 1 and 5 years (4–7). These results are encouraging, however, studies have not performed an analysis of the DCD cohort compared to DBD over time including a profile of DCD lung donors and recipients in the United States.

Given the importance of DCD lungs in expanding the donor pool and some evidence of their equivalence to traditional DBD organs, a more thorough analysis is warranted. We hypothesized that geographic and individual center variation in the usage of DCD lungs still exists and that differences exist between DBD and DCD donors. Further, we anticipate that the profile of the recipients of DCD organs has changed over time and represents a more heterogeneous cohort than early experiences with DCD lung transplantation. In this study, we sought to characterize the evolving nature of the use of DCD organs for lung transplant, and compare donor, recipient, and operative characteristics with traditional DBD organs.

METHODS

Data Source and Patient Population

This retrospective cohort study utilized data from the United Network for Organ Sharing/Organ Procurement and Transplant Network (UNOS/OPTN) STAR file. The UNOS/OPTN STAR file is a well validated dataset of patients undergoing transplantation in the United States (1, 8). The study was submitted for Ohio State IRB approval (protocol: 2018H0079) and deemed exempt. The STAR file was queried from 5/1/2005 to 4/30/2020 to include all DCD and DBD lung transplants after implementation of the lung allocation score (LAS) in 2005. DCD and DBD recipient outcomes were collected using the identifier of NON_HRT_DON in the STAR file. Pediatric patients (age <18), those with a previous lung transplant, and multi-organ transplants were excluded from analysis. Three "eras" were constructed based on date of transplantation in 5-year increments: 5/1/2005-4/30/2010 (**era 1**), 5/1/2010-4/30/2015 (**era 2**), and 5/1/2015-4/30/2020 (**era 3**). We included all instances of DCD lung donation, including controlled and uncontrolled DCD. For purposes of geographic variations, the state of recipient permanent address was utilized to identify where usage of a DCD organ occurred.

Statistical Analysis

Data was analyzed for normality using the Kolmogorov-Smirnov test and missingness for each variable was calculated (**Supplementary Table S1**). Continuous data was compared with an analysis of variance (ANOVA) or the Kruskal-Wallis test for parametric and non-parametric data respectively. Categorical variables were compared using the Chi-Square test. Survival rates were calculated simultaneously across all 3 eras using the Kaplan-Meier method and the log-rank test. An additional Kaplan-Meier survival analysis examined each era of recipients of DCD lungs as compared to recipients of DBD lungs. A Cox proportional hazard model was created to examine the independent effect of era and DCD donors on survival. This model utilized the following covariates which were selected a priori: DCD status, era, LAS, age, body mass index (BMI), sex, waitlist time, diabetes, smoking history, pre-operative hospitalization status, yearly center volume, organ ischemic time, organ distance traveled, donor BMI, and donor age. De-identified recipient center ID numbers were used to determine center DCD lung transplantation volume. DCD utilization by state was determined according to recipient permanent address.

Missing data was excluded from analysis and no imputation was performed. In all cases $p < 0.05$ was considered significant. All statistical analysis was performed using R version 3.6.2 (Vienna, Austria).

RESULTS

Recipient, Donor and Operative Characteristics of DCD and DBD Organs

A total of 27,205 DBD organ, and 728 DCD organ lung transplants were identified from 5/1/2005 to 4/30/2020. Recipients of DCD organs were slightly older (61 vs. 60 years old, $p < 0.01$), more likely to be in the ICU prior to transplant (13.9% vs. 10.7%, $p < 0.01$) and more likely to require pre-operative ECMO (6.9% vs. 3.7%, $p < 0.01$) (**Table 1**). DCD organ donors were older (39 vs. 33 years, $p < 0.01$), more commonly Caucasian (81.7% vs. 61.2%, $p < 0.01$), had higher BMI (26.3 vs. 25.3, $p < 0.01$), and lower mean PaO2:FiO2 (PF) ratio (423 vs. 436, $p < 0.01$) than their DBD counterparts. In the DCD cohort, the inciting event leading to becoming a donor was more likely to be anoxia (39.3% vs. 21.6%, $p < 0.01$) and less likely trauma (31.2% vs. 43.6%, $p < 0.01$) than in the DBD organ cohort (**Table 2**).

Transplants utilizing DCD organs were more commonly bilateral lung transplants (76.9% vs. 69.8%, $p < 0.01$), had longer total ischemic time (6.3 vs. 5.1 h, $p < 0.01$), longer post-operative length of stay (21 vs. 16 days, $p < 0.01$), more commonly required dialysis (11.1% vs. 6.5%, $p < 0.01$), and were more likely to use Ex-vivo lung perfusion (EVLP) (27.2% vs. 3.7%, $p < 0.01$). DCD organ transplants also more commonly occurred at centers with higher annual total lung transplant volume (55.6 vs. 39.2 average yearly center volume for centers utilizing DCD lungs vs. centers only performing DBD lung transplantation, $p < 0.01$) (**Table 3**).

Recipient, Donor and Operative Characteristics of DCD Organs by Era

A total of 728 transplants using DCD lungs were identified across 3 eras with 73 transplants in era 1, 127 in era 2, and 528 in era 3. 2

TABLE 1 | Recipient demographics and baseline characteristics.

Variable	Overall	DBD	DCD	P-value
Cohort Size	*27,933*	*27,205*	*728*	
Age (y)	60 (51, 65)	60 (51, 65)	61 (53, 66)	0.01*
Male Sex	16,645 (59.6%)	16,194 (59.5%)	451 (62%)	0.20
Ethnicity				0.02*
Caucasian	22,779 (81.5%)	22,163 (81.5%)	616 (84.6%)	
African-American	2,524 (9%)	2,459 (9%)	65 (8.9%)	
Other	2,630 (9.4%)	2,583 (9.5%)	47 (6.5%)	
BMI (kg/m2)	25.7 (22, 29)	25.7 (22, 29)	25.4 (22, 28.7)	0.28
Former Smoker	16,551 (60.3%)	16,119 (60.3%)	432 (59.8%)	0.81
Diabetes	5,229 (18.8%)	5,088 (18.8%)	141 (19.4%)	0.74
GFR (mL/min/1.73m2)	92.8 (73.4, 120.4)	92.8 (73.4, 120.3)	96.1 (72.8, 122.1)	0.33
Diagnosis				0.70
Cystic Fibrosis/Immunodeficiency	3,028 (10.8%)	2,952 (10.9%)	76 (10.4%)	
Obstructive Lung disease	8,360 (29.9%)	8,128 (29.9%)	232 (31.9%)	
Pulmonary Vascular disease	1,076 (3.9%)	1,050 (3.9%)	26 (3.6%)	
Restrictive Lung disease	15,469 (55.4%)	15,075 (55.4%)	394 (54.1%)	
Blood Group				0.11
A	11,164 (40%)	10,861 (39.9%)	303 (41.6%)	
B	3,107 (11.1%)	3,039 (11.2%)	68 (9.3%)	
AB	1,082 (3.9%)	1,063 (3.9%)	19 (2.6%)	
O	12,580 (45%)	12,242 (45%)	338 (46.4%)	
Medical Condition				0.03*
Not Hospitalized	22,140 (80.1%)	21,578 (80.2%)	562 (77.2%)	
Hospitalized	2,508 (9.1%)	2,443 (9.1%)	65 (8.9%)	
In ICU	2,985 (10.8%)	2,884 (10.7%)	101 (13.9%)	
Functional Status				0.08
ADL With No Assistance	6,140 (22.5%)	5,986 (22.6%)	154 (21.5%)	
ADL With Assistance	10,379 (38.1%)	10,078 (38%)	301 (42%)	
Disabled/Hospitalized	10,741 (39.4%)	10,480 (39.5%)	261 (36.5%)	
On Ventilator	1,567 (5.6%)	1,523 (5.6%)	44 (6%)	0.66
LAS	40.2 (34.8, 51.8)	40.2 (34.8, 51.8)	39.1 (34.2, 51.7)	0.10
PRA	0 (0, 1)	0 (0, 1)	0 (0, 0)	0.02*
Days on Waitlist	59 (17, 184)	60 (17, 184)	49 (14, 175)	0.05*
Previous ECMO/on ECMO	1,051 (3.8%)	1,001 (3.7%)	50 (6.9%)	0.01*

*Data displayed as mean ± standard deviation (SD) median (interquartile range) for parametric or non-parametric continuous variables respectively and number (percent of total) for categorical variables. BMI, body mass index; DBD, donation after brain death; DCD, donation after circulatory death; GFR, glomerular filtration rate; ICU, intensive care unit; ADL, activities of daily living; LAS, lung allocation score; PRA, percent reactive antibodies; ECMO, extracorporeal membrane oxygenation. * indicates* $p < 0.05$.

donors (0.3%) were identified as uncontrolled DCD, and the remaining were controlled DCD. Median recipient age increased from 56 years (era 1) to 62 years (era 3) ($p < 0.01$) and there was an increase in disabled/hospitalized pre-operative functional status after the first era (era 1–15.5%, era 2–36.0%, era 3–39.4%; $p < 0.01$). Eras 2 and 3 had increased LAS ($p < 0.01$) and reduced waitlist time ($p < 0.01$). Additionally, later eras were associated with increases in transplant for restrictive lung disease ($p < 0.01$) (**Table 4**).

Regarding DCD donors, median age did not differ by era ($p = 0.90$), however in era 3, donors were less likely to have a significant smoking history ($p < 0.01$) and more likely to have a clinically diagnosed infection ($p < 0.01$). Median donor PF ratio also decreased after era 1 ($p = 0.03$). Other donor characteristics were similar amongst all eras (**Table 4**, **Supplementary Table S2**). The fraction of all lung transplants using a DCD donor increased from 1.1% of donors in era 1, to 1.5% in era 2 and 4.2% in era 3 ($p = 0.04$). The fraction of all organ donors that are DCD, including those in whom the lungs were not used, has significantly increased from 9.8% in era 1, to 13.9% in era 2, and 19.8% in era 3 ($p = 0.04$). DCD lung donor utilization calculated as the fraction of all DCD donors where a lung was procured and transplanted also significantly increased from 1.3% in era 1, to 2.2% in era 2, and 5% in era 3 ($p < 0.05$). Regarding transplant characteristics, there was an increase in the total ischemic time from 5.6 h in Era 1 to 6.5 h in Era 3 ($p < 0.01$). There was also an increase in post-transplant length of stay from 17 days in Era 1 to 22 days in Era 3 ($p = 0.03$).

Survival Analysis

With regard to survival there was no significant difference on unadjusted analysis between DCD and DBD organ recipients (**Figure 1**). Actuarial survival of DCD lung recipients at 3 years was 69.0% (95% CI: 65.1–73.3%) across all eras, 68.5% (CI: 95% 58.6–80.0%) in era 1, 66.8% (95% CI: 59.0–75.5%) in era 2 and 69.8% (95% CI: 65.6–75.4%) in era 3 ($p = 0.85$) (**Figure 1A**). There was no significant difference in survival between donor organs procured following brain death or circulatory death in all eras (**Figures 1B–D**). Cox proportional hazard model demonstrated usage of a DCD organ in lung transplantation was not associated with increased mortality (Hazard Ratio [HR] 1.04, 95% CI 0.91–1.19, $p = 0.55$), and transplant in more recent eras was associated with improved survival (era 2 HR 0.91, $p <$

TABLE 2 | Donor characteristics.

Variable	Overall	DBD	DCD	P-Value
Cohort Size	27,933	27,205	728	
Age	33 (23, 46)	33 (22, 46)	39 (28, 48)	< 0.01*
Male Sex	16,888 (60.5%)	16,455 (60.5%)	433 (59.5%)	0.61
Ethnicity				< 0.01*
Caucasian	17,243 (61.7%)	16,648 (61.2%)	595 (81.7%)	
African-American	5,177 (18.5%)	5,128 (18.8%)	49 (6.7%)	
Other	5,513 (19.8%)	5,429 (20.0%)	84 (11.6%)	
BMI (kg/m^2)	25.3 (22.4, 28.9)	25.3 (22.4, 28.9)	26.3 (23, 31)	< 0.01*
Coronary artery disease	1,487 (5.4%)	1,479 (5.5%)	8 (1.1%)	< 0.01*
Smoking History	2,595 (9.4%)	2,542 (9.5%)	53 (7.4%)	0.07
Recent cocaine Use	4,168 (15.2%)	4,022 (15.1%)	146 (20.2%)	0.01*
Diabetes	2,041 (7.3%)	1,990 (7.4%)	51 (7%)	0.80
Hypertension	6,540 (23.6%)	6,355 (23.5%)	185 (25.6%)	0.21
Inciting Event Leading to Donation				< 0.01*
Anoxia	6,154 (22%)	5,868 (21.6%)	286 (39.3%)	
CVA	8,856 (31.7%)	8,657 (31.8%)	199 (27.3%)	
Head Trauma	12,099 (43.3%)	11,872 (43.6%)	227 (31.2%)	
CNS Tumor	174 (0.6%)	173 (0.6%)	1 (0.1%)	
Other	649 (2.3%)	634 (2.3%)	15 (2.1%)	
Donor Bloodstream Infection	2,076 (7.4%)	2,019 (7.4%)	57 (7.8%)	0.73
Donor Clinical Infection	18,278 (66.4%)	17,785 (66.3%)	493 (68.1%)	0.34
Donor Pulmonary Infection	16,214 (58%)	15,778 (58%)	436 (59.9%)	0.33
PaO2/FiO2 Ratio	435.9 (373, 492)	436 (374, 492)	423 (360, 481)	< 0.01*

*Data displayed as mean ± standard deviation (SD) median (interquartile range) for parametric or non-parametric continuous variables respectively and number (percent of total) for categorical variables. BMI, body mass index; CVA, cerebrovascular accident; CNS, central nervous system. * indicates* p *< 0.05.*

TABLE 3 | Operative characteristics and postoperative outcomes.

Variable	Overall	DBD	DCD	P-Value
Cohort Size	*27933*	*27205*	*728*	
Type of Transplant				< 0.01
Bilateral	19,544 (70%)	18,984 (69.8%)	560 (76.9%)	
Single	8,389 (30%)	8,221 (30.2%)	168 (23.1%)	
Distance Traveled (nautical miles)	140 (26, 313)	142 (26, 313)	113.5 (16, 325.2)	0.284
Ischemia Time (hours)	5.1 (4.1, 6.2)	5.1 (4.1, 6.2)	6.3 (5.1, 8.2)	< 0.01
Length of Stay (days)	16 (11, 27)	16 (11, 27)	21 (14, 37)	< 0.01
Postop Dialysis	1,821 (6.6%)	1,740 (6.5%)	81 (11.1%)	<0.01
Postop Stroke	618 (2.3%)	603 (2.3%)	15 (2.1%)	0.83
Postop Airway Dehiscence	414 (1.5%)	398 (1.5%)	16 (2.2%)	0.16
In-Hospital Mortality	1,255 (4.6%)	1,213 (4.6%)	42 (5.9%)	0.12
Acute Rejection Before Discharge				0.01
Yes & Treated with Immunosuppressant	1,999 (7.3%)	1,926 (7.2%)	73 (10%)	
Yes & Not Treated with Immunosuppressant	303 (1.1%)	293 (1.1%)	10 (1.4%)	
No	25,270 (91.7%)	24,625 (91.7%)	645 (88.6%)	
Rejection Treatment Within One Year	5,657 (26.5%)	5,514 (26.4%)	143 (28.6%)	0.30
Lung Perfusion Used	330 (4.8%)	241 (3.7%)	89 (27.2%)	<0.01

*Data displayed as mean ± standard deviation (SD) median (interquartile range) for parametric or no-parametric continuous variables respectively and number (percent of total) for categorical variables. Lung perfusion data available from 2/28/2018-4/30/2020. * indicates* p *< 0.05.*

0.01, and era 3 HR 0.85, $p < 0.01$) compared to era 1. Diabetes, poorer pre-operative health status, and donor smoking were all also associated with reduced survival in this model (**Figure 2**).

Center Volume Trends and Geographic Variation in DCD Organ Use

41 different centers transplanted a lung from a DCD donor since 2005. The total number of centers utilizing DCD lungs for transplantation increased from 14 in era 1 to 24 in era 2 to 38 in era 3 (**Table 4**). Of all U.S. centers performing lung transplantation, 21.2% performed a DCD lung transplant in era 1, 33.8% in era 2, and 54.3% in era 3. Within centers that used DCD lungs, the total DCD lung volume was stable between era 1 and 2 before increasing in era 3 (**Figure 3**). Of all centers participating in DCD lung usage, in era 1 64.3% transplanted 1-5 DCD lungs, in era 2 this grew to 79.2%, and shrank to 39.5% in era 3. However, in era 3, 28.9% of centers transplanted >15 DCD lungs, compared to 7.1% in era 1. There was also geographic variation observed in DCD use over time (**Figure 4**; **Supplementary Table S3**). When weighted by population, Ohio

TABLE 4 | Selected DCD characteristics by era.

Variable	Overall	Era 1	Era 2	Era 3	P-value
Date Range	*5/1/05 to 4/30/20*	*5/1/05 to 4/30/10*	*5/1/10 to 4/30/15*	*5/1/15 to 4/30/20*	
Cohort Size	*728*	*73*	*127*	*528*	
Recipient Characteristics					
Age (y)	61 (53, 66)	56 (46, 62)	60 (49.5, 64)	62 (55, 67)	<0.01*
Male sex (%)	451 (62%)	48 (65.8%)	81 (63.8%)	322 (61%)	0.66
Diagnosis					<0.01*
Cystic Fibrosis/Immunodeficiency	76 (10.4%)	12 (16.4%)	17 (13.4%)	47 (8.9%)	
Obstructive Lung disease	232 (31.9%)	35 (47.9%)	33 (26%)	164 (31.1%)	
Pulmonary Vascular disease	26 (3.6%)	3 (4.1%)	3 (2.4%)	20 (3.8%)	
Restrictive Lung disease	394 (54.1%)	23 (31.5%)	74 (58.3%)	297 (56.2%)	
Medical Condition					0.13
Not Hospitalized	562 (77.2%)	61 (83.6%)	89 (70.1%)	412 (78%)	
Hospitalized	65 (8.9%)	6 (8.2%)	17 (13.4%)	42 (8%)	
In ICU	101 (13.9%)	6 (8.2%)	21 (16.5%)	74 (14%)	
Functional Status					<0.01*
ADL With No Assistance	154 (21.5%)	36 (50.7%)	18 (14.4%)	100 (19.2%)	
ADL With Assistance	301 (42%)	24 (33.8%)	62 (49.6%)	215 (41.3%)	
Disabled/Hospitalized	261 (36.5%)	11 (15.5%)	45 (36%)	205 (39.4%)	
On Ventilator	44 (6%)	5 (6.8%)	13 (10.2%)	26 (4.9%)	0.08
LAS	39.1 (34.2, 51.7)	36 (33.2, 41.8)	42.8 (35, 59.5)	39.1 (34.3, 51.7)	<0.01*
PRA	0 (0, 0)	0 (0, 3)	0 (0, 2.5)	0 (0, 0)	<0.01*
Days on Waitlist	49 (14, 175)	138 (47, 368)	54 (12.5, 198)	44 (14, 138.5)	<0.01*
Previous ECMO/on ECMO	50 (6.9%)	4 (5.5%)	11 (8.7%)	35 (6.6%)	0.64
Donor Characteristics					
Age	39 (28, 48)	41 (29, 47)	39 (26.5, 49)	38 (28, 48)	0.90
Male sex (%)	433 (59.5%)	40 (54.8%)	90 (70.9%)	303 (57.4%)	0.02*
Smoking History	53 (7.4%)	12 (16.4%)	9 (7.1%)	32 (6.1%)	<0.01*
Anoxia Cause of Brain Injury	286 (39.3%)	24 (32.9%)	44 (34.6%)	218 (41.3%)	<0.01*
Donor Pulmonary Infection	436 (59.9%)	23 (31.5%)	70 (55.1%)	343 (65%)	< 0.01*
PaO2/FiO2 Ratio	416.1 ± 88.3	443.1 ± 84.2	416.5 ± 87.7	412.4 ± 88.6	0.03*
DCD Donor Lung Utilization (%)[A]	3.3%	1.3%	2.2%	5.0%	0.05*
Percentage of organ donors that are DCD[B]	15.1% (20,396/ 135,521)	9.8% (3,883/ 39,755)	13.9% (5,745/ 41,450)	19.8% (10,768/ 54,316)	0.04*
DCD Fraction of all Lung Transplants (%)	2.6%	1.1%	1.5%	4.2%	0.04*
Operative Characteristics and Outcomes					
Single Lung Transplant	168 (23.1%)	18 (24.7%)	46 (36.2%)	104 (19.7%)	<0.01*
Centers with DCD Lung transplant (% of all Lung Transplant Centers)	41 (51.3%)	14 (21.2%)	24 (33.8%)	38 (54.3%)	
Center DCD Volume	4 (2, 12)	3 (1, 6.75)	3 (1.75, 4.25)	10 (3.25, 18)	<0.01*
Ischemia Time (hours)	6.3 (5.1, 8.2)	5.6 (4.6, 6.6)	5.8 (4.7, 7.6)	6.5 (5.3, 8.7)	<0.01*
Length of Stay (days)	21 (14, 37)	17 (12, 29)	21 (14, 37)	22 (14, 38)	0.03*
Postop Dialysis	81 (11.1%)	8 (11%)	14 (11%)	59 (11.2%)	0.99

*Data displayed as mean ± standard deviation (SD) median (interquartile range) for parametric or non-parametric continuous variables respectively and number (percent of total) for categorical variables. BMI, body mass index; GFR, glomerular filtration rate; ICU, intensive care unit; ADL, activities of daily living; LAS, lung allocation score; PRA, percent reactive antibodies; ECMO, extracorporeal membrane oxygenation. * indicates* $p < 0.05$. *[A] "DCD Donor Lung Utilization (%)" calculated as fraction of DCD donors where a lung was procured and transplanted divided by all DCD donors regardless of which organ was donated. [B] "Percentage of all Organ Donors that are DCD" calculated as all DCD donors regardless of which organ was donated divided by all organ donors (DBD and DCD).*

increased the most from 1.2 DCD donors per million population (PMP) in era 1 to 6.8 DCD donors pmp in era 3, followed by Vermont and Minnesota. The largest absolute increase was observed in Ohio, which increased its use of DCD from 14 in era 1 to 80 in era 3. Other states with large increases were New York and Texas.

DISCUSSION

In this analysis we demonstrated that lung transplant recipients of DCD organs were older, more likely to be in the ICU or on ECMO pre-operatively and had shorter waitlist time compared to recipients of DBD lungs. However, DCD organs also had greater ischemic time, and recipients had a greater post-operative length of stay and use of dialysis. Despite these differences, DCD recipients continue to have similar survival to recipients of DBD lungs, on both unadjusted and adjusted survival analyses. There has been expansion in DCD use, but the overall number of DCD lungs used for lung transplantation remains low. Together these data characterize DCD lung characteristics and outcomes in the LAS era in the United States.

Recipient outcomes from DCD donors were equivalent to DBD donors and remained so throughout each era. Since era 1, the DCD recipient population became older and sicker, mirroring similar changes that have occurred in the lung transplant population as a whole (1). Waitlist time also decreased across

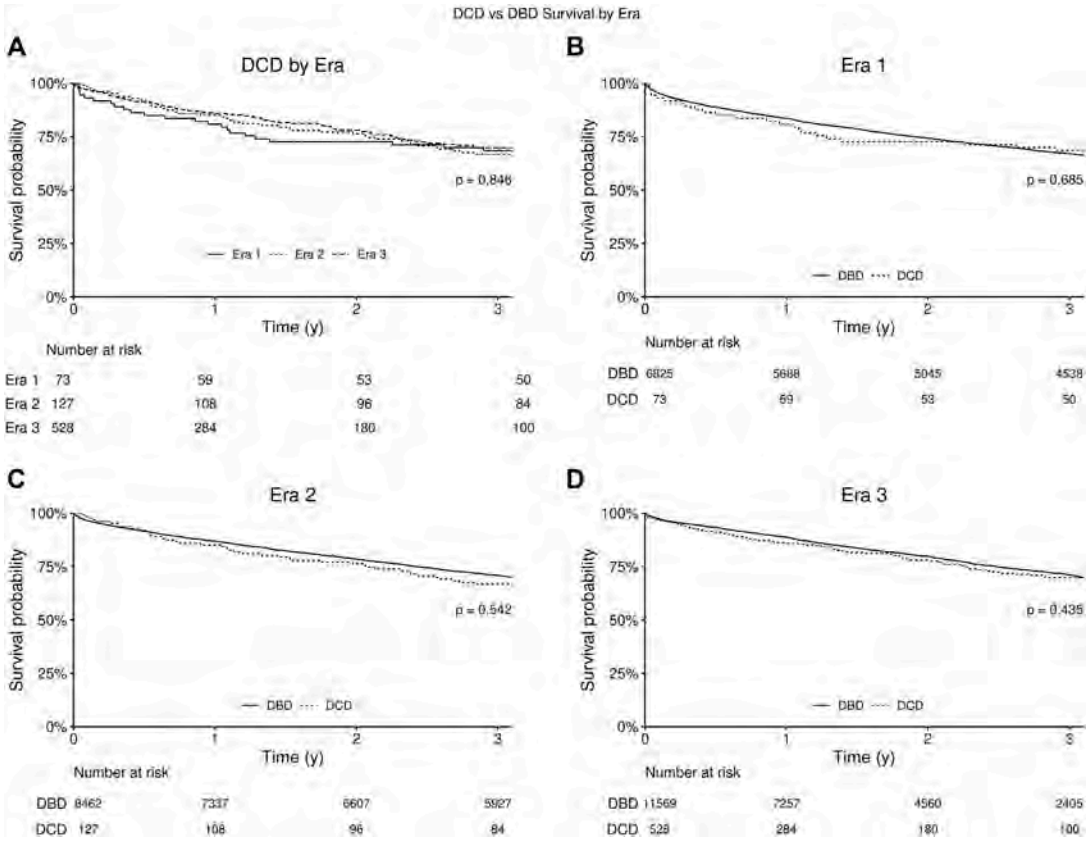


FIGURE 1 | Kaplan-Meier survival curve for recipients of lungs from circulatory death donors (DCD) and brain dead donors (DBD). P-value for log-rank test comparing all three eras. **(A)** Kaplan-Meier survival curves for all eras from date of lung transplantation. **(B)** Kaplan-Meier survival curves for Era 1 (2005–2010). **(C)** Kaplan-Meier survival curves for Era 2 (2010–2015). **(D)** Kaplan-Meier survival curves for Era 3 (2015–2020).

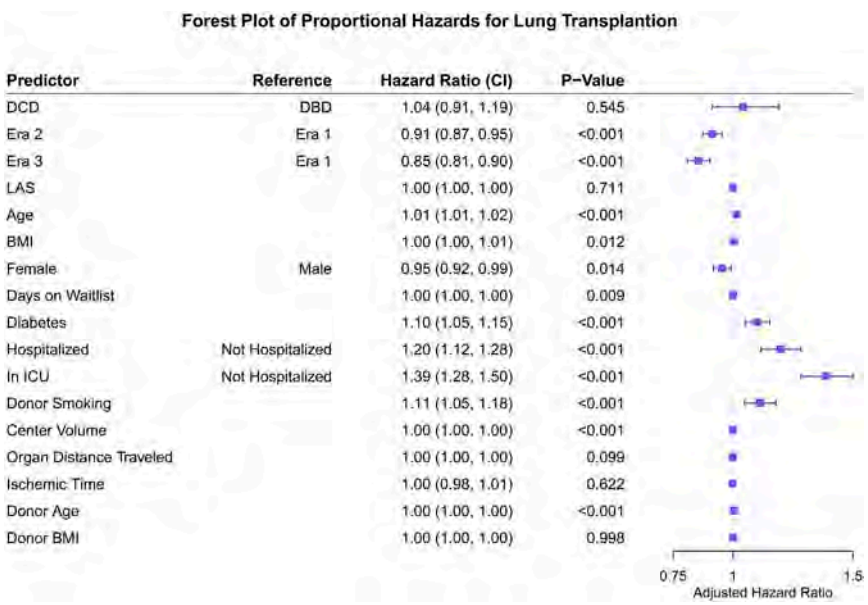


FIGURE 2 | Forest plot for hazard ratio of death for covariates of interest following lung transplantation. DCD, donation after circulatory death; DBD, donation after brain death; LAS, lung allocation score; BMI, body mass index. ICU, intensive care unit.

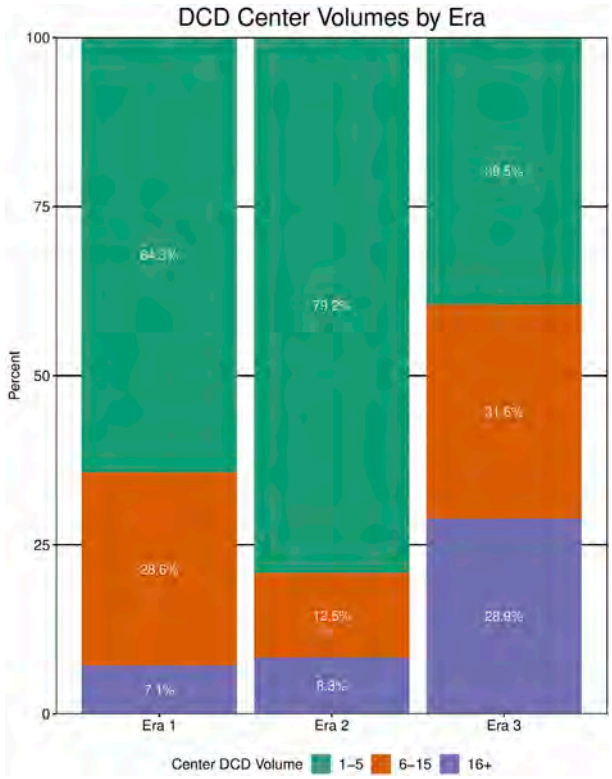


FIGURE 3 | Center volume of donation after circulatory death (DCD) lung transplantation by era. Data for center volumes from any center performing >1 DCD lung transplant within that era.

each era and was lower in recipients of DCD lungs compared to DBD lungs. These are promising changes as they may represent an increasing acceptance of DCD lungs as a robust means to expand the donor pool and may demonstrate a greater overall sense of comfort in the use of DCD organs by transplant teams.(6, 9) Though DCD use has increased >7-fold since era 1, it still only comprises 4.2% of all lung transplants in the United States. Additionally, our data shows that donation after circulatory death comprises ~20% of all organ donation (across all types of organ donation) in the United States. This suggests that DCD use for lung transplant has room for further growth. In the most recent era DCD organ use comprised only 4.2% of all lung transplants. This small percentage of usage relative to the number of DCD organs available represents an opportunity for lung transplant centers, particularly for those with longer waitlist times or increased waitlist mortality rates. Globally, the experience with DCD usage is different than in the United States (10). In a European survey of DCD use in lung transplantation, 1,381 DCD lung transplants were identified from 2008 to 2016 (11). This exceeds the 728 DCD lung transplants that we identified in the United States from 2005 to 2020. Moreover, in 2016 the same European state consortium reported 218 controlled DCD lung transplants, and 15 uncontrolled DCD lung transplants, out of 2,549 total lung transplants. DCD lungs composed 9.3% of all lung transplants that year, compared to 4.2% in the US in the most recent era. In certain countries such as Australia, United Kingdom, and Netherlands, the use of DCD for lung transplantation is 30–50% (11, 12). This could be due to greater standardization and more explicit regulations around what is and is not allowed during procurement (some European states allow pre-mortem interventions such as administration of heparin or cannulation). Or it could be due to differences in consenting processes (i.e., opt-in or out-out consent for organ donation after death) (13).

Several barriers exist that may slow the growth of DCD use in the United States. Utilization of DCD lungs requires transplant programs to view the organs as viable and equivalent to traditional DBD organs. This study adds to the growing foundation of literature supporting this concept. Our data indicate that over the past 15 years, the proportion of lung transplant centers using DCD lungs has increased from 21.2% to 54.3%. Retrospective studies and meta-analyses have previously shown inconsistent results regarding long-term survival and operative complications using DCD lungs, possibly slowing the rate of DCD adoption by new centers. A 2020 systematic review and meta-analysis found no difference in 1-year survival or PGD between DCD and DBD organs, but observed an increase in airway complications and a reduction in 5-year survival in DCD organs (14). However, due to the relatively small overall DCD cohort size and single-center nature of most of the included studies, the study mentions a high likelihood of allocation bias. Two additional meta-analyses found no difference in 1-year survival between DBD and DCD lungs.(4, 15) In a database analysis of the International Society for Heart and Lung Transplantation (ISHLT) registry using unadjusted and multivariable analyses, there was no difference in 5-year survival between DBD and DCD organs, though a survival benefit was associated with era of transplant (2003–2009 vs. 2010–2016) (16). Similarly our analysis using 15 years of data and over 27,000 lung transplant cases in the United States, did not demonstrate a survival difference between DBD and DCD lungs, but found a survival benefit associated with more recent era of transplant. In addition to uncertainty about graft viability and survival (17), transplantation teams prefer to have intraoperative organ assessment prior to transplantation. Prior to a DCD procurement there is no opportunity for determination of intraoperative PF ratio, something commonly performed in DBD donors following lung recruitment where the chest has already been opened. Additionally, as the patient is not deceased, the workup (i.e., scans, bronchoscopy, etc.) of the donor may be less comprehensive than DBD donors. Our data suggests teams used a more conservative donor PF ratio in earlier eras, as DCD donor PF ratio decreased from era 1 (443) to era 3 (412). EVLP, however, provides a technique allowing for pre-implantation assessment of donor lung allografts that may help alleviate concerns of organ functional assessment prior to transplantation (18). SRTR began collecting data on organ perfusion prior to transplantation on 2/28/2018. Our data indicate that EVLP use in era 3 was 27.3% in DCD lungs compared to 3.8% of DBD lungs. This suggests practitioners are preferentially utilizing EVLP for assessment of DCD lungs. As availability of EVLP continues to increase (including third party services that can be contracted for EVLP use), this may help to

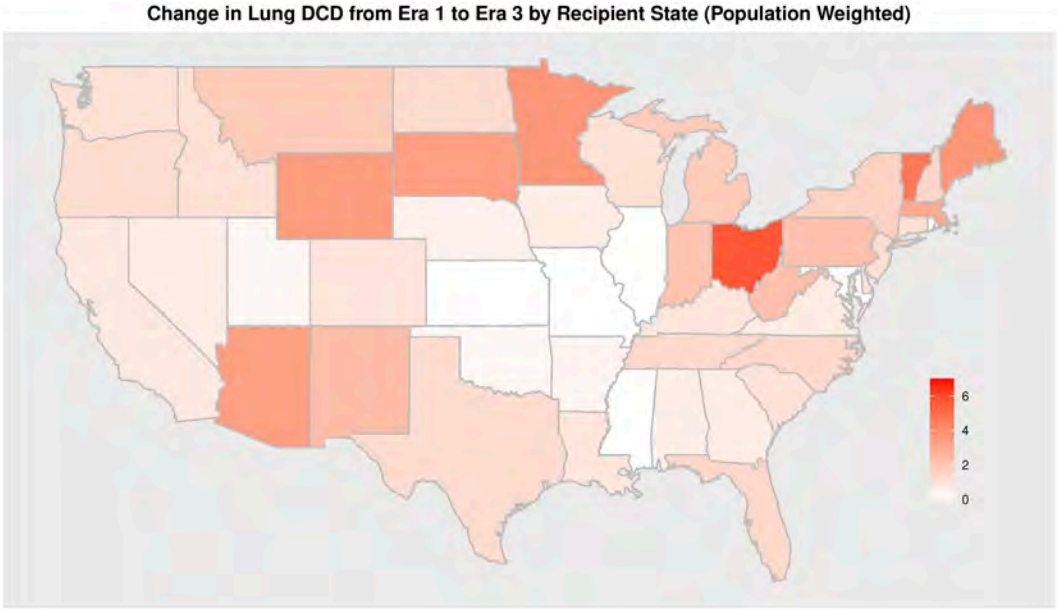


FIGURE 4 | Change in Absolute Number of DCD Lung Transplantation from Era 1 to Era 3 by Recipient State per 1 million inhabitants.

alleviate further center barriers to DCD organ adoption. As additional evidence accumulates around the efficacy of DCD lungs, the volume of DCD lungs should continue to expand in the pursuit of reducing waitlist time and mortality.

DCD lung transplantation has expanded in its utilization, however, not uniformly across all centers. Over time, an increasing number of centers have elected to use DCD lungs and the DCD volume within those centers has increased. Despite this increase, only about 50% of all centers have used a DCD organ for lung transplantation. From era 1 to era 2, the number of centers utilizing a DCD lung increased, but the median DCD volume at those centers remained unchanged. However, as DCD expansion continued into era 3, there was an increase in both the number of centers using DCD and median DCD volume. This may reflect a transition in DCD lung transplantation from an experimental novelty to a real avenue for growth in transplant volume. We also identified geographic variation in DCD use. We observed that DCD use constitutes a larger percentage (~15%) of all lung recipients from certain states and reliance on DCD organs for lung transplant is generally concentrated in the northern portion of the United States (**Supplementary Figures S1, S2**). A recent analysis of DCD usage by OPO confirms a similar geographic pattern of use (19). Our analysis helps to provide more granularity to these previously published findings as well as add context through an analysis of donor and recipient profiles. Several potential elements may determine why certain states and centers increasingly rely on DCD lungs. During procurement for a DCD organ, the patient is extubated and a pre-determined time is allotted for declaration of death. This process of how death is declared and the time frame for progression to death varies by center and jurisdiction (20). There is no universal protocol for sedation or allotted post-extubation time, and variations in these factors (within an OPO or hospital) has the potential to impact whether a donor organ can be procured (21). Other differences in logistical management by centers and OPOs create variability (22). For example, rules regulating when surgical teams are allowed in the OR, when heparin is administered, how long procuring teams wait for declaration of death, and protocols governing comfort care surrounding withdrawal commonly differ amongst hospitals and OPOs. Additional factors include an OPO or donor hospital's willingness to perform recruitment maneuvers, bronchoscopies, and CT scans on potential DCD donors. Lastly, resource and labor availability also affect a center's likelihood of sending a team for a DCD lung procurement, as it has a potentially lower chance of conversion to transplant than a DBD procurement. The use of local procurement teams (as is commonly done for kidney procurement) could help address this issue, though the importance of intraoperative assessment of lungs such as compliance, unlike kidneys, may limit centers enthusiasm. In comparison to protocols and consensus statements for organ procurement following DBD, DCD organ procurement is less standardized (23). Given the variability observed in the United States we propose working towards more-refined consensus statements and idealized protocols for DCD lung procurement, which may impact increased utilization.

Limitations

There are some limitations to our findings. Our large dataset is multi-center and retrospective and is therefore subject to information and selection bias. Longer survival data are necessary to compare to DBD and DCD lungs (17), especially for the most recent era where a larger number of DCD transplants were performed. Furthermore, we did not analyze the association of DCD or DBD organs with chronic lung allograft dysfunction, nor we did not investigate the cause of DCD organs rejected for transplantation (24). Additionally,

geographic analysis was conducted at the recipient level, not where donor procurement took place or by implanting institution. Our data is also subject to selection bias as we are only analyzing DCD organs that were transplanted and not assessing organs that were deemed unsuitable for transplantation following attempted procurement. In analyzing the PaO2/FiO2 ratio, UNOS does not specify the timing of sample collection. It is possible samples are taken from the ICU before procurement or even in the operating room after full lung recruitment. Finally, we do not have EVLP use data prior to 2018, due to lack of data in the STAR file, which limits better characterization of DCD organs prior to transplantation.

CONCLUSION

In summary, use of DCD lungs has increased over time, with similar long-term survival compared to DBD lungs despite higher ischemic time. Continued increases in DCD volume will help expand the lung donor pool, particularly for recipients with limitations on size and antibody profile. Given the heterogeneous geographic distribution in DCD utilization further investigation into limiting factors for utilization is warranted and may justify protocol standardization for these 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

This study was submitted to Ohio State IRB for approval (protocol: 2018H0079) and deemed exempt. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

Participated in research design: CB, BW, and AG. Participated in the writing of the paper: CB, BW, MH, NM, BK, JR, and AG. Participated in the performance of the research: CB and AG. Contributed new reagents or analytic tools: CB, BW, BK, JR, and AG. Participated in data analysis: CB, BW, MH, NM, BK, JR, and AG.

CONFLICT OF INTEREST

AG served as a consultant for Abbvie Pharmaceuticals. BW is a consultant for Abbott Laboratories and serves on the Clinical Events Committee of TransMedics OCS; BW is partially supported through National Institutes of Health (NIH) National Heart Lung and Blood Institute grant R01HL143000 and Department of Defense (DOD) Army Medical Research Acquisition Activity grant PR170989. NM is a consultant and investigator for Abbott, Medtronic, Carmat, Xylocor and SynCardia.

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.2022.10172/full#supplementary-material

REFERENCES

1. Valapour M, Lehr CJ, Skeans MA, Smith JM, Uccellini K, Goff R, et al. OPTN/SRTR 2018 Annual Data Report: Lung. *Am J Transpl* (2020) 20(Suppl. s1): 427–508. doi:10.1111/ajt.15677
2. Gulack BC, Ganapathi AM, Speicher PJ, Meza JM, Hirji SA, Snyder LD, et al. What Is the Optimal Transplant for Older Patients with Idiopathic Pulmonary Fibrosis? *Ann Thorac Surg* (2015) 100(5):1826–33. doi:10.1016/j.athoracsur.2015.05.008
3. Chancellor WZ, Charles EJ, Mehaffey JH, Hawkins RB, Foster CA, Sharma AK, et al. Expanding the Donor Lung Pool: How many Donation after Circulatory Death Organs Are We Missing? *J Surg Res* (2018) 223:58–63. doi:10.1016/j.jss.2017.09.029
4. Krutsinger D, Reed RM, Blevins A, Puri V, De Oliveira NC, Zych B, et al. Lung Transplantation from Donation after Cardiocirculatory Death: a Systematic Review and Meta-Analysis. *J Heart Lung Transplant* (2015) 34(5):675–84. doi:10.1016/j.healun.2014.11.009
5. Levvey B, Keshavjee S, Cypel M, Robinson A, Erasmus M, Glanville A, et al. Influence of Lung Donor Agonal and Warm Ischemic Times on Early Mortality: Analyses from the ISHLT DCD Lung Transplant Registry. *J Heart Lung Transplant* (2019) 38(1):26–34. doi:10.1016/j.healun.2018.08.006
6. Reeb J, Keshavjee S, Cypel M. Expanding the Lung Donor Pool. *Curr Opin Organ Transpl* (2015) 20(5):498–505. doi:10.1097/mot.0000000000000233
7. Cypel M, Levvey B, Van Raemdonck D, Erasmus M, Dark J, Love R, et al. International Society for Heart and Lung Transplantation Donation after Circulatory Death Registry Report. *J Heart Lung Transplant* (2015) 34(10): 1278–82. doi:10.1016/j.healun.2015.08.015
8. Leppke S, Leighton T, Zaun D, Chen S-C, Skeans M, Israni AK, et al. Scientific Registry of Transplant Recipients: Collecting, Analyzing, and Reporting Data on Transplantation in the United States. *Transplant Rev* (2013) 27(2):50–6. doi:10.1016/j.trre.2013.01.002
9. Inci I. Donors after Cardiocirculatory Death and Lung Transplantation. *J Thorac Dis* (2017) 9(8):2660–9. doi:10.21037/jtd.2017.07.82
10. Ceulemans LJ, Inci I, Van Raemdonck D. Lung Donation after Circulatory Death. *Curr Opin Organ Transpl* (2019) 24(3):288–96. doi:10.1097/mot.0000000000000627
11. 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
12. Levvey BJ, Harkess M, Hopkins P, Chambers D, Merry C, Glanville AR, et al. Excellent Clinical Outcomes from a National Donation-After-Determination-

Of-Cardiac-Death Lung Transplant Collaborative. *Am J Transpl* (2012) 12(9): 2406–13. doi:10.1111/j.1600-6143.2012.04193.x
13. Lewis A, Koukoura A, Tsianos G-I, Gargavanis AA, Nielsen AA, Vassiliadis E. Organ Donation in the US and Europe: The Supply vs Demand Imbalance. *Transplant Rev* (2021) 35(2):100585. doi:10.1016/j.trre.2020.100585
14. Palleschi A, Rosso L, Musso V, Rimessi A, Bonitta G, Nosotti M. Lung Transplantation from Donation after Controlled Cardiocirculatory Death. Systematic Review and Meta-Analysis. *Transplant Rev* (2020) 34(1):100513. doi:10.1016/j.trre.2019.100513
15. Zhou J, Chen B, Liao H, Wang Z, Lyu M, Man S, et al. The Comparable Efficacy of Lung Donation after Circulatory Death and Brain Death: A Systematic Review and Meta-Analysis. *Transplantation* (2019) 103(12):2624–33. doi:10.1097/tp.0000000000002888
16. Van Raemdonck D, Keshavjee S, Levvey B, Cherikh WS, Snell G, Erasmus M, et al. Donation after Circulatory Death in Lung Transplantation-Five-Year Follow-Up from ISHLT Registry. *J Heart Lung Transplant* (2019) 38(12): 1235–45. doi:10.1016/j.healun.2019.09.007
17. Mulvihill MS, Gulack BC, Ganapathi AM, Speicher PJ, Englum BR, Hirji SA, et al. The Association of Donor Age and Survival Is Independent of Ischemic Time Following Deceased Donor Lung Transplantation. *Clin Transpl* (2017) 31(7). doi:10.1111/ctr.12993
18. Erasmus ME, van Raemdonck D, Akhtar MZ, Neyrinck A, de Antonio DG, Varela A, et al. DCD Lung Donation: Donor Criteria, Procedural Criteria, Pulmonary Graft Function Validation, and Preservation. *Transpl Int* (2016) 29(7):790–7. doi:10.1111/tri.12738
19. Hirji SA, Halpern AL, Helmkamp LJ, Roberts SH, Houk AK, Osho A, et al. Geographic and Temporal Patterns of Growth in the Utilization of Donation after Circulatory Death Donors for Lung Transplantation in the United States. *J Heart Lung Transplant* (2020) 39(11):1313–5. doi:10.1016/j.healun.2020.08.015
20. Wind J, Faut M, van Smaalen TC, van Heurn EL. Variability in Protocols on Donation after Circulatory Death in Europe. *Crit Care* (2013) 17(5):R217. doi:10.1186/cc13034
21. den Hartogh G. When Are You Dead Enough to Be a Donor? Can Any Feasible Protocol for the Determination of Death on Circulatory Criteria Respect the Dead Donor Rule? *Theor Med Bioeth* (2019) 40(4):299–319. doi:10.1007/s11017-019-09500-0
22. Choi AY, Jawitz OK, Raman V, Mulvihill MS, Halpern SE, Barac YD, et al. Predictors of Nonuse of Donation after Circulatory Death Lung Allografts. *J Thorac Cardiovasc Surg* (2021) 161(2):458–66. doi:10.1016/j.jtcvs.2020.04.111
23. Copeland H, Hayanga JWA, Neyrinck A, MacDonald P, Dellgren G, Bertolotti A, et al. Donor Heart and Lung Procurement: A Consensus Statement. *J Heart Lung Transplant* (2020) 39(6):501–17. doi:10.1016/j.healun.2020.03.020
24. Speicher PJ, Ganapathi AM, Englum BR, Gulack BC, Osho AA, Hirji SA, et al. Single-lung Transplantation in the United States: what Happens to the Other Lung? *J Heart Lung Transplant* (2015) 34(1):36–42. doi:10.1016/j.healun.2014.08.018

ESOT | Transplant International

ORIGINAL RESEARCH
published: 14 April 2022
doi: 10.3389/ti.2022.10048

Lung Volume Reduction Followed by Lung Transplantation in Emphysema—A Multicenter Matched Analysis

Alexis Slama[1,2†], Laurens J. Ceulemans[3,4†], Celia Hedderich[1], Panja M. Boehm[5], Jan Van Slambrouck[3,4], Stefan Schwarz[5], Christelle M. Vandervelde[3], Markus Kamler[2], Peter Jaksch[5], Dirk Van Raemdonck[3,4], Konrad Hoetzenecker[5†] and Clemens Aigner[1,2†]*

[1]Department of Thoracic Surgery and Thoracic Endoscopy, University Medicine Essen, Ruhrlandklinik, Essen, Germany, [2]West German Center for Lung Transplantation, University Medicine Essen, Essen, Germany, [3]Department of Thoracic Surgery, University Hospitals Leuven, Leuven, Belgium, [4]Department of Chronic Diseases and Metabolism, Laboratory of Respiratory Diseases and Thoracic Surgery (BREATHE), KU Leuven, Leuven, Belgium, [5]Clinic of Thoracic Surgery, Medical University of Vienna, Vienna, Austria

Objective: The impact of previous lung volume reduction surgery (LVRS) or endoscopic lung volume reduction (ELVR) on lung transplantation (LuTX) remains unclear. This study assesses the risk of previous lung volume reduction on the outcome of a later LuTX.

Methods: Patients suffering from emphysema who underwent bilateral LuTX were included in this multicenter analysis. Study groups were defined as: previous LVRS, previous ELVR, controls. Imbalances were corrected by coarsened exact matching for center, gender, age, diagnosis, and BMI. A comparative analysis of intraoperative characteristics, perioperative outcome and long-term survival was performed.

Results: 615 patients were included (LVRS = 26; ELVR = 60). Compared to controls, LVRS patients had a higher rate of postoperative ECMO (15.4 vs. 3.9%; $p = 0.006$), whereas ELVR patients suffered more often from wound infections (8.9% vs. 2.5%; $p = 0.018$). Perioperative outcome, duration of ventilation, ICU stay, and hospital stay were comparable between groups. Bacterial colonization of the airway differed significantly between both LVR groups and controls in pre- and post-LuTX cultures. Survival was not impacted (1-/3-/5-year survival for LVRS: 92.3%/85.7%/77.1%; controls: 91.3%/82.4%/76.3%; $p = 0.58$ | ELVR: 93.1%/91%/91%; controls 91.2%/81.7%/75.3%; $p = 0.17$).

Conclusion: Lung volume reduction does not impact short and long-time survival after bilateral LuTX. Due to differences in airway colonization after LVR, caution to prevent infectious complications is warranted.

Keywords: ELVR, emphysema, LVRS, lung transplantation, lung volume reduction

OPEN ACCESS

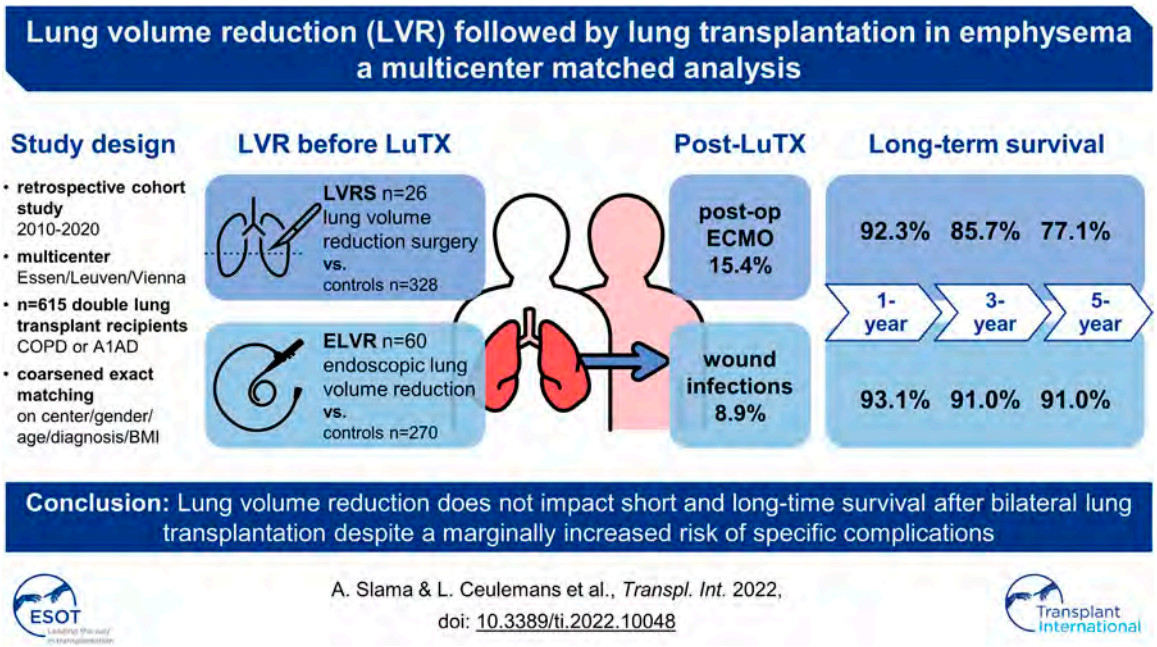
***Correspondence:**
Alexis Slama
alexis.slama@uk-essen.de

†These authors have contributed equally to this work

***Received:** 22 September 2021*
***Accepted:** 24 March 2022*
***Published:** 14 April 2022*

Citation:
Slama A, Ceulemans LJ, Hedderich C, Boehm PM, Van Slambrouck J, Schwarz S, Vandervelde CM, Kamler M, Jaksch P, Van Raemdonck D, Hoetzenecker K and Aigner C (2022) Lung Volume Reduction Followed by Lung Transplantation in Emphysema—A Multicenter Matched Analysis. Transpl Int 35:10048. doi: 10.3389/ti.2022.10048

Abbreviations: BMI, body mass index; CEM, coarsened exact matching; CLAD, chronic lung allograft dysfunction; COPD, chornic obstructive pulmonary desease; CPB, cardio pulmonary bypass; CVA, cerebro-vascular accident; ECMO, extra-corporeal membrane oxygenation; ECS, extra corporeal support; ELVR, endoscopic lung volume reduction; ICU, intensive care unit; LAS, lung allocation score; LuTX, lung transplantation; LVR, lung volume reduction; LVRS, lung volume reduction surgery; MOF, multi-organ failure; 6MWT, six minutes walking test; PGD, primary graft dysfunction; QOL, quality of life; VATS, video assisted thoracic surgery.



GRAPHICAL ABSTRACT |

INTRODUCTION

Patients with end-stage pulmonary emphysema have limited therapeutic options. Lung volume reduction (LVR) and lung transplantation (LuTX) have been shown to improve lung function, quality of life (QOL) and survival, despite an associated perioperative risk (1, 2). Lung volume reduction surgery (LVRS) has gained popularity in the nineties, however, remained undervalued and underused after the large NETT trial (3–5). In more recent years, endoscopic lung volume reduction (ELVR) techniques by implantation of valves or coils, thermal ablation, or instillation of chemical sealants emerged (6). However, the effect of all LVR procedures is counteracted by the progression of disease usually leading to a decay in lung function after several months to years following treatment, leaving LuTX as the last option (7). Due to the protracted clinical course of COPD in comparison to other end-stage lung diseases, the best timing for LuTX referral remains debated. Guidelines for LuTX selection recommend the use of the BODE score, which is a good predictor for pre-LuTX mortality and post-LuTX survival benefit (8).

With increased use of LVR in highly impaired "low FEV1" patients (9, 10), and the more apparent overlap of patients eligible for both LVR and LuTX, the use of LVR procedures as a "bridge to transplant" has gained acceptance (7).

Simultaneous referral of patients for both LVR and LuTX is always recommended, and the decision should be taken in an interdisciplinary emphysema board with access to all treatment options (8). Those therapies are not mutually exclusive, and most combinations have been reported, of which, in most cases, LuTX was considered the last resort when all other previous therapies failed.

In a previous review, twelve published reports on LVRS and one on ELVR preceding LuTX were identified (11). North American papers showed that LVRS before LuTX can negatively affect survival (12, 13). Other publications demonstrated only an increased perioperative risk with no impact on survival (14–16). Nevertheless, these conclusions were not supported by the most recent and largest single institution report, in which no adverse effect of a previous LVRS was observed (17).

The increased surgical risk after LVRS can potentially be attributed to a higher occurrence of adhesions and thereby a longer operation time, more bleeding complications, and a higher need for blood transfusions. Also, a higher risk of injury to the phrenic nerve during adhesiolysis has been hypothesized but was never confirmed in the available reports (11).

For ELVR prior to LuTX, available data is even more scarce, with only a single institutional analysis available (18). In 20 ELVR patients, outcome was comparable to a matched control group, although ELVR was associated with a higher occurrence of bacterial airway colonization. This observation has been recently confirmed in a larger cohort outside the scope of LuTX (19).

Given the rarity and controversy of available evidence on the impact of LVRS and ELVR on a later lung LuTX, this study aimed to further determine the short- and long-term outcomes in these patients.

METHODS

We conducted a multicenter retrospective analysis of post-LuTX outcomes in patients suffering from emphysema. Data was collected and anonymized before transmission between

participating centers. This study was approved by the ethics committee of the Medical Faculty of the University of Duisburg-Essen (21-9856-BO).

Study Population

Data was collected from three European high volume LVR and LuTX centers (Essen, Leuven, Vienna). All patients who underwent a bilateral LuTX for COPD or α-1 antitrypsin deficiency were included. The timespan for inclusion was defined individually for each center ranging from their first patient undergoing LuTX after previous LVR according to recent treatment algorithms (Essen: 2/2015; Vienna: 1/2015, Leuven: 1/2010) until August 2020. Patients with re-LuTX, unilateral LuTX or preoperative ECMO support were excluded from the analysis.

Recipient Characteristics

Variables routinely used for listing, lung allocation score (=LAS) calculation, and organ allocation (dependent on center-specific approaches) were collected and used for analysis: age, waiting time, pack years, functional parameters, supplemental oxygen need, and pulmonary arterial pressure. LAS-data was only available for two centers. Intra-operative data included the need for size reduction (wedge or lobar LuTX), intraoperative cardiopulmonary support (ECMO, no support, CPB), duration of surgery, and cold ischemic time of both lungs independently.

Endpoints

The primary endpoint was 1-year survival after LuTX. Secondary endpoints included duration of mechanical ventilation, time to discharge from ICU and from hospital. Surgical and medical complications were categorized and the need for postoperative ECMO was assessed. Data collection allowed for the entry of two causes of death and survival was compared to unmatched unweighted data.

Bacterial Samples

An additional secondary endpoint of the study was to assess potential differences in airway and bronchial colonization. All positive cultures of the respiratory tract obtained while waiting for LuTX were recorded. Sputum cultures, bronchoalveolar lavage samples and swabs of the explanted recipient lung were considered. In one center, the information for every sample taken after transplantation could also be included and was used for a subgroup analysis.

Matching

A matching algorithm was applied to reduce imbalance between groups of treated patients and controls, and to thereby improve the estimation of causal effects by statistical testing. For this purpose, "coarsened exact matching" (=CEM) was used (20). CEM has the capacity to approximate a fully blocked randomized trial unlike propensity score matching (PSM), being a less data efficient and more biased completely randomized approach (21). Coarsening was achieved by considering 5 covariates: center, gender, diagnosis, age, and BMI, the latter two with defined cut points (24, 45, 55, 65 years and 18.5, 25, 30 BMI). LAS was not used for CEM stratification as it is dependent on three of the five included variables (age, diagnosis and BMI).

Statistics

Preprocessing of data to generate matched groups was caried out by means of R (R-Foundation for Statistical Computing, Vienna, Austria), SPSS (IBM Corp., Armonk, NY) and CEM-Extension bundle (Matthew Blackwell). All statistical analyses were conducted with SPSS v.25. Variables were assumed to be non-parametric and are reported as median and range and compared by Mann-Whitney-U tests. Nominal data were compared by means of chi-squared test. Cases in the control groups were weighted by the CEM algorithm and frequency weight data values were rounded to the nearest integers if needed by analysis or in tables. Patient survival between the groups was compared by log-rank (Mantel-cox) tests on unmatched groups and unweighted matched data. Two-sided P values <0.05 were considered statistically significant. No adjustment for multiple testing was used.

RESULTS

615 patients [320 (52%) female and 295 (48%) male] were included in this study. Of those, 26 (4.2%) underwent LVRS before LuTX and 60 (9.8%) had ELVR prior to transplantation. Mean age was 58 ± 5.9 years. Indications for LuTX were COPD in 572 (93%) cases and alpha-1 antitrypsin deficiency emphysema in 43 (7%) cases. In 24 (92.3%) LVRS patients, surgery took place before listing ($\tilde{x}$: 3.8 years; range: 0.6–32.7) whereas in two cases (7.7%), LVRS was performed while patients were on the waitlist for LuTX (2.2 and 4.4 years after listing). Median time from LVRS to LuTX was 4.0 (1.1-32.7) years. LVRS was either unilateral (15; 58%) or bilateral (11; 42%). Surgical access for LVRS was VATS in 11 (44%) cases and open surgery via thoracotomy or sternotomy in 14 (56%) cases. In one patient this information was missing. Most LVRS were performed by parenchymal stapling (n = 22; 85%). The remaining 4 patients underwent lobectomy (15%).

Out of 60 patients with previous ELVR, 54 (90%) had the intervention before being listed for LuTX ($\tilde{x}$: 2.2 years range: 0.5–6.0) and 6 (10%) on the waiting list ($\tilde{x}$: 2.5 years; range 0.4–6.8 after listing). Time from ELVR to LuTX was 2.7 (0.02–7.4) years. 50 (83.3%) patients had unilateral interventions and 10 (16.7%) bilateral. The procedures were: valves: 50 (83.3%), coils: 9 (15%) and a combination of both in 1 (1.7%) case. One of the patients treated with valves had hydrogel foam instilled in the contralateral apical lobe. In 20 out of 51 (39%) patients with valves, later re-intervention became necessary to either reposition or remove the valves because of unsuccessful treatment.

All 26 patients with a history of LVRS (T_{LVRS}) were matched to 328 weighted controls (C_{LVRS}). In patients with ELVR, 56 patents (T_{ELVR}) remained in the analysis (n = 4 unmatched by lack of partners) and 270 weighted controls (C_{ELVR}). Metrics on matching are presented in the supplementary file (**Table 1**).

Pre-LuTX characteristics of treatment groups and matched controls are presented in **Table 1**. After coarsened exact matching, groups were balanced throughout demographic variables. Patients undergoing LuTX after previous LVRS had a lower median LAS at time of LuTX (32.4 vs. 33.4; p = 0.038) and lesser need for oxygen (2 vs. 3 L/min; p = 0.033); however, this was not considered of clinical relevance.

TABLE 1 | Demographics and patient characteristics ahead of LuTX.

		T_{LVRS} (n = 26)	C_{LVRS} (n = 328)	p =	T_{ELVR} (n = 56)	C_{ELVR} (n = 270)	p =
Gender	Female	13 (50.0%)	50.0%	1.000	29 (51.8%)	51.8%	0.988
	Male	13 (50.0%)	50.0%		27 (48.2%)	48.2%	
Diagnosis	α1-AT def.	2 (7.7%)	7.7%	0.990	3 (5.4%)	5.4%	0.963
	COPD	24 (92.3%)	92.3%		53 (94.6%)	94.6%	
Age at LuTX (y)		59 (42–70)	57 (45–74)	0.649	60 (45–72)	58 (42–74)	0.946
Waiting time (d)		180 (6–2161)	203 (2–4326)	0.506	156 (1–2932)	176 (2–3962)	0.590
BMI		21.1 (18.5–27.6)	22.5 (16.2–29.7)	0.101	22.5 (16.0–30.9)	21.6 (12.6–31.7)	0.184
Pack years		30 (0–56)	30 (0–100)	0.145	39 (0–120)	37 (0–110)	0.634
6MWT (m)		310 (20–492)	250 (0–611)	0.066	235 (0–480)	231 (0–530)	0.339
rTLC (L)		7.25 (4.03–11.1)	8 (2.89–12.1)	0.191	7.91 (4.1–12.1)	7.99 (3.26–12.6)	0.922
pTLC (L)		6.25 (4.38–7.9)	5.83 (3.65–8.2)	0.948	5.63 (3.63–7.86)	5.71 (3.98–8.67)	0.782
FEV1 (%)		21 (9.9–66)	19.6 (10–94)	0.088	19.1 (10–41)	19 (9.9–85)	0.338
PAP mmHg		32 (21–59)	32 (8–94)	0.612	34 (18–70)	31 (8–94)	0.368
LAS at listing		31.8 (29.9–35.1)	32.9 (27.8–69.7)	0.076	32.0 (29.6–38.5)	32.8 (27.8–69.7)	0.185
LAS at LuTX		32.4 (29.9–40.9)	33.4 (27.8–87.2)	***0.038***	32.6 (29.6–90.2)	33.0 (27.8–87.2)	0.223
O2 Therapy (L/min)		2 (0–6)	3 (0–15)	***0.033***	2 (0–8)	2 (0–15)	0.696
pre-LuTX hospitalization	No	25 (96.2%)	89.9%	0.306	50 (89.3%)	92.5%	0.435
	Yes	1 (3.8%)	10.1%		6 (10.7%)	7.5%	
pre-LuTX MV	No	19 (73.1%)	69.2%	0.680	41 (73.2%)	62.7%	0.297
	Noninvasive	7 (26.9%)	30.8%		15 (26.8%)	36.4%	
	ET intubation					0.9%	

Numbers are median (range) or counts (%); control columns include weighted data; significant p-values are bold; T_{LVRS}, patients with previous LVRS; C_{LVRS}, matched controls; T_{ELVR}, patients with previous ELVR; C_{ELVR}, matched controls; α1-AT def., alpha-1 antitrypsin deficiency emphysema; BMI, body mass index 6MWT, 6-min walking test; rTLC, measured total lung capacity; pTLC, predicted total lung capacity; FEV1, forced expiratory volume; PAP, pulmonary arterial pressure; LAS, lung allocation score; MV, mechanical ventilation; ET, endotracheal.

TABLE 2 | Intra-operative characteristics of LuTX.

		T_{LVRS} (n = 26)	C_{LVRS} (n = 328)	p =	T_{ELVR} (n = 56)	C_{ELVR} (n = 270)	p =
Size reduction of the graft	No	19 (73.1%)	78.4%	0.532	41 (73.2%)	74.9%	0.802
	Yes	7 (26.9%)	21.6%		15 (26.8%)	25.1%	
Which size reduction	Wedge unilat.	2 (7.7%)	7.3%	0.785	2 (3.6%)	7.4%	0.512
	Wedge. bilat.	5 (19.2%)	14.2%		13 (23.2%)	15.9%	
	Lobe unilat.		0.1%			0.5%	
	Lobe bilat.					1.2%	
Intra-OP ECS	None	9 (36.0%)	33.5%	0.069	12 (21.4%)	18.1%	0.747
	CPB		5.7%		1 (1.8%)	2.4%	
	vaECMO	15 (60.0%)	60.4%		43 (76.8%)	77.9%	
	vvECMO	1 (4.0%)	0.3%			1.5%	
LuTX duration (min)		348 (137–705)	323 (150–743)	0.296	283 (150–660)	288 (137–705)	0.703
TIT 1st implanted side (min)		295 (173–577)	293 (158–812)	0.816	285 (175–542)	299 (158–698)	0.051
TIT 2nd implanted side (min)		465 (218–639)	403 (235–960)	0.101	360 (235–692)	380 (218–769)	0.180

Data expressed as median (range) or counts (%); control columns consist of weighted data; T_{LVRS}, patients with previous LVRS; C_{LVRS}, matched controls; T_{ELVR}, patients with previous ELVR; C_{ELVR}, matched controls; ECS, extra corporeal support; CPB, cardio-pulmonary bypass; vaECMO, veno-arterial extracorporeal membrane oxygenation; vvECMO, veno-venous ECMO; TIT, total ischemic time of the graft. Significant p *values are highlited in bold italic.*

14.5% of all allocated grafts were size reduced during LuTX to match the recipient chest. Size reduction was performed either by wedge resection (n = 83; 13.5%) or by lobar transplantation (n = 6; 1.0%). 44.3% of patients had intraoperative extra corporeal support (ECS), either by ECMO (42.7%) or to a lesser extent by means of cardiopulmonary bypass (1.6%). The use of size reduction or ECS was comparable between groups. More detailed intraoperative data of both treatment groups and their weighted controls are presented in **Table 2**.

Verify that all the equations and special characters are displayed correctly.In patients with previous LVRS (T_{LVRS}) duration of LuTX (348 vs. 323 min; p = 0.296), as well as total ischemic time (TIT) of both donor lungs (465 vs. 403 min; p = 0.101) was statistically comparable to controls. Patients with previous ELVR did not exhibit significant differences in transplant duration and ischemic times, compared to their matched controls (283 vs. 288 min; p = 0.703 and 360 vs. 380 min; p = 0.180).

Short-term perioperative results (reoperation rates, intubation time, time on ICU and time in hospital) were excellent throughout treatment groups (T_{LVRS} and T_{ELVR}). When compared to controls no clinically relevant differences were

TABLE 3 | post-LuTX outcomes.

	T_{LVRS} (n = 26)	C_{LVRS} (n = 328)	p =	T_{ELVR} (n = 56)	C_{ELVR} (n = 270)	p =
Surgical revision	5 (19.2%)	17.7%	0.843	8 (14.3%)	16.2%	0.708
Successful weaning	24 (96.0%)	95.5%	0.894	55 (98.2%)	98.3%	0.973
Days ventilated	2 (0.5–23)	2 (0.5–79)	0.159	2 (0.5–19)	2 (0.5–79)	0.563
Post-OP ECMO	4 (15.4%)	3.8%	***0.006***	1 (1.8%)	6.4%	0.179
Post-OP ECMO (days)	7 (3–9)	4 (2–10)	0.078	2 (2–2)	5 (2–10)	0.250
Days on ICU	7 (3–213)	6 (2–152)	0.149	6 (2–107)	7 (2–152)	0.127
Days to transfer to normal ward	11 (5–213)	10 (3–118)	0.154	9 (2–107)	9 (2–118)	0.382
Days to dismissal from hospital	35 (15–105)	37 (9–152)	0.717	42 (18–109)	35 (8–152)	0.158
Death before dismissal	2 (7.7%)	8.0%	0.963	1 (1.8%)	5.8%	0.203

Control columns consist of weighted data; T_{LVRS}, patients with previous LVRS; C_{LVRS}, matched controls; T_{ELVR}, patients with previous ELVR; C_{ELVR}, matched controls; surgical revisions include all later surgical interventions on the chest and the lungs; ICU, intensive care unit. Significant p *values are highlited in bold italic.*

TABLE 4 | post-LuTX complications and causes of death.

	T_{LVRS} (n = 26; †: n = 6)	C_{LVRS} (n = 328; †: n = 70*)	p =	T_{ELVR} (n = 56; †: n = 6)	C_{ELVR} (n = 270; †: n = 62*)	p =
Complications			*0.754*			*0.444*
Pleural effusion	1 (3.8%)	10.1%		1 (1.8%)	6.8%	
Empyema/lung abscess	2 (7.7%)	1.2%	***0.014***	3 (5.4%)	3.1%	
Hemothorax	3 (11.5%)	6.7%		4 (7.1%)	6.5%	
Pneumothorax/air leak		2.7%			2.1%	
Pneumonia		2.3%		1 (1.8%)	3.0%	
Phrenic nerve injury/diaph. palsy		1.2%			2.5%	
Wound	1 (3.8%)	2.7%		5 (8.9%)	2.5%	***0.018***
Abdominal	2 (7.7%)	4.8%		3 (5.4%)	4.6%	
Arrhythmia	1 (3.8%)	2.5%		1 (1.8%)	1.1%	
ECMO related		1.1%		1 (1.8%)	1.8%	
Chest wall		1.2%			1.6%	
Sepsis		0.8%			1.8%	
PGD 3	3 (11.5%)	8.3%			2.9%	
Thrombosis. embolism		0.8%		1 (1.8%)	1.3%	
Renal failure	1 (3.8)	3.6%			1.6%	
Causes of death			*0.881*			***0.031***
Unknown	1 (16.7%)	26.9%		1 (16.7%)	14.1%	
Sepsis	1 (16.7%)	13.0%			25.0%	
Pneumonia	2 (33.3%)	18.2%		2 (33.3%)	11.1%	
MOF	1 (16.7%)	7.3%			24.1%	
GI bleeding/ischemia	1 (16.7%)	3.3%			10.6%	
Resp. insufficiency	1 (16.7%)	10.2%			25.9%	
Bleeding		2.4%			6.4%	
Graft failure	1 (16.7%)	1.9%	***0.044***	1 (16.7%)	1.5%	***0.034***
Kidney failure		1.9%			3.9%	
Malignancy		1.9%			2.2%	
Cardiac arrest/failure		3.9%		1 (16.7%)	6.9%	
CLAD		14.5%		1 (16.7%)	5.0%	
Acute/humoral rejection		6.0%			3.6%	
Pulmonary embolism					1.9%	
Euthanasia		1.1%			0.3%	
ECMO-failure		1.5%		1 (16.7%)		
Ischemic CVA		2.1%			0.2%	
Myelopathy		0.3%				

Multiple answers were allowed; complications are expressed as percentage of whole group, causes of death in relation of total deaths; T_{LVRS}, patients with previous LVRS; C_{LVRS}, matched controls; T_{ELVR}, patients with previous ELVR; C_{ELVR}, matched controls; T_{LVRS}: n = *8 patients with complications (vs.* n = *92*) and* n = *6 patients died (vs.* n = *70*); T_{ELVR}:* n = *13 with complications (vs.* n = *56*) and* n = *6 died (vs.* n = *62*); PGD, primary graft dysfunction; MOF, multi organ failure; GI, gastro-intestinal; CLAD, chronic lung allograft dysfunction; CVA, cerebrovascular accident; *control columns are calculated on weighted data; in Wilcoxon–Mann–Whitney comparison of a single factor between two groups, p values >0.05 are not reported for improved readability. Significant* p *values are highlited in bold italic.*

observed (**Table 3**). Nevertheless, in patients with previous LVRS a significantly higher rate of post-LuTX ECMO use was recorded (15.4% vs. 3.8%; p = 0.006).

In T_{LVRS} complications occurred in 8 (30.8%) and in T_{ELVR} in 13 (23.2%) patients. Slight differences in the spectrum of complications were identified as LVRS patients had a higher

TABLE 5 | Microbiological colonization before LuTX.

	T_{LVRS} (n = 26)	C_{LVRS} (n = 328)	p (%) =	T_{ELVR} (n = 56)	C_{ELVR} (n = 270)	p (%) =
Colonization pre-LuTX			***0.009***			***0.010***
None	15 (57.7%)	68.9		34 (60.7%)	67.6	
Candida sp. or YLF	8 (30.8%)	13.7		14 (25.0%)	16.8	
Aspergillus spp.	1 (3.8%)	7.5		4 (7.1%)	1.7	***0.022***
Pseudomonas spp.		6.8		1 (1.8%)	5.2	
Staphylococcus aureus	1 (3.8%)	5.7		4 (7.1%)	3.2	
Klebsiella spp.	1 (3.8%)	4.0		3 (5.4%)	2.1	
Escherichia coli	1 (3.8%)	1.8		2 (3.6%)	5.1	
Serratia marcescens	1 (3.8%)	3.1		2 (3.6%)	3.9	
Stenotrophomonas maltophilia	1 (3.8%)	1.4		1 (1.8%)	4.9	
Pasteurella multocida	1 (3.8%)			1 (1.8%)		
Achromobacter spp.	1 (3.8%)	0.7			0.9	
Enterobacter cloacae complex	1 (3.8%)	0.4	***0.037***		3.0	
Streptococcus spp.	1 (3.8%)	0.3	***0.027***		4.3	
Slow growing NTM		2.6		1 (1.8%)	1.9	

Species with occurrence <3% in all groups were omitted from table; control columns consist of weighted data; T_{LVRS}, patients with previous LVRS; C_{LVRS}, matched controls; T_{ELVR}, patients with previous ELVR; C_{ELVR}, matched controls; YLF, yeast like fungi; NTM, nontuberculous mycobacteria. Significant p *values are highlited in bold italic.*

TABLE 6 | Microbiological cultures after LuTX in one center.

	T_{LVRS} (n = 11)	C_{LVRS} (n = 138)	p (%) =	T_{ELVR} (n = 26)	C_{ELVR} (n = 126)	p (%) =
Colonization post-LuTX			***0.005***			***0.021***
None	5 (45.5%)	27.2		6 (23.1%)	32.2	
Enterococcus spp.	5 (45.5%)	51.5		13 (50.0%)	32.3	
Slow growing NTM	3 (27.3%)	2.5	***0.000***	3 (11.5%)	5.1	
Candida spp. or YLF	3 (27.3%)	43.0		14 (53.8%)	41.3	
Aspergillus species	2 (18.2%)	14.5		7 (26.9%)	28.5	
Staphylococcus epidermidis	1 (9.1%)			2 (7.7%)		
Enterobacter cloacae complex		2.5		3 (11.5%)	3.3	
Stenotrophomonas maltophilia		9.3		2 (7.7%)	11.1	
Escherichia coli		4.2		1 (3.8%)	2.6	
Klebsiella spp.		2.5		1 (3.8%)	9.8	
Achromobacter spp.		3.4			2.6	
Staphylococcus aureus		3.2			7.8	
Citrobacter freundii		0.8			3.3	
Rapid growing NTM		0.8			3.3	
Pseudomonas spp.		10.3			18.2	

Percentages <3% in all groups were omitted; control columns consist of weighted data; T_{LVRS}, patients with previous LVRS; C_{LVRS}, matched controls; T_{ELVR}, patients with previous ELVR; C_{ELVR}, matched controls; NTM, nontuberculous mycobacteria; YLF, yeast like fungi. Significant p *values are highlited in bold italic.*

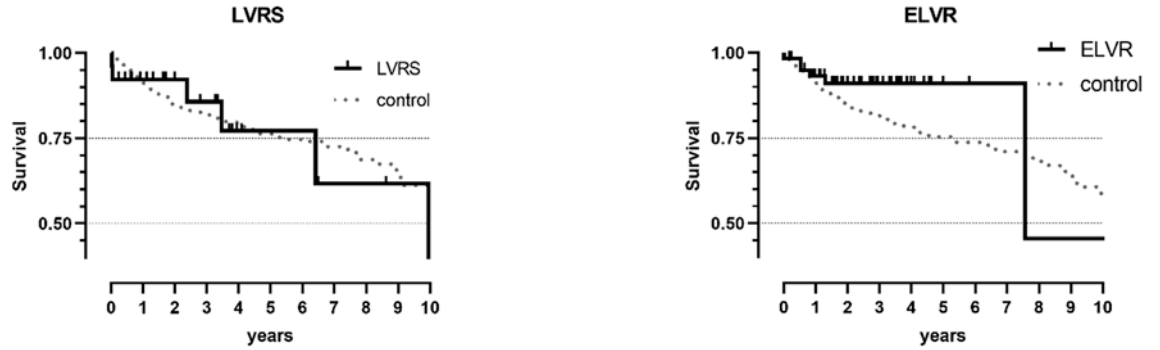
occurrence of post-operative empyema (n = 2; 7.7% vs. 1.2%; p = 0.014). On the other hand, ELVR patients had a higher rate of wound infections (n = 5; 8.9% vs. 2.5%; p = 0.018). In deceased patients, graft failure was reported more often as cause of death in both treatment groups compared to controls (LVRS: 16.7% vs. 1.9; p = 0.044 | ELVR: 16.7 vs. 1.5%; p = 0.034). All recorded complications and causes of death are presented in **Table 4**.

In patients with previous LVR treatment, microbiologic colonization or infection was more often detected and the spectrum of positive cultures differed significantly (LVRS: 42.3% vs. 31.1%; p = 0.009 | ELVR: 39.3% vs. 32.4%; p = 0.01) as shown in **Table 5**. After LuTX and immunosuppression, the rate of positive cultures increased (LVRS: 54.5% vs. 72.8%; p = 0.005 | ELVR: 76.9% vs. 76.8%; p = 0.021) and as expected certain species became more prevalent as the microbiome changed (*enterococcus* spp., yeasts, mycobacteria, *aspergillus*; **Table 6**).

Short and long-term survival after LuTX was excellent across groups and controls and consistently comparable with the control groups (1-/5-year: LVRS: 92.3%/77.1%/p = 0.583|ELVR: 98.3%/91.0%/p = 0.174). Median survival after LuTX was: LVRS: 9.95 years and ELVR: 7.56 years. Median survival for controls was not calculated as more than 50% of those patients were still alive at time of analysis (**Figure 1**).

DISCUSSION

This multi-center matched retrospective cohort study, assessed post-operative outcomes of emphysema patients, who underwent surgical or endoscopic LVR prior to LuTX. After matching, baseline indicators



# at risk	1-year	3-year	5-years	10-years
T_{LVRS}	20	13	6	1
$C_{controls}$	481	289	155	15

# at risk	1-year	3-year	5-years	10-years
T_{ELVR}	49	21	4	2
$C_{controls}$	452	281	157	14

FIGURE 1 | Kaplan Meier survival between treatment groups and controls. Data is unmatched and unweighted; T_{LVRS}: patients with previous L_{VRS}; C_{LVRS}: matched controls; T_{ELVR}: patients with previous E_{LVR}. C_{ELVR}: matched controls; no significant differences in Log-Rank tests.

and intraoperative modalities were comparable between groups and controls. The analysis showed that both previous LVRS and ELVR were associated with a different spectrum of bacterial colonization prior to LuTX which must be considered to prevent infectious complications. LVR did not impact short- and long-term survival, which was equally good in all groups.

Interestingly we observed that patients with previous LVRS had a higher rate of post-operative ECMO need after LuTX. This might be explained by a longer and more difficult preparation due to pleural adhesions. Hence, potentially leading to longer surgery durations/ischemic times and an increased need for blood transfusions, all known risk factors for early mortality and PGD (22–24). Three cases of PGD 3 within 72 h occurred in the LVRS group. In previous publications, an association of previous LVRS with a higher pulmonary arterial pressure and an increased risk of phrenic nerve palsy was postulated (13, 15). These assumptions are not corroborated by our data as median PAP was 32 mmHg and not a single case of phrenic nerve injury was observed in the LVRS group. Additionally, it is noteworthy that in this cohort, the outcome in LVRS patients was statistically comparable to the outcome in patients with previous non-LVRS intrathoracic surgery.

To the best of our knowledge, this study is the largest published series about ELVR prior to LuTX. Those patients had equally good perioperative outcomes as controls although a higher occurrence of wound infections (8.9% vs. 2.5%; p = 0.018) was observed. Out of those five patients, four had positive sputum cultures (*Candida/Aspergillus/Klebsiella*) before LuTX. After LuTX, these patients suffered also from empyema $n = 3$ and pneumonia $n = 1$. Although the association between ELVR and post-LuTX wound infection is not fully understood, we hypothesize that a different spectrum of pre-transplant colonization and an increased exposure to antibiotics might make them more susceptible to hospital acquired infections and multi-drug resistant bacteria.

This study is the first to assess extensively airway colonization of LuTX recipients who underwent previous LVRS or ELVR. It showed that LVR was associated with a distinct airway microbiome both before and after LuTX. In comparison to other LuTX-indications COPD has a lower risk of bacterial infections (25). It was unexpected that the number of colonized patients were as high even before LuTX (LVRS: 42.3%; ELVR: 39.3%). Unfortunately, data on colonization after LuTX was only available for one of the three participating centers. In this subgroup, colonization rates of 54% in LVRS patients and 76.9% in ELVR patients were seen after LuTX.

In LVRS patients a higher rate of slow growing mycobacteria was observed after LuTX in comparison to the control group (27.3% vs. 2.5%; $p < 0.001$), an observation which cannot be readily explained. On the other hand, ELVR has been previously associated with pathological colonization as implanted valves and coils impede mucus clearance (26). Although a predominance of *stenotrophomonas maltophilia* (40%) after ELVR and LuTX has been described (18), this relationship could not be confirmed by our data in which only 7.7% patients presented with *s.maltophilia* after LuTX.

The strength of this study and its conclusion is given by its design. Three high volume centers experienced in both LVR and LuTX provided data on all patients recently transplanted for emphysema at their institution. To correct for selection bias and differences in patient characteristics, LVRS and ELVR patients were matched to weighted control groups. This led to highly balanced groups.

The observations of this study are mostly in line with a recent single-center analysis comparing 52 LVRS+LuTX patients to 65 unmatched controls (17). However, our findings differed markedly from those of a recent UNOS-database analysis (12) which included 106 LVRS+LuTX patients (from 37 LuTX centers), propensity matched to 106 controls without previous intrathoracic surgery (from 67 LuTX centers). This UNOS analysis identified a significantly increased risk of death (HR: 1.72; CI: 1.13-2.6; p = 0.01) after LVRS+LuTX, which was

surprisingly not associated with the total number of LuTX (HR: 0.99) or the total number of LVRS+LuTX (HR: 0.99) of individual centers. Furthermore, the observed median survival was significantly worse in LVRS patients in comparison to matched controls (3.4 vs. 6.5; $p = 0.038$).

The present study has several limitations. First, no donor specific characteristics apart from ischemic times of the donor lungs were taken into consideration. Secondly, there is substantial heterogeneity in how LVRS was performed. LVRS nowadays is routinely performed by a bilateral video-thoracoscopic approach and we can hypothesize that such a minimally invasive approach would have a lesser associated risk in a latter LuTX. In this cohort, sternotomy, thoracotomy, VATS, pleurectomy, pleurodesis and pleural tenting were in use and inevitably lead to pleural adhesions, albeit to different extent. By the sample size of 26 LVRS patients, this cannot be sufficiently considered. A similar limitation applies to different ELVR approaches (valves, coils, foam, vapor) having a different risk profile (pneumonia, exacerbation and pneumothorax) (27) and suggesting that their impact on a later LuTX may differ. A possible observer bias must be addressed with regards to the microbiological cultures. Although recipient bronchi were all sampled during LuTX, patients who underwent previous ELVR had supposedly more bronchoscopies and therefore more samples taken before transplant.

Most patients had LVR before being listed for transplantation ($n = 78$). "Bridging to LuTX" only took place in 8 patients who were already on the waiting list. Waiting time for LuTX was comparable throughout groups as presented in **Table 1** (LVRS 180 days vs. 203 days in controls; ELVR 156 vs. 176 days in controls).

Additionally, critically ill patients who underwent LVR and were not later referred to LuTX (because of improvement, complications, or clinical misjudgment) were not considered. Hence, this study cannot predict the impact of LVR as an alternative to LuTX. It did not account for functional improvements while waiting, nor for changes in LAS scores and impact on waiting times. The crucial question about QOL, functional/survival benefits, and timing of LVR before LuTX cannot be answered and the authors recommend further prospective investigation to answer it.

CONCLUSION

This study clearly demonstrates that patients who underwent previous surgical or endoscopic LVR can safely be considered for later LuTX. Although a marginally increased risk of specific complications and differences in airway colonization after LuTX were observed, short- and long-term survival was very good.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committee of the Medical Faculty of the University of Duisburg-Essen (21-9856-BO). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

All authors meet ICMJE criteria as they contributed substantially to this study. Conception: AS. Data collection: AS, CH, PB, JV, SS and CV. Data analysis and interpretation: AS, LC, CH, KH and CA. Drafting of the article: AS and CA. Critical revision of the article: LC, DV, KH, CA, MK and PJ. Final approval: AS, LC, CH, PB, JV, SS, DV, KH, CA, CV, MK and PJ.

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 wish to express their gratitude to the nurses, coordinators, assistants, anesthesiologists, intensive care physicians, pneumologists and surgeons involved in the programs of the participating centers. In particular, following persons are thanked for their commitment and their expert advice: Vasiliki Bessa, Kaid Darwiche, Christian Taube, Stephane Collaud, Achim Koch, Anna Frick, Alberto Benazzo, Nikolaus Pizanis. We acknowledge financial support by the Open Access Publication Fund of the University of Duisburg-Essen.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10048/full#supplementary-material

REFERENCES

1. van Agteren JE, Carson KV, Tiong LU, Smith BJ. Lung Volume Reduction Surgery for Diffuse Emphysema. *Cochrane Database Syst Rev* (2016) 10: CD001001. doi:10.1002/14651858.CD001001.pub3
2. Singer L, Singer J. Quality of Life in Lung Transplantation. *Semin Respir Crit Care Med* (2013) 34:421–30. doi:10.1055/s-0033-1348470
3. Criner GJ, Cordova F, Sternberg AL, Martinez FJ. The National Emphysema Treatment Trial (NETT). *Am J Respir Crit Care Med* (2011) 184:881–93. doi:10.1164/rccm.201103-0455ci
4. Naunheim KS, Wood DE, Mohsenifar Z, Sternberg AL, Criner GJ, DeCamp MM, et al. Long-term Follow-Up of Patients Receiving Lung-Volume-Reduction Surgery versus Medical Therapy for Severe Emphysema by the National Emphysema Treatment Trial Research Group. *Ann Thorac Surg* (2006) 82:431–43. doi:10.1016/j.athoracsur.2006.05.069
5. Fishman A, Martinez F, Naunheim K, Piantadosi S, Wise R, Ries A, et al. A Randomized Trial Comparing Lung-Volume-Reduction Surgery with Medical Therapy for Severe Emphysema. *N Engl J Med* (2003) 348:2059–73. doi:10.1056/NEJMoa030287
6. Gompelmann D, Eberhardt R, Herth FJF. Endoscopic Lung Volume Reduction. A European Perspective. *Ann ATS* (2013) 10:657–66. doi:10.1513/annalsats.201301-003fr
7. Darwiche K, Aigner C. Clinical Management of Lung Volume Reduction in End Stage Emphysema Patients. *J Thorac Dis* (2018) 10:S2732–S2737. doi:10.21037/jtd.2018.02.69
8. Weill D, Benden C, Corris PA, Dark JH, Davis RD, Keshavjee S, et al. A Consensus Document for the Selection of Lung Transplant Candidates: 2014-An Update from the Pulmonary Transplantation Council of the International Society for Heart and Lung Transplantation. *J Heart Lung Transplant* (2015) 34:1–15. doi:10.1016/j.healun.2014.06.014
9. Darwiche K, Karpf-Wissel R, Eisenmann S, Aigner C, Welter S, Zarogoulidis P, et al. Bronchoscopic Lung Volume Reduction with Endobronchial Valves in Low-FEV1 Patients. *Respiration* (2016) 92:414–9. doi:10.1159/000452629
10. Trudzinski FC, Höink AJ, Leppert D, Fähndrich S, Wilkens H, Graeter TP, et al. Endoscopic Lung Volume Reduction Using Endobronchial Valves in Patients with Severe Emphysema and Very Low FEV1. *Respiration* (2016) 92: 258–65. doi:10.1159/000448761
11. Slama A, Taube C, Kamler M, Aigner C. Lung Volume Reduction Followed by Lung Transplantation-Considerations on Selection Criteria and Outcome. *J Thorac Dis* (2018) 10:S3366–S3375. doi:10.21037/jtd.2018.06.164
12. Krishnan A, Chidi A, Merlo CA, Shah PD, Ha J, Higgins RSD, et al. Lung Volume Reduction Surgery Prior to Lung Transplantation: A Propensity-Matched Analysis. *Ann Thorac Surg* (2021) 113(2):491–7. doi:10.1016/j.athoracsur.2021.02.009
13. Backhus L, Sargent J, Cheng A, Zeliadt S, Wood D, Mulligan M. Outcomes in Lung Transplantation after Previous Lung Volume Reduction Surgery in a Contemporary Cohort. *J Thorac Cardiovasc Surg* (2014) 147:1678–83. doi:10.1016/j.jtcvs.2014.01.045
14. Burns KEA, Keenan RJ, Grgurich WF, Manzetti JD, Zenati MA. Outcomes of Lung Volume Reduction Surgery Followed by Lung Transplantation: a Matched Cohort Study. *Ann Thorac Surg* (2002) 73:1587–93. doi:10.1016/s0003-4975(02)03499-9
15. Shigemura N, Gilbert S, Bhama JK, Crespo MM, Zaldonis D, Pilewski JM, et al. Lung Transplantation after Lung Volume Reduction Surgery. *Transplantation* (2013) 96:421–5. doi:10.1097/tp.0b013e31829853ac
16. Wisser W, Deviatko E, Simon-Kupilik N, Senbaklavaci Ö, Huber ER, Wolner E, et al. Lung Transplantation Following Lung Volume Reduction Surgery. *J Heart Lung Transplant* (2000) 19:480–7. doi:10.1016/s1053-2498(00)00085-1
17. Inci I, Iskender I, Ehrsam J, Caviezel C, Hillinger S, Opitz I, et al. Previous Lung Volume Reduction Surgery Does Not Negatively Affect Survival after Lung Transplantation†. *Eur J Cardiothorac Surg* (2018) 53:596–602. doi:10.1093/ejcts/ezx318
18. Fuehner T, Clajus C, Fuge J, Jonigk D, Welte T, Haverich A, et al. Lung Transplantation after Endoscopic Lung Volume Reduction. *Respiration* (2015) 90:243–50. doi:10.1159/000434685
19. Sarmand N, Gompelmann D, Kontogianni K, Polke M, Herth FJ, Eberhardt R. New Bacterial Growth in Bronchial Secretions after Bronchoscopic Valve Implantation. *Int J Chron Obstruct Pulmon Dis* (2018) 13:565–70. doi:10.2147/copd.s148196
20. Iacus SM, King G, Porro G. Cem: Software for Coarsened Exact Matching. *J Stat Softw* (2009) 30:1–27. doi:10.18637/jss.v030.i09
21. King G, Nielsen R. Why Propensity Scores Should Not Be Used for Matching. *Polit Anal* (2019) 27:435–54. doi:10.1017/pan.2019.11
22. Altun GT, Arslantaş MK, Cinel İ. Primary Graft Dysfunction after Lung Transplantation. *Turk J Anaesthesiol Reanim* (2015) 43:418–23. doi:10.5152/TJAR.2015.16443
23. Borders CF, Suzuki Y, Lasky J, Schaufler C, Mallem D, Lee J, et al. Massive Donor Transfusion Potentially Increases Recipient Mortality after Lung Transplantation. *J Thorac Cardiovasc Surg* (2017) 153:1197–203. doi:10.1016/j.jtcvs.2016.12.006
24. Weber D, Cottini SR, Locher P, Wenger U, Stehberger PA, Fasshauer M, et al. Association of Intraoperative Transfusion of Blood Products with Mortality in Lung Transplant Recipients. *Perioper Med* (2013) 2:20. doi:10.1186/2047-0525-2-20
25. Paglicci L, Borgo V, Lanzarone N, Fabbiani M, Cassol C, Cusi MG, et al. Incidence and Risk Factors for Respiratory Tract Bacterial Colonization and Infection in Lung Transplant Recipients. *Eur J Clin Microbiol Infect Dis* (2021) 40(6):1271–82. doi:10.1007/s10096-021-04153-1
26. Fruchter O, Rosengarten D, Goldberg E, Ben-Zvi H, Tor R, Kramer MR. Airway Bacterial Colonization and Serum C-Reactive Protein Are Associated with Chronic Obstructive Pulmonary Disease Exacerbation Following Bronchoscopic Lung Volume Reduction. *Clin Respir J* (2016) 10:239–45. doi:10.1111/crj.12211
27. Ramaswamy AA, Puchalski J. Bronchoscopic Lung Volume Reduction: Recent Updates. *J Thorac Dis* (2018) 10:2519–27. doi:10.21037/jtd.2018.02.72

ESOT | Transplant International

ORIGINAL RESEARCH
published: 13 April 2022
doi: 10.3389/ti.2022.10228

Three-Year Outcomes in Kidney Transplant Recipients Switched From Calcineurin Inhibitor-Based Regimens to Belatacept as a Rescue Therapy

*Antoine Morel[1], Léa Hoisnard[2,3†], Caroline Dudreuilh[1†], Anissa Moktefi[4,5], David Kheav[6], Ana Pimentel[1], Hamza Sakhi[1], David Mokrani[1], Philippe Attias[1], Karim El Sakhawi[1], Cécile Maud Champy[7], Philippe Remy[1,5], Emilie Sbidian[2,3,8,9], Philippe Grimbert[1,2,5,10] and Marie Matignon[1,5]**

[1]Nephrology and Renal Transplantation Department, AP-HP (Assistance Publique-Hôpitaux de Paris), Hôpitaux Universitaires Henri Mondor, Créteil, France, [2]AP-HP (Assistance Publique-Hôpitaux de Paris), Hôpitaux Universitaires Henri Mondor, Centre d'Investigation Clinique and Fédération Hospitalo-Universitaire TRUE (InnovaTive theRapy for immUne disordErs), Créteil, France, [3]Université Paris Est Créteil (UPEC), EpiDermE (Epidemiology in Dermatology and Evaluation of therapeutics), Créteil, France, [4]Groupe Hospitalier Henri-Mondor/Albert-Chenevier, Pathology Department, AP-HP (Assistance Publique-Hôpitaux de Paris), Créteil, France, [5]Institut National de la Santé et de la Recherche Médicale (INSERM) U955, Institut Mondor de Recherche Biomédicale (IMRB), Université Paris-Est Créteil, Créteil, France, [6]AP-HP (Assistance Publique-Hôpitaux de Paris), Laboratoire Régional d'histocompatibilité, Hôpital Saint Louis, Vellefaux, Paris, [7]Groupe Hospitalier Henri-Mondor/Albert Chenevier, Urology department, AP-HP (Assistance Publique-Hôpitaux de Paris), Hôpitaux Universitaires Henri Mondor, Créteil, France, [8]Department of Dermatology, AP-HP (Assistance Publique-Hôpitaux de Paris), Hôpitaux Universitaires Henri Mondor, Créteil, France, [9]INSERM, Centre d'Investigation Clinique 1430, Créteil, France, [10]AP-HP (Assistance Publique-Hôpitaux de Paris), Hôpitaux Universitaires Henri Mondor, CIC biotherapy, Créteil, France

Background: The long-term benefits of conversion from calcineurin inhibitors (CNIs) to belatacept in kidney transplant recipients (KTr) are poorly documented.

Methods: A single-center retrospective work to study first-time CNI to belatacept conversion as a rescue therapy [eGFR <30 ml/min/1.73 m^2, chronic histological lesions, or CNI-induced thrombotic microangiopathy (TMA)]. Patient and kidney allograft survivals, eGFR, severe adverse events, donor-specific antibodies (DSA), and histological data were recorded over 36 months after conversion.

Results: We included N = 115 KTr. The leading cause for switching was chronic histological lesions with non-optimal eGFR (56.5%). Three years after conversion, patient, and death-censored kidney allograft survivals were 88% and 92%, respectively, eGFR increased significantly from 31.5 ± 17.5 to 36.7 ± 15.7 ml/min/1.73 m^2 ($p < 0.01$), the rejection rate was 10.4%, OI incidence was 5.2 (2.9–7.6) per 100 person-years. Older age was associated with death, eGFR was not associated with death nor allograft loss. No patient developed *dn*DSA at M36 after conversion. CNI-induced TMA disappeared in all cases without eculizumab use. Microvascular inflammation and chronic lesions remained stable.

Conclusion: Post-KT conversion from CNIs to belatacept, as rescue therapy, is safe and beneficial irrespective of the switch timing and could represent a good compromise facing organ shortage. Age and eGFR at conversion should be considered in the decision whether to switch.

Keywords: kidney transplantation, transplant outcomes, belatacept conversion, rescue therapy, CNI

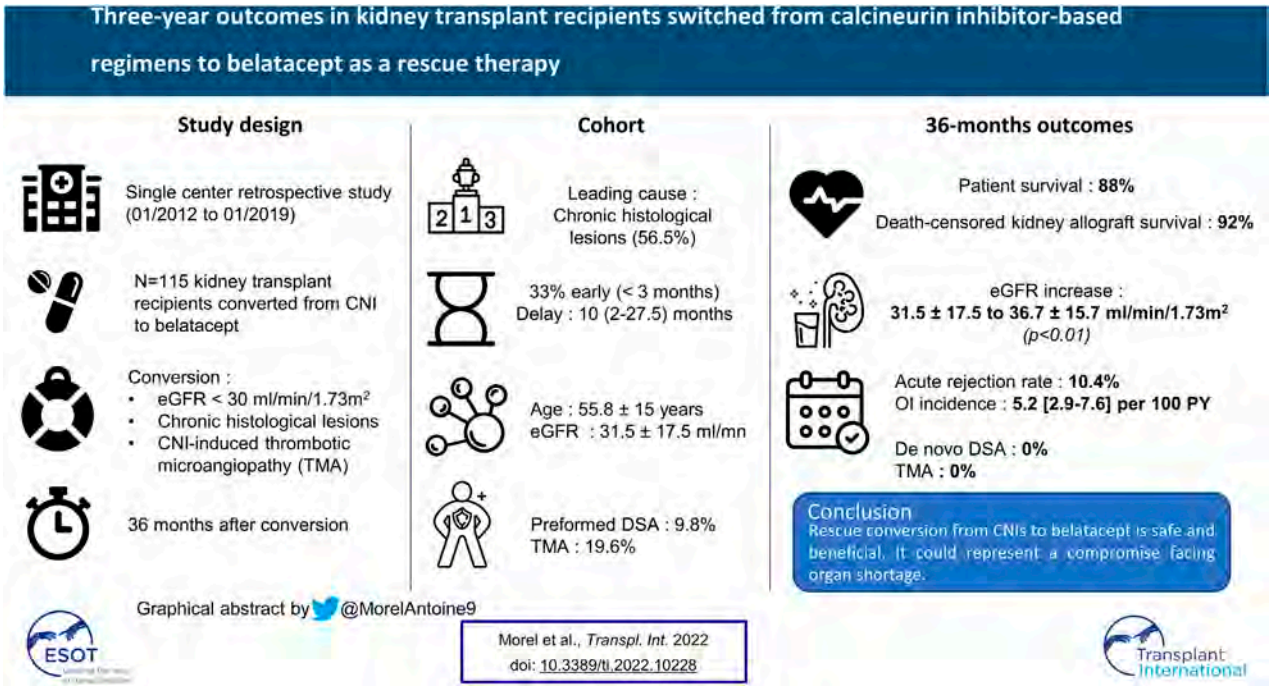
OPEN ACCESS

***Correspondence:**
Marie Matignon
marie.matignon@aphp.fr

†These authors have contributed equally to this work

***Received:** 13 November 2021*
***Accepted:** 18 March 2022*
***Published:** 13 April 2022*

Citation:
Morel A, Hoisnard L, Dudreuilh C, Moktefi A, Kheav D, Pimentel A, Sakhi H, Mokrani D, Attias P, El Sakhawi K, Champy CM, Remy P, Sbidian E, Grimbert P and Matignon M (2022) Three-Year Outcomes in Kidney Transplant Recipients Switched From Calcineurin Inhibitor-Based Regimens to Belatacept as a Rescue Therapy. Transpl Int 35:10228. doi: 10.3389/ti.2022.10228



GRAPHICAL ABSTRACT |

INTRODUCTION

Despite the improvement of kidney allograft short-term survival with conventional immunosuppressive agents, allograft long-term survival has not increased as expected (1). One of the main reasons is the growing proportion of expanded criteria donors (ECD) in kidney transplantation (KT) (2). Calcineurin inhibitors (CNIs) are the standard long-term immunosuppression therapy in kidney transplant recipients (KTr), albeit they could contribute to acute and chronic impairment of kidney allograft function, especially in patients with chronic histological damage (2–5,6,8). Therefore, new immunosuppressive strategies are needed to preserve kidney allograft function and improve the graft long-term survival (6).

Belatacept is a CD80/CD86—CD28 T-cell selective costimulation blocker developed to counteract CNI-induced nephrotoxicity. Two prospective randomized trials (BENEFIT and BENEFIT-EXT study) reported long-term safety and efficacy of *de novo* belatacept treatment coupled with the improvement of estimated glomerular filtration rate (eGFR), and similar patient or kidney allograft survival in comparison with cyclosporine (8–11).

Since, growing evidence has suggested shifting KTr from CNIs-based regimen to belatacept, especially in those with low graft function or chronic histological lesions where belatacept is used as a rescue therapy (12–15). Additionally, it might be effective in sensitized kidney allograft recipients with preformed DSA (13–16). There is very little knowledge on the outcome of patients switched to belatacept after 1 year of follow-up, and what data there is often come from small sample cohorts, without DSA nor histological evolution analysis after the switch (8, 16).

In this study, we assessed the safety and tolerability of belatacept treatment as a rescue therapy up to 3 years after switching from CNIs. Additionally, we analyzed kidney allograft function, patient and kidney allograft survival, and major outcomes after switching to belatacept and its effects on both DSA and kidney allograft histology changes.

METHODS

Patients and Study Design

In this retrospective monocentric study, we included all adults KTr converted for the first time from CNI-based immunosuppressive regimen to belatacept from January 2012 to January 2019. Patients who tested negative for EBV before transplantation, pregnant women, or women not on any contraceptive methods were not included since they were not eligible to receive belatacept treatment.

The KTr Cohort Was Approved by IRB #00003835.

Interventions

Early and late conversion groups were defined according to the time of conversion from KT to first belatacept infusion: < 3 months or >3 months, respectively. In early-stage conversion, CNIs were stopped at day 1, and KTr were given 10 mg/kg belatacept infusions at days 1, 5, 14, and 28, and weeks 8 and 12, and then 5 mg/kg from week 16 onwards, every 4 weeks. In late-stage conversion, CNIs were stepped down to 50% at day 14

and stopped at day 28 after conversion, and belatacept infusions were given at 5 mg/kg at day 1, 14, 28, and then every 4 weeks thereafter (8).

Study Endpoints

The primary endpoint was the safety and tolerability of belatacept treatment. Major adverse events were defined as patient death and kidney allograft loss. Follow-up continued till August 30, 2021 or the date when a major adverse event occurred. Other severe adverse events (SAE) included community-acquired infections requiring hospitalization, OIs, acute rejections, and neoplasia.

Secondary endpoints were: 1) eGFR and urine protein/creatinine ratio (UPCR) evolution up to 3 years after conversion, 2) identification of different clusters of eGFR trajectory after conversion, 3) metabolic parameters (LDL, HDL-cholesterol, triglycerides concentration, HbA1C) and blood pressure profile evolution, 4) CMV or BK viremia, 5) pre-existing and *dn*DSA evolution, and 6) histological lesions evolution.

Community-Acquired Infection, Opportunistic Infection Definitions, and Anti-microbial Prophylaxis

Community-acquired infections were considered only in case of hospitalization. Opportunistic Infection (OIs) were defined according to the current literature and international guidelines (18). OIs caused by the following pathogens were considered:

- Bacteria: *Mycobacterium* sp., *Listeria* monocytogenes, and *Nocardia* sp.
- Viruses: Cytomegalovirus (CMV), Varicella-Zoster virus (VZV), Human Herpes Virus-8 (HHV8), Norovirus, BK virus nephropathy, and JC virus.
- Fungi: Candida spp., Cryptococcus spp., invasive molds, and Pneumocystis jirovecii.
- Parasites: Toxoplasma gondii, Microsporidium sp., Cryptosporidium sp., Leishmania sp.

Patients were screened for BK viremia once a month during the first 3 months after KT, then every 3 months till the end of the first year, and every year till the end of year 5. After switching to belatacept, BKV was monitored every 3 months during the first year then once a year up to 5 years after KT. CMV prophylaxis followed the international guidelines: valganciclovir for 6 months in high-risk patients CMV D^+/R^- and 3 months in intermediate-risk patients CMV D^+/R^+ or CMV D^-/R^+ (19). Pneumocystis prophylaxis (Trimethoprim + Sulfamethoxazole) was administered during the first post-KT year.

Variables

Demographic characteristics, medical data, and laboratory samples, in particular eGFR, UPCR, and DSA, were collected at the time of transplantation, at the time of conversion, and during belatacept treatment (3, 12, 24, 36, 48, and 60 months).

GFR was estimated using the Modification of diet in renal disease (MDRD) formula (20). Indications for switching to belatacept were recorded. Chronic histological lesions associated with suboptimal allograft function was defined as eGFR <30 ml/min/1.73 m^2 and histological lesions associating ci + ct ≥ 3 and/or cv + ah ≥ 2.

Delayed graft function (DGF) was defined as the need for dialysis within 7 days after transplantation (21). Allograft loss was defined as the need for long-term dialysis and/or retransplantation.

Anti-HLA Antibody Screening

High-resolution DNA typing was performed in donors and recipients (HLA-A, HLA-B, Cw, HLA-DR, HLA-DQ, or HLA-DP) at the time of KT. All serum samples were assessed for the presence of circulating preformed DSA and *de novo* DSA (*dn*DSA) on all HLA loci (HLA-A, HLA-B, Cw, HLA-DR, HLA-DQ, or HLA-DP) at the time of KT, at conversion, at 3, 12, 24, 36, 48, and 60 months using high-resolution Luminex SAB assay technology (One Lambda, Inc., Canoga Park, CA, United States) on Luminex platform. All beads with MFI >1,000 were considered positive (22). *Dn*DSA were considered positive if MFI was higher than 1,000 at two time points.

Naturally existing DSA antibodies (i.e., presence of DSA in patients with no past immunizing events such as transfusion or pregnancy or having a previous transplant at the time of KT), as well as IgM DSA, were not considered in our study (23).

Histological Analysis

Patients underwent for-cause or protocol kidney allograft biopsies. Acute and chronic histological lesions were described according to the updated Banff classification (24).

Statistical Analysis

Continuous variables were presented in mean (standard deviation, SD) or median (Interquartile range, IQR) as appropriate, and categorical variables in number and percentage. We used *t*-test or Wilcoxon test for continuous variables, and Chi-2 or Fisher exact tests for categorical variables. Paired *t*-test was used to compare quantitative variables at two different time points. In patients who had at least two kidney biopsies (before and after conversion), paired comparisons of histological lesions were performed using Mc Nemar test or binomial test.

Time to death and to allograft loss, and survival without rejection after conversion (censored for death, kidney allograft loss, and belatacept withdrawal) were displayed with Kaplan Meier curves. Hazard ratios were estimated by the Cox regression model. Incident rates of SAEs were estimated per 100 person-years (PY) with their confidence interval and the inter-group ratio of such incidence rate.

Sensitivity analyses of eGFR and proteinuria evolution after conversion to belatacept were performed with imputations of missing data regarding allograft loss (as 6 ml/min/1.73 m^2) alone then death and allograft loss together. However, data missed because of belatacept treatment interruption over the 3-year period were not imputed since the causes of interruption were multiple.

To identify clusters of eGFR trajectories, we used the *k*-means method relying on expectation-maximization algorithms.

TABLE 1 | Clinical and biological characteristics at the time of transplantation.

Variables	Whole Cohort, N = 115	Late Switch, N = 77	Early Switch, N = 38
Recipient characteristics			
Age, mean ± SD	55.8 (15.0)	53.9 (15.0)	60.0 (14.3)
Gender (Male), N (%)	76 (66.1)	51 (66.2)	25 (65.8)
Hemodialysis, N (%)	106 (92.2)	74 (96.1)	32 (84.2)
Previous KT, N (%)	15 (13.0)	13 (16.9)	2 (5.3)
Initial nephropathy			
Glomerulopathy, N (%)	24 (20.9)	15 (19.5)	9 (23.7)
Diabetes mellitus, N (%)	18 (15.7)	9 (11.7)	9 (23.7)
Nephroangiosclerosis, N (%)	11 (9.6)	8 (10.4)	3 (7.9)
Genetic, N (%)	10 (8.7)	8 (10.4)	2 (5.3)
Autoimmune disease, N (%)	4 (3.5)	3 (3.9)	1 (2.6)
Other, N (%)	22 (19.1)	15 (19.5)	7 (18.4)
Undetermined, N (%)	26 (22.6)	19 (24.7)	7 (18.4)
Donor			
Age, mean ± SD	61.5 (15)	60.6 (14.29)	63.4 (16.39)
Living donor, N (%)	7 (6.1)	5 (6.5)	2 (5.3)
Extended criteria donor, N (%)	69 (60)	46 (59.7)	23 (60.5)
Donor/recipient CMV status			
D+/R+, N (%)	51 (44.3)	36 (48.8)	15 (39.5)
D+/R-, N (%)	22 (19.1)	16 (20.8)	6 (15.8)
D-/R+, N (%)	34 (29.6)	21 (27.3)	13 (34.2)
D-/R-, N (%)	8 (7)	4 (5.2)	4 (10.5)
Kidney transplant characteristics			
Anti HLA donor specific antibodies, N (%)	11 (9.8)	6 (7.9)	5 (13.9)
Cold ischemia time, hours N = 112, N (%)	18.1 (5.7)	18.2 (5.5)	17.7 (6)
Delayed graft function, N (%)	51 (44.3)	31 (40.3)	20 (52.6)
Induction immunosuppressive therapy			
Anti-interleukin 2 receptor, N (%)	61 (53)	42 (54.5)	19 (50)
Antithymocyte globulin, N (%)	54 (47)	35 (45.5)	19 (50)
Maintenance immunosuppressive therapy			
Calcineurin inhibitors, N (%)			
Cyclosporine	20 (17.4)	16 (20.8)	4 (10.5)
Tacrolimus	95 (82.6)	61 (79.2)	34 (89.5)
Mycophenolic acid (MPA), N (%)	95 (82.6)	66 (85.7)	29 (76.3)
mTOR inhibitors, N (%)	19 (16.5)	11 (14.3)	8 (21.1)
Steroids	115 (100)	77 (100)	38 (100)

KT, Kidney transplantation; mTOR, Mammalian target of rapamycin.

Sensitivity analyses were performed for mean eGFR at different time points and eGFR trajectories. Missing data due to graft loss and/or death were imputed as 6 ml/min/1.73 m^2.

A p-value <0.05 was considered significant. Tests were two-tailed. Statistical analyses were carried out using R 3.6.2.

RESULTS

From January 2012 to 01/2019, 115 patients underwent first-time switch from CNI to belatacept, of whom 38 (33%) had an early-stage switch, and 76 patients (66.1%) were men.

At the time of transplantation (**Table 1**), the mean age was 55.8 ± 15 years old. Almost all donors were deceased [N = 108 (93.9%)], mainly ECD [N = 69 (60%)], and 61.5 ± 15 years old. All recipients who received induction immunosuppressive therapy, such as anti-interleukin-2 receptor [N = 61/115 (53%)], and N = 11/115 (9.8%) had pretransplant DSA. Maintenance immunosuppressive therapy included CNIs (100%), mycophenolic acid (MPA) (82.6%), and steroids (100%). Of more, N = 22/115 (19.1%) patients were at high risk for CMV transmission (D^+/R^-).

At the time of conversion (**Table 2**), 10 (2–27.5) months after KT, class I and II DSA were detected in 8/115 (7%) and 12/115 (10.4%) patients, respectively. In the late-switch group, the main cause of conversion was chronic vascular histological lesions associated with non-optimal kidney allograft function (71.4%), whereas it was prolonged DGF (55.3%) in the early-

TABLE 2 | Clinical and biological characteristics at the time of conversion.

Variables	Whole Cohort, N = 115	Late Switch, N = 77	Early Switch, N = 38
Conversion time from KT, months, median (IQR)	10 (2–27.5)	17 (10–67)	1 (1–2)
Age, mean ± SD	58.6 (14.4)	57.5 (14.5)	60.8 (14.2)
Reasons for switching			
Prolonged delayed graft function, N (%)	23 (20)	2 (2.6)	21 (55.3)
Chronic histological lesions associated with suboptimal allograft function (ci + ct ≥ 3 and/or cv + ah ≥ 2), N (%)	65 (56.5)	55 (71.4)	10 (26.3)
Thrombotic microangiopathy, N (%)	21 (18.3)	17 (22.1)	4 (10.5)
Other renal causes, N (%)	4 (3.5)	1 (1.3)	3 (7.9)
Undetermined issues, N (%)	2 (1.7)	2 (2.6)	0 (0)
Kidney allograft function			
eGFR (mL/min/1.73 m^2), mean ± SD	31.7 (17.8)	33.9 (16.9)	27.3 (19)
Urine protein/creatinine ratio >100 mg/mmol, N (%)	29 (25.9)	15 (19.7)	14 (38.9)
Drugs			
Antihypertensive drugs, median (IQR)	2 (1–2)	2 (1–2)	2 (1–2)
MPA, N (%)	104 (90.4)	69 (89.6)	35 (92.1)
500 mg per day, N (%)	12 (11.5)	11 (15.9)	1 (2.9)
1,000 mg per day, N (%)	40 (38.5)	38 (55.1)	2 (5.7)
2,000 mg per day, N (%)	40 (38.5)	9 (13.0)	31 (88.6)
Other dose, N (%)	12 (11.5)	11 (15.9)	1 (2.9)
mTOR inhibitors, N (%)	10 (8.7)	7 (9.1)	3 (7.9)
T0 level (ng/ml), median (IQR)	5.2 (4.3–6.1)	4.5 (4.2–5.3)	6.3 (5.8–6.9)
Corticosteroids, N (%)	115 (100)	77 (100)	38 (100)
5 mg per day, N (%)	84 (73.0)	75 (97.4)	9 (23.7)
10 mg per day, N (%)	31 (27.0)	2 (2.6)	29 (76.3)
Anti HLA donor specific antibodies			
Class I, N (%)	8 (7)	6 (7.8)	2 (5.3)
Class II, N (%)	12 (10.4)	12 (15.6)	0 (0)
Both class I and class II, N (%)	2 (1.7)	2 (2.6)	0 (0)
None, N (%)	97 (84.3)	61 (79.2)	36 (94.7)
Kidney biopsy (Banff lesions score)	N = 102	N = 77	N = 25
Biopsy to conversion time, days, median (IQR)	35 (92–12)	48 (118–26)	8.5 (19.8–6.2)
Acute tissue injury			
Banff lesions score ≥1 in at least one compartment, N (%)	50 (48.5)	37 (48.1)	13 (50)
Acute tubular necrosis, N (%)	20 (19.2)	11 (14.3)	9 (33.3)
Glomerulitis (g), N (%)	7 (6.7)	7 (9.1)	0 (0)
Interstitial inflammation (i), N (%)	3 (2.9)	3 (3.9)	0 (0)
Tubulitis (t), N (%)	10 (9.6)	9 (11.7)	1 (3.7)
Peri-tubular capillaritis (cpt), N (%)	3 (2.9)	2 (2.6)	1 (3.7)
Vascular inflammation (v), N (%)	0 (0)	0 (0)	0 (0)
Thrombotic microangiopathy, N (%)	21 (19.6)	17 (22.1)	4 (13.3)
g + cpt (≥2), N (%)	9 (8.7)	8 (10.4)	1 (3.7)
Chronic tissue injury			
Banff lesions score ≥1 in at least one compartment, N (%)	97 (97)	74 (98.7)	23 (92)
Transplant glomerulopathy (cg), N (%)	8 (7.8)	8 (10.4)	0 (0)
Interstitial fibrosis (ci), N (%)	89 (87.3)	68 (89.5)	21 (80.8)
Total inflammation (ti), N (%)			
Tubular atrophy (ct), N (%)	84 (82.4)	66 (86.8)	18 (69.2)
Chronic vasculopathy (cv), N (%)	67 (65.7)	48 (63.2)	19 (73.1)
Arteriolar hyalinization (ah), N (%)	80 (79.2)	61 (81.3)	19 (73.1)
IFTA (ci + ct), N (%)	93 (92.1)	71 (93.4)	22 (88.0)
ci + ct + cg + cv, median (IQR)	4 (2–5)	4 (3–6)	4 (2–4)

KT, Kidney transplantation; eGFR, Estimated glomerular filtration rate; MPA, Mycophenolic acid; mTOR, Mammalian target of rapamycin; IFTA, Interstitial fibrosis and tubular atrophy.

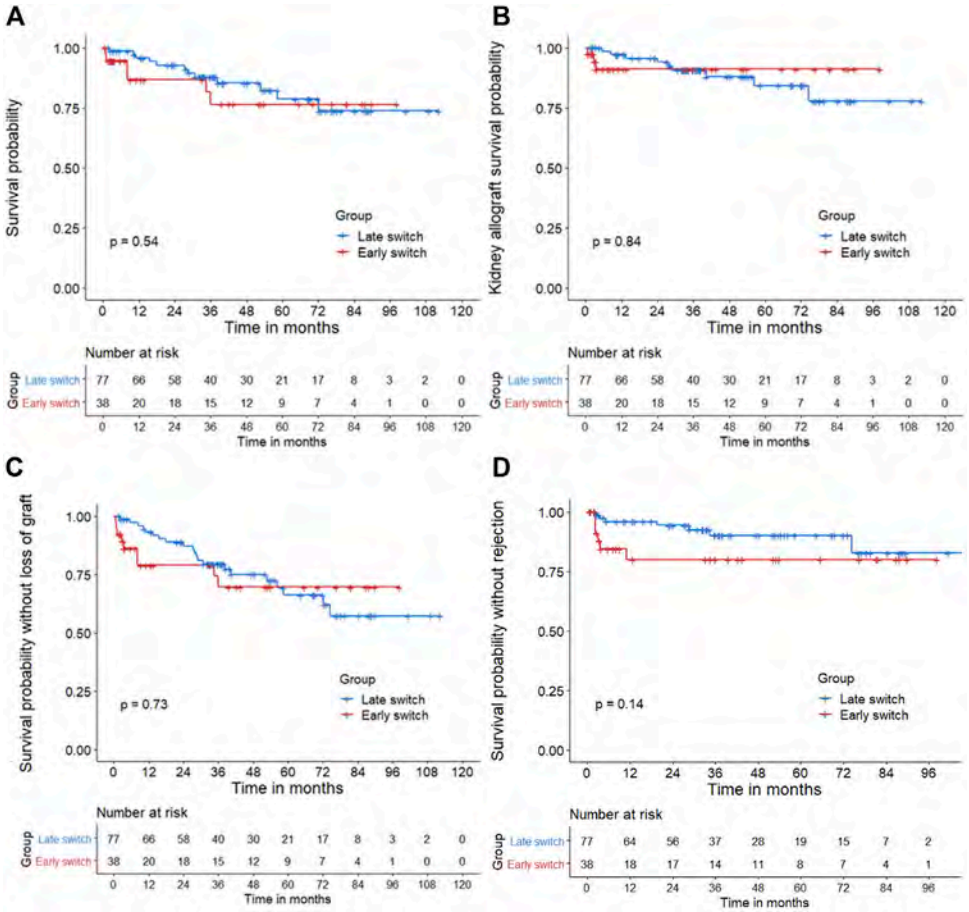


FIGURE 1 | (A): Patient survival—**(B)**: Death-censored kidney allograft survival—**(C)**: Global survival (using a composite outcome of the patient and death-censored kidney allograft survivals)—**(D)**: Survival without acute rejection (censored for death, kidney allograft loss, and belatacept withdrawal). Kaplan-Meier method was used to assess patient survival from time of belatacept conversion (time 0). *p*-values were measured from the log-rank test. *X*-axis: Post-conversion months. The blue curve represents the late switch group, whereas the red curve represents the early switch group. There was no statistical difference of patient, kidney allograft, global survival or survival probability without rejection between early and late conversion groups using Cox analysis ($p = 0.54$, $p = 0.84$, $p = 0.73$, and $p = 0.14$, respectively).

switch group. Concomitant immunosuppression is provided in **Table 2**. A median number of anti-hypertensive drugs was 2 (1–2), levels of HbA1c, LDL, and HDL-cholesterol were 5.9 ± 0.5%, 2.1 ± 0.5 g/L, and 1 ± 0.3 g/L, respectively, (**Supplementary Table S3**)).

Analysis at Month 36

The last follow-up checking was on August 30, 2021. Recipients were followed over 40.2 ± 30.1 months after conversion and $N = 58/115$ (51%) completed 36 months of follow-up. Of the remaining 57 patients who did not reach the third year time point, N = 26/57 discontinued belatacept (alive with functional kidney allograft), N = 13/57 died, N = 9/57 lost their KT, N = 8/57 did not complete 36 months, and N = 1/57 was lost to follow-up. Three of the study patients (N = 115; 2.6%) aged more than 70 years old were treated for less than 3 months. The first developed BK virus nephropathy a month after conversion (blood BK virus replication >6 log at the time of switch), hence the interruption of belatacept. The other two patients were switched to belatacept for arterial thrombosis and primary non-function. Both developed rapid kidney allograft failure requiring renal replacement therapy and interruption of belatacept.

Three years after conversion, patient's and death-censored kidney allograft survival rates were respectively 88% and 92%, which dropped down to 81% and 89% at year 5 (**Figures 1A,B**). Overall graft survival was similar between groups (**Figure 1C**). Age was the only significant risk factor for death after conversion in the univariate analysis [HR: 1.05 (1.01–1.1)]. None of the other factors (conversion time from KT, gender, or eGFR) was significantly associated with death or allograft loss (**Supplementary Table S1**). Estimated GFR significantly increased during the 36 months after conversion, from 31.5 ± 17.5 to 36.7 ±

TABLE 3 | Serious adverse events after conversion, incidence rates per 100 person-years (PY) of treatment exposure.

Events	Whole Cohort N = 115 N (%)	Whole Cohort N = 115 incidence per 100 PY [95% CI]	Late Switch N = 77	Early Switch N = 38	Incidence Rate Ratio [95% CI]
Rejections	12 (10.4)	3.5 [1.6–5.5]	2.6 [1.0–5.3]	6.1 [2.2–13.3]	2.36 [0.66–8.21]
Borderline	1 (0.9)				
Mixed	2 (1.8)				
Acute TCMR	5 (4.3)				
Acute ABMR	1 (0.9)				
Chronic ABMR	3 (2.6)				
Infections					
Community acquired infections	47 (40.9)	15.6 [11.1–20.0]	12.3 [8.2–17.8]	25.7 [15.5–40.1]	2.08 [1.1–2.62]
Opportunistic infections	19 (16.5)	5.2 [2.9–7.6]	5.4 [3.1–8.8]	4.8 [1.8–10.5]	0.89 [0.25–2.62]
CMV disease	7 (6.1)				
Pneumocystosis	5 (4.3)				
VZV	4 (3.5)				
Other OI	3 (2.6)				
Neoplasia	14 (12.2)	3.9 [1.9–6.0]	3.8 [1.8–7.0]	4.3 [1.2–10.9]	1.12 [0.26–3.88]
Solid malignancy	8 (7.0)				
Non-melanoma skin cancer	5 (4.3)				
Post-transplant lymphoproliferative disorder	1 (1.0)				

TCMR, T cell-mediated rejection; ABMR, Antibody-mediated rejection; CMV, Cytomegalovirus; VZV, Varicella-Zoster-Virus; OI, Opportunistic infection; PY, Person-year; CI, Confidence interval.

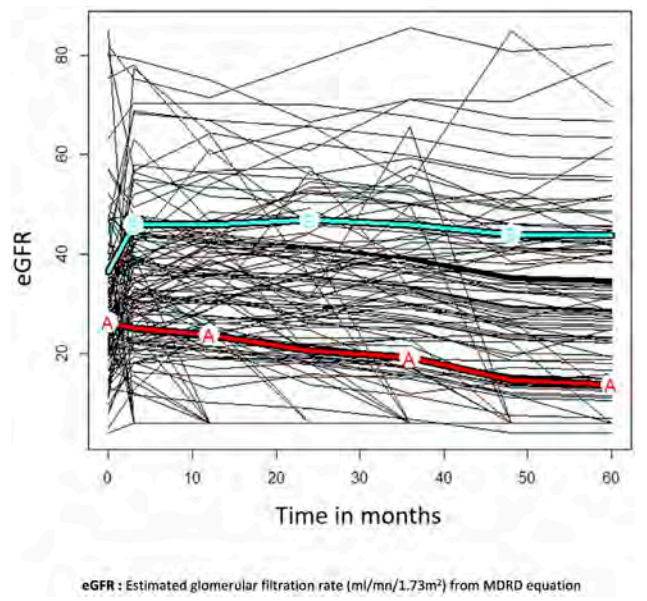


FIGURE 2 | Clusters of estimated glomerular filtration rate trajectories **(A,B)** in a subgroup of N = 114 patients with at least 1 year of follow-up. Missing data were imputed at 6 ml/min for patients who died or lost their allograft. Trajectory A is represented by the red curve and trajectory B by the cyan curve. eGFR rapidly improved at three months and remained stable overtime in trajectory **(B)**. In trajectory **(A)**, eGFR progressively decreased after conversion.

15.7 ml/min/1.73 m^2 ($p < 0.01$). This significant increase was confirmed in the sensitivity analysis ($p = 0.05$; **Supplementary Table S2**). UPCR remained stable after conversion without and with sensitivity analysis (**Supplementary Table S2**). HbA1c, HDL, and LDL-c serum levels significantly decreased over the 36 months after conversion ($p < 0.01$ in all parameters; **Supplementary Table S3**). The number of anti-hypertensive drugs and the triglycerides level remained stable ($p = 0.87$ and $p = 0.39$, respectively) (**Supplementary Table S3**).

Major Adverse Events at the End of Follow-Up

At the end of follow-up, 18/115 (16%) patients died, 12/115 (14%) had allograft failure, and 31/115 (26.9%) discontinued their treatment. The main causes of death were infection (N = 11/115, 9.5%), including three cases of COVID-19, followed by cardiovascular diseases (N = 6/115, 5.2%), and neoplasia (N = 1/115, 0.9%). Allograft loss was mainly due to chronic allograft dysfunction (N = 7/115, 6.1%); other causes implied primary non-function (N = 3/115, 2.6%), chronic antibody-mediated rejection (ABMR) (N = 1/115, 0.9%), and BK virus nephritis (N = 1/115, 0.9%).

The leading cause of belatacept discontinuation was OIs episodes (n = 10/31, 32.3%), albeit no patient discontinued because of allograft loss or death. None of the 31 patients who had their treatment interrupted died and N = 6/31 (19.3%) lost their kidney allograft within 1 year after belatacept interruption. Reasons for kidney allograft loss in those were as follows: N = 4/6 chronic dysfunction, N = 1/6 acute ABMR, and N = 1/6 severe focal and segmental glomerulosclerosis (FSGS).

TABLE 4 | Preformed and *de novo* DSA evolution after conversion.

	Switch N (%) or Median (Q1-Q3)	M3	M12	M24	M36	M48	M60
Available data DSA	115	107	86	77	56	40	34
Pre-existing DSA	18 (15.7)	17/107 (17.2)	18/86 (20.9)	14/77 (18.2)	10/56 (17.9)	8/40 (20)	8/34 (23.5)
Class I	8 (7)	7/107 (6.5)	4/86 (4.6)	5/77 (6.5)	2/56 (3.6)	2/40 (5)	3/34 (8.8)
Class I MFI max	2,188 (1,601–2,844)		1903 (1,288–2,569)		3,302 (2,876–3,728)		
Class I MFI sum	2,388 (1807–3,841)		2,453 (2090–2,569)		3,302 (2,876–3,728)		
Class II	12 (10.4)	12/107 (11.2)	16/86 (18.6)	12/77 (15.6)	9/56 (16.2)	7/40 (1.8)	7/34 (20.6)
Class II MFI max	1769 (1,433–2,951)		1,472 (1,201–3,314)		3,273 (1957–5,092)		
Class II MFI sum	2,920 (1,642–3,178)		2027 (1,292–4,457)		3,674 (1857–5,277)		
*dn*DSA appearance	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

dnDSA, De novo Donor Specific Antibodies; MFI, Mean fluorescence intensity.

SAE at the End of Follow-Up

At the end of the follow-up, the incidence of acute rejection was 10.4% (N = 12/115). Two patients developed another rejection episode. The most common rejection mechanism was acute T-cell mediated (TCMR) (N = 5/115, 4.3%), occurring within the first 3 months after conversion. Incidence was similar in early and late switch groups (20.0% and 9.8% respectively; p = 0.14) (**Figure 1D**). Evolution after rejection was as follows: 1) no patient died, 2) all discontinued belatacept infusion except one case with borderline lesions, and 3) one kidney allograft loss within 1 year after conversion (refractory acute ABMR).

Incidences rates of OI and community-acquired infections were 5.2 (2.9–7.6) and 15.6 (11.1–20) per 100 PY, respectively. The 19 OIs happened 10 (2–17) months after conversion and were mainly CMV disease (N = 7/115, 6.1%) and pneumocystis pneumonia (N = 5/115, 4.3%) (**Table 3**). BK viremia was reported in N = 11/115 (10.8%) patients and CMV reactivation in N = 27/115 (26.5%), especially in early conversion group (38.9% vs. 17.1% in late conversion group, p = 0.012). Among the N = 19 OI patients, the infection caused the death of N = 4/19 (21%), but no allograft loss was reported.

Malignancies were reported in 14/115 (12.2%) recipients, the incidence rate was 3.9 (1.9–6.0) per 100 PY. Most of them had solid malignancy (N = 8/115, 7%) and non-melanoma skin cancers (N = 5/115, 4.3%). We documented one case of post-transplant lymphoproliferative disorder. Of the 14 cancer patients, none stopped belatacept treatment, one died of esophagus neoplasia within 6 months of diagnosis, and two lost their kidney allograft after chronic progressive kidney allograft dysfunction.

The incidence rate ratio between late and early switch groups was similar for all of the studied SAE (e.g., acute rejection, infections, and malignancies) (**Table 3**).

TABLE 5 | Comparison of histological lesions before and after conversion.

Variables	Before Switch, *n* = 48	After Switch, *n* = 48	*p*-value
Time in days (median, IQR)	28 (9–71)	378 (182–802)	—
Acute tissue injury			
Banff lesions score ≥1 in at least one compartment, N (%)	23 (47.9)	30 (62.5)	—
Acute tubular necrosis, N (%)	8 (16.7)	8 (16.7)	1
Glomerulitis (g), N (%)	4 (8.3)	5 (10.4)	1
Interstitial inflammation (*i*), N (%)	3 (6.2)	7 (14.6)	0.34
Tubulitis (*t*), N (%)	3 (6.2)	10 (20.8)	0.07
Peri-tubular capillaritis (cpt), N (%)	3 (6.2)	8 (16.7)	0.13
MVI (g + cpt ≥2), N (%)	0 (0)	1 (2.1)	1
Thrombotic microangiopathy, N (%)	11 (22.9)	0 (0)	<0.001
Chronic lesions			
Banff lesions score ≥1 in at least one compartment, N (%)	47 (97.9)	48 (100)	—
Transplant glomerulopathy (cg), N (%)	4 (8.3)	6 (12.5)	0.5
Interstitial fibrosis (ci), N (%)	43 (89.6)	48 (100)	0.06
Total inflammation (ti), N (%)	7 (14.6)	6 (12.5)	1
Tubular atrophy (ct), N (%)	40 (83.3)	47 (97.9)	0.04
Chronic vasculopathy (cv), N (%)	33 (68.8)	39 (81.2)	0.18
Arteriolar hyalinization (ah), N (%)	33 (68.8)	40 (83.3)	0.07
IFTA (ci + ct), median (IQR)	3 (2–3)	3 (2–3.25)	0.17

MVI, Micro-vascular inflammation.

eGFR Trajectories After Conversion

Two distinct eGFR trajectories were identified in the N = 114 recipients after conversion (**Figure 2**): trajectory A in N = 64/114 (56.1%) KTr and trajectory B in N = 50/114 (43.9%). eGFR rapidly improved at 3 months and remained stable over time in trajectory B. In trajectory A, eGFR progressively decreased after conversion. Cluster A recipients were more likely to have renal replacement therapy before KT ($p < 0.01$), previous KT ($p = 0.01$), eGFR <30 ml/min/1.73 m^2 at the time of conversion (<0.01) (**Supplementary Table S4**). Other characteristics (i.e., early or late switch, recipients' age, and histological characteristics before conversion) did not differ significantly between trajectory clusters.

Pre-Existing and *de novo* DSA Analysis

Before the switch, DSA was detected in N = 18/115 (15.7%) patients whose number remained stable over time after conversion (**Table 4**), though one developed chronic ABMR [N = 1/18 (5.6%)]. No patient developed *dn*DSA at the end of the follow-up.

Histological Analysis

Among the 48 patients who underwent paired kidney allograft biopsies (**Table 5**), the second biopsy was performed 378 (182–802) days after conversion and 60.1% were for-cause biopsies. Regarding acute tissue injuries, all CNI-associated acute thrombotic microangiopathy (TMA) lesions disappeared after conversion ($p < 0.001$). Microvascular inflammation (MVI) remained stable. As for the chronic lesions, all remained stable over time apart from the significant increase in tubular atrophy ($p = 0.04$).

DISCUSSION

We herein report a large monocentric cohort in a real-life situation where KT recipients were switched from CNI-based regimen to belatacept and followed for 3 years. In this cohort, belatacept safety was confirmed. At year 3, the recipient and death-censored kidney allograft survivals reached almost 90%. Estimated GFR improved significantly over the 36-month period after conversion, regardless of the switch early or late timing. To our knowledge, our study is the second-largest cohort studying CNIs-to-belatacept conversion as a rescue therapy with at least 3-years outcomes assessment, the longest follow-up currently available in this indication.

We observed a long-term benefit of CNIs-to-belatacept switch with significant improvement of kidney allograft function up to 3 years after the switch. Like other studies, eGFR significantly improved over the first 3 months, probably after suspending the hemodynamic effect of CNIs (8, 13, 14, 25), and remained stable up to year 5 after conversion in our study. Long-term benefits of belatacept in kidney allograft recipients treated with *de novo* belatacept and no CNIs are well known (9, 10). Recently, a similar benefit has been demonstrated at 24 months after the switch in kidney allograft recipients, regardless of time after transplant and cause of switch (9–11, 26, 27). However, our KTr were older, sourced their grafts mainly from ECD, and had lower eGFR (<35 ml/min/1.73 m^2) (26). UPCR remained stable after conversion without worsening as already described in short-term follow-up studies (13). We also observed a long-term improvement of metabolic parameters such as the reduction in LDL cholesterol and HbA1C with stabilization of triglycerides concentration. Blood pressure remained stable after belatacept conversion. Other studies have already described such metabolic benefits (11, 28) and their short-term stability after CNI-to-belatacept conversion, our results confirmed the long-term stability (13). The clinical outcome of these metabolic changes needs to be further investigated in much longer-term studies.

In the whole cohort and in the late switch group, the leading cause of conversion was histological chronic vascular lesions associated with non-optimal kidney allograft function, whereas in the early switch group it was prolonged DGF. This real-life study design is different from that used in other studies which relied only on patients with stable eGFR (35–75 ml/min/1.73 m^2) (16). Here we confirmed that belatacept is a useful immunosuppressive agent at any time after transplantation and for any cause, even in patients with poor prognostic clinical features.

Recipient and kidney allograft survivals were up to 90% at year 3 after conversion and belatacept safety remained acceptable. Early and late switch groups had similar survivals, suggesting that belatacept could increase kidney allograft survival at any time after transplantation as in KTr with severe vascular lesions (15). Survival results reported in other studies varied according to KT recipients' characteristics (14, 16). In ours, age was the sole significant post-switch risk factor for death. eGFR level at switch was not a risk factor for neither death nor for graft loss, suggesting that conversion could be beneficial in all patients irrespective of their eGFR level. Age should be considered in the clinical decision and further research is warranted to investigate the effects of belatacept conversion in the elderly (i.e., > 70 years old).

OIs incidence in our cohort was comparable to previously published cohorts (29, 30). Alike for OIs leading causes: CMV disease and pneumocystis pneumonia (29,30). Accordingly, we suggest maintaining CMV and Pneumocystis pneumonia prophylaxis in early conversion, close monitoring of CMV viremia, and considering pneumocystis pneumonia prophylaxis in case of lymphopenia (lymphocytes count <1,000/mm^3) (31). Infectious risk should always be considered upon deciding to switch. Similar to other studies, the incidence of malignancies, as well as the low occurrence of PTLD, confirm the low risk of malignancies after belatacept treatment (16).

This is the largest cohort that focuses on kidney allograft histological evolution after conversion from CNI to belatacept. Around 60% of the second biopsies were for-cause. We observed TMA disappearance with no development of ABMR. The usefulness of CNI-to-belatacept conversion in patients with TMA has been described in a few case reports (32–35). In our work, more than 10 TMA lesions vanished after switching to belatacept, suggesting that the latter alone might satisfy cost-effectiveness standards and be a safer strategy than if coupled with Eculizumab in recipients with TMA lesions (33). Post-switch

biopsies showed no worsening in MVI (g + cpt ≥2) but precautions should be taken given the higher risk of allograft loss in these patients (36). Regarding chronic damages, we did not find significant variation over time except for tubular atrophy alone. Nevertheless, interstitial fibrosis and tubular atrophy (IFTA) remained stable, contrary to their tendency to worsen as described in a cohort of post-switch surveillance biopsies (37). Clinical outcomes such as eGFR might be a better predictor of graft outcomes as compared with IFTA (p = 0.031), which is consistent with eGFR improvement after belatacept conversion in our cohort (38). More data on CNI group comparison are still needed to assess kidney allograft histological modifications after conversion.

Considering immunological risks, switching our KTr to belatacept appeared to be safe as the prevalence of rejection was 10%. Similar results were observed in former studies even in sensitized patients (14, 16). Acute rejection appeared quickly after conversion and almost all episodes were TCMR. Short CNI association could be considered especially in early conversion to avoid acute TCMR rejection risk (39). Despite the acute rejection, allograft renal function improved significantly after 3 years. We also confirmed the low incidence of ABMR in recipients with preformed DSA treated with belatacept. DSA detected before the switch remained stable irrespective of other parameters and the incidence rate of *dn*DSA was null over 5 years after conversion. A similar 7-years incidence has been reported in BENEFIT and BENEFIT-EXT studies with higher MFI thresholds (i.e., > 2,000) (40, 41). The post-switch incidence of *dn*DSA was similar to the former study (27). DSA detection techniques and thresholds vary and can explain the differences in results (16, 17, 42). The low incidence of *dn*DSA with belatacept might be explained by the modulation of B-cell function, directly and at the level of B cell-Tfh interaction, incurring impairment of germinal center formation and improper antibody response in belatacept-treated KTr (43).

Despite some limitations including the monocentric, retrospective design and lack of control cohort, our study has several strengths: 1) 3-years post-switch follow-up in a real-life study design, 2) including recipients with impaired kidney allograft function who were potentially not eligible for randomized studies, 3) extensive data collection for each patient, from clinical characteristics to histological and DSA evolution. Data collection was exhaustive over a long follow-up interval.

In conclusion, we showed that in real-life conditions, conversion from CNIs to belatacept, as rescue therapy, is safe and beneficial in terms of long-term kidney allograft preserved function. Patient and kidney allograft survivals were excellent 36 months after conversion with a low incidence of SAE (acute rejection or infections). The immunological risk remained stable after conversion. CNI-to-belatacept switch should also be considered in CNI-treated recipients who develop TMA without ABMR, and could stabilize chronic histological lesions. Prospective studies are warranted to confirm those results.

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 KTr cohort was approved by our local institutional review board no. 00003835 (Mondor Institute for Biomedical Research). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MM, LH and AM conceptualized study and analyzed data. AM, LH, MM, and PG wrote the paper. AM, LH, CD, AM, DK, HS, DM, PA, KE, CM, PR, ES, PG, and MM participated in research design and data collecting.

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

We thank Ms. Tiphanie Londero, the manager of our local KTr follow-up database.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10228/full#supplementary-material

REFERENCES

1. Coemans M, Süsal C, Döhler B, Anglicheau D, Giral M, Bestard O. Analyses of the Short- and Long-Term Graft Survival after Kidney Transplantation in Europe between 1986 and 2015. *Kidney Int*. 2018;94(5):964–73.doi:10.1016/j.kint.2018.05.018
2. Liefeldt L, Brakemeier S, Glander P, Waiser J, Lachmann N, Schönemann C, et al. Donor-specific HLA Antibodies in a Cohort Comparing Everolimus with Cyclosporine after Kidney Transplantation. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2012) 12(5):1192–8. doi:10.1111/j.1600-6143.2011.03961.x
3. Gaston RS. Chronic Calcineurin Inhibitor Nephrotoxicity: Reflections on an Evolving Paradigm. *Clin J Am Soc Nephrol CJASN* (2009) 4(12):2029–34. doi:10.2215/cjn.03820609

4. Nankivell BJ, Borrows RJ, Fung CL-S, O'Connell PJ, Allen RDM, Chapman JR. The Natural History of Chronic Allograft Nephropathy. *N Engl J Med* (2003) 349(24):2326–33. doi:10.1056/nejmoa020009
5. Opelz G, Döhler BCollaborative Transplant Study. Influence of Immunosuppressive Regimens on Graft Survival and Secondary Outcomes after Kidney Transplantation. *Transplantation* (2009) 87(6):795–802. doi:10.1097/tp.0b013e318199c1c7
6. Bamoulid J, Staeck O, Halleck F, Khadzhynov D, Brakemeier S, Dürr M, et al. The Need for Minimization Strategies: Current Problems of Immunosuppression. *Transpl Int* (2015) 28(8):891–900. doi:10.1111/tri.12553
7. Leal R, Tsapepas D, Crew RJ, Dube GK, Ratner L, Batal I. Pathology of Calcineurin and Mammalian Target of Rapamycin Inhibitors in Kidney Transplantation. *Kidney Int Rep* (2017) 3(2):281–90. doi:10.1016/j.ekir.2017.10.010
8. Rostaing L, Massari P, Garcia VD, Mancilla-Urrea E, Nainan G, del Carmen Rial M, et al. Switching from Calcineurin Inhibitor-Based Regimens to a Belatacept-Based Regimen in Renal Transplant Recipients: a Randomized Phase II Study. *Clin J Am Soc Nephrol CJASN* (2011) 6(2):430–9. doi:10.2215/cjn.05840710
9. Vincenti F, Larsen CP, Alberu J, Bresnahan B, Garcia VD, Kothari J, et al. Three-year Outcomes from BENEFIT, a Randomized, Active-Controlled, Parallel-Group Study in Adult Kidney Transplant Recipients. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2012) 12(1):210–7. doi:10.1111/j.1600-6143.2011.03785.x
10. Pestana JOM, Grinyo JM, Vanrenterghem Y, Becker T, Campistol JM, Florman S, et al. Three-year Outcomes from BENEFIT-EXT: a Phase III Study of Belatacept versus Cyclosporine in Recipients of Extended Criteria Donor Kidneys. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2012) 12(3):630–9. doi:10.1111/j.1600-6143.2011.03914.x
11. Vincenti F, Rostaing L, Grinyo J, Rice K, Steinberg S, Gaite L, et al. Belatacept and Long-Term Outcomes in Kidney Transplantation. *N Engl J Med* (2016) 374(4):333–43. doi:10.1056/nejmoa1506027
12. Le Meur Y, Aulagnon F, Bertrand D, Heng AE, Lavaud S, Caillard S, et al. Effect of an Early Switch to Belatacept Among Calcineurin Inhibitor-Intolerant Graft Recipients of Kidneys from Extended-Criteria Donors. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2016) 16(7):2181–6. doi:10.1111/ajt.13698
13. Brakemeier S, Kannenkeril D, Dürr M, Braun T, Bachmann F, Schmidt D, et al. Experience with Belatacept rescue Therapy in Kidney Transplant Recipients. *Transpl Int Off J Eur Soc Organ Transpl* (2016) 29(11):1184–95. doi:10.1111/tri.12822
14. Darres A, Ulloa C, Brakemier S, Garrouste C, Bestard O, Bestard ADB, et al. Conversion to Belatacept in Maintenance Kidney Transplant Patients: A Retrospective Multicenter European Study. *Transplantation* (2018) 102(9):1545–52. doi:10.1097/tp.0000000000002192
15. Bertrand D, Cheddani L, Etienne I, François A, Hanoy M, Laurent C, et al. Belatacept Rescue Therapy in Kidney Transplant Recipients with Vascular Lesions: A Case Control Study. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2017) 17(11):2937–44. doi:10.1111/ajt.14427
16. Grinyó J, Del Carmen RIal M, Alberu J, Steinberg S, Manfro M, Manfro GN, et al. Safety and Efficacy Outcomes 3 Years after Switching to Belatacept from a Calcineurin Inhibitor in Kidney Transplant Recipients: Results from a Phase 2 Randomized Trial. *Am J Kidney Dis Off J Natl Kidney Found* (2016) 69(5):587–94. doi:10.1053/j.ajkd.2016.09.021
17. Ulloa CE, Anglicheau D, Snanoudj R, Scemla A, Martinez F, Timsit M-O, et al. Conversion from Calcineurin Inhibitors to Belatacept in HLA-Sensitized Kidney Transplant Recipients with Low-Level Donor-specific Antibodies. *Transplantation* (2019) 103(10):2150–6. doi:10.1097/tp.0000000000002592
18. Attias P, Melica G, Boutboul D, De Castro N, Audard V, Stehlé T, et al. Epidemiology, Risk Factors, and Outcomes of Opportunistic Infections after Kidney Allograft Transplantation in the Era of Modern Immunosuppression: A Monocentric Cohort Study. *J Clin Med* (2019) 8(5). doi:10.3390/jcm8050594
19. Kotton CN, Kumar D, Caliendo AM, Huprikar S, Chou S, Danziger-Isakov L, et al. The Third International Consensus Guidelines on the Management of Cytomegalovirus in Solid-Organ Transplantation. *Transplantation* (2018) 102(6):900–31. doi:10.1097/tp.0000000000002191
20. Levey AS, Stevens LA, Schmid CH, Zhang Y, Lucy)Castro AF, et al. A New Equation to Estimate Glomerular Filtration Rate. *Ann Intern Med* (2009) 150(9):604–12.
21. Mannon RB. Delayed Graft Function: The AKI of Kidney Transplantation. *Nephron* (2018) 140(2):94–8.
22. Lefaucheur C, Loupy A, Hill GS, Andrade J, Nochy D, Antoine C, et al. Preexisting Donor-specific HLA Antibodies Predict Outcome in Kidney Transplantation. *J Am Soc Nephrol JASN* (2010) 21(8):1398–406. doi:10.1681/asn.2009101065
23. Ziemann M, Altermann W, Angert K, Arns W, Bachmann A, Bakchoul T, et al. Preformed Donor-specific HLA Antibodies in Living and Deceased Donor Transplantation. *Clin J Am Soc Nephrol CJASN* (2019) 14(7):1056–66. doi:10.2215/cjn.13401118
24. Haas M, Loupy A, Lefaucheur C, Roufosse C, Glotz D, Seron D, et al. The Banff 2017 Kidney Meeting Report: Revised Diagnostic Criteria for Chronic Active T Cell-Mediated Rejection, Antibody-Mediated Rejection, and Prospects for Integrative Endpoints for Next-Generation Clinical Trials. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2018) 18(2):293–307. doi:10.1111/ajt.14625
25. Dürr M, Lachmann N, Zukunft B, Schmidt D, Budde K, Brakemeier S. Late Conversion to Belatacept after Kidney Transplantation: Outcome and Prognostic Factors. *Transpl Proc* (2017) 49(8):1747–56.e1. doi:10.1016/j.transproceed.2017.05.013
26. Gupta G, Raynaud M, Kumar D, Sanghi P, Chang J, Kimball P, et al. Impact of Belatacept Conversion on Kidney Transplant Function, Histology, and Gene Expression - a Single-center Study. *Transpl Int Off J Eur Soc Organ Transpl* (2020).
27. Budde K, Prashar R, Haller H, Rial MC, Kamar N, Agarwal A, et al. Conversion from Calcineurin Inhibitor– to Belatacept-Based Maintenance Immunosuppression in Renal Transplant Recipients: A Randomized Phase 3b Trial. *J Am Soc Nephrol* (2021) 32(12):3252–64. doi:10.1681/asn.2021050628
28. Durrbach A, Pestana JM, Florman S, Del Carmen Rial M, Rostaing L, Kuypers D, et al. Long-Term Outcomes in Belatacept- versus Cyclosporine-Treated Recipients of Extended Criteria Donor Kidneys: Final Results from BENEFIT-EXT, a Phase III Randomized Study. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2016) 16(11):3192–201. doi:10.1111/ajt.13830
29. Bertrand D, Terrec F, Etienne I, Chavarot N, Sberro R, Gatault P, et al. Opportunistic Infections and Efficacy Following Conversion to Belatacept-Based Therapy after Kidney Transplantation: A French Multicenter Cohort. *J Clin Med* (2020). doi:10.3390/jcm9113479
30. Bertrand D, Chavarot N, Gatault P, Garrouste C, Bouvier N, Grall-Jezequel A, et al. Opportunistic Infections after Conversion to Belatacept in Kidney Transplantation. *Nephrol Dial Transpl Off Publ Eur Dial Transpl Assoc - Eur Ren Assoc.* (2020) 35(2):336–45. doi:10.1093/ndt/gfz255
31. Kaminski H, Belliere J, Couzi L, Kamar N, Merville P. Reply to Coussement et al. *Clin Infect Dis* (2021) 73(4):753–4. doi:10.1093/cid/ciab022
32. Ashman N, Chapagain A, Dobbie H, Raftery MJ, Sheaff MT, Yaqoob MM. Belatacept as Maintenance Immunosuppression for Postrenal Transplant De Novo Drug-Induced Thrombotic Microangiopathy. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2009) 9(2):424–7. doi:10.1111/j.1600-6143.2008.02482.x
33. Merola J, Yoo PS, Schaub J, Smith JD, Rodriguez-Davalos MI, Tichy E, et al. Belatacept and Eculizumab for Treatment of Calcineurin Inhibitor-Induced Thrombotic Microangiopathy after Kidney Transplantation: Case Report. *Transpl Proc* (2016) 48(9):3106–8. doi:10.1016/j.transproceed.2016.04.005
34. Cicora F, Paz M, Mos F, Roberti J. Use of Belatacept as Alternative Immunosuppression in Three Renal Transplant Patients with De Novo Drug-Induced Thrombotic Microangiopathy. *Case Rep Med* (2013) 2013:260254. doi:10.1155/2013/260254
35. Koppula S, Yost SE, Sussman A, Bracamonte ER, Kaplan B. Successful Conversion to Belatacept after Thrombotic Microangiopathy in Kidney Transplant Patients. *Clin Transpl* (2013) 27(4):591–7. doi:10.1111/ctr.12170
36. Choi M, Bachmann F, Wu K, Lachmann N, Schmidt D, Brakemeier S, et al. Microvascular Inflammation Is a Risk Factor in Kidney Transplant Recipients with Very Late Conversion from Calcineurin Inhibitor-Based Regimens to Belatacept. *BMC Nephrol* (2020) 21(1):354. doi:10.1186/s12882-020-01992-6

37. Gupta G, Raynaud M, Kumar D, Sanghi P, Chang J, Kimball P, et al. Impact of Belatacept Conversion on Kidney Transplant Function, Histology, and Gene Expression - a Single-center Study. *Transpl Int* (2020) 33(11):1458–71. doi:10.1111/tri.13718
38. Loupy A, Aubert O, Orandi BJ, Naesens M, Bouatou Y, Raynaud M, et al. Prediction System for Risk of Allograft Loss in Patients Receiving Kidney Transplants: International Derivation and Validation Study. *BMJ* (2019) 366:l4923. doi:10.1136/bmj.l4923
39. Adams AB, Goldstein J, Garrett C, Zhang R, Patzer RE, Newell KA, et al. Belatacept Combined with Transient Calcineurin Inhibitor Therapy Prevents Rejection and Promotes Improved Long-Term Renal Allograft Function. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2017) 17(11):2922–36. doi:10.1111/ajt.14353
40. Bray RA, Gebel HM, Townsend R, Roberts ME, Polinsky M, Yang L, et al. De Novo donor-specific Antibodies in Belatacept-Treated vs Cyclosporine-Treated Kidney-Transplant Recipients: Post Hoc Analyses of the Randomized Phase III BENEFIT and BENEFIT-EXT Studies. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2018) 18(7):1783–9. doi:10.1111/ajt.14721
41. Bray RA, Gebel HM, Townsend R, Roberts ME, Polinsky M, Yang L, et al. Posttransplant Reduction in Preexisting Donor-specific Antibody Levels after Belatacept- versus Cyclosporine-Based Immunosuppression: Post Hoc Analyses of BENEFIT and BENEFIT-EXT. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2018) 18(7):1774–82. doi:10.1111/ajt.14738
42. Gombos P, Opelz G, Scherer S, Morath C, Zeier M, Schemmer P, et al. Influence of Test Technique on Sensitization Status of Patients on the Kidney Transplant Waiting List. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2013) 13(8):2075–82. doi:10.1111/ajt.12332
43. Leibler C, Thiolat A, Hénique C, Samson C, Pilon C. Control of Humoral Response in Renal Transplantation by Belatacept Depends on a Direct Effect on B Cells and Impaired T Follicular Helper-B Cell Crosstalk. *J Am Soc Nephrol JASN* (2018) 29(3):1049–62. doi:10.1681/asn.2017060679

ESOT | Transplant International

ORIGINAL RESEARCH
published: 04 April 2022
doi: 10.3389/ti.2022.10117

Association Between Side of Living Kidney Donation and Post-Transplant Outcomes

Ellen L. K. Dobrijevic[1]*, *Eric H. K. Au*[1,2], *Natasha M. Rogers*[1,2,3,4], *Philip A. Clayton*[5,6,7], *Germaine Wong*[1,2,3] and *Richard D. M. Allen*[1,2,8]

[1]Faculty of Medicine and Health, University of Sydney, Sydney, NSW, Australia, [2]The Centre for Transplant and Renal Research, Westmead Institute of Medical Research, Westmead, NSW, Australia, [3]Department of Renal and Transplant Medicine, Westmead Hospital, Westmead, NSW, Australia, [4]Starzl Transplant Institute, University of Pittsburgh, Pittsburgh, PA, United States, [5]Central and Northern Adelaide Renal and Transplantation Services, Adelaide, SA, Australia, [6]Australia and New Zealand Dialysis and Transplant (ANZDATA) Registry, Adelaide, SA, Australia, [7]Discipline of Medicine, University of Adelaide, Adelaide, SA, Australia, [8]Department of Transplantation Surgery, Westmead Hospital, Westmead, NSW, Australia

Background: Right-sided living donor kidneys have longer renal arteries and shorter veins that make vascular anastomosis more challenging. We sought to determine whether recipients of right-sided living donor kidneys have worse outcomes than left-sided kidney recipients.

Methods: An observational analysis of the Australia and New Zealand Dialysis and Transplant Registry (ANZDATA) was undertaken. We used adjusted logistic regression to determine the association between side and delayed graft function (DGF) and time-stratified adjusted cox regression models for graft and patient survivals.

Results: Between 2004 and 2018, 4,050 living donor kidney transplants were conducted with 696 (17.2%) using right kidneys. With reference to left kidneys, the adjusted OR (95% CI) for DGF was 2.01 (1.31–3.09) for recipients with right kidneys. Within 30 days, 46 allografts (1.4%) were lost, with major causes of overall graft loss being technical, primary non-function and death. Recipients of right donor kidneys experienced a greater risk of early graft loss (aHR 2.02 [95% CI 1.06–3.86], $p = 0.03$), but not beyond 30 days (aHR 0.97 [95% CI 0.80–1.19], $p = 0.8$]).

Conclusion: Technical challenge is the most common cause of early graft loss. The risk of early graft loss among recipients who received right kidneys is doubled compared to those who received left living donor kidneys.

Keywords: patient survival, kidney transplant, living donor, graft survival, delayed graft function

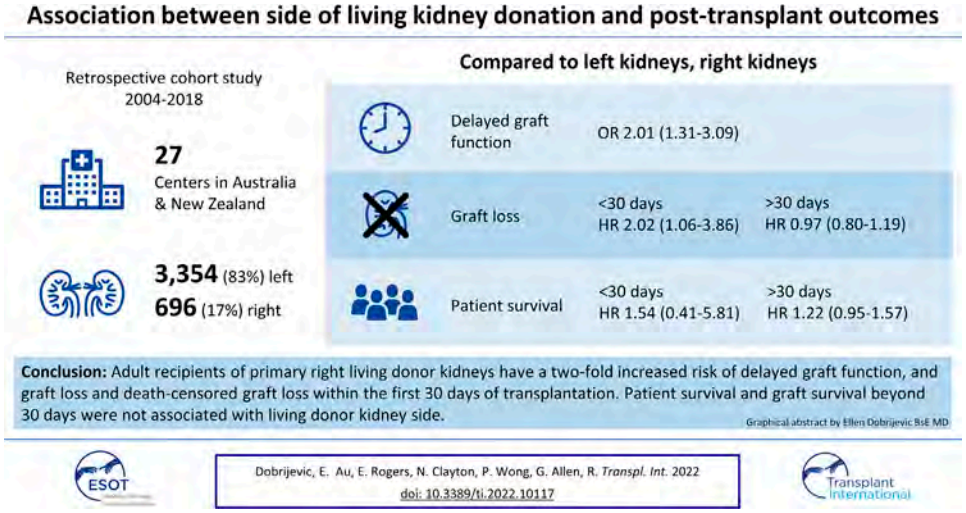
OPEN ACCESS

***Correspondence:**
Ellen L. K. Dobrijevic
edob6931@uni.sydney.edu.au
orcid.org/0001-7865-0642

Received: *15 October 2021*
Accepted: *03 March 2022*
Published: *04 April 2022*

Citation:
Dobrijevic ELK, Au EHK, Rogers NM, Clayton PA, Wong G and Allen RDM (2022) Association Between Side of Living Kidney Donation and Post-Transplant Outcomes. Transpl Int 35:10117. doi: 10.3389/ti.2022.10117

Abbreviations: aHR, adjusted hazard ratio; ANZDATA, Australia and New Zealand Dialysis and Transplant Registry; CMV, cytomegalovirus; DGF, delayed graft function; EBV, Epstein-Barr virus; GN, glomerulonephritis; HLA, human leukocyte antigen; PCKD, polycystic kidney disease; PNF, primary non-function.



GRAPHICAL ABSTRACT |

INTRODUCTION

The effect of the side of the living donor kidney on graft and recipient outcomes remains a subject of debate. Transplant surgeons prefer left-sided living donor kidneys because the longer renal vein facilitates implantation of the donor kidney to the deeply situated recipient right iliac vein (1–8). Compared with the use of right living donor kidneys, both the tension on the venous anastomosis and the potential kinking of a longer right renal artery are minimized when using left kidneys (4–6, 9). International registry and cohort studies demonstrate that more left kidneys are transplanted than right, particularly following the introduction of laparoscopic nephrectomy (1, 7, 10, 11). A multicentre analysis of the Organ Procurement and Transplantation Network (OPTN) database between 2000 and 2009 showed approximately 14% of living donor kidneys transplants were right-sided and, with a downward trend over time (2).

The increased technical difficulty of implanting a right donor kidney may predict the greater risk of thrombosis, delayed graft function (DGF) and graft loss for recipients of right compared to left kidneys (2, 9, 12–14). This trend is also observed for deceased donor kidneys (9, 15, 16). A recent systematic review and meta-analysis of observational studies comparing left and right living donor laparoscopic nephrectomies reported that left living donor kidneys had approximately 30% lower rates of DGF and thrombosis compared to right living donor kidneys (13). However, the certainty of the evidence is low, most studies were of small, single centres with substantial heterogeneity in study design, and almost all were judged to have high risk of bias in domains of selection, confounding, and outcomes reporting (13). Furthermore, sensitivity analysis did not demonstrate any significant difference in outcomes between left and right living donor recipients (13). Therefore, this study aimed to assess the association between the side of living donor kidney and patient outcomes including delayed graft function (DGF), early allograft loss and patient survival using data from a large national cohort of kidney transplant recipients.

METHODS

Ethics approval was granted by the Western Sydney Local Health District Human Research Ethics Committee ((6063) 2019/ETH09846) and the ANZDATA executive. This manuscript was prepared following the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) guidelines (17).

Population

An observational analysis of the Australia and New Zealand Dialysis and Transplant (ANZDATA) registry was undertaken from January 2004 to the end of 2018. Paediatric recipients (404, 8%) and non-primary grafts (482, 10%) were excluded from the analysis, as these populations are expected to have different characteristics and outcome profiles that may be inadequately captured by measured characteristics. Cases with missing data on the key exposure, kidney side, were not included (85, 2%) (**Figure 1**).

Data Collection

The key exposure of interest was the side, left or right, of the living donor kidney. Donor baseline characteristics included for analysis were age, sex, ethnicity, smoking, family history of diabetes mellitus, diabetes mellitus, hypertension, and body mass index (BMI).

Recipient baseline characteristics included age, sex, ethnicity, primary kidney disease, smoking, diabetes mellitus, body mass index, cytomegalovirus (CMV) and Epstein-Barr virus (EBV) status, history of chronic lung disease, cardiovascular disease,

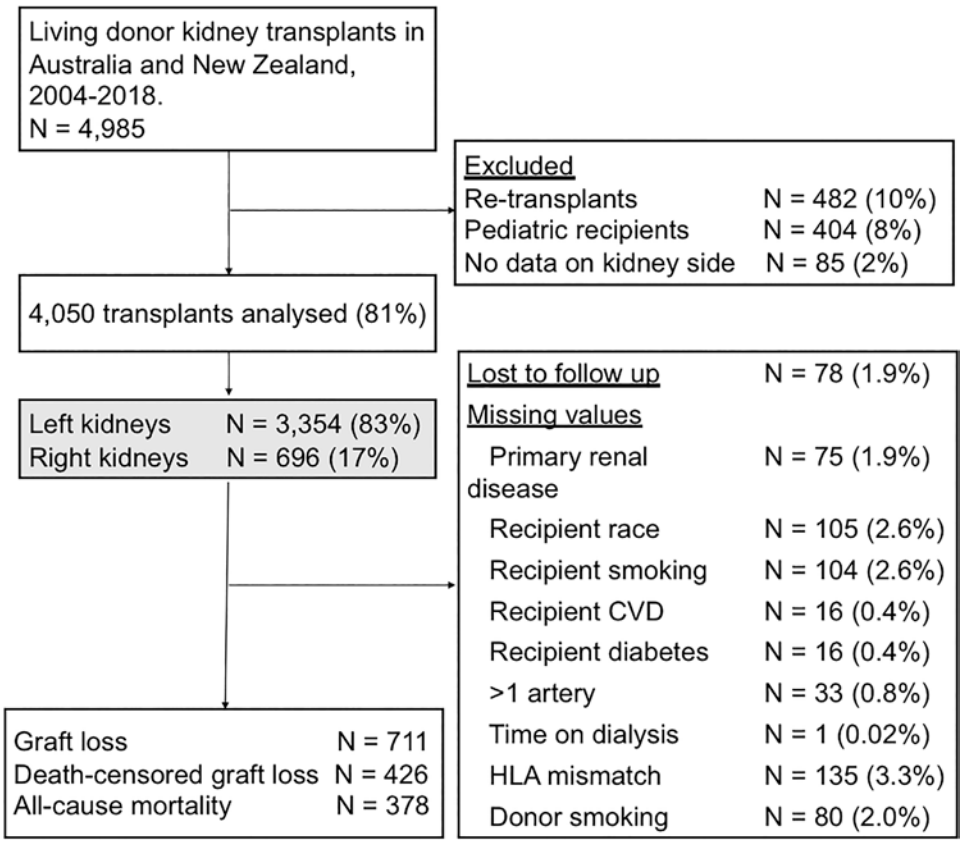


FIGURE 1 | Flow diagram outlining the study cohort and exclusions. CVD, cardiovascular disease; HLA, human leukocyte antigen.

hepatitis or cancer, and time on dialysis before transplantation. The primary kidney disease of the recipient was classified into glomerulonephritis, polycystic kidney disease, reflux nephropathy, vascular, diabetes mellitus and other. Cardiovascular disease was defined as a history of any one of coronary artery disease, peripheral vascular disease or cerebrovascular disease. Kidney donor surgery and implantation characteristics included the procedure date, donor and recipient relationship, human leukocyte (HLA) mismatches, total ischemia time, number of renal arteries and veins anastomosed, number of ureters, operation type and approach. The relationship between the donor and recipient was classified as related or unrelated. Total ischaemia time was the sum of warm and cold ischemia times, from donor renal artery interruption to the release of the renal artery in the recipient. The information collected by ANZDATA on DGF changed in 2017, from recording grafts requiring dialysis within 72 h to grafts requiring dialysis within 7 days after transplantation. Our analysis therefore defined DGF as recipients who required dialysis within 7 days of transplantation.

Outcomes

The patient relevant outcomes included in these analyses were overall graft loss, death censored graft loss, all-cause death and DGF. We also sought to compare the cause of early graft loss in the first 30 days after transplantation, between left and right living donor kidneys. Overall graft loss was defined as transplant nephrectomy, recommencing long term dialysis, re-transplantation or death from any cause. Time to graft loss was the period from the date of transplantation until the date of graft failure or death, with cases censored for loss to follow-up and the end of the study period. For death-censored graft loss, recipients were censored at the time of death, loss to follow-up or the end of the study period, whichever one came first. Patient survival was defined as the time from transplantation until patient death, censored for loss to follow up and the end of the study period.

Statistical Analysis

Categorical variables are presented as counts with percentages and compared using Pearson chi-square tests. Non-normally distributed continuous variables are presented as medians with interquartile ranges and compared using Mann-Whitney U tests. A Pearson's product-moment correlation was performed to determine the relationship between the total number of transplants performed at each centre and the percentage of right kidneys transplanted. The 17 centres that performed transplants in 2018 were included to capture the centres that are well established and currently active. p-values less than 0.05 were considered statistically significant.

TABLE 1 | Donor baseline characteristics.

Factor	Left (*n* = 3,354)	Right (*n* = 696)	*p*-value
Age, median (IQR)	51 (43–58)	51 (43–59)	0.50
Sex, male (n, %)	1,396 (41.6)	282 (40.5)	0.59
Ethnicity (n, %)			
Caucasian	2,887 (86.7)	615 (89.3)	0.44
Aboriginal or Torres Strait Islander Peoples	72 (2.2)	10 (0.3)	
Maori	71 (2.1)	10 (0.3)	
Pacific Islander	41 (1.2)	8 (0.2)	
Asian	202 (6.0)	39 (1.2)	
Other	55 (1.7)	7 (0.2)	
Smoking (n, %)			
Never	1976 (60.0)	423 (62.3)	0.36
Current	196 (6.0)	44 (6.5)	
Former	1,119 (34.0)	212 (31.2)	
Diabetes mellitus (n, %)			
Nil	3,284 (99.3)	677 (99.0)	0.44
Type 1	2	0	
Type 2—requiring insulin	1	1	
Type 2—non-insulin requiring	10	1	
Gestational	10	4	
Family history of diabetes mellitus (n, %)	592 (19.3)	128 (20.4)	0.53
Hypertension (n, %)	362 (10.9)	71 (10.3)	0.68
BMI (kg/m^2), median (IQR)	26.5 (23.9–29.2)	26.2 (23.9–29.0)	0.75

Association Between Side of the Kidney and Delayed Graft Function

Adjusted binomial logistic modelling was used to determine the association between donor kidney side and DGF. Variables with a *p*-value <0.25 on univariate analysis were included in the initial model, as well as kidney side. We then used a step-wise backward elimination process until the variables with $p < 0.05$ remained in the final model. To examine the effect of the change in definition of DGF in 2017, a two-level categorical variable representing the different periods was constructed from the year of transplant (2004–2016, 2017–2018) and added to the final multivariable model. The binomial logistic regression analysis was then fitted using a random effect model to account for clustering of DGF within centres.

Overall Graft Survival, Death-Censored Graft Survival and All-Cause Death

Time to event outcomes (overall graft survival, death-censored graft survival and all-cause death) were analysed using the Kaplan-Meier method and differences in survival curves were compared using the log-rank test.

Association Between Sides of the Kidneys, Overall Graft Loss, Death-Censored Graft Loss and All-Cause Death

Adjusted cox regression modelling was used to assess the association between the side of the kidney and allograft outcomes. For each outcome, the initial multivariable model included variables with a *p*-value of less than 0.25 on univariable analysis. The least significant variables were then removed from the base model using a step-wise backward elimination process until only variables with $p < 0.05$ remained in the final parsimonious model. The linearity of continuous variables was assessed by dividing into categories and examining the trend.

The proportional hazards assumptions of the Cox models considering the whole study period (from 2004 to 2018) were tested and the Schoenfeld residuals were plotted for each variable. There was no deviation from the assumption with the key exposure (side of kidney) for overall graft loss, death-censored graft loss and overall mortality. The models were then fitted with the predetermined division at 30 days. Thirty days was selected to elucidate the differences between early and late recipient outcomes and as a clinically relevant timepoint used in studies investigating early kidney graft loss (2, 18, 19). For each outcome two Cox regression models were fitted. The first model analysed events in the first 30 days after transplantation, censoring events from day 31 onwards. The second model analysed events occurring between day 31 to the end of the study period. Compared to the models analysing the whole time period, the models with the division at 30 days had a better fit by comparing the negative 2 log likelihood values.

To assess the robustness of our results, the Cox proportional hazard regression analyses were also fitted using a random effect model (frailty model) to account for clustering of graft loss and mortality risk within centres. Additionally, a three-level categorical variable for transplant year was constructed (2004–2008, 2009–2013, 2014–2018) and added to the final Cox models to assess for era effects, this term was removed from the model if it was not significant.

Analyses were performed using IBM SPSS v25 (IBM Corp., Armonk, NY, United States) and RStudio (RStudio, PBC. Boston, MA, United States).

TABLE 2 | Recipient and transplant characteristics.

Factor	Left (*n* = 3,354)	Right (*n* = 696)	*p*-value
Age, median (IQR)	47.0 (35.0–57.0)	50.0 (36.0–60.0)	0.0011
Sex, male (n, %)	2,155 (64.3)	419 (60.2)	0.045
Primary kidney disease (n, %)			
Glomerulonephritis	1,512 (46.0)	306 (44.6)	0.025
Polycystic	585 (17.8)	115 (16.8)	
Reflux	313 (9.5)	57 (8.3)	
Vascular	157 (4.8)	51 (7.4)	
Diabetes mellitus	274 (8.3)	72 (10.5)	
Other	448 (13.6)	85 (12.4)	
Ethnicity (n, %)			
Caucasian	2,683 (82.2)	576 (84.2)	0.30
Aboriginal or Torres Strait Islander Peoples	27 (0.8)	8 (1.2)	
Maori	92 (2.8)	20 (2.9)	
Pacific Islander	89 (2.7)	22 (3.2)	
Asian	310 (9.5)	47 (6.9)	
Other	61 (1.9)	11 (1.6)	
Smoking (n, %)			
Never	2049 (62.8)	419 (61.5)	0.14
Current	211 (6.5)	33 (4.9)	
Former	1,005 (30.8)	229 (33.6)	
Diabetes mellitus (n, %)			
Nil	2,775 (83.1)	546 (78.6)	0.0019
Type 1	78 (2.3)	20 (2.9)	
Type 2—requiring insulin	234 (7.0)	47 (6.8)	
Type 2—non-insulin requiring	251 (7.5)	82 (11.8)	
Cardiovascular disease (n, %)	516 (15.5)	102 (14.7)	0.60
Chronic lung disease (n, %)	154 (4.6)	41 (5.9)	0.15
Hepatitis (n, %)	30 (1.0)	6 (0.9)	0.89
Cancer ever (n, %)	175 (5.2)	42 (6.0)	0.39
BMI, median (IQR)	25.9 (22.8–29.4)	26.2 (22.8–29.6)	0.40
Time on RRT (years), median (IQR)	6.93 (0–20.7)	6.26 (0–21.6)	0.89
Donor-recipient relationship			
Related	1726 (51.5%)	359 (51.6%)	0.96
Unrelated	1,628 (48.5%)	337 (48.4%)	
Ischemia time (hours), median (IQR)	3.00 (2.00–4.00)	3.00 (2.00–4.00)	0.11
Number of arteries			
1	2,765 (82.9%)	560 (82.0%)	0.050
2	532 (16.0%)	107 (15.7%)	
3	35 (1.1%)	14 (2.1%)	
4	2 (0.1%)	2 (0.3%)	
Number of veins			
1	3,233 (97.1%)	619 (90.6%)	<0.001
2	95 (2.9%)	59 (8.6%)	
3	2 (0.1%)	4 (0.6%)	
4	0	1 (0.1%)	
Number of ureters			
1	3,291 (98.9%)	678 (99.3%)	0.41
2	36 (1.1%)	5 (0.7%)	
Operation type			
Hand assisted laparoscopic	1,145 (34.2%)	278 (40.4%)	<0.001
Laparoscopic	1938 (57.9%)	299 (43.5%)	
Open	267 (8.0%)	111 (16.1%)	
Operation approach			
Extraperitoneal	633 (19.5%)	131 (19.5%)	0.10
Transperitoneal	2,618 (80.5%)	542 (80.5%)	
HLA-A mismatch			
0	747 (23.1%)	161 (23.6%)	0.88
1	1729 (53.4%)	366 (53.7%)	
2	764 (23.6%)	155 (22.7%)	
HLA-B mismatch			
0	463 (14.3%)	113 (16.6%)	0.31
1	1,618 (50.0%)	329 (48.2%)	
2	1,158 (35.8%)	240 (35.2%)	
HLA-DR mismatch			

(Continued on following page)

TABLE 2 | (*Continued*) Recipient and transplant characteristics.

Factor	Left (*n* = 3,354)	Right (*n* = 696)	*p*-value
0	672 (20.8%)	154 (22.6%)	0.48
1	1733 (53.5%)	349 (51.3%)	
2	833 (25.7%)	177 (26.0%)	

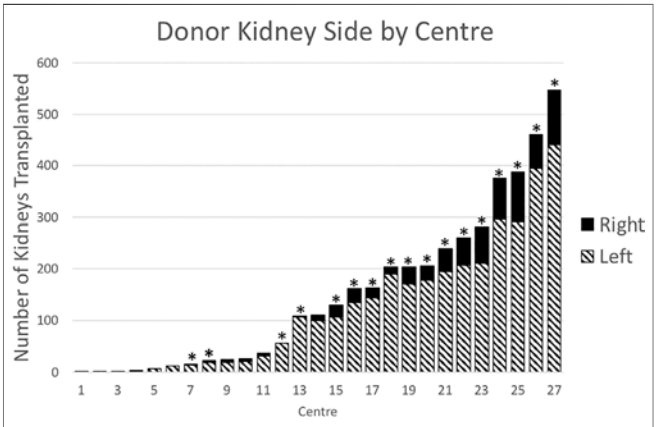


FIGURE 2 | Donor kidney side by transplant centre, 2004–2018. Bars with asterisks (n = 17) indicate centres that performed transplants in 2018.

RESULTS

There were 4,985 living donor transplants between 2004 and 2018. After excluding paediatric recipients, non-primary grafts, and donor kidneys with missing data on kidney side, the recipient cohort of 4,050 living donor transplants included 3,354 (82.8%) left kidneys and 696 (17.2%) right kidneys (**Figure 1**). The baseline characteristics of the donors, recipients and the transplant procedures are shown in **Tables 1**, **2**.

There were no differences between left and right kidney donors (**Table 1**). Recipients of right living donor kidneys were more likely to be older (median age 47 for left compared to 50 years for right kidneys), female and have diabetes mellitus (**Table 2**). Compared to left donor nephrectomies, right donor nephrectomies were more commonly hand-assisted laparoscopic procedures (34.2% left kidneys compared to 40.4% right kidneys) and open procedures (8.0% left kidneys compared to 16.1% right kidneys) (**Table 2**).

There was variation in the proportion of right kidneys transplanted between the 27 transplant centres in this study (Pearson chi-square $p < 0.01$) (**Figure 2**). There was also a positive correlation between the total number of transplants performed at each centre and the percentage of right kidneys transplanted (Pearson's product-moment correlation r = 0.55, p = 0.02). During the time period of the study, 10 of the 27 transplant centres closed or merged with others. Between 2004 and 2018, the proportion the transplanted kidneys each year that were right sided was stable (mean = 17.1%, standard deviation = 2.2%).

Association Between Kidney Side and Delayed Graft Function

DGF was reported in 3.0% of transplants. Recipients of right kidneys were more likely to experience DGF with 86 recipients (2.6%) of left kidneys compared to 34 (4.9%) of right kidneys affected (p = 0.001). Right kidneys were associated with an increased risk of DGF (adjusted odds ratio (OR) (95% CI) 2.01 [1.31–3.09]), adjusting for total ischemia time, time on

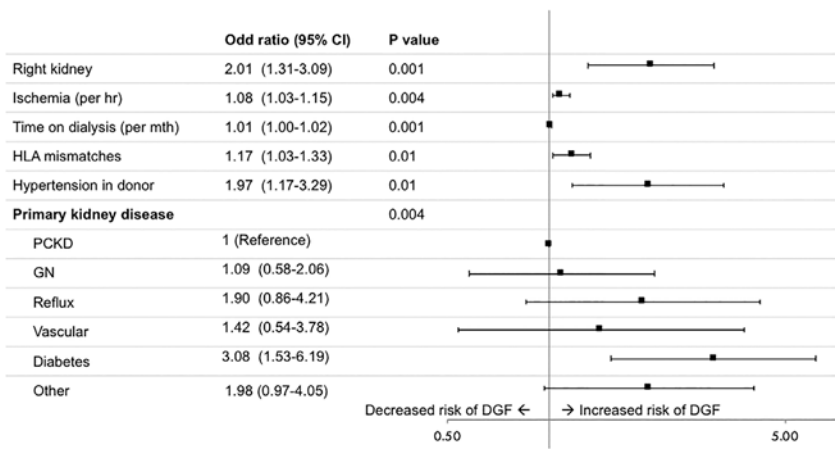


FIGURE 3 | Risk factors for delayed graft function (DGF), defined as the need for dialysis within 7 days of transplantation. GN, glomerulonephritis; PCKD, polycystic kidney disease.

TABLE 3 | Causes of graft loss over the study period (from 2004 to 2018) and over the first 30 days after transplant.

Time period	First 30 days after transplantation				Study period (2004–18)			
Side of kidney	Left		Right		Left		Right	
Total	32		14		587		134	
Death with function	6	18.8%	2	14.3%	229	39.0%	57	42.5%
Chronic allograft nephropathy	—	—	—	—	183	31.2%	39	29.1%
Recurrent glomerulonephritis	1	3.1%	0	0%	61	10.4%	8	6.0%
Acute rejection	6	18.8%	3	21.4%	30	5.1%	11	8.2%
Technical	16	50.0%	4	28.6%	25	4.3%	4	3.0%
Primary non-function	3	9.4%	5	35.7%	3	0.5%	5	3.7%
Other	—	—	—	—	56	9.5%	10	7.5%
Pearson chi-square *p*-value				0.2				0.02

dialysis before transplant, number of HLA mismatches, donor hypertension and primary kidney disease (**Figure 3**). In the frailty model, clustering for transplant centre, the adjusted OR (95% CI) for receiving a right compared to left kidney was 2.09 [1.34–3.24]. The risk factor profile for DGF was not significantly altered after accounting for the change in definition of DGF in 2017, with the adjusted OR (95% CI) for receiving a right compared to left kidney being 1.89 (1.24–2.87).

Causes of All-Cause Graft Loss and Death

Between 2004 and 2018, 736 recipients lost their allografts, with the cause documented in 721 (98.0%) cases (**Table 3**). During the first 30 days after transplantation, 46 grafts were lost. Right kidneys accounted for 14 (30.4%) of the grafts lost in the first 30 days, despite representing only 17.2% of all transplants. Technical causes (including haemorrhage, renal artery or vein thrombosis and renal artery stenosis) accounted for 50.0% of left kidneys and 28.6% of right kidneys lost. Primary non-function accounted for 9.4% of left kidneys and 35.7% of right kidneys lost in the first 30 days after transplantation (**Table 3**). After the first 30 days, the main causes of graft loss were death with functioning graft (39.0% left and 42.5% right), followed by chronic allograft nephropathy (31.2% left and 29.1% right) and recurrent glomerulonephritis (10.4% left and 6.0% right) (**Table 3**). A total of 391 patients died in the study period. The main causes of death were cardiovascular disease (25%), malignancy (23%) and infection (16%). Eleven patients died within the first 30 days of transplantation. Eight were recipients of left LDKs and three were recipients of right kidneys. The main causes of death were cardiac (3 three cases of cardiac arrest of uncertain cause and one case of myocardial infarct) and septicaemia (2 cases).

Kaplan-Meier Estimates of Graft, Death-Censored Graft and Patient Survivals

Graft survival was lower for right living donor kidney recipients in the 30 days after transplantation (left 99.1% vs. right 97.7%, log rank *p*-value = 0.005) (**Figure 4A**). Overall graft survival at 1 and 5 years was 97.4% (95% CI 96.9–97.9) and 89.6% (95% CI 88.5–90.6%) respectively. One-year overall graft survival was 97.7% (95% CI 97.1–98.2) for left kidneys and 96.1% (95% CI 94.3–97.3) for right kidneys. At 5 years, graft survival was 89.8% (95% CI 88.6–90.9) for left kidneys and 88.8% (95% CI 86.0–91.1) for right kidneys (**Figure 4B**).

Death-censored graft survival was lower for right kidney recipients in the first 30 days after transplantation (99.3% for left vs. 98.1% for right, log rank *p*-value = 0.005) (**Figure 4C**). The overall death-censored graft survival at 1 and 5 years was 98.4% (95% CI 98.0–98.8) and 93.7% (95% CI 92.8–94.4%) respectively. At 1 and 5 years, there was no significant difference between the death-censored graft survival of left and right kidney transplants (**Figure 4D**).

Patient survival was not significantly different between left and right kidney recipients at 30 days, 1 year or 5 years. Thirty days after transplantation, the survival of recipients of left and right kidneys was 99.7% (95% CI 99.5–99.9%) and 99.5% (95% CI 98.7–99.9%) respectively (**Figure 4E**). Overall patient survival at 1 and 5 years was 98.8% (95% CI 98.4–99.1) and 94.9% (95% CI 94.1–95.6%) respectively.

Association Between Sides of the Kidney and Overall Graft Loss

With reference to the left kidney, the adjusted HR of overall graft loss within 30 days of transplantation was 2.02 [95% CI 1.06–3.86], $p = 0.03$ (**Supplementary Figure S1A**). Other risk factors for graft loss in the first 30 days included having more than one renal artery (aHR 2.05 [95%CI 1.07–3.91], $p = 0.03$) and the recipient having type 1 diabetes mellitus (aHR 4.26 [95% CI 1.51–12.04], $p = 0.03$) (**Supplementary Figure S1A**). After 30 days, the adjusted HR for overall graft loss among recipients who received right kidneys compared to the left was 0.97 [95% CI 0.80–1.19], $p = 0.8$ (**Supplementary Figure S1B**). In the frailty analysis, clustering for centres, the adjusted HR for left kidneys compared to right kidneys was 2.02 [95% CI 1.06–3.87] within 30 days and 0.97 [95% CI 0.80–1.19] after 30 days. The adjusted HRs for left kidneys compared to right kidneys, both within and after 30 days of transplantation, are unchanged after sensitivity analysis to account for the effect of transplant centres. Furthermore, both within and after 30 days of transplantation, the 5-year era in which the transplant occurred was not associated with the risk of graft loss.

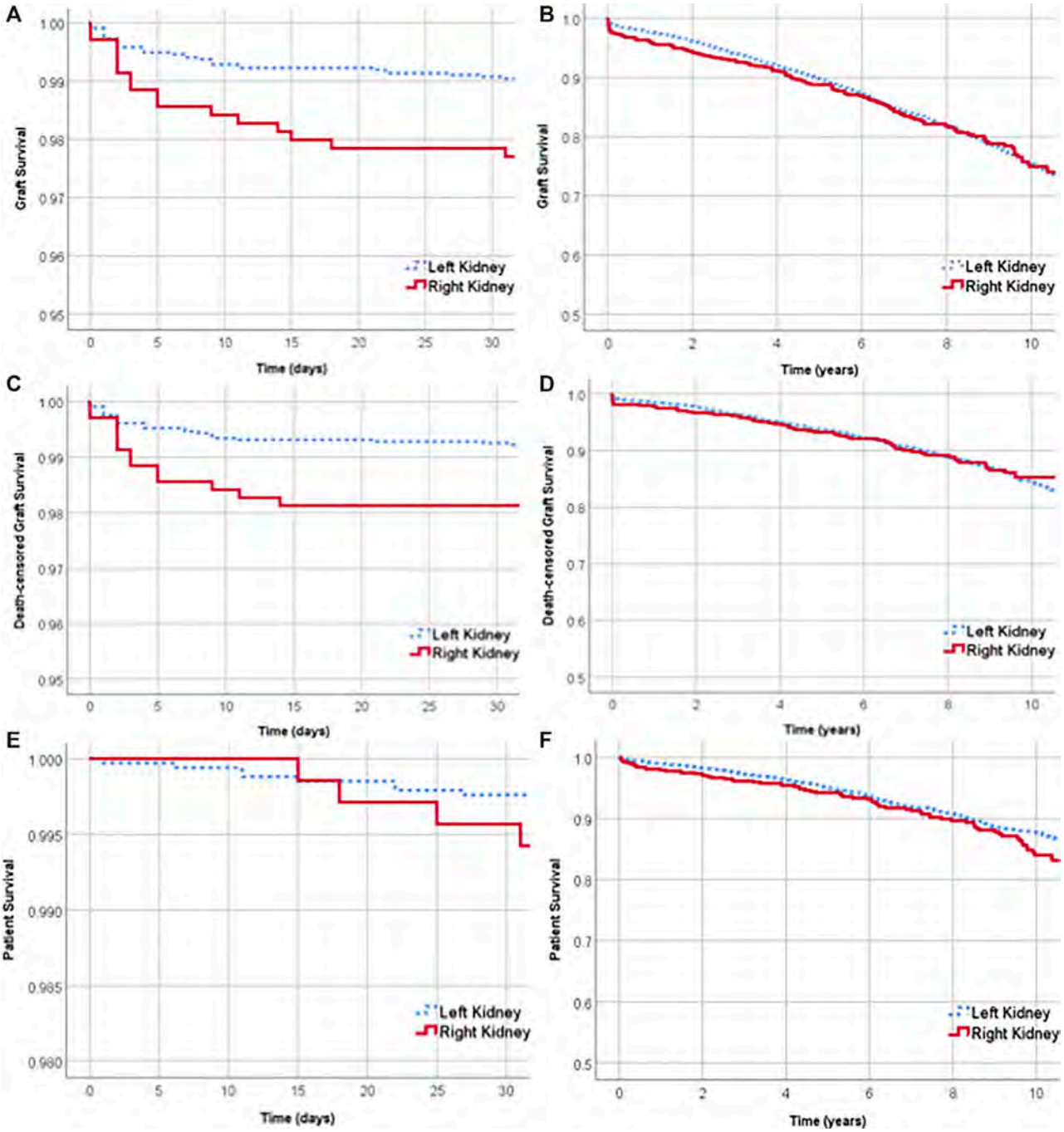


FIGURE 4 | Graft survival of left and right kidneys over the first 30 days after transplant **(A)** and over 10 years **(B)**. Death-censored graft survival over the first 30 days after transplant **(C)** and over 10 years **(D)**. Survival of recipients of left and right living donor kidneys over the first 30 days after transplant **(E)** and over 10 years **(F)**.

Association Between Sides of the Kidney and Death-Censored Graft Loss

Within the first 30 days after transplant, the adjusted HR for death-censored graft loss among recipients of right kidneys compared to left was aHR 2.14 [95% CI 1.05–4.34], $p = 0.04$) (**Supplementary Figure S2A**). Grafts with more than one renal artery were at increased risk of death-censored graft loss (aHR 2.11 [95% CI 1.04–4.28], $p = 0.04$) (**Supplementary Figure S2A**). After 30 days, right kidneys and more than one renal artery were no longer an independent risk factors for death-censored graft loss (**Supplementary Figure S2B**). In the frailty analysis, clustering for centres, the adjusted HR for left kidneys

compared to right kidneys was 2.17 [95% CI 1.06–4.41] within 30 days and 0.89 [95% CI 0.67–1.16] after 30 days. Both within and after 30 days of transplantation, the 5-year era in which the transplant occurred was not associated with the risk of death-censored graft loss.

Association Between Sides of the Kidney and All-Cause Death

Side was not an independent risk factor for patient survival either within or after the first 30 days (within the first 30 days, aHR 1.54 [95% CI 0.41–5.81], $p = 0.5$, and after the first 30 days aHR 1.22 [95% CI 0.95–1.57], $p = 0.1$) (**Supplementary Figures S3A,S3B**). In the frailty analysis, clustering for centres, the adjusted HR for left kidneys compared to right kidneys was 1.54 [95% CI 0.41–5.81] within 30 days and 1.22 [95% CI 0.94–1.57] after 30 days. Both within and after 30 days of transplantation, the 5-year era in which the transplant occurred was not associated with the risk of patient survival.

DISCUSSION

This large registry-based study demonstrates that adult recipients of primary right living donor kidneys have a two-fold increased risk of DGF, and graft loss and death-censored graft loss within the first 30 days of transplantation. Primary non-function accounted for 9% of left kidneys and 36% of right kidneys that were lost in the first 30 days after transplantation. Patient survival and graft survival beyond 30 days were not associated with living donor kidney side.

The association between living donor kidney side and recipient outcomes has mostly been studied previously in small, single-centre studies with disparate results, complicating the debate as to whether more right-sided nephrectomies should be undertaken. An OPTN registry-based retrospective analysis from 2000 to 2009 reached similar conclusions to our study, with lower effect sizes; right living donor kidneys experienced a 1.4 (95% CI 1.2–1.5) increased risk of DGF and a 1.1 (95% CI 0.85–1.5) increased risk of graft loss (2). An earlier ANZDATA analysis investigating DGF identified right sided kidneys as a risk factor (14). In contrast, two meta-analyses have shown that right laparoscopic living donor kidneys were not associated with increased rates of DGF, after sensitivity analysis, or graft loss at 1 year (10, 13).

Our multi-centre and registry-based study had sufficient power to examine differences in DGF, patient survival and graft survival. Furthermore, the findings of our study corroborated prior work that compared outcomes between transplanted left and right deceased donor kidneys (9). The inferior results of transplanted right deceased donor kidneys in that study may be attributed to technical challenges, with a recommendation that transplanting teams optimise allocation of surgical expertise (9). However, in this study the inferior outcomes of transplanting right-sided living donor kidneys could not be proven to directly relate to surgical challenges associated with its transplantation. There are some important differences to note, for example, unlike deceased donor procedures, the transplantation of living donor kidneys are typically undertaken as elective day-time procedures in optimally prepared recipients and carefully selected donors. Furthermore, the low incidence of graft loss in the first 30 days after transplant likely limited our analysis of cause-specific graft loss. Equally, this low rate of graft loss by international registry standards, reflects the good outcomes of kidney transplantation in Australia and New Zealand (20, 21).

However, the demonstrated higher incidence of DGF and PNF-related graft loss in right living donor kidneys in the first 30 days after transplantion supports increased surgical challenges associated with transplanting right living donor kidneys. In the first 30 days, 64.3% of right kidneys that were lost were lost due to either primary non-function or technical causes compared to 59.4% of left kidneys that were lost. Expanding data collection to include important factors such as vascular anastomosis times, and intra-operative and post-operative complications could help determine if there are increased surgical challenges with right living donor kidney transplantation. For example, although anastomosis times were not captured by the ANZDATA registry, right deceased donor kidneys have been shown to have longer anastomosis times (22).

Our study has a number of limitations. Indication bias remains a possibility. We were unable to account for inter-centre decision-making variations that might influence outcomes such as indications for right donor nephrectomies in preference to left. This clinical decision evaluates the risk of surgery on either side and aims to maximise the residual renal function of the donor. However, the shared frailty models demonstrated minimal changes to the estimates when accounting for centre-specific random effects. Even though there were multiple confounding factors adjusted for in the analyses, there are likely to be several unmeasured and residual confounders, such as differential kidney function of the living donor kidneys and individual surgeons' volumes and expertise. The definition of DGF changed from the need for dialysis within 72 h after transplantation to the need for dialysis within 7 days in 2017. We defined DGF as the need for dialysis within 7 days, therefore this may lead to an underestimation of the overall incidence of DGF. Adjusting for the era of the transplantation in the model did not change the risk factor profile. However, only 2 years of data were captured using the revised ANZDATA Registry definition. Additionally, the outcome ascertainment bias is unlikely to be differential between recipients of left and right living donor kidneys, as the proportion of right kidneys transplanted each year was relatively stable. Strengths of this study are the large cohort with few missing values and cases lost to follow up and the minimal risk of selection bias as the study population represents all primary adult recipients of kidney transplants in Australia and New Zealand.

The time period of this ANZDATA based study corresponded to the progressive uptake of laparoscopic donor nephrectomy. The driving force behind this was a consumer-driven preference by prospective living kidney donors and their referring nephrologists to avoid open surgery where possible. In the 15 year study period, 10 of 27 centres ceased to provide a

living donor kidney transplantation service, likely driven by their inability to provide laparoscopic surgical expertise. The higher percentage of right nephrectomies performed by open or hand assisted laparoscopic surgery and low overall rates of right nephrectomies likely reflected the increasing uptake of laparoscopic nephrectomies and hesitancy to undertake right laparoscopic nephrectomies during this learning phase, particularly in the early part of the study. Importantly, this careful approach resulted in equivalent recipient outcomes for laparoscopic and open donor surgery, as the type of operation was not a risk factor for graft or patient survival. Furthermore, the era of transplantation was not a risk factor for inferior recipient outcomes. Centres with low volumes of transplants were not excluded in the study, as they remained important data points, and despite the variation in transplant centre volume, the shared frailty models demonstrated minimal changes to the estimates when accounting for centre-specific random effects. Equally, the use of right living donor kidneys (17.2%), which is high by international standards, suggests that it would have been uncommon for recipients in Australia and New Zealand to be denied the opportunity to be transplanted with a living donor kidney because their transplant centre had been reluctant to tackle either donation or transplantation of a right-sided donor kidney.

Robot-assisted surgery may have an emerging role in living kidney transplantation and the impact of the side of the living donor kidney should be studied in this context. There has been increasing uptake of robot-assisted kidney transplantation with initial studies indicating that it is non-inferior to open kidney transplantation (23) and feasible with multiple vessel grafts (24). The shorter renal vein of right kidneys is particularly an issue in obese recipients and recipients with narrow pelvises in the setting of the traditional open approach. The magnification and dexterity possible with the robotic platform is particularly advantageous in these situations as it facilitates the formation of tension-free vascular anastomosis even in the case of short renal veins. However, implementation of this technique requires appropriate training and a team with extensive experience in both robotic surgery and open transplantation.

In summary, our results indicate that recipients of right living donor kidneys may have an increased risk of DGF and graft loss in the first 30 days after transplantation. The implication is that the technical challenges of transplanting a right living donor kidney are real, but not to the extent that right-sided kidneys should be excluded, particularly in light of the limitations addressed above. The prospective donor of a right kidney and the recipient should be informed but also reassured that the differences between left and right living donor kidneys are relatively small, confined to the early post-operative period and are similar to those seen in recipients of left or right deceased donor kidneys (9). Nevertheless, the increased risks associated with receiving a right kidney should be factored into trial-based analyses and published living donor kidney transplant outcomes of individual transplant centres. The underlying mechanisms of the observed findings of this study may be clarified by prospective studies or analyses of data at large transplant centres, with the availability of additional variables such as anastomosis times, pre-operative differential kidney function, intra-operative complications and other provider-related factors. Overall, a patient in need of kidney transplantation should not be denied this opportunity only because of reluctance to use a right-sided living donor kidney.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: Raw data were generated at the Australia and New Zealand Dialysis and Transplant Registry (ANZDATA). Data can be accessed through application to ANZDATA. Requests to access these datasets should be directed to requests@anzdata.org.au.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Western Sydney Local Health District Human Research Ethics Committee ((6063) 2019/ETH09846). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

RA conceived the study with input from PC and ED. ED lead the data analysis with input from EA and GW. ED and RA wrote the manuscript with input and critical feedback from EA, NR, and GW. All authors read and approved the article.

FUNDING

The data reported here were supplied by the ANZDATA.

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.2022.10117/full#supplementary-material

REFERENCES

1. Liu K-L, Chiang Y-J, Wu C-T, Lai W-J, Wang H-H, Chu S-H. Why We Consistently Use the Left Donor Kidney in Living Related Transplantation: Initial Experience of Right Laparoscopic Donor Nephrectomy and Comparison with Left Nephrectomy. *Transplant Proc* (2006) 38(7):1977–9. doi:10.1016/j.transproceed.2006.06.023
2. Khalil A, Mujtaba MA, Taber TE, Yaqub MS, Goggins W, Powelson J, et al. Trends and Outcomes in Right vs. Left Living Donor Nephrectomy: an Analysis of the OPTN/UNOS Database of Donor and Recipient Outcomes - Should We Be Doing More Right-Sided Nephrectomies? *Clin Transpl* (2016) 30:145–53. doi:10.1111/ctr.12668
3. Kay MD, Brook N, Kaushik M, Harper SJF, Bagul A, Nicholson ML, et al. Comparison of Right and Left Laparoscopic Live Donor Nephrectomy. *BJU Int* (2006) 98:843–4. doi:10.1111/j.1464-410x.2006.06429.x
4. Khan TT, Ahmad N, Siddique K, Fourtounas K. Implantation of Right Kidneys: Is the Risk of Technical Graft Loss Real? *World J Surg* (2018) 42(5):1536–41. doi:10.1007/s00268-017-4314-y
5. Mandal AK, Cohen C, Montgomery RA, Kavoussi LR, Ratner LE. Should the Indications for Laparascopic Live Donor Nephrectomy of the Right Kidney Be the Same as for the Open Procedure? Anomalous Left Renal Vasculature Is Not a Contraindication to Laparoscopic Left Donor Nephrectomy1. *Transplantation* (2001) 71(5):660–4. doi:10.1097/00007890-200103150-00015
6. Swartz DE, Cho E, Flowers JL, Dunkin BJ, Ramey JR, Bartlett ST, et al. Laparoscopic Right Donor Nephrectomy. *Surg Endosc* (2001) 15(12):1390–4. doi:10.1007/s00464-001-8135-5
7. Tsoulfas G, Agorastou P, Ko D, Hertl M, Elias N, Cosimi AB, et al. Laparoscopic Living Donor Nephrectomy: Is There a Difference between Using a Left or a Right Kidney? *Transplant Proc* (2012) 44(9):2706–8. doi:10.1016/j.transproceed.2012.09.019
8. Crane C, Lam VWT, Alsakran A, Vasilaras A, Lau H, Ryan B, et al. Are There Anatomical Barriers to Laparoscopic Donor Nephrectomy? *ANZ J Surg* (2010) 80:781–5. doi:10.1111/j.1445-2197.2010.05439.x
9. Vacher-Coponat H, McDonald S, Clayton P, Loundou A, Allen RDM, Chadban SJ. Inferior Early Posttransplant Outcomes for Recipients of Right versus Left Deceased Donor Kidneys: an ANZDATA Registry Analysis. *Am J Transplant* (2013) 13(2):399–405. doi:10.1111/j.1600-6143.2012.04312.x
10. Wang K, Zhang P, Xu X, Fan M. Right versus Left Laparoscopic Living-Donor Nephrectomy: a Meta-Analysis. *Exp Clin Transpl* (2015) 13(3):214–26. doi:10.6002/ect.2014.0227
11. Lind MY, Hazebroek EJ, Hop WCJ, Weimar W, Jaap Bonjer H, Ijzermans JNM. Right-sided Laparoscopic Live-Donor Nephrectomy: Is Reluctance Still Justified? *Transplantation* (2002) 74:1045–7. doi:10.1097/00007890-200210150-00025
12. Minnee RC, Bemelman WA, Maartense S, Bemelman FJ, Gouma DJ, Idu MM. Left or Right Kidney in Hand-Assisted Donor Nephrectomy? A Randomized Controlled Trial. *Transplantation* (2008) 85(2):203–8. doi:10.1097/tp.0b013e3181601486
13. Liu N, Wazir R, Wang J, Wang K-J. Maximizing the Donor Pool: Left versus Right Laparoscopic Live Donor Nephrectomy-Systematic Review and Meta-Analysis. *Int Urol Nephrol* (2014) 46(8):1511–9. doi:10.1007/s11255-014-0671-8
14. Mogulla MR, Bhattacharjya S, Clayton PA. Risk Factors for and Outcomes of Delayed Graft Function in Live Donor Kidney Transplantation - a Retrospective Study. *Transpl IntNov* (2019) 32(11):1151–60. doi:10.1111/tri.13472
15. Domingo H, Rufino M, Armas S, González A, Gutiérrez P, Barbero P, et al. Retrospective Analysis of Surgical Complications Following Cadaveric Kidney Transplantation in the Modern Transplant Era. *Nephrol Dial Transplant* (2006) 21(10):2908–15. doi:10.1093/ndt/gfl338
16. Lechevallier E, Dussol B, Luccioni A, Thirion X, Vacher-Copomat H, Jaber K, et al. Posttransplantation Acute Tubular Necrosis: Risk Factors and Implications for Graft Survival. *Am J Kidney Dis* (1998) 32(6):984–91. doi:10.1016/s0272-6386(98)70073-3
17. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: Guidelines for Reporting Observational Studies. *The Lancet* (2007) 370(9596):1453–7. doi:10.1016/s0140-6736(07)61602-x
18. Phelan PJ, O'Kelly P, Tarazi M, Tarazi N, Salehmohamed MR, Little DM, et al. Renal Allograft Loss in the First post-operative Month: Causes and Consequences. *Clin Transpl* (2012) 26(4):544–9. doi:10.1111/j.1399-0012.2011.01581.x
19. Hamed MO, Chen Y, Pasea L, Watson CJ, Torpey N, Bradley JA, et al. Early Graft Loss after Kidney Transplantation: Risk Factors and Consequences. *Am J Transpl* (2015) 15(6):1632–43. doi:10.1111/ajt.13162
20. ERA-EDTA Registry. *ERA-EDTA Registry Annual Report 2017 Amsterdam UMC*. Amsterdam: Department of Medical Informatics (2019).
21. OPTN. Kidney Kaplan-Meier Graft Survival Rates For Transplants Performed: 2008 - 2015 2021 (2021). [updated February 12 2021. Available from: https://optn.transplant.hrsa.gov/data/view-data-reports/national-data/# (Accessed April 27, 2021).
22. Heylen L, Pirenne J, Samuel U, Tieken I, Naesens M, Sprangers B, et al. The Impact of Anastomosis Time During Kidney Transplantation on Graft Loss: A Eurotransplant Cohort Study. *Am J Transpl* (2017) 17:724–32. doi:10.1111/ajt.14031
23. Musquera MA-O, Peri L, Ajami T, Campi RA-O, Tugcu VA-O, Decaestecker K, et al. Robot-assisted Kidney Transplantation: Update from the European Robotic Urology Section (ERUS) Series. *BJU Internation* (2021) 127:222. doi:10.1111/bju.15199
24. Siena G, Campi R, Decaestecker K, Tuğcu V, Sahin S, Alcaraz A, et al. Robot-assisted Kidney Transplantation with Regional Hypothermia Using Grafts with Multiple Vessels After Extracorporeal Vascular Reconstruction: Results from the European Association of Urology Robotic Urology Section Working Group. *Eur Urol Focus* (2018) 4:175. doi:10.1016/j.euf.2018.07.022

ESOT | Transplant International

ORIGINAL RESEARCH
published: 11 April 2022
doi: 10.3389/ti.2022.10128

Dynamic Behaviour of Donor Specific Antibodies in the Early Period Following HLA Incompatible Kidney Transplantation

*Mason Phillpott[1], Sunil Daga[2,3,4], Rob Higgins[3], David Lowe[5], Nithya Krishnan[6], Daniel Zehnder[3,7], David Briggs[5,8] and Natalia Khovanova[1]**

[1]School of Engineering, University of Warwick, Coventry, United Kingdom, [2]St James's University Hospital, LTHT NHS Trust, Leeds, United Kingdom, [3]Warwick Medical School, University of Warwick, Coventry, United Kingdom, [4]NIHR Leeds In-Vitro Diagnostics Co-operative, Leeds, United Kingdom, [5]Histocompatibility and Immunogenetics, NHS Blood and Transplant, Birmingham, United Kingdom, [6]University Hospitals Coventry & Warwickshire NHS Trust, Coventry, United Kingdom, [7]North Cumbria Integrated Care NHS Trust, Carlisle, Cumbria, United Kingdom, [8]Institute of Cancer and Genomic Sciences, University of Birmingham, Birmingham, United Kingdom

In HLA-incompatible kidney transplantation, monitoring donor-specific antibodies (DSA) plays a crucial role in providing appropriate treatment and increases kidney survival times. This work aimed to determine if early post-transplant DSA dynamics inform graft outcome over and above other predictive factors. Eighty-eight cases were classified by unsupervised machine learning into five distinct DSA response groups: no response, fast modulation, slow modulation, rise to sustained and sustained. Fast modulation dynamics gave an 80% rate for early acute rejection, whereas the sustained group was associated with the lowest rejection rates (19%). In complete contrast, the five-year graft failure was lowest in the modulation groups (4–7%) and highest in the sustained groups (25–31%). Multivariable analysis showed that a higher pre-treatment DSA level, male gender and absence of early acute rejection were strongly associated with a sustained DSA response. The modulation group had excellent five-year outcomes despite higher rates of early rejection episodes. This work further develops an understanding of post-transplant DSA dynamics and their influence on graft survival following HLA-incompatible kidney transplantation.

Keywords: kidney transplantation, antibody dynamics, 5 years graft failure, donor specific antibody, dynamic patterns, clustering

OPEN ACCESS

Correspondence:
Natalia Khovanova
n.khovanova@warwick.ac.uk

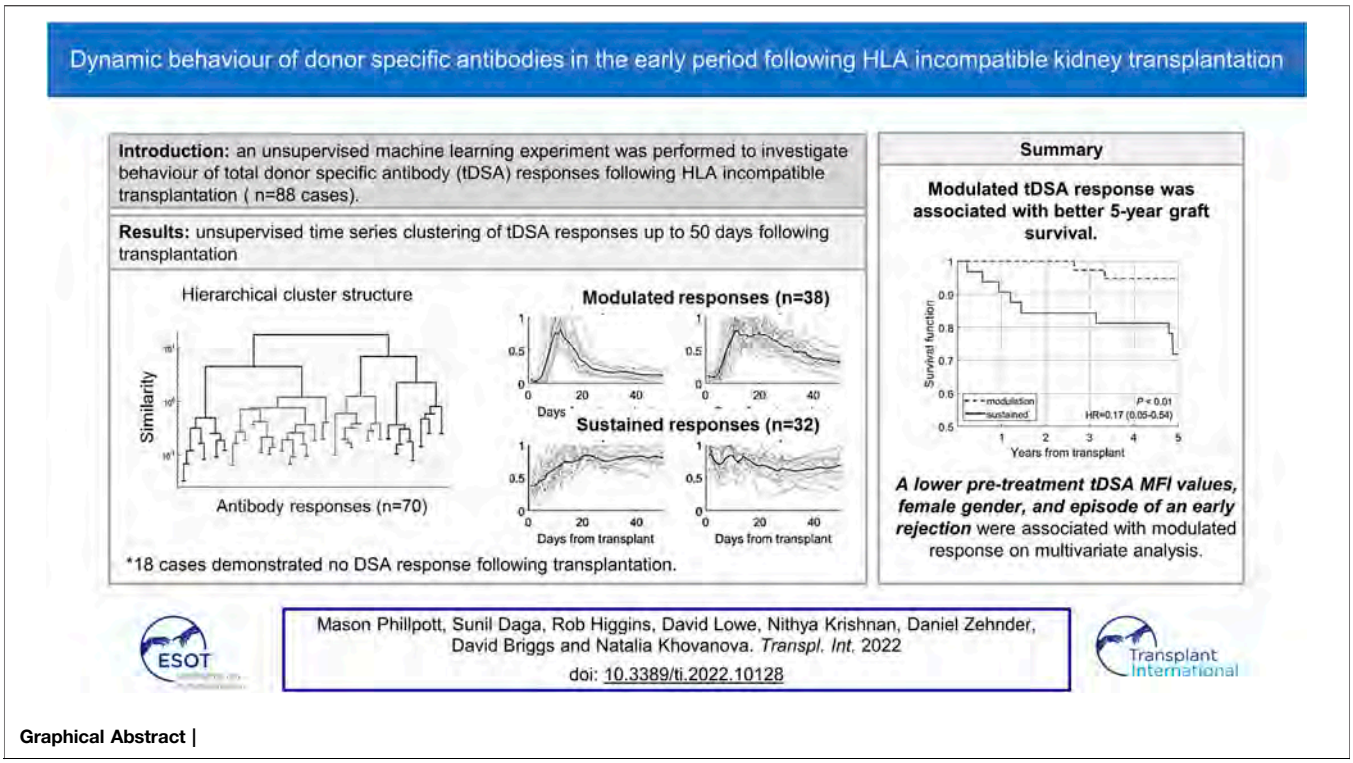
Received: *20 October 2021*
Accepted: *03 March 2022*
Published: *11 April 2022*

Citation:
Phillpott M, Daga S, Higgins R, Lowe D, Krishnan N, Zehnder D, Briggs D and Khovanova N (2022) Dynamic Behaviour of Donor Specific Antibodies in the Early Period Following HLA Incompatible Kidney Transplantation. Transpl Int 35:10128. doi: 10.3389/ti.2022.10128

INTRODUCTION

Despite the advances in the identification of acceptable mismatch programmes (1), better allocation of deceased donor kidneys (2), and advances in kidney sharing protocols for those with living kidney donors (3), there is still a role for HLA-incompatible transplants, especially when lower-risk scenarios could be identified (4) and those at the highest end of the sensitisation spectrum still do not have equal access to transplantation (5).

Abbreviations: ACR, acute cellular rejection; AMR, antibody mediated rejection; AUC, area under curve; CDC, complement-dependent cytotoxic; DSA, donor specific antibodies; DFPP, double filtration plasmapheresis; DTW, dynamic time warping; ESRF, end stage renal failure; FC, flow cytometry; GF, graft failure; HLA, human leukocyte antigen; LR, logistic regression; MFI, mean fluorescence intensity; MAR, mixed acute rejection; PR, precision-recall; ROC, receiver operator characteristic; RRT, renal replacement therapy; SAB, single antigen bead; tDSA, total DSA.



Graphical Abstract |

It is well-recognised that the presence of donor HLA specific antibodies (DSA) both before and after kidney transplantation correlates strongly with poorer graft outcomes (6–9). However, the monitoring and characterisation of the early post-transplant DSA response and how this may inform outcome and transplant management is still a developing area (10). Previous research has shown that DSA measurements pre-transplantation or at the time of transplantation can be a powerful tool for predicting graft outcome, but the sensitivity and specificity obtained varied with different DSA cut-off values (11–15).

Post-transplantation tools, such as protocol biopsies, in the early period to guide management and predict outcomes are limited and often not acceptable to patients. Early episodes of antibody mediated rejection (AMR) may be associated with recurrent rejection, chronic AMR and poor graft survival (16). The presence of DSA post-transplantation was associated with an increased likelihood of AMR (17–19) and graft failure (12, 20, 21). Recent studies (22, 23) suggest AMR may not always be associated with poor middle- or long-term graft failure (GF).

Studies on monitoring DSA immediately following transplantation are usually limited in the number of post-transplant samples (10, 17, 18, 24). The early post-transplant period (first 2 weeks) is a critical time for B-cell anamnestic memory and dynamic DSA behaviour and the occurrence of accelerated AMR episodes. The behaviour of DSA in the first month after transplantation and their associations with immediate/short term transplant outcomes has been previously described (25, 26). With access to up to 50 days post-transplant DSA measurements, our work looks at the medium-term outcomes and aims to determine how different dynamic DSA patterns relate to 5-year graft survival.

PATIENTS AND METHODS

Patients

133 patients referred from multiple centres in the United Kingdom and Republic of Ireland for HLA-incompatible kidney transplantation between 2003 and 2014 at the University Hospitals of Coventry and Warwickshire NHS Trust were considered. Of these cases, 88 were used in the final analysis. Twenty-four cases were excluded for the following reasons: no consent to use of data (n = 1), not proceeding to transplantation (n = 7), also ABO-incompatible (n = 16), early death or early graft failure (n = 5), insufficient follow up data (n = 9), antibody assay saturated (n = 2), less than 5 years follow-up (n = 5). Within 5 years following transplantation, graft failure occurred in 13 out of the 88 cases, and all failed due to immunological reasons. Study approval was obtained from the local ethics committee (CREC-055/01/03 and 13/WM/0090).

HLA Testing

HLA Class I and Class II specific antibodies were identified before transplantation by bead assay (One Lambda Inc. Canoga Park, CA), initially using HLA phenotype beads (N = 19) and subsequently with single antigen beads (SAB) as previously described for this programme (20). HLA typing of patients and donors was performed by a DNA probe assay (Lifecodes HLA SSO, Immucor) at a resolution comparable to the antibody identification, allowing identification of all donor-specific antibodies corresponding to HLA-A, -B, -Cw, -DRB1/3/4/5, -DQ, and -DP.

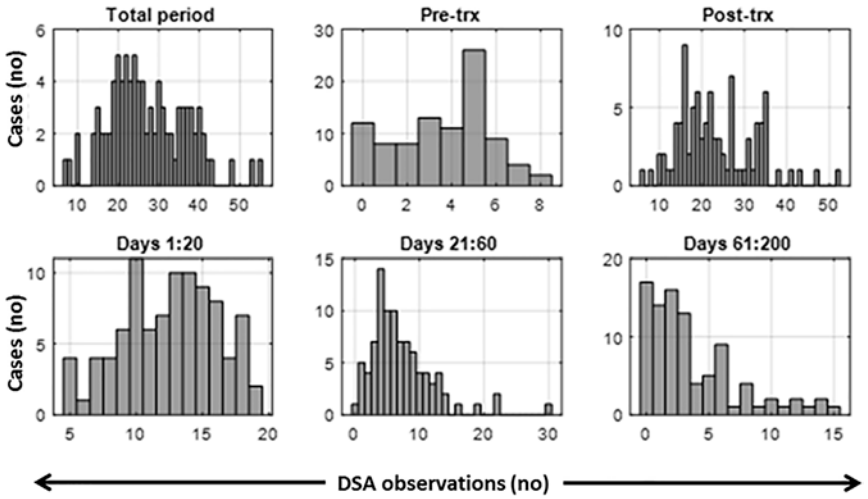


FIGURE 1 | Histograms of the observed number of data points per measurement period. Total period includes pre-transplant and post-transplant period. Trx, transplant.

Desensitisation and Immunosuppressive Protocol

68/88 patients required several sessions of pretransplant double filtration plasmapheresis (DFPP) with a target of negative FC cross-match or cumulative DSA median fluorescence intensity (MFI) <3,000 pre-transplantation. The maximum number of sessions administered was seven, with patients typically receiving five. In some cases where the DFPP sessions could not achieve a negative FC cross-match, patients were transplanted in the presence of higher DSA levels. IVIg was given in three cases. Twenty cases proceeded to transplant without DFPP because the total DSA MFI values were below 3,000 (n = 14) or with higher levels in cases of deceased donors' kidney transplantation where pretransplant antibody reduction was not logistically possible (n = 7). For these deceased donor cases, DSA were predominantly specific from HLA-DP mismatches.

Typical immunosuppression consisted of 1,000 mg mycophenolate mofetil twice daily, starting 10 days before transplant with dosage reduced if white cell count dropped below 4.0 x 10^9 per litre. Daily administrations of tacrolimus were commenced 4 days before transplantation. Dosages were given at 0.15 mg/kg/day in increments with a target trough level of 10–15 μg/L in the first month. At the point of surgery, a single 500 mg methylprednisolone dose was provided intravenously, and 20 mg basiliximab induction was given twice on day zero and day four post-transplant. Oral prednisolone was given at 20 mg/day and tapered to 5 mg/day after 30 days.

Monitoring and Management Following HLA-Incompatible Kidney Transplantation

Apart from on-table post perfusion samples, a biopsy was done for cause only, i.e., in cases of graft function deterioration or creatinine stuck as described previously (27). Acute rejection (n = 41) episodes occurring before 30 days post-transplant were identified at incidence under the most recent BANFF guidelines (28). In six instances where the biopsy was not possible, for example, for patients on anticoagulation therapy or during weekends, a rapid rise of HLA DSA MFI values alongside a drop in urine output and increase in creatinine (with one case with delayed graft function on dialysis) was defined as clinical rejection. All 41 rejection cases were treated with a course of pulse methylprednisolone 500 mg once a day for 3 days. In thirty cases, a lymphocyte-depleting agent (ATG, OKT3, or Campath) was administrated. DFPP treatment was performed in thirteen cases, of which five were given IVIg. One case had ecluzimab in addition to rescue therapy (see **Supplementary Table S1**). DFPP was only given in cases with rejection and not pre-emptively, with DSA levels going up in the presence of good urine output and stable renal function.

Pre-Processing of HLA DSA Data

The total number of DSAs in our cohort of 88 cases was 211, with between 1 to 7 for each patient. In this work, the levels in each case were considered. For the following analyses, we calculated the sum of individual HLA DSA MFIs to give a total DSA (tDSA) for each time point.

Post-transplant antibody testing involved more frequent testing during the early phase, with the majority (71/88) being sampled ten or more times during the first 20 days, after which the rate of sampling declined (**Figure 1**). Cases that had at least 21 days of DSA monitoring data points were included in this study. Variation in sampling days presents a challenge to clustering algorithms requiring uniform sampling rates. A linear interpolation was used within the 50-day time frame to fill missing values which were few in the first 2 weeks post-transplantation and increased with time (**Figure 1**). At the 2 week mark, the median length of interpolated values is 0

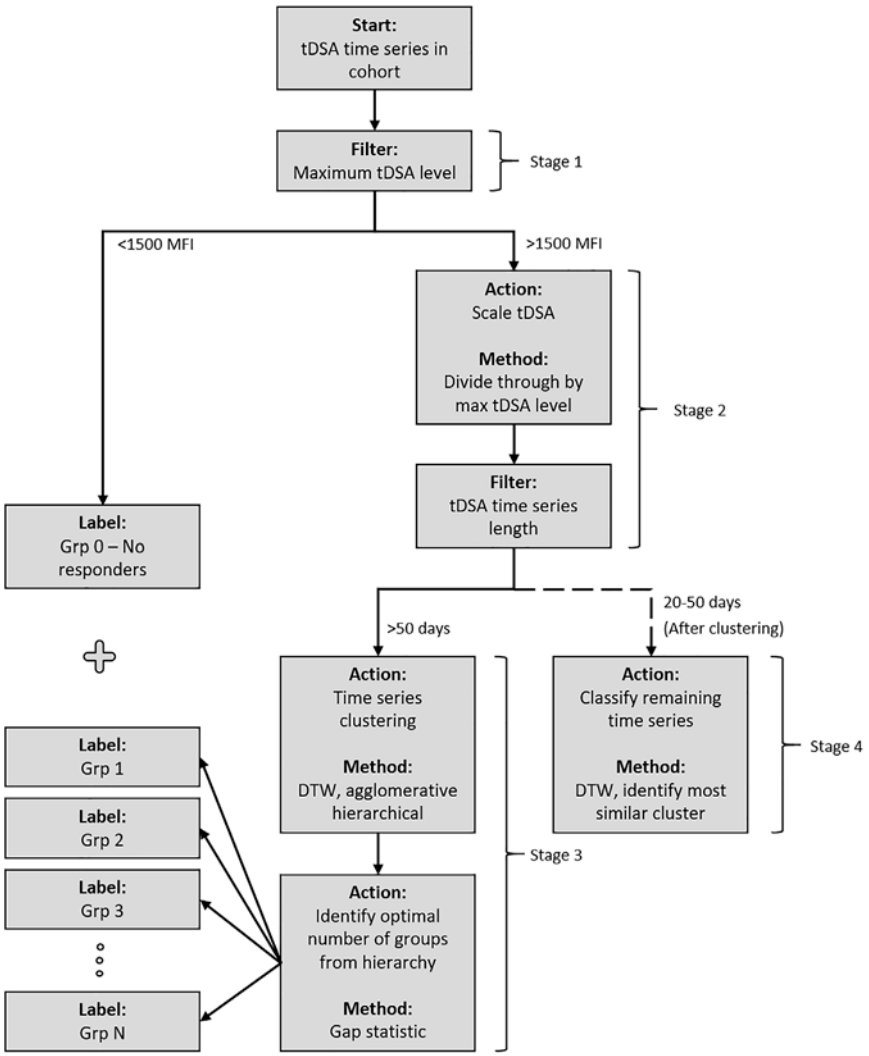


FIGURE 2 | Post-transplant DSA dynamics clustering methodology. The cohort is passed through the system and sorted into groups based on the similarity of post-transplant DSA dynamics. Stage 1 separates DSA into a pre-designated "no response" group if their maximum post-transplant tDSA titre level is less than 1500 MFI. Stage 2 scales the remaining DSA based on maximum post-transplant tDSA titre level before an additional filter is applied, separating the DSA based on time series length. All data which have 50 days are passed to Stage 3 and subsequently clustered using DTW distance measure under an agglomerative hierarchical structure. The optimal numbers of groups within the structure are identified using the gap statistic. Stage 4 classifies the remaining DSA, which have 20–50 days of data, into the groups identified in Stage 3.

days; at 4 weeks, this extends to 2 days, at 50 days to 5 days, and at 100 days to 16 days. We, therefore, included up to 50 post-transplant days in the study; this extends well-passed the period involving the DSA rebound and avoids the times with high levels of missing data.

Clustering of DSA Time Series

The DSA data are a time series comprising successive DSA measurements for 88 patients for up to 50 days post-transplant. These were investigated to identify possible classes of early post-transplant antibody behaviour (such as rebound and modulation). They could then be tested for association with pretransplant parameters and post-transplant events. The classification was performed using unsupervised machine learning clustering (29). The four-stage procedure, illustrated in **Figure 2**, was as follows.

Stage 1 | Grouping of no-response data. No-response cases consistently demonstrated low tDSA, i.e., below 1500 MFI (N = 18). These cases are not used in the clustering algorithm due to the disruptive influence on the analysis once scaled. The DSA levels in this group also have a significant uncertainty (29, 30, 31), which leaves dynamics in this MFI range unidentifiable. Instead, they are considered as a separate no-response group 0.

Stage 2 | Pre-processing for DSA time series clustering. Some post-transplant DSAs displayed little dynamic activity but high MFI levels. To distinguish such DSA dynamic patterns from one

TABLE 1 | Comparison of baseline characteristics for GF and no-GF groups (N = 88).

Graft Failure (GF)	No (75)	Yes (13)	p-value	Odds (95% CI)
Continuous, md(r)				
Age, years	43 (36–49)	33 (23–40)	<**0.01**	
Established renal failure, years	13 (3–18)	9 (1.5–15)	0.24	
Pre-Tx DSA levels, kMFI	5.5 (2.9–9.4)	11.6 (3.6–27.8)	0.07	
Categorical, n (%)				
Gender				
Male	28 (37)	6 (46)		
Female	47 (63)	7 (54)	0.55	0.7 (0.21–2.3)
Living donor				
No	7 (9)	0 (0)		
Yes	68 (91)	13 (100)	0.59	n/a
Previous transplants				
No	29 (39)	3 (23)		
Yes	46 (61)	10 (77)	0.36	2.1 (0.53–8.3)
Early acute rejection				
No	41 (55)	5 (38)		
Yes	33 (45)	8 (62)	0.37	1.9 (0.58–6.4)
Crossmatch status				
CDC (-) FC(-) SAB (+)	18 (24)	5 (38)		
CDC (-) FC(+) SAB (+)	44 (59)	3 (23)	0.10	0.25 (0.05–1.1)
CDC (+) FC(+) SAB (+)	13 (17)	5 (38)	0.72	1.4 (0.33–5.8)
DSA HLA class type				
Class I	27 (36)	4 (31)		
Class II	15 (20)	4 (31)	0.46	1.8 (0.39–8.3)
Class I and II	33 (44)	5 (38)	1.00	1 (0.25–4.2)
DSA count				
≤3	45 (60)	8 (62)		
≥4	30 (40)	5 (38)	1.00	0.94 (0.28–3.1)
HLA (A,B,DR) mismatches				
≤3	54 (72)	10 (77)		
≥4	21 (28)	3 (23)	1.00	0.77 (0.19–3.1)
Pre-treatment DFPP				
No	18 (24)	2 (15)		
Yes	57 (76)	11 (85)	0.72	1.7 (0.35–8.6)
Post-transplant DFPP				
No	66 (88)	9 (69)		
Yes	9 (12)	4 (31)	0.10	3.3 (0.83–13)
Post-transplant lymphodepletion				
No	53 (71)	7 (54)		
Yes	22 (29)	6 (46)	0.33	2.1 (0.62–6.8)

N (%) = number of cases (% of cases); md(r) = median (interquartile range). Continuous and ordinal data, e.g., patient's age at transplantation, treatment duration, etc., were compared using Wilcoxon rank-sum test. The Fisher two-tailed exact test was applied to binary data, e.g., gender, previous transplant (yes/no), etc. Significant variables (p < 0.05) in univariate analysis are displayed in bold.

another without affecting the clustering algorithm, the time series were scaled by their corresponding maximal DSA levels (29). Additionally, many temporal clustering techniques require equal length time series, which in this case required establishing a defined period for clustering analysis. Picking a shorter time series length for analysis would allow for the inclusion of more cases in analysis, although at the compromise of losing potentially valuable data obtained from clustering longer time intervals. DSA time series length is inconsistent in our cohort, and a steady drop in measurements/samples is observed as time progresses. A period of 50 days provided the best compromise for analysis. The remaining cases were split based on their length: up to 50-days data available (longer time series) and 20–50 days (shorter time series).

Stage 3 | DSA clustering and validation of the longer DSA time series (n = 47). Unsupervised clustering was completed via a dynamic time warping (DTW) distance measure (29) and agglomerative hierarchical approach (32). Cluster links were formed via the mean of the two joining time series. The algorithm identified the two most similar time series at each iteration based upon the distance measure and merged to form a cluster. The identified time series were then replaced by the newly formed cluster for which distance measures were newly calculated. For the unsupervised clustering, the number of groups was not known *a priori*. Although this value can be identified through visual inspection in some cases, additional certainty in estimation was required due to time series complexity. Here the gap statistic was implemented, which is a measure that estimates the correct number of groups, k, by comparing the within-cluster dispersion by that expected under a reference/surrogate set (33). The reference set consisted of 47 artificially formed time series and was created

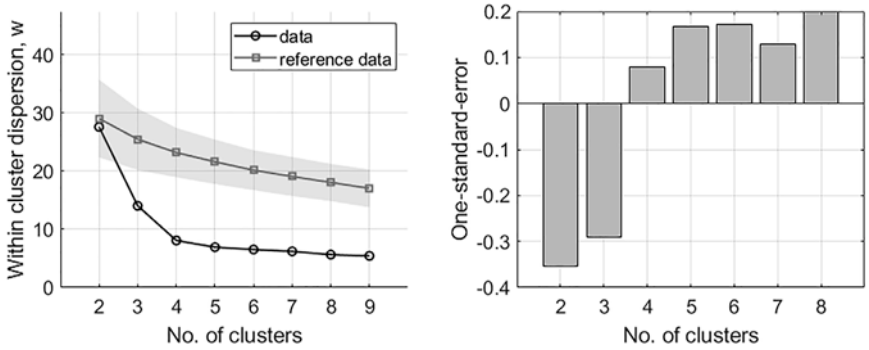


FIGURE 3 | Results of the gap analysis for DSA time series. Left: within-cluster dispersion, the standard deviation is indicated for reference data via the shaded region. Right: one-standard-error measurements. The optimal number of clusters is indicated by the lowest k on the left in combination with positive one-standard-error measurement on the right; in this case, $k = 4$.

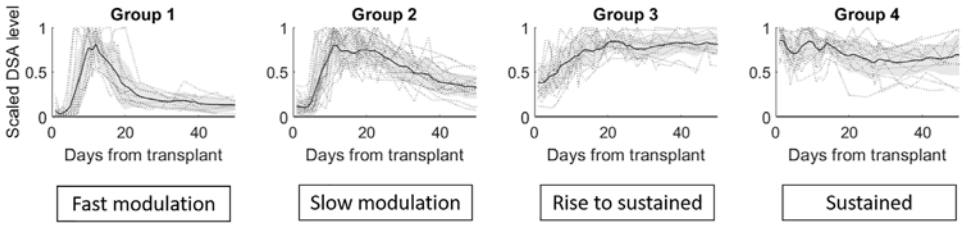


FIGURE 4 | The four groups identified via the gap statistic from DTW agglomerative hierarchical clustering on the DSA dataset. Cluster linkages, i.e., means of the clusters, are shown in dark grey lines. The shaded region displays the standard deviation for each cluster, and individual profiles are displayed in grey dotted lines.

by a random selection of DSA time series segments of 10 time points in length (block bootstrapping). One hundred reference sets are shown to be sufficient in implementations of the gap statistic (34). Within-cluster dispersion calculated for the original DSA dataset and for the mean of the reference data set were compared. For each value of k, a one-standard-error term was calculated. It was used to identify the lowest k for which the difference in within-cluster dispersion between the original and reference data sets stopped increasing. The lowest value of k was subsequently given as the optimal number of clusters.

Stage 4 | The remaining shorter time series ($n = 23$) were classified, using the DTW distance measure, into the clusters identified in Stage 3.

Logistic Regression Analysis

Logistic regression (LR) classification analysis (Matlab, stepwiseglm tool) was performed to consider the associations of identified groups with 5-years graft outcomes whilst accounting for the other potentially confounding variables. Each model was formed via a stepwise LR algorithm with the F-test (35), comparing the fit of two models at each step and determining if variables should be introduced (F-test, $p < 0.05$) into the model or removed (F-test, $p > 0.1$). Due to class imbalance in data, i.e., 75 no-GFs in negative class versus 13 GFs in positive/minority class, two area under curve (AUC) measures were calculated for each model to evaluate LR performance: the receiver operator characteristic (ROC) AUC and precision-recall (PR) AUC. The former is a standard model performance measure but tends to be optimistic on imbalanced classification problems with fewer samples in the minority class. The latter is focused on the minority class and, thus, helps evaluate the model on it. Finally, odds ratios (OR) with corresponding confidence intervals (CI) were evaluated by the t-test (35).

RESULTS

Patients Cohort Characteristics

Of the 88 cases, 48% experienced acute rejection (see **Supplementary Table S1** for types) within the first 30-days, and 15% experienced GF within 5 years following transplantation (**Table 1**). The number of cases rises to almost a quarter experiencing GF when looking at the cases with early rejection in isolation. Of the 13 GF cases, eight experienced an acute rejection episode (**Table 1**). On univariate analysis, only younger age at the time of receiving transplantation was associated with worse 5-year graft outcome, but no significant difference was found across different baseline characteristics, e.g., cross-match types, DSA class or DSA count (**Table 1**).

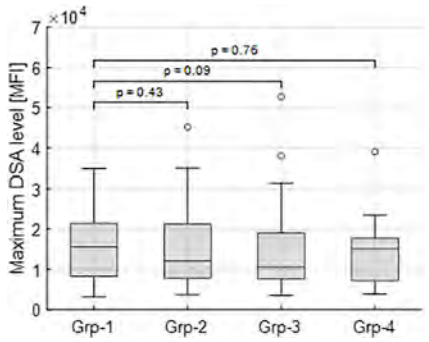


FIGURE 5 | Maximum tDSA level distributions for each group. Group 1 has the highest median value (16,263 MFI) and is compared to groups 2–4 for a significant difference. In each case, no significant differences are identified between groups highlighting how different dynamics occur across all observed DSA ranges.

Post-Transplant DSA Response Types and Patients Characteristics Within the Identified Groups

The DTW agglomerative hierarchical clustering of 47 tDSA time series with 50-time points following transplantation are presented in **Figure 3**. **Figure 3** (left figure) shows that at $k = 4$, the difference within-cluster dispersion between the original and reference data stops increasing, suggesting that the optimal number of clusters is four. This assessment is reinforced when observing the subsequent one-standard error measurement (**Figure 3**, rightmost figure), whose first positive instance is also at $k = 4$. A breakdown of the four clusters/groups for post-transplant tDSAs is shown in **Figure 4**. A description of each group is given, including the additional group of non-responders identified separately in Stage 1 of clustering analysis:

a) No-response group (group 0): low post-transplantation tDSA levels <1500 MFI.
b) Fast modulation (group 1): sharp rise followed by a sharp decrease is tDSA MFI values; the mean peak is day 13 post-transplant. The dynamic behaviour can be summarised as having a short peak duration, typically 3–4 days, before experiencing a sharp drop and settling at the pre-peak DSA level. DSA are typically inactive for up to 5 days following transplant.
c) Slow modulation (group 2): sharp rise followed by a gradual decline in tDSA values; the mean peak day is 13 post-transplant. In this cohort, peak duration is not easily defined and gradually reduces to approximately 30% of peak levels by the 50th-day post-transplant. DSA is typically inactive for up to 5 days following transplant. There are large oscillations in DSA values, not seen in group 1.
d) Rise to sustained (group 3): slower rise followed by sustained high levels; mean peak at day 21 post-transplant, and DSA levels remain consistently high from this point onwards. DSA are typically at a higher baseline than in groups 1 and 2 up to 5 days post-transplant.
e) Sustained (group 4): no substantial rise or fall but tDSA is persistently above 1500 MFI.

Inclusion of the remaining 23 shorter DSA time series (Stage 4), classified into the closest of the selected groups 1–4 gave the total numbers of dynamic patterns: group 0 ($n = 18$), group 1 ($n = 15$), group 2 ($n = 23$), group 3 ($n = 16$) and group 4 ($n = 16$). The highest median MFI value (16,263) is observed in group 1; however, there is no significant difference in the maximal DSA MFI values between this group and groups 2–4 (**Figure 5**).

The patient characteristic analysis of these five groups is illustrated in **Table 2**. There were no statistically significant differences in the occurrence of pre or post-transplant DFPP (Fisher exact test). Groups were assessed for their associated rates of acute rejection and graft failure. The fast modulation responses

TABLE 2 | Characteristics of groups based on clustering.

	Cluster/group					
	0 No response ($n = 18$)	1 Fast modulation ($n = 15$)	2 Slow modulation ($n = 23$)	3 Rise to sustained ($n = 16$)	4 Sustained ($n = 16$)	All ($n = 88$)
Age (years, mean)	43.94	44.87	40.17	37.13	39.69	41.10
ESRF (years, mean)	13.08	7.33	10.65	12.16	14.23	11.48
Female (%)	55.56	93.33	78.26	31.25	43.75	61.36
LDKT (%)	94.44	100	95.65	93.75	75	92.05
Previous tx (%)	72.22	26.67	60.87	81.25	75	63.64
Crossmatch+ (%)	55.56	73.33	69.57	93.75	81.25	73.86
CDC+ (%)	11.11	13.33	17.39	37.5	25	20.45
Out of all CDC+, CDC>1:2 (%)	0	0	0	100	50	55.56
Pre Tx-DFPP(%)	66.7	80.0	82.6	93.7	62.5	77.27
Average tDSA value	2062	7023	7105	14813	12674	8735
DSA (n)	2	3	3	3	3	3
Rejection within first 30-days, %	16.67	80.0	56.52	56.25	18.75	47.73
Post Tx-DFPP (%)	5.56	33.33	13.04	18.75	6.25	14.77
Lymphocyte-depleting agent (%)	5.56	40	47.83	50	12.5	31.82
GF-5 years (%)	11.11	6.67	4.35	31.25	25	14.77

ESRF, end stage renal failure; LDKT, living donor kidney transplant; Tx, transplant; DFPP, double filtration plasmapheresis; CDC, Complement dependent cytoxicity.

TABLE 3 | Univariate analysis: comparison of patients' characteristics for modulation and sustained groups (n = 70).

	tDSA dynamic group		p-value	OR (95% CI)
	Modulation (38)	**Sustained (32)**		
Continuous, md (r)				
Age (years)	44 (36–49)	40 (33–43)	**0.04**	
ESRF (years)	7 (2–16)	15 (7–18)	0.06	
Pre-treatment tDSA level	5200 (3300–9400)	9800 (6600–16000)	<**0.01**	
Maximum tDSA level	12000 (7800–21000)	13000 (7000–18000)	0.87	
Categorical, n (% in each group)				
Gender				
Male	6 (16)	20 (63)		
Female	32 (84)	12 (37)	<**0.001**	0.11 (0.04–0.35)
Living donor				
No	1 (3)	5 (16)		
Yes	37 (97)	27 (84)	0.09	0.15 (0.02–1.3)
Previous transplants				
No	20 (53)	7 (22)		
Yes	18 (47)	25 (78)	**0.01**	4 (1.4–11)
CDC crossmatch				
CDC(-) FC(-) SAB(+)	11 (29)	4 (13)		
CDC(-) FC(+) SAB(+)	21 (55)	18 (56)	0.23	2.4 (0.64–8.7)
CDC(+) FC(+) SAB(+)	6 (16)	10 (31)	0.07	4.6 (0.99–21)
DSA HLA class				
Class I	12 (32)	10 (31)		
Class II	6 (16)	7 (22)	0.73	1.4 (0.35–5.5)
Class I and II	20 (53)	15 (47)	1.00	0.9 (0.31–2.6)
DSA count				
≤3	21 (55)	17 (53)		
≥4	17 (45)	15 (47)	1.00	1.1 (0.42–2.8)
HLA (A, B and DR) mismatches				
≤3	22 (58)	28 (87)		
≥4	16 (42)	4 (13)	<**0.01**	0.2 (0.06–0.67)
Pre-treatment DFPP				
No	7 (18)	7 (22)		
Yes	31 (81)	25 (78)	0.77	0.81 (0.25–2.6)
Early acute rejection				
No	11 (29)	20 (63)		
Yes	27 (71)	12 (37)	<**0.01**	0.24 (0.09–0.67)
5 years graft failure				
No	36 (94)	23 (72)		
Yes	2 (6)	9 (28)	0.02	7 (1.4–36)
Post-transplant DFPP				
No	30 (79)	28 (87)		
Yes	8 (21)	4 (13)	0.53	0.54 (0.15–2)
Post-transplant lymphodepletion				
No	21 (55)	22 (69)		
Yes	17 (45)	10 (31)	0.33	0.56 (0.21–1.5)

n (%) = number of cases (% of cases); md(r) = median (interquartile range). Variables that demonstrated significantly different distributions (p < 0.05) within the 2 groups are highlighted in bold.

(group 1) demonstrated the highest rejection rate of 80%, the sustained group showed the lowest rate at 19%, whereas the slow modulation and rise to sustained groups had rejection rates around 56%. This is in contrast to 5-year GF rates, with 4–7% in the two modulation groups (groups 1 and 2) and 25–31% in the two sustained groups (groups 3 and 4).

Modulated Versus Sustained DSA Responses

Because of their similarity in GF rates, the two modulation groups were combined, and the two sustained groups were combined for further analysis. **Table 3** illustrates the results of a univariate analysis with significant variables ($p < 0.05$) highlighted in bold. Younger patients and those with previous transplants were more likely to produce a sustained response. In contrast, female gender, more than 4 HLA mismatches and an episode of early acute rejections were associated with a modulated response. The higher pre-treatment tDSA levels were strongly associated with sustained dynamics. Multivariable LR analysis (**Table 4**), accounting for ten confounding variables with $p < 0.2$ in univariate analysis (**Table 3**), showed higher pre-treatment tDSA levels, male gender, and the absence of an episode of early acute rejection were all strongly associative of a sustained response (ROC-AUC/

TABLE 4 | Multivariable LR model showing association of selected ($p < 0.2$) variables from **Table 3** with a sustained dynamic response.

	OR	95% CI		p-value
Intercept	1.36	2.85	5.97	0.24
Pre-treatment DSA level (↑1000 MFI)	1.08	1.13	1.19	**<0.01**
Gender (Female)	0.06	0.12	0.24	**<0.01**
Acute rejection (Yes)	0.07	0.14	0.28	**<0.01**
Cases		70		
of which are Sustained		32 (46%)		
PR-AUC (PR-AUC/baseline)		0.84 (1.83)		
ROC-AUC (ROC-AUC/baseline)		0.86 (1.59)		

Significant p-values (p < 0.05) are highlighted in bold.

TABLE 5 | Multivariable LR model showing association of DSA response type (modulation and sustained) with 5-year GF while accounting for confounding variables, $p < 0.2$, identified from univariate analysis in **Table 3**.

	OR	95% CI		p-value
Intercept	8.29	1.32	52.20	0.25
Age (↑)	0.90	0.86	0.95	**0.03**
DSA response (sustained)	10.05	3.75	26.92	**0.02**
Post-transplant DFPP (no)	0.15	0.05	0.39	**0.05**
Cases		70		
of which are GF		11 (16%)		
PR-AUC (PR-AUC/baseline)		0.60 (3.83)		
ROC-AUC (ROC-AUC/baseline)		0.85 (1.00)		

Significant p-values (p < 0.05) are highlighted in bold.

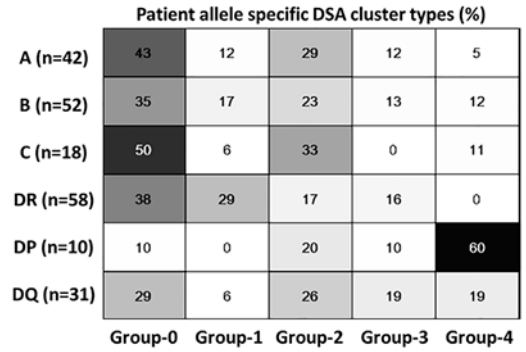


FIGURE 6 | Dynamic cluster patterns at DSA-HLA specific allele levels. Class 1 and DR specific DSA has predominantly modulating response compared to HLA DP; HLA DQ has mixed dynamics.

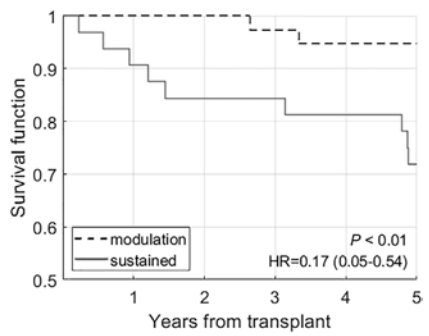


FIGURE 7 | Kaplan-Meier analysis comparing the survival rates in modulation (groups 1 and 2) and sustained (groups 3 and 4) groups.

baseline = 1.59, PR-AUC/baseline = 1.83). A sub-group analysis of cases who experienced early rejection found no statistically significant difference in treatment proportions between modulated and sustained DSA dynamic responses (p = ns Fischer 2-tail test). The dynamic response varied with DSA specificities with more modulation response for HLA-A, -B and -DR specificities and sustained against HLA-DP specificities (**Figure 6**), though given patient there were DSA specificities that followed mixed dynamics.

Kaplan-Meier analysis (**Figure 7**) confirms that the sustained group has a worse 5-year GF rate. A multivariable logistic regression model was also developed (**Table 5**) to look at the association of the dynamic patterns of the identified groups, combined with confounding variables, namely age, cross-match status and post-transplant DFPP (from **Table 3**, $p < 0.2$), with GF. The model in **Table 5** shows significant associations of younger age, sustained tDSA response and post-transplant DFPP, with GF (PR-AUC/baseline = 3.83).

DISCUSSION

Following kidney transplantation, DSA monitoring can be a useful surrogate marker as allorecognition and memory responses to the re-exposure of HLA is associated with DSA rise and rejection (36). The response can be variable (37) and may depend on the type of sensitisation events (38), age, baseline immunosuppressant, time since sensitisation and level of cross-match before transplantation. T-cell help (39) is required to reactivate memory B-cells (40, 41), and successful treatment of rejection with OKT3/ATG in the study supports this. Equally, a rising DSA trend is often considered more worrying and clinically useful than a steady state or drop in the MFI values.

As a result, most transplant centres and national guidelines suggest post-transplant DSA monitoring (42). However, recommendations vary, and there is much uncertainty over how to perform such monitoring. We have previously described dynamic patterns seen in individual DSA and third party HLA antibodies using the visual description from the same dataset (25), including more complex mathematical modelling of a modulatory type of dynamic behaviour (26). In this study, we have, for the first time, applied an unsupervised clustering (33) approach to the DSA MFI time series to describe the overall dynamic trends and patterns of DSA following HLA-incompatible kidney transplantation and their associations with transplant outcomes, in particular with 5-year graft survival. Four dynamic patterns were identified after separating the non-responder group, demonstrating heterogeneous dynamic behaviour. The total

DSA levels remained notably subdued up to the first five to 10 days following transplantation, particularly in fast (group 1) and slow (group 2) modulation groups. This effect in the first 4 days following transplant has been noted in several studies before (18, 25, 43), in part caused by adsorption of HLA antibodies onto the kidney allograft (25). As the use of post-transplant DFPP was not associated with a particular dynamic pattern/group (**Tables 2**, **3**), it suggests that other mechanisms are responsible for differential behaviour.

Our data show that the more sensitised cases at baseline, i.e., those with the higher DSA MFI levels and CDC titre>1:2, are likely to develop the sustained post-transplant response. These, in turn, have the poorest five-year survival. We have previously reported poor survival in the higher titre baseline cases (23). Others have also shown that pre-transplant higher total DSA associates with persistent high total DSA post-transplantation (44) and that sustained total DSA levels associate with the worse outcome than resolved DSA (44, 45, 46). Unlike previous study (45), a single point day 30 DSA levels (tDSA >2000 MFI) in a multivariable model was not an independently significant predictor for graft outcome in our study. We found that early modulation cases are likely to have a very different outcome, despite a significantly higher incidence of early AMR. This all points to different levels of immune regulation in the two broad antibody dynamic groups that we have identified. The very rapid modulation seems in part to be AMR-dependent (87.7% incidence in the fast modulators, Group 1) which implies an active immunological process. Notably, the early antibody dynamics were not significantly associated with the use of post-transplant antibody removal. In some cases, we observed no sustained fall in MFI values over a course of DFPP, and in others, we saw spontaneously MFI falls without DFPP use. A recent study (22) also showed that declining DSA levels following AMR associated with the good longer-term outcome, but the two cohorts are not directly comparable and with different approaches to AMR treatment, so it is unclear whether it is the treatment itself as opposed to the AMR process (and its successful resolution) that determines the outcome.

This study allows some observations to be made that might assist further investigations. First, the "decisions" made by the immune system whether or not to increase DSA levels and then whether or not to have a sustained response or a fall in DSA levels seem to be made in the first 2 weeks or so after transplantation. We did not see late shifts in trajectory, though we cannot exclude the possible impacts of events such as non-adherence and pregnancy. Second, the sustained falls in DSA levels followed initial rises. There was no "sustained to fall" group. This could mean that initial activation of the immune system was required before there could be elimination or suppression of antibody production. Lastly, the rate of fall in DSA levels in our slow modulation group is broadly in line with the known half-life of IgG1 (about 23 days) (47), while the disappearance of DSA in the fast modulation group (half-life in the order of 5 days) seems to imply an active mechanism of DSA removal. However, while the fast disappearance of DSA may be useful, it was not by itself a requirement for good long term outcomes rather the "decision" to modulate was paramount. These observations are speculative but provide many opportunities to drive targeted investigations in the future.

The results of our study reflect specific immunosuppressive protocol, management and monitoring protocols, and case selections, limiting generalizability to broader patient groups. Ideally, a larger multi-centre study is required to confirm the findings. SAB are the best available option for determining DSA levels and have improved our ability to identify and manage allosensitised transplant patients (48, 49). They provide a semi-quantitative measurement of DSA in the form of MFI, with has limitations at larger DSA levels such as the prozone and saturation effects (49–51). While we have made the best efforts to identify and address the prozone and saturation effect occurrences within the cohort, it is still recognised that MFI levels cannot accurately represent true antibody strength. Other limitations include cases discharged back to parent units, a management protocol that may have influenced long term outcomes, and we did not employ protocol biopsies, so we cannot comment on the possible relationship of these to sustained antibody levels. Despite these limitations we made an in-depth and detailed description of DSA dynamic responses. This work may help in future tailoring of treatment so that lower risk HLA-incompatible patients are not subjected to over-immunosuppression even if they have had early acute rejection and that high-risk patients can be looked at more carefully even if they haven't had an early acute rejection.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because they are available on request and for collaborative research only. Requests to access the datasets should be directed to Natalia Khovanova n.khovanova@warwick.ac.uk.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by CREC-055/01/03 and 13/WM/0090. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MP–design, analysis, writing and editing; SD–data collection, design, analysis, writing and editing; RH–data collection, design, writing and editing; DL–data collection, design; NKr–data collection, editing; DZ–data collection, editing; DB–data collection, design, writing and editing; NKh–design, analysis, writing and editing.

FUNDING

We acknowledge EPSRC UK (EP/K02504X/1) support, which helped initiate this research and UHCW NHS Trust -Renal

Transplant research funds for funding the project. SD is supported by the National Institute for Health Research Leeds *In Vitro* Diagnostics Co-operative.

AUTHOR DISCLAIMER

The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

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

We are grateful to patients who consented and provided data and materials, healthcare staff at the renal transplant centre at UHCW NHS Trust and scientists at H&I, NHSBT Birmingham. We also would like to thank colleagues from referral centres across the United Kingdom and the Republic of Ireland.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10128/full#supplementary-material

Supplementary Table S1 | Breakdown of acute rejection types (ACR, acute cellular rejection; AMR, antibody mediated rejection; MAR, mixed acute rejection), number of cases (No) and corresponding treatment. [*lymphodepeleting agent–11–OKT3 and 17–ATG (including one with Alemtuzumab)].

REFERENCES

1. Mumford L, Fuggle SV, Martorell J, Slavcev A, Iniotaki A, Haasnoot GW, et al. A Europe Wide Acceptable Mismatch Program Will Enable Transplantation of Long Waiting Highly Sensitised Patients with a Compatible Donor. *Transpl Immunol* (2021) 64:101354. doi:10.1016/j.trim.2020.101354
2. Jackson KR, Motter JD, Kernodle A, Desai N, Thomas AG, Massie AB, et al. How Do Highly Sensitized Patients Get Kidney Transplants in the United States? Trends over the Last Decade. *Am J Transpl* () 20:2101–12. doi:10.1111/ajt.15825
3. Manook M, Johnson R, Robb M, Burnapp L, Fuggle SV, Mamode N. Changing Patterns of Clinical Decision Making: Are Falling Numbers of Antibody Incompatible Transplants Related to the Increasing success of the UK Living Kidney Sharing Scheme? A National Cohort Study. *Transpl Int* (2021) 34:153–62. doi:10.1111/tri.13776
4. Schinstock CA, Smith BH, Montgomery RA, Jordan SC, Bentall AJ, Mai M, et al. Managing Highly Sensitized Renal Transplant Candidates in the Era of Kidney Paired Donation and the New Kidney Allocation System: Is There Still a Role for Desensitization? *Clin Transpl* (2019) 33:e13751. doi:10.1111/ctr.13751
5. Stewart DE, Wilk AR, Toll AE, Harper AM, Lehman RR, Robinson AM, et al. Measuring and Monitoring Equity in Access to Deceased Donor Kidney Transplantation. *Am J Transpl* (2018) 18(8):1924–35. doi:10.1111/ajt.14922
6. Cravedi P, Heeger PS. Immunologic Monitoring in Transplantation Revisited. *Curr Opin Organ Transplant* (2012) 17:26–32. doi:10.1097/mot.0b013e32834ee402
7. Loupy A, Hill GS, Jordan SC. The Impact of Donor-specific Anti-HLA Antibodies on Late Kidney Allograft Failure. *Nat Rev Nephrol* (2012) 8:348–57. doi:10.1038/nrneph.2012.81
8. Mannon RB. Immune Monitoring and Biomarkers to Predict Chronic Allograft Dysfunction. *Kidney Int Suppl* (2010) 78:S59–65. doi:10.1038/ki.2010.425
9. Terasaki PI, Ozawa M, Castro R. Four-year Follow-Up of a Prospective Trial of HLA and MICA Antibodies on Kidney Graft Survival. *Am J Transpl* (2007) 7:408–15. doi:10.1111/j.1600-6143.2006.01644.x
10. Tait BD, Süsal C, Gebel HM, Nickerson PW, Zachary AA, Claas FHJ, et al. Consensus Guidelines on the Testing and Clinical Management Issues Associated with HLA and Non-HLA Antibodies in Transplantation. *Transplantation* (2013) 95:19–47. doi:10.1097/tp.0b013e31827a19cc
11. Gloor JM, Winters JL, Cornell LD, Fix LA, DeGoey SR, Knauer RM, et al. Baseline Donor-specific Antibody Levels and Outcomes in Positive Crossmatch Kidney Transplantation. *Am J Transplant* (2010) 10:582–9. doi:10.1111/j.1600-6143.2009.02985.x
12. Lefaucheur C, Loupy A, Hill GS, Andrade J, Nochy D, Antoine C, et al. Preexisting Donor-specific HLA Antibodies Predict Outcome in Kidney Transplantation. *JASN* (2010) 21:1398–406. doi:10.1681/asn.2009101065
13. Malheiro J, Tafulo S, Dias L, Martins LS, Fonseca I, Beirão I, et al. Analysis of Preformed Donor-specific Anti-HLA Antibodies Characteristics for Prediction of Antibody-Mediated Rejection in Kidney Transplantation. *Transpl Immunol* (2015) 32:66–71. doi:10.1016/j.trim.2015.01.002
14. Salvadé I, Aubert V, Venetz J-P. Clinically-relevant Threshold of Preformed Donor-specific Anti-HLA Antibodies in Kidney Transplantation. *Hum Immunol* (2016) 77:483–9.
15. Ziemann M, Altermann W, Angert K, Arns W, Bachmann A, Bakchoul T, et al. Preformed Donor-specific HLA Antibodies in Living and Deceased Donor Transplantation. *CJASN* (2019) 14:1056–66. doi:10.2215/cjn.13401118
16. Dunn TB, Noreen H, Gillingham K, Maurer D, Ozturk OG, Pruett TL, et al. Revisiting Traditional Risk Factors for Rejection and Graft Loss after Kidney Transplantation. *Am J Transplant* (2011) 11:2132–43. doi:10.1111/j.1600-6143.2011.03640.x
17. Amrouche L, Aubert O, Suberbielle C, Rabant M, Van Huyen J-PD, Martinez F, et al. Long-term Outcomes of Kidney Transplantation in Patients with High Levels of Preformed DSA. *Transplantation* (2017) 101:2440–8. doi:10.1097/tp.0000000000001650
18. de Castro MCR, Barbosa EA, Souza RP, Agena F, de Souza PS, Maciel G, et al. The Kinetics of Anti-HLA Antibodies in the First Year after Kidney Transplantation: in Whom and when Should They Be Monitored? *J Transpl* (2018) 2018:8316860. doi:10.1155/2018/8316860
19. Shaikhina T, Lowe D, Daga S, Briggs D, Higgins R, Khovanova N. Decision Tree and Random forest Models for Outcome Prediction in Antibody Incompatible Kidney Transplantation. *Biomed Signal Process Control* (2019) 52:456–62. doi:10.1016/j.bspc.2017.01.012
20. Khovanova N, Daga S, Shaikhina T, Krishnan N, Jones J, Zehnder D, et al. Subclass Analysis of Donor HLA -specific IgG in Antibody-incompatible Renal Transplantation Reveals a Significant Association of IgG 4 with Rejection and Graft Failure. *Transpl Int* (2015) 28:1405–15. doi:10.1111/tri.12648
21. Mihaylova A, Baltadjieva D, Boneva P, Ivanova M, Penkova K, Marinova D, et al. Clinical Relevance of Anti-HLA Antibodies Detected by Flow-Cytometry Bead-Based Assays-Single-Center Experience. *Hum Immunol* (2006) 67:787–94. doi:10.1016/j.humimm.2006.07.011
22. Lichvar AB, Tremblay S, Leino AD, Shields AR, Cardi MA, Abu Jawdeh BG, et al. Reducing Donor-specific Antibody during Acute Rejection Diminishes Long-Term Renal Allograft Loss: Comparison of Early and Late Rejection. *Transplantation* (2020) 104:2403–14. doi:10.1097/tp.0000000000003145
23. Krishnan N, Abimbola A, Machan N, Daga S, Gopalakrishnan K, Lam F, et al. HLA Antibody Incompatible Renal Transplantation: Long-Term Outcomes

Similar to Deceased Donor Transplantation. *TransplantationTransplant Direct* (2021) 7(8):e732. doi:10.1097/txd.0000000000001183
24. Vo AA, Aubert O, Haas M, Huang E, Zhang X, Choi J, et al. Clinical Relevance of Posttransplant DSAs in Patients Receiving Desensitization for HLA-Incompatible Kidney Transplantation. *Transplantation* (2019) 103:2666–74. doi:10.1097/tp.0000000000002691
25. Higgins R, Lowe D, Hathaway M, Lam F, Kashi H, Tan LC, et al. Rises and Falls in Donor-specific and Third-Party HLA Antibody Levels after Antibody Incompatible Transplantation. *Transplantation* (2009) 87:882–8. doi:10.1097/tp.0b013e31819a6788
26. Zhang Y, Briggs D, Lowe D, Mitchell D, Daga S, Krishnan N, et al. A New Data-Driven Model for post-transplant Antibody Dynamics in High Risk Kidney Transplantation. *Math Biosciences* (2017) 284:3–11. doi:10.1016/j.mbs.2016.04.008
27. Krishnan N, Abimbola A, Machan N, Daga S, Gopalakrishnan K, Lam F, et al. HLA Antibody Incompatible Renal Transplantation: Long-Term Outcomes Similar to Deceased Donor Transplantation. *Transplant Direct* (2021) 7:e732. doi:10.1097/txd.0000000000001183
28. Solez K, Racusen LC. The Banff Classification Revisited. *Kidney Int* (2013) 83:201–6. doi:10.1038/ki.2012.395
29. Warren Liao T. Clustering of Time Series Data-A Survey. *Pattern Recognition* (2005) 38:1857–74. doi:10.1016/j.patcog.2005.01.025
30. Gebel HM, Bray RA. HLA Antibody Detection with Solid Phase Assays: Great Expectations or Expectations Too Great? *Am J Transplant* (2014) 14(9):1964–75. doi:10.1111/ajt.12807
31. Reed EF, Rao P, Zhang Z, Gebel H, Bray RA, Guleria I, et al. Comprehensive Assessment and Standardization of Solid Phase Multiplex-Bead Arrays for the Detection of Antibodies to HLA. *Am J Transplant* (2013) 13:1859–70. doi:10.1111/ajt.12287
32. Kaufman L, Rousseeuw PJ. *Finding Groups in Data: An Introduction to Cluster Analysis*. John Wiley & Sons (2009).
33. Tibshirani R, Walther G, Hastie T. Estimating the Number of Clusters in a Data Set via the gap Statistic. *J R Stat Soc* (2001) 63:411–23. doi:10.1111/1467-9868.00293
34. Maechler M, Rousseeuw P, Struyf A, Hubert M, Hornik K. Cluster Analysis Basics and Extensions. *R Package Version 2.0* (20172019) 6.
35. Hardle WK, Simar L. *Applied Multivariate Statistical Analysis*. Springer (2019).
36. Lúcia M, Luque S, Crespo E. Preformed Circulating HLA-specific Memory B Cells Predict High Risk of Humoral Rejection in Kidney Transplantation. *Kidney Int* (2015) 88:874–87.
37. Ahmed R, Gray D. Immunological Memory and Protective Immunity: Understanding Their Relation. *Science* (1996) 272:54–60. doi:10.1126/science.272.5258.54
38. Higgins R, Lowe D, Daga S, Hathaway M, Williams C, Lam FT, et al. Pregnancy-induced HLA Antibodies Respond More Vigorously after Renal Transplantation Than Antibodies Induced by Prior Transplantation. *Hum Immunol* (2015) 76:546–52. doi:10.1016/j.humimm.2015.06.013
39. Mohammed MT, Cai S, Hanson BL, Zhang H, Clement RL, Daccache J, et al. Follicular T Cells Mediate Donor-specific Antibody and Rejection after Solid Organ Transplantation. *Am J Transpl* (2021) 21(5):1893–901. doi:10.1111/ajt.16484
40. Crotty S. A Brief History of T Cell Help to B Cells. *Nat Rev Immunol* (2015) 15:185–9. doi:10.1038/nri3803
41. de Graav GN, Dieterich M, Hesselink DA, Boer K, Clahsen-van Groningen MC, Kraaijeveld R, et al. Follicular T Helper Cells and Humoral Reactivity in Kidney Transplant Patients. *Clin Exp Immunol* (2015) 180:329–40. doi:10.1111/cei.12576
42. British Transplant Society. *Guidelines for Antibody Incompatible Transplantation* (2015).
43. Burns JM, Cornell LD, Perry DK, Pollinger HS, Gloor JM, Kremers WK, et al. Alloantibody Levels and Acute Humoral Rejection Early after Positive Crossmatch Kidney Transplantation. *Am J Transplant* (2008) 8:2684–94. doi:10.1111/j.1600-6143.2008.02441.x
44. Senev A, Lerut E, Van Sandt V, Coemans M, Callemeyn J, Sprangers B, et al. Specificity, Strength, and Evolution of Pretransplant Donor-specific HLA Antibodies Determine Outcome after Kidney Transplantation. *Am J Transpl* (2019) 19:3100–13. doi:10.1111/ajt.15414
45. Shaffer D, Feurer ID, Crowe D, Schaefer H. Early and Sustained Reduction in Donor-specific Antibodies in Desensitized Living Donor Kidney Transplant Recipients. *Transpl Direct* (2016) 2(2):e62. doi:10.1097/txd.0000000000000570
46. Zachary AA, Montgomery RA, Leffell MS. Factors Associated with and Predictive of Persistence of Donor-specific Antibody after Treatment with Plasmapheresis and Intravenous Immunoglobulin. *Hum Immunol* (2005) 66:364–70. doi:10.1016/j.humimm.2005.01.032
47. Mankarious S, Lee M, Fischer S, Pyun KH, Ochs HD, Oxelius VA, et al. The Half-Lives of IgG Subclasses and Specific Antibodies in Patients with Primary Immunodeficiency Who Are Receiving Intravenously Administered Immunoglobulin. *J Lab Clin Med* (1988) 112(5):634–40.
48. Ellis TM. Interpretation of HLA Single Antigen Bead Assays. *Transplant Rev* (2013) 27:108–11. doi:10.1016/j.trre.2013.07.001
49. Gu Y, Koh RWK, Lai ML, Pochinco D, Teo RZC, Chan M, et al. Defining the Structural Basis for Human Leukocyte Antigen Reactivity in Clinical Transplantation. *Sci Rep* (2020) 10:18397. doi:10.1038/s41598-020-75355-4
50. Garcia-Sanchez C, Usenko CY, Herrera ND, Tambur AR. The Shared Epitope Phenomenon-A Potential Impediment to Virtual Crossmatch Accuracy. *Clin Transpl* (2020) 34:e13906. doi:10.1111/ctr.13906
51. Koh RWK, Lai ML. Defining the Structural Basis for Human Leukocyte Antigen Reactivity in Clinical Transplantation. *Sci Rep* (2020) 10:18397. doi:10.1038/s41598-020-75355-4

ESOT | Transplant International

ORIGINAL RESEARCH
published: 14 April 2022
doi: 10.3389/ti.2022.10212

Creating a Single Inflow Orifice From Living Donor Kidney Allografts With Multiple Renal Arteries

*Marina M. Tabbara[1,2], Giselle Guerra[3,2], Juliano Riella[1,2], Phillipe Abreu[1,2], Angel Alvarez[2], Rodrigo Vianna[1,2], Linda Chen[1,2], Mahmoud Morsi[1,2], Jeffrey J. Gaynor[1,2], Javier Gonzalez[4] and Gaetano Ciancio[1,2,5]**

[1]Department of Surgery, Miller School of Medicine, University of Miami, Miami, Florida, [2]Miami Transplant Institute, Miller School of Medicine, University of Miami, Jackson Memorial Hospital, Miami, Florida, [3]Division of Nephrology, Department of Medicine, Miller School of Medicine, University of Miami, Miami, Florida, [4]Department of Urology, Hospital General Universitario Gregorio Marañón, Madrid, Spain, [5]Department of Urology, Miller School of Medicine, University of Miami, Miami, Florida

Background: Multiple renal arteries (MRA) are often encountered during living-donor kidney transplantation (LDKT), requiring surgeons to pursue complex renovascular reconstructions prior to graft implantation. With improvements in reconstruction and anastomosis techniques, allografts with MRA can be successfully transplanted with similar outcomes to allografts with a single renal artery. Here, we describe in detail various surgical techniques for reconstruction of MRA grafts with the intent of creating a single arterial inflow.

Methods: We retrospectively reviewed the medical records of all LDKT recipients with laparoscopically procured MRA kidneys between March 2008 and July 2021. Recipient and donor characteristics, operative data, type of reconstruction, and recipient outcomes were analyzed. The primary outcomes were the incidence of developing delayed graft function (DGF) and/or a vascular or urological complication within 12 months post-transplant.

Results: Seventy-three LDKT recipients of MRA donor allografts were evaluated. Two renal arteries (RA) were encountered in 62 allografts (84.9%) and three RA in 11 allografts (15.1%). Renal artery reconstruction was performed in 95.8% (70/73) of patients. Eighteen different reconstruction techniques of MRA were utilized, the most common being side-to-side anastomosis in allografts with two RA (N = 44) and side-to-side-to-side anastomosis in allografts with three RA (N = 4). Interposition grafting was performed in seven cases (9.6%). A single ostium was created in 69 cases (94.5%), and the median warm ischemia time was 27 (range 20–42) minutes. None of the patients developed DGF or post-operative vascular or urological complications. Median creatinine at 3, 6, and 12 months post-transplant remained stable at 1.1 mg/dl. With a median follow-up of 30.4 months post-transplant, only one graft failure has been observed–death-censored graft survival was 98.6%.

Conclusion: Complex reconstruction techniques to create a single renal artery ostium for graft implantation anastomosis in allografts with MRA show acceptable warm ischemic times, with no increased risk of post-operative vascular or urological complications.

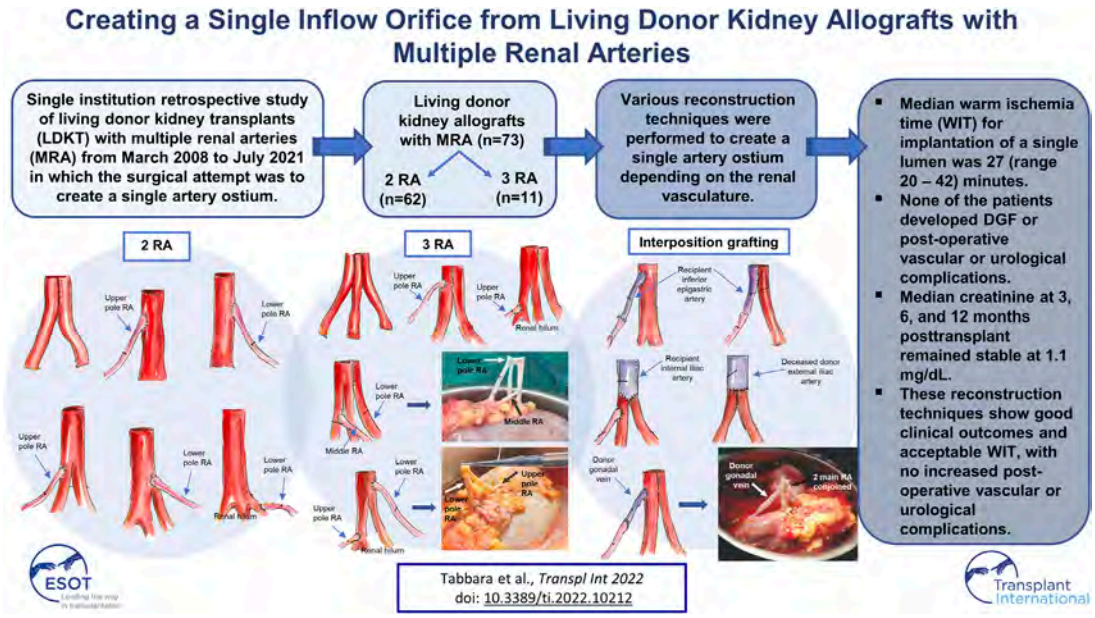
Keywords: multiple renal blood vessels, surgical innovation, living donors, renal transplantation, kidney allografts

OPEN ACCESS

***Correspondence:**
Gaetano Ciancio
gciancio@med.miami.edu

Received: *10 November 2021*
Accepted: *08 March 2022*
Published: *14 April 2022*

Citation:
Tabbara MM, Guerra G, Riella J, Abreu P, Alvarez A, Vianna R, Chen L, Morsi M, Gaynor JJ, Gonzalez J and Ciancio G (2022) Creating a Single Inflow Orifice From Living Donor Kidney Allografts With Multiple Renal Arteries. Transpl Int 35:10212. doi: 10.3389/ti.2022.10212



Graphical Abstract |

INTRODUCTION

With a widening gap between supply and demand of organs, living-donor kidney transplantation (LDKT) has substantially increased in efforts to expand the donor pool. This has led to a surge in living-donor kidneys (LDK) with anatomical variations, specifically, multiple renal arteries (MRA) (1, 2). Kidneys with MRA are common in renal vascular anatomy, occurring at an incidence of 18–43% in potential kidney donors (3). When encountered during LDKT, they often require complex back-table reconstructions, which has been associated with a higher risk of post-transplant vascular and urologic complications (1, 4, 5). However, with improvements in reconstruction and anastomosis techniques, allografts with MRA have been shown to be successfully transplanted with similar surgical and clinical outcomes compared to allografts with a single renal artery (6–8). Examples of these improvements include the use of interposition grafting (9, 10) and side-to-side anastomoses to create a wide lumen (11, 12). Additionally, routine use of low-molecular weight dextran and optical magnification have helped to minimize postoperative complications and made it easier to construct microvascular anastomosis during LDKT (6–8).

Although long-term graft and patient survival have been shown to be similar for single and multiple arteries, the impact of the type of arterial reconstruction method for MRA has rarely been investigated and warrants additional study (12, 13). Performing multiple anastomoses is often associated with poor visibility, difficult suturing (14), thrombosis, and bleeding (15). Additionally, multiple anastomoses are associated with a prolonged warm ischemia time (WIT), which has been shown to have a detrimental effect on both early graft function and long-term graft survival in LDKT (16–21). In this study, we describe in detail 18 different surgical techniques for reconstruction of MRA during LDKT, with the main goal of creating a single renal artery ostium for allograft implantation in efforts to facilitate construction of the *in situ* vascular anastomosis, minimize recipient WIT, and reduce post-operative complications. We evaluated recipient and donor demographics, operative data, early outcomes such as delayed graft function (DGF), development of any post-operative vascular, urological, or other complication within 12 months post-transplant, and graft survival.

METHODS

We retrospectively reviewed the medical records of all LDKT recipients with laparoscopically procured MRA kidneys at our institution between March 2008 and July 2021. This study was approved by the University of Miami Institutional Review Board and follows the ethical principles (as revised in 2013) of the Helsinki Declaration. All patients gave written informed consent prior to enrollment.

All donors underwent comprehensive nephrologic evaluation including their medical history, physical examination, renal function assessment, and urinalysis. Evaluation of the donor renal vascular anatomy was performed using computed tomography angiography (CTA). Thus, the presence of multiple vessels was known before surgery. All donors referred to us were considered suitable based on their vasculature. The approach for reconstruction of MRA and the availability of deceased donor vessels for interposition grafting reconstruction were determined before surgery.

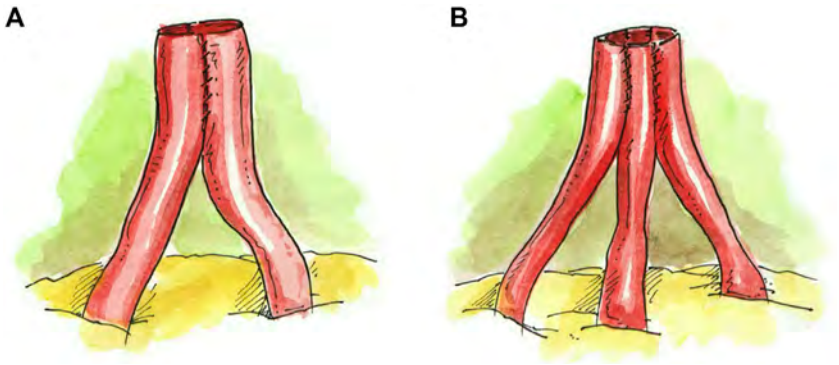


FIGURE 1 | Conjoined anastomosis techniques. **(A)** Single ostium side-to-side anastomosis. **(B)** Single ostium side-to-side-to-side anastomosis.

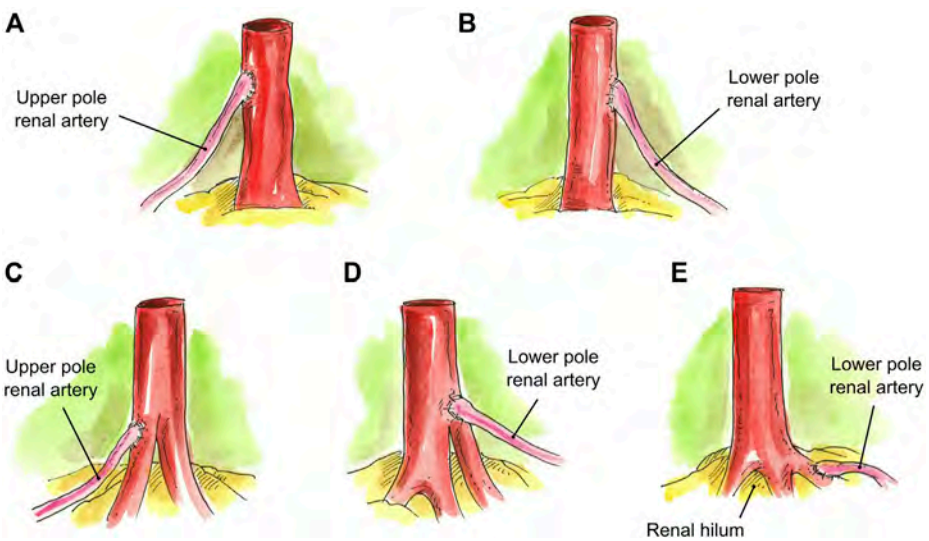


FIGURE 2 | Techniques for grafting a main RA and an accessory pole artery. **(A)** UPRA anastomosed end-to-side to main RA. **(B)** LPRA anastomosed end-to-side to main RA. **(C)** Short UPRA anastomosed end-to-side to a branch of the main RA. **(D)** Short LPRA anastomosed end-to-side to a branch of the main RA. **(E)** Short LPRA anastomosed end-to-side to a branch of the main RA inside the hilum.

All recipients began Aspirin 81 mg daily on post-operative day 3 and remained on this regimen indefinitely. To monitor development of vascular and/or urological complications, baseline Doppler Ultrasound (DU) was performed after surgery, and then repeated at 1, 3, and 12 months post-operatively. If there were any vascular or urological concerns, further imaging with magnetic resonance angiography and/or Tc99m MAG-3 renal scintigraphy was performed.

Statistical Analysis

Analyzed baseline variables included date of transplant, recipient age, recipient gender, recipient race/ethnicity, recipient BMI, recipient pre-transplant history of diabetes mellitus, kidney retransplant status, donor type, donor kidney location (left or right), number of donor arteries, type of vascular reconstruction, whether or not a single renal artery ostium was used, living donor type (related/unrelated), double-J ureteral stent insertion, JP drain insertion, total operative time, cold ischemia time (CIT), and warm ischemia time (WIT) for single and multiple anastomoses. Recipient outcomes included development of DGF (requirement for dialysis during the first post-operative week), length of hospital stay, development of a post-operative vascular, urological, or other complication within 12 months posttransplant, and graft loss (return to permanent dialysis or death). Estimated glomerular filtration rate (eGFR) was

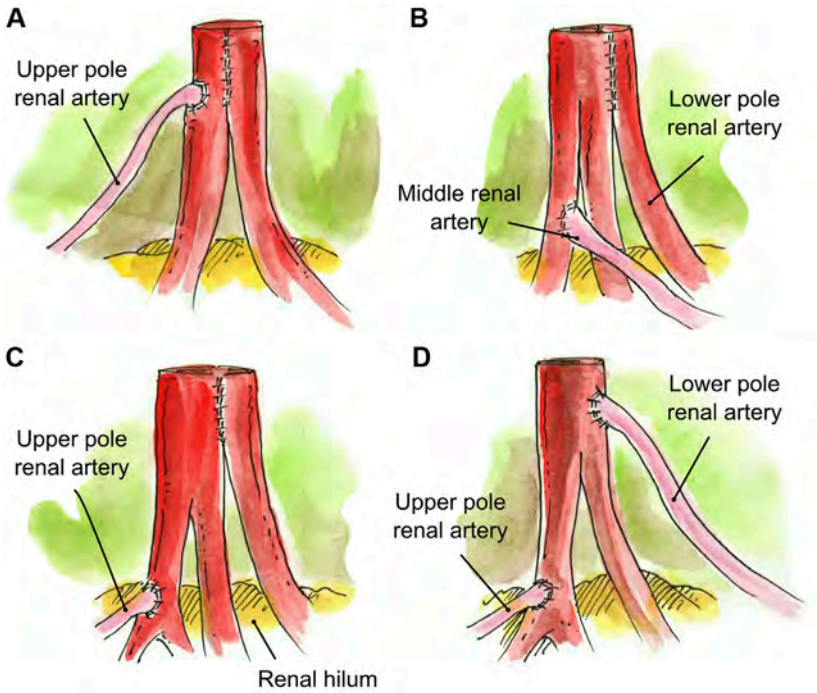


FIGURE 3 | Creation of a single inflow orifice for grafts with 3RA. **(A)** Two main RA conjoined side-to-side and UPRA anastomosed end-to-side to the upper main RA. **(B)** LPRA conjoined in a single lumen with the main RA, and short middle RA anastomosed end-to-side to the upper branch of the main RA. **(C)** Two RA conjoined side-to-side in a single lumen and short UPRA anastomosed end-to-side to a branch of the upper renal artery inside the hilum. **(D)** Short UPRA anastomosed end-to-side to a branch of the main RA inside the hilum, and the LPRA was anastomosed end-to-side to main RA.

calculated using the Chronic Kidney disease Epidemiology Collaboration Equation. Percentages of patients having selected baseline characteristics were determined as well as means, standard errors, medians, and ranges of values for baseline continuous variables.

Surgical Techniques

A hand-assisted laparoscopic donor nephrectomy was performed in a standard fashion with special attention given to the renal hilum and preservation of the length of the renal vessels (22). The vessels were stapled using the Ethicon Echelon Flex Powered Stapler with the 45-mm vascular linear cutter. In the case of early bifurcation, we used the Ethicon Echelon Flex Powered Stapler with a 35-mm vascular linear cutter to avoid having two renal vessels. The graft was flushed with cold Histidine-tryptophan-ketoglutarate until the effluent was clear. The renal arteries and veins were dissected from the surrounding perivascular lymphatics and fat. The donor and recipient vessels were prepared by trimming any redundant length of the vessels to prevent kinking during anastomosis. The ureter with its blood supply and the periureteric tissue were preserved, and all remaining redundant perinephric fat was trimmed.

Ex-vivo reconstructions were performed during bench surgery according to the case-specific anatomy. Surgical loupes at 3.5x magnification were used for the reconstructions. All the vascular reconstructions were performed with 8–0 Prolene.

In the case of two renal arteries (RA) of similar length ($N = 43$), the preferred approach was a single ostium side-to-side anastomosis, which was created by spatulating the two arteries medially and conjoining them into a single lumen (**Figure 1A**). This technique was extrapolated in the case of three RA of similar length ($N = 4$), where a single renal artery ostium was created by conjoining the arteries in a side-to-side-to-side manner (**Figure 1B**). If the additional renal artery was <1 mm in length and not suitable for anastomosis, it was tied off, and the remaining two RA were conjoined together into a single lumen (N = 1) (**Figure 1A**).

In the case of a graft with a main RA and an accessory upper pole renal artery (UPRA) ($N = 4$) or lower pole renal artery (LPRA) ($N = 3$), an end-to-side anastomosis to the main RA was created in a running fashion (**Figures 2A,B**). If there was a short UPRA ($N = 1$) or short LPRA ($N = 1$), it was anastomosed end-to-side to one of the branches of the main RA (**Figures 2C,D**). In one case, the short LPRA was anastomosed to a branch of the main RA inside the hilum ($N = 1$) (**Figure 2E**).

In the case of three RA, several approaches were taken to create a single ostium. One approach was to conjoin the two main RA side-to-side and then anastomose the UPRA end-to-side to the upper main RA ($N = 1$) (**Figure 3A**). In one case, the LPRA was conjoined in a single lumen with the main RA, and the middle RA was anastomosed end-to-side to the upper branch of the main RA ($N = 1$) (**Figures 3B**, **6A**). In another approach, the two main RA were anastomosed side-to-side in a single lumen and the short

UPRA was anastomosed end-to-side to a branch of the RA inside the hilum ($N = 1$) (**Figure 3C**). Finally, there was one case where the short UPRA was anastomosed end-to-side to a branch of the main RA inside the hilum, and the LPRA was anastomosed end-to-side to main RA ($N = 1$) (**Figures 3D**, **6B**).

When an accessory pole artery was located too far from the renal artery(s) and creation of a single ostium was not feasible, two separate arterial anastomoses were implanted ($N = 4$). In three of these cases, there were grafts with two RA, with a short LPRA located too far from the main RA to perform a reconstruction. One of these was a case of 2-year-old pediatric recipient in which the LPRA was anastomosed end-to-side to the external iliac artery, and the main RA was anastomosed end-to-side to the common iliac artery. The two remaining cases had a short LPRA that was 8 cm from the main RA. The short LPRA was anastomosed end-to-end to the recipient inferior epigastric artery, and the main RA was anastomosed end-to-side to the external iliac artery (**Figure 4A**). In the final case of a graft with three RA, a LPRA was 7 cm from the two main RA. The two main RA were conjoined together side-to-side into a single ostium, and the short LPRA was anastomosed end-to-end to the recipient ipsilateral inferior epigastric artery, which was fully mobilized and dissected from the abdomen (**Figure 4B**).

Interposition grafting was utilized as various conduits for short renal arteries. A segment of recipient inferior epigastric artery (RIEA) was used in four renal grafts two 2 RA; a short UPRA was anastomosed end-to-end to the RIEA, and then anastomosed side-to-side ($N = 3$) or end-to-side ($N = 1$) to the main RA (**Figures 5A,B**). In a graft with three RA, the two main RA were anastomosed end-to-end to a segment of the recipient internal iliac artery, and the short UPRA was anastomosed end-to-side to one of the main RA ($N = 1$) (**Figure 5C**). A segment of deceased donor external iliac artery was used to extend two short RA conjoined in a single lumen (N = 1) (**Figure 5D**). Finally, a segment of donor gonadal vein was used to extend a short UPRA in a graft with three RA, which was anastomosed end-to-side to the one of the two main RA that were conjoined into single ostium ($N = 1$) (**Figures 5E**, **6C**).

Once the reconstructions were complete, grafts were anastomosed end-to-side to the recipient external iliac artery and vein. After reperfusion, an extravesical ureteroneocystostomy was performed (23).

Of note, while diameter sizes of donor arteries were measured pre-operatively by CTA (upper and lower pole arteries measured approximately 2 mm in diameter), the diameter of the ostium of the reconstructed arteries was not measured. However, its diameter was the combined size of the two or three conjoined RA.

Immunosuppression

All recipients received immunosuppressant therapy according to our center's protocols (24) with induction consisting of intravenous antithymocyte globulin 1 mg/kg, basiliximab 20 mg, and methylprednisolone 500 mg administered intraoperatively before organ reperfusion. Maintenance immunosuppression included a steroid-free regimen consisting of tacrolimus and mycophenolate mofetil, starting on postoperative day 1.

RESULTS

Recipient and donor baseline demographics and operative data appear in **Table 1**. Seventy-three LDKT recipients of MRA donor allografts were evaluated. Median recipient age was 48.8 (range 2.3–77.1) years, and 67.1% (49/73) recipients were male. Black and Hispanic participants comprised 12.3% (9/73) and 42.5% (31/73) of the transplant recipients, respectively. The majority of transplant recipients, 93.2% (68/73), received a primary kidney transplant; only 6.8% (5/73) were retransplants. The percentage of recipients who received a left donor kidney was 94.5% (69/73); 5.5% (4/73) received a right donor kidney. The percentage who received a kidney with two RA and three RA was 83.6% (61/73) and 16.4% (12/73), respectively. A double-J ureteral stent was placed in only 4.1% (3/73) of the patients. A JP drain was placed in 20.5% (15/73) of the patients. Median total operative time was 296 (range 206–483) minutes. The median warm ischemia time for anastomosis of a single artery ostium was 27 (range 20–42) minutes, and for two separate anastomoses it was 31.5 (range 21–33) minutes. Median estimated blood loss was 40 (range 10–300) ml.

The types of reconstruction are detailed in **Table 2**. Renal artery reconstruction was performed in 95.8% (70/73) of patients; reconstruction was not performed in three patients. A single renal artery ostium was created in 94.5% (69/73) of patients. Two separate renal artery anastomoses were implanted in 5.5% (4/73) of patients. Interposition grafting was performed in seven cases (9.6%).

Recipient outcomes are listed in **Table 3**. Median length of hospital stay was 4 (range 3–67) days. Median follow-up among 67 patients who were alive with a functioning graft as of the last follow-up date (31 July 2021) was 30.4 (range: 0.3–151.2) months post-transplant. Median preoperative creatinine was 6.0 (range 0.9–22.6) mg/dl, which decreased to 1.1 (range 0.25–2.0) mg/dl at 3 months. At 6 and 12 months post-transplant, the median creatinine remained stable at 1.1 mg/dl.

None of the 73 patients had DGF or developed a postoperative vascular or urological complication. Since the main concern with lower pole artery reconstruction is the risk of developing a postoperative urological complication, it was reassuring that no such complication was observed in any of the patients. Thus, there were no differences in clinical outcomes between those who received an upper pole artery vs. lower pole artery reconstruction.

Two patients (2.7%) developed a nonsurgical post-operative complication during the first 30 days (12 months) post-transplant, including *C. difficile* colitis/sepsis at 4 days post-transplant ($N = 1$) and acute respiratory distress syndrome (ARDS) at 6 days post-transplant ($N = 1$). The patient who developed *c. difficile* colitis/sepsis died of that infection (with a functioning graft) at 0.8 months post-transplant. The patient who developed ARDS did not experience graft loss.

One patient (1.4%) developed graft failure due to acute T-cell-mediated rejection (TCMR) at 41.8 months post-transplant. Five patients have died with a functioning graft: cardiovascular event in two patients (at 4.4 and 7.9 months post-transplant, respectively), infection in two patients (death due to *C. difficile* colitis/sepsis in one patient at 0.8 months post-transplant, and death due to sepsis in one patient at 125.2 months post-transplant), and ruptured aortic aneurysm in one patient (at 5.2 months post-transplant).

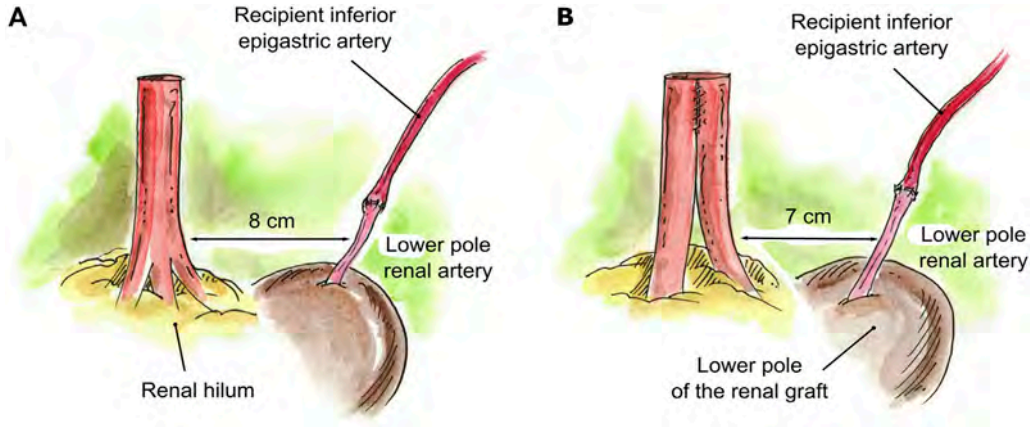


FIGURE 4 | Creation of two separate anastomoses for implantation. **(A)** Short LPRA (8 cm from the main RA) anastomosed end-to-end to the recipient inferior epigastric artery. **(B)** Two RA were conjoined in a single lumen and the LPRA (7 cm from the 2 RA) anastomosed end-to-end to the recipient ipsilateral inferior epigastric artery.

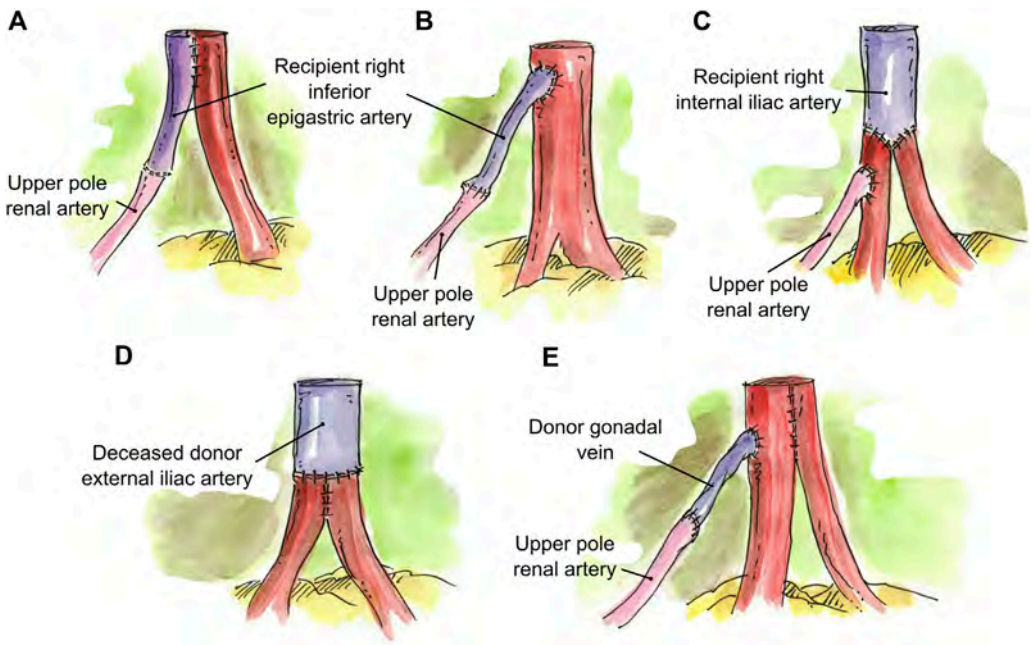


FIGURE 5 | Interposition grafting. **(A)** Segment of recipient inferior epigastric artery anastomosed end-to-end to the short UPRA, and then anastomosed side-to-side to the main RA. **(B)** Segment of recipient inferior epigastric artery anastomosed end-to-end to the short UPRA, and then anastomosed end-to-side to the main RA. **(C)** Segment of recipient internal iliac artery anastomosed end-to-end to the two main RA, and the short UPRA anastomosed end-to-side to one of the main RA. **(D)** Segment of deceased donor external iliac artery anastomosed end-to-end to two short RA conjoined in a single lumen. **(E)** Short UPRA extended with a segment of donor gonadal vein, then anastomosed end-to-side to the one of the 2 main RA that were conjoined in single ostium.

DISCUSSION

Kidney transplantation is the treatment of choice for patients with ESRD. However, donor organ shortage has prevented the wider application of this treatment. This has prompted surgeons to utilize each donor organ they encounter in a maximal and favorable manner, such as kidney grafts with MRA (7, 25). Up until recently, renal artery multiplicity was viewed as a contraindication for transplantation due to its greater technical demand and association with a higher incidence of vascular and urological complications (1, 4, 5). Additionally, prolonged total operative times and ischemia times were thought to add unnecessary risk to the recipient (7, 26). However, with recent advances in surgical reconstruction and

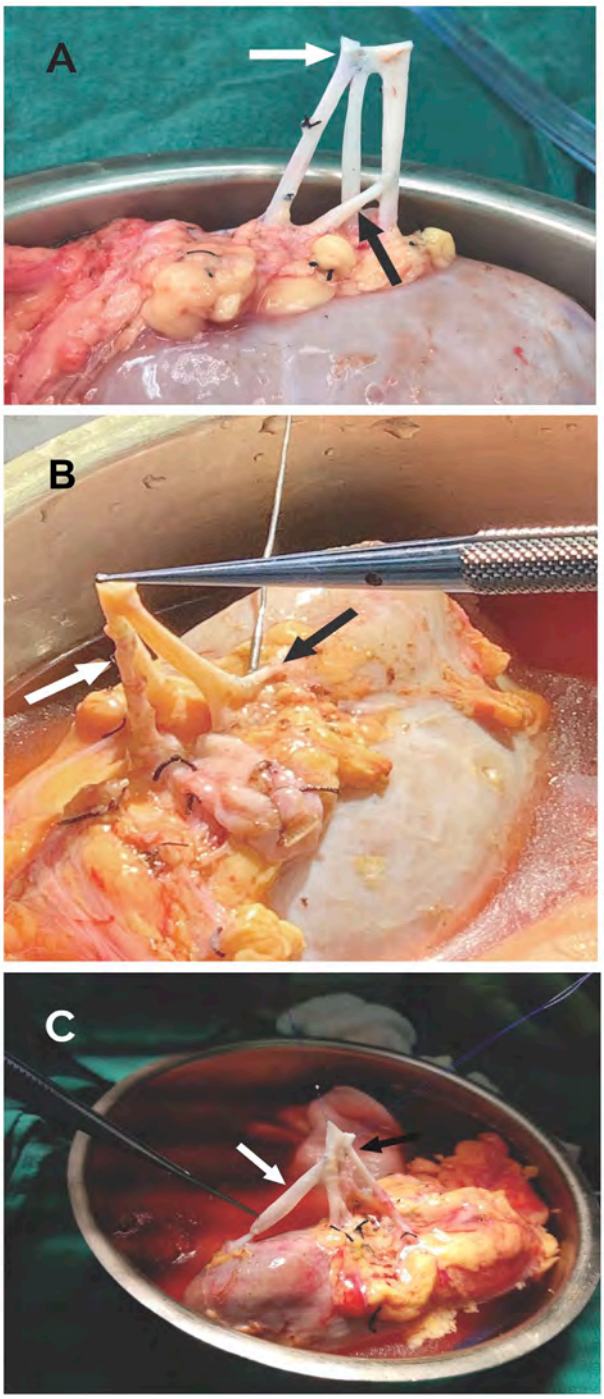


FIGURE 6 | (A) LPRA (white arrow) anastomosed side-to-side the main RA with 8–0 Prolene, middle RA anastomosed end-to-side to the main RA (black arrow) with 8–0 Prolene. **(B)** LPRA (white arrow) anastomosed end-to-side to the main RA with 8–0 Prolene. The UPRA was short, so it was anastomosed end-to-side to one of the branches of the main RA inside the hilum. **(C)** UPRA anastomosed end-to-end to the donor gonadal vein with 8–0 Prolene, then end-to-side with 8–0 Prolene to the main RA (white arrow). The 2 RA were conjoined side-to side with 8–0 Prolene (black arrow).

TABLE 1 | Distributions of recipient and donor demograpghics and of recipient operative data.

Baseline variable	Mean ± SE if continuous (geometric mean ± SE for variables with skewed distributions); Percentage with characteristic if categorical
Recipient age (year)	47.2 ± 1.9 (N = 73)
—	(Median = 48.8, Range: 2.3–77.1)
Recipient age (year)	—
<18	6.8% (5/73)
≥18, <50	43.8% (32/73)
≥50	49.3% (36/73)
Recipient Gender	—
Female	32.9% (24/73)
Male	67.1% (49/73)
Recipient race/Ethnicity	—
Black (non-Hispanic)	12.3% (9/73)
Hispanic	42.5% (31/73)
White (non-Hispanic)	41.1% (30/73)
Other	4.1% (3/73)
Recipient BMI (kg/m^2)	26.3 ± 0.7 (*N* = 73)
	(Median = 26.0, Range: 16.0–42.4)
Recipient pretransplant diabetes mellitus	—
No	76.7% (56/73)
Yes	23.2% (17/73)
Retransplant	—
No	93.2% (68/73)
Yes	6.8% (5/73)
Donor type	—
Living related	57.5% (42/73)
Living unrelated	42.5% (31/73)
Kidney	—
Left	94.5% (69/73)
Right	5.5% (4/73)
Number of Donor arteries	—
2	83.6% (61/73)
3	16.4% (12/73)
JP drain placed	—
No	79.5% (58/73)
Yes	20.5% (15/73)
Double-J ureteral stent placed	—
No	95.9% (70/73)
Yes	4.1% (3/73)
Total Operative Time (min)	309.2 ± 8.1 (*N* = 73)
—	(Median = 296, Range: 206–483)
CIT (min)	77.8 ± 2.9 (*N* = 73)
—	(Median = 73, Range: 15–190)
WIT (min)	28.2 ± 0.6 (*N* = 73)
—	(Median = 27, Range: 20–42)
WIT single anastomosis (min)	28.1 ± 0.6 (*N* = 69)
—	(Median = 27, Range: 20–42)
WIT two anastomosis (min)	29.3 ± 2.8 (*N* = 4)
—	(Median = 31.5, Range: 21–33)
EBL (ml)	37.9 */1.09 (*N* = 73)
—	(Median = 40.0, Range: 10–300)

anastomoses techniques, transplantation of allografts with MRA is no longer considered to be a surgical restriction and has been shown to provide comparable post-operative and

TABLE 2 | Types of reconstruction.

2 RA (*N* = 61)	**N (%)**
None[a] (**Figure 4A** and one pediatric case not illustrated)	3 (4.9%)
Conjoined, side-to-side (**Figure 1A**)	43 (70.4%)
Accessory pole RA end-to-side to main RA (**Figures 2A, 2B**)	7 (11.5%)
Accessory pole RA end-to-side to branch of main RA (**Figures 2C, 2D**)	2 (3.3%)
Accessory RA end-to-side to branch of main RA inside the hilum (**Figure 2E**)	1 (1.5%)
UPRA end-to-end to Recipient IEA,[b]then either side-to-side or end-to-side to main RA (**Figures 5A, 5B**)	4 (6.6%)
2 conjoined RA end-to-end to a segment of Deceased Donor EIA[b] (**Figure 5D**)	1 (1.5%)
3 RA (*N* = 12)	
Accessory pole <1 mm ligated, 2 remaining RA conjoined side-to-side (**Figure 1A**)	1 (1.5%)
Conjoined, side-to-side-to-side (**Figure 1B**)	4 (36.4%)
2 RA conjoined, UPRA end-to-side to main RA (**Figure 3A**)	1 (8.3%)
LPRA and main RA conjoined, middle RA end-to-side to upper branch of main RA (**Figures 3B**, **6A**)	1 (8.3%)
2 RA conjoined side-to-side, UPRA end-to-side to branch of upper RA inside the hilum (**Figure 3C**)	1 (8.3%)
LPRA end-to-side to main RA, UPRA end-to-side to branch of RA inside the hilum (**Figures 3D**, **6B**)	1 (8.3%)
2 RA conjoined, and LPRA end-to-end to recipient IEA[a] (**Figure 4B**)	1 (8.3%)
2 main RA end-to-end to a segment of Recipient IIA, then UPRA end-to-side to one of the main RA (**Figure 5C**)	1 (8.3%)
UPRA end-to-end to a segment of Donor gonadal vein,[b] then end-to-side to 2 conjoined RA (**Figures 5E**, **6C**)	1 (8.3%)

[a]*Two separate anastomosis.*
[b]*Interposition grafting.*
Abbreviations: IEA = inferior epigastric artery; EIA = external iliac artery; UPRA= upper pole renal artery; LPRA = lower pole renal artery; IIA = internal iliac artery.

clinical outcomes to allografts with a single renal artery (5, 7, 8, 27, 28).

Several reconstruction techniques of MRA have been described in the literature with the common goal of minimizing ischemic insult and avoiding vascular complications. Transplantation of MRA in LDKT is often achieved by performing multiple arterial anastomoses without reconstruction. In a retrospective study by Hwang et al, sequential arterial anastomoses of MRA were performed in 81.1% of their cases with MRA; the remaining grafts with MRA were implanted with single anastomosis by either conjoining the renal arteries into a single lumen or ligating the accessory polar artery (29). Vaccarisi et al explained that in cases of MRA, they did not consider the opportunity to perform vascular reconstruction to unify the ostium, and all anastomoses were created separately in succession without kidney reperfusion (30). Popov et al mentioned that when dealing with two arteries of unequal size, it is preferable to anastomose them separately rather than to perform bench surgery, thereby decreasing the risk of compromising the lumen of the larger renal artery (31).

Although multiple anastomoses techniques like those described can provide good long-term outcomes, they are often associated with poor visibility and difficult suturing (14). We believe it is advantageous to create a single arterial lumen from MRA while in cold preservation, as it facilitates *in situ* vascular anastomosis and minimizes recipient warm ischemia time (WIT). Additionally, we prefer to revascularize simultaneously, because sequential revascularization requires added WIT and increases the risk of troublesome bleeding (14).

Prolonged WIT has been shown to have a detrimental effect on early graft function and long-term graft survival in LDKT (16–21). A study by Khan et al showed that WIT greater than 45 min was a risk factor for poor early graft function; they also reported that longer WIT was likely attributed to performance of multiple anastomoses in MRA donors (19). Similarly, Marzouk et al reported that an anastomosis time greater than 29 min was associated with an increased need for dialysis and length of stay, as well as slower recovery of kidney function (20). Additionally, Weissenbacher et al demonstrated that an anastomosis time greater than 30 min significantly affects long-term graft outcome and leads to inferior patient survival (21). In this current study, we describe in detail 18 different techniques for reconstruction of MRA in LDKT with the goal of minimizing both WIT and the risks associated with performing these complex anastomoses. Surgical loupes at 3.5× magnification were used for the reconstructions, which have been shown to increase the ease of performing anastomosis and yield better results in living-donor transplantation (32).

Of the reconstructions where a single renal artery lumen was created ($N = 69$), we report a median WIT of 27 min. In the four cases where vessels were implanted with two arterial anastomoses, the median WIT was 31.5 min. Our median WIT for creating a single inflow orifice is acceptable compared to the reported published literature (19–21, 33). We report no incidence of DGF nor vascular or urological complications in any of our patients during the first 12 months post-transplant.

Our main goal of the study was not reached in these four cases, because the accessory polar artery was located too far from the main renal artery to be safely reconstructed into a single lumen. Therefore, the accessory polar artery was anastomosed separately to other suitable vessels located a shorter distance away from it compared with the main renal artery. The use of interposition grafting to extend the length of the polar arteries (which we implemented in seven cases of short arteries) was not an option for achieving a single lumen in these specific cases, as it would have required too long of a graft, increasing the risk of complications. Nevertheless, the use of interposition grafting in LDKT has been shown to be a useful standard method for grafts with MRA (9, 10, 34). A study by Hiramitsu et al (10) describe the usefulness of arterial reconstruction using the recipient's own internal iliac artery for MRA grafts. They report no significant differences in complication incidence or perioperative and postoperative graft

TABLE 3 | Recipient outcomes.

Outcome variable	Mean ± SE if continuous (geometric mean ± SE for variables with skewed distributions); Percentage with characteristic if categorical
Length of hospital stay (days)	4.71 ± 1.06 (*N* = 73)
—	(Median = 4, Range: 3–67)
Developed delayed graft function (DGF)	—
No	100.0% (73/73)
Yes	0.0% (0/73)
Developed a post-operative complication (vascular, urological, or surgical) (within 12 months post-transplant)[a]	—
No	97.3% (71/73)
Yes	2.7% (2/73)
	—
Serum Cr at DOT (mg/dl)	6.9 ± 1.07 (*N* = 73)
—	(Median = 6.0, Range: 0.9–22.6)
Serum Cr at 3 months post-tx (mg/dl)	1.1 ± 1.04 (*N* = 67)
—	(Median = 1.1, Range: 0.25–2.0)
Serum Cr at 6 months post-tx (mg/dl)	1.1 ± 1.04 (*N* = 65)
—	(Median = 1.1, Range: 0.3–2.0)
Serum Cr at 12 months post-tx (mg/dl)	1.2 ± 1.05 (N = 60)
—	(Median = 1.1, Range: 0.3–4.9)
eGFR at 3 months post-tx (ml/min/1.73 m^2)	78.4 ± 3.4 (*N* = 67)
—	(Median = 76.8, Range: 34.8–234.5)
eGFR at 6 months post-tx (ml/min/1.73 m^2)	76.5 ± 3.3 (*N* = 65)
—	(Median = 74.2, Range: 38.2–217.2)
eGFR at 12 months post-tx (ml/min/1.73 m^2)	76.2 ± 3.7 (*N* = 60)
—	(Median = 70.9, Range: 15.6–216.5)
eGFR at 36 months post-tx (ml/min/1.73 m^2)	66.8 ± 4.2 (*N* = 30)
—	(Median = 66.6, Range: 12.0–114.0)
eGFR at 60 months post-tx (ml/min/1.73 m^2)	62.6 ± 6.2 (*N* = 18)
—	(Median = 67.5, Range: 6.2–107.7)
Graft failure, (i.e., return to permanent dialysis or retransplanted) (as of the Last follow-up date)[b]	—
No	98.6% (72/73)
Yes	1.4% (1/73)
Death with a functioning graft (as of the last follow-up date)[b]	—
No	93.2% (68/73)
Yes	6.8% (5/73)
Graft Loss (death uncensored) (as of the last follow-up date)[b]	—
No	91.8% (67/73)
Yes	8.2% (6/73)

[a]*Among the 2 patients who developed a post-operative complication during the first 12 months post-transplant, the following complications were observed: acute respiratory distress syndrome (ARDS) (N = 1), and* C. difficile *colitis/sepsis (*N = *1).*

[b]*The date of last follow-up for this study was 31 July 2021. Median follow-up among 67 patients who were alive with a functioning graft as of the last follow-up date was 30.4 (range: 0.3–151.2) months post-transplant. The single cause and time-to-graft failure (return to permanent dialysis) was as follows (listed chronologically by time to graft failure): Acute TCMR, at 41.8 months post-transplant. The 5 causes of death with a functioning graft and times-to-death were as follows: Cardiovascular Event in 2 patients (at 4.4- and 7.9-months post-transplant, respectively), Infection in 2 patients (death due to* C. difficile *colitis/sepsis in 1 patient at 0.8 months post-transplant, and death due to infection/sepsis in 1 patient at 125.2 months post-transplant), and Ruptured Aortic Aneurysm in 1 patient (at 5.2 months post-transplant).*

function of the interposition group at 60 months of follow-up compared to the conjoined group and the end-to-side method group. A few reports in the literature describe the use of donor gonadal vein as a conduit for renal arteries in LDKT with no vascular complications noted during short-term follow-up of these cases; however, long-term patency and safety remain unclear (9, 35–37). In our cohort, interposition grafting was performed in seven cases with various conduits such as recipient inferior epigastric artery, recipient internal iliac artery, deceased donor external iliac artery, and donor gonadal vein with no observed vascular or post-operative complications as of last follow-up.

When dealing with deceased donor kidney grafts with MRA, we also perform vascular reconstructions with the goal of creating a single arterial orifice in efforts to minimize ischemic insult. We commonly transplant MRA from deceased donors with the use of a Carrel aortic patch. If the renal arteries are located too far apart from the aorta and result in a case of long Carrel patch, we trim the Carrel patch and anastomosis it end-to-end to create a shorter carrel patch (38), or we perform a back-table vascular reconstruction into a single ostium for the same reasons as indicated in this manuscript.

Limitations of our study include the lack of comparison to outcomes for LDKT of single renal arteries. Additionally, sample sizes for certain subgroups of patients were relatively small, limiting our ability to show significant differences between the WIT of single and multiple arterial anastomoses. Another limitation of our study includes the fact that this was an

evaluation of consecutively transplanted living donor recipients performed at a single center by a single, highly experienced transplant surgeon. While the chances of achieving such successful anastomoses without post-operative complications being an issue requires a surgeon who is highly experienced in performing such techniques, these techniques can be easily duplicated and incorporated by other transplant surgeons to expand their surgical armamentarium.

CONCLUSION

Complex reconstruction techniques to create a single renal artery ostium for graft implantation anastomosis in allografts with MRA shows good clinical outcomes and acceptable WIT, with no increased post-operative vascular or urological complications. These techniques can be applied by other transplant surgeons when faced with vessel multiplicity to avoid potential complications associated with multiple arterial implantations.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Approved by the University of Miami Institutional Review Board.

AUTHOR CONTRIBUTIONS

MMT, GG, JR, PA, AA, RV, LC, MM, JJG, JG and GC contributed to conception and design of the study. MMT, JJG, GC organized the database. JJG and MMT performed the statistical analysis. MMT and GC wrote the first draft of the manuscript. MMT, JR, PA, MM, JJG, JG and GC wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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. Carter JT, Freise CE, McTaggart RA, Mahanty HD, Kang S-M, Chan SH, et al. Laparoscopic Procurement of Kidneys with Multiple Renal Arteries Is Associated with Increased Ureteral Complications in the Recipient. *Am J Transpl* (2005) 5(6):1312–8. doi:10.1111/j.1600-6143.2005.00859.x
2. Hevia V, Gómez V, Hevia M, Lorca J, Santiago M, López-Plaza J, et al. Troubleshooting Complex Vascular Cases in the Kidney Graft: Multiple Vessels, Aneurysms, and Injuries during Harvesting Procedures. *Curr Urol Rep* (2020) 21(1):5. doi:10.1007/s11934-020-0955-8
3. Chedid MF, Muthu C, Nyberg SL, Lesnick TG, Kremers WK, Prieto M, et al. Living Donor Kidney Transplantation Using Laparoscopically Procured Multiple Renal Artery Kidneys and Right Kidneys. *J Am Coll Surgeons* (2013) 217(1):144–52. discussion 152. doi:10.1016/j.jamcollsurg.2013.04.010
4. Fuller TF, Deger S, Büchler A, Roigas J, Schönberger B, Schnorr D, et al. Ureteral Complications in the Renal Transplant Recipient after Laparoscopic Living Donor Nephrectomy. *Eur Urol* (2006) 50(3):535–41. discussion 540-1. doi:10.1016/j.eururo.2006.03.025
5. Kadotani Y, Okamoto M, Akioka K, Ushigome H, Ogino S, Nobori S, et al. Management and Outcome of Living Kidney Grafts with Multiple Arteries. *Surg Today* (2005) 35(6):459–66. doi:10.1007/s00595-004-2967-2
6. Gawish AE, Donia F, Samhan M, Halim MA, Al-Mousawi M. Outcome of Renal Allografts with Multiple Arteries. *Transplant Proc* (2007) 39(4):1116–7. doi:10.1016/j.transproceed.2007.03.067
7. Saidi R. Living Donor Kidney Transplantation with Multiple Arteries. *Arch Surg* (2009) 144(5):472–5. doi:10.1001/archsurg.2009.49
8. Sevmis M, Demir ME, Merhametsiz O, Aktas S, Sevmis S, Uyar M. Grafts with Multiple Renal Arteries in Kidney Transplantation. *Transplant Proc* (2021) 53(3):933–40. doi:10.1016/j.transproceed.2020.07.019
9. Tomizawa M, Hori S, Nishimura N, Omori C, Nakai Y, Miyake M, et al. Arterial Reconstruction Using the Donor's Gonadal Vein in Living Renal Transplantation with Multiple Renal Arteries: a Case Report and a Literature Review. *BMC Nephrol* (2020) 21(1):190. doi:10.1186/s12882-020-01848-z
10. Hiramitsu T, Futamura K, Okada M, Yamamoto T, Tsujita M, Goto N, et al. Impact of Arterial Reconstruction with Recipient's Own Internal Iliac Artery for Multiple Graft Arteries on Living Donor Kidney Transplantation. *Medicine (Baltimore)* (2015) 94(43):e1811. doi:10.1097/md.0000000000001811
11. Dogan SM, Dogan G, Simsek C, Okut G, Berktas B, Simsek A, et al. Transplantation Using Renal Grafts with Multiple Renal Arteries: A Putative Study on the Impact of Arterial Reconstruction Technique and Site of Implantation on Outcomes. *Transplant Proc* (2021) 53(3):920–6. doi:10.1016/j.transproceed.2020.08.024
12. Yamanaga S, Rosario A, Fernandez D, Kobayashi T, Tavakol M, Stock PG, et al. Inferior Long-Term Graft Survival after End-To-Side Reconstruction for Two Renal Arteries in Living Donor Renal Transplantation. *PLoS One* (2018) 13(7):e0199629. doi:10.1371/journal.pone.0199629
13. Scheuermann U, Rademacher S, Wagner T, Lederer A, Hau HM, Seehofer D, et al. Influence of Multiple Donor Renal Arteries on the Outcome and Graft Survival in Deceased Donor Kidney Transplantation. *J Clin Med* (2021) 10(19):4395. doi:10.3390/jcm10194395
14. Makiyama K, Tanabe K, Ishida H, Tokumoto T, Shimmura H, Omoto K, et al. Successful Renovascular Reconstruction for Renal Allografts with Multiple Renal Arteries. *Transplantation* (2003) 75(6):828–32. doi:10.1097/01.tp.0000054461.57565.18
15. Ali-El-Dein B, Osman Y, Shokeir AA, Shehab El-dein AB, Sheashaa H, Ghoneim MA. Multiple Arteries in Live Donor Renal Transplantation: Surgical Aspects and Outcomes. *J Urol* (2003) 169(6):2013–7. doi:10.1097/01.ju.0000067637.83503.3e
16. Hellegering J, Visser J, Kloke HJ, D'Ancona FCH, Hoitsma AJ, van der Vliet JA, et al. Deleterious Influence of Prolonged Warm Ischemia in Living Donor Kidney Transplantation. *Transplant Proc* (2012) 44(5):1222–6. doi:10.1016/j.transproceed.2012.01.118
17. Brennan TV, Freise CE, Fuller TF, Bostrom A, Tomlanovich SJ, Feng S. Early Graft Function after Living Donor Kidney Transplantation Predicts Rejection but Not Outcomes. *Am J Transpl* (2004) 4(6):971–9. doi:10.1111/j.1600-6143.2004.00441.x
18. Nogueira JM, Haririan A, Jacobs SC, Weir MR, Hurley HA, Al-Qudah HS, et al. The Detrimental Effect of Poor Early Graft Function after Laparoscopic Live Donor Nephrectomy on Graft Outcomes. *Am J Transpl* (2009) 9(2):337–47. doi:10.1111/j.1600-6143.2008.02477.x

19. Khan TFT, Ahmad N, Serageldeen AS, Fourtounas K. Implantation Warm Ischemia Time in Kidney Transplant Recipients: Defining its Limits and Impact on Early Graft Function. *Ann Transpl* (2019) 24:432–8. doi:10.12659/aot.916012
20. Marzouk K, Lawen J, Alwayn I, Kiberd BA. The Impact of Vascular Anastomosis Time on Early Kidney Transplant Outcomes. *Transpl Res* (2013) 2(1):8. doi:10.1186/2047-1440-2-8
21. Weissenbacher A, Oberhuber R, Cardini B, Weiss S, Ulmer H, Bösmüller C, et al. The Faster the Better: Anastomosis Time Influences Patient Survival after Deceased Donor Kidney Transplantation. *Transpl Int* (2015) 28(5):535–43. doi:10.1111/tri.12516
22. Shirodkar SP, Sageshima J, Bird VG, Martínez JM, Chen L, Burke GW, et al. Living Donor Nephrectomy: University of Miami Technique and Current Results. *Arch Esp Urol* (2010) 63(3):163–70. doi:10.4321/s0004-06142010000300001
23. Ciancio G, Farag A, Gonzalez J, Vincenzi P, Gaynor JJ. Results of a Previously Unreported Extravesical Ureteroneocystostomy Technique without Ureteral Stenting in 500 Consecutive Kidney Transplant Recipients. *PLoS One* (2021) 16(1):e0244248. doi:10.1371/journal.pone.0244248
24. Ciancio G, Gaynor JJ, Guerra G, Sageshima J, Roth D, Chen L, et al. Randomized Trial of rATg/Daclizumab vs. rATg/Alemtuzumab as Dual Induction Therapy in Renal Transplantation: Results at 8years of Follow-Up. *Transpl Immunol* (2017) 40:42–50. doi:10.1016/j.trim.2016.11.004
25. Lee L-Y, Pham TA, Melcher ML. Living Kidney Donation: Strategies to Increase the Donor Pool. *Surg Clin North America* (2019) 99(1):37–47. doi:10.1016/j.suc.2018.09.003
26. Paragi PR, Klaassen Z, Stephen Fletcher H, Tichauer M, Chamberlain RS, Wellen JR, et al. Vascular Constraints in Laparoscopic Renal Allograft: Comparative Analysis of Multiple and Single Renal Arteries in 976 Laparoscopic Donor Nephrectomies. *World J Surg* (2011) 35(9):2159–66. doi:10.1007/s00268-011-1168-6
27. Chabchoub K, Mhiri MN, Bahloul A, Fakhfakh S, Ben Hmida I, Hadj Slimen M, et al. Does Kidney Transplantation with Multiple Arteries Affect Graft Survival? *Transplant Proc* (2011) 43(9):3423–5. doi:10.1016/j.transproceed.2011.09.027
28. Ghazanfar A, Tavakoli A, Zaki MR, Pararajasingam R, Campbell T, Parrott NR, et al. The Outcomes of Living Donor Renal Transplants with Multiple Renal Arteries: a Large Cohort Study with a Mean Follow-Up Period of 10 Years. *Transplant Proc* (2010) 42(5):1654–8. doi:10.1016/j.transproceed.2009.12.067
29. Hwang JK, Kim SD, Park SC, Choi BS, Kim JI, Yang CW, et al. The Long-Term Outcomes of Transplantation of Kidneys with Multiple Renal Arteries. *Transplant Proc* (2010) 42(10):4053–7. doi:10.1016/j.transproceed.2010.09.075
30. Vaccarisi S, Bonaiuto E, Spadafora N, Garrini A, Crocco V, Cannistrà M, et al. Complications and Graft Survival in Kidney Transplants with Vascular Variants: Our Experience and Literature Review. *Transplant Proc* (2013) 45(7):2663–5. doi:10.1016/j.transproceed.2013.07.007
31. Popov Z, Stankov O, Stavridis S, Saidi S, Ivanovski O, Spasovski G, et al. Management of Multiple Renal Arteries and Unusual Venous Anatomy during Kidney Transplant: From a Simple Technical Problem to a Graft-Saving Procedure. *Exp Clin Transpl* (2020) 18(7):763–70. doi:10.6002/ect.2019.0314
32. Li P-C, Thorat A, Jeng L-B, Yang H-R, Li M-L, Yeh C-C, et al. Hepatic Artery Reconstruction in Living Donor Liver Transplantation Using Surgical Loupes: Achieving Low Rate of Hepatic Arterial Thrombosis in 741 Consecutive Recipients-Tips and Tricks to Overcome the Poor Hepatic Arterial Flow. *Liver Transpl* (2017) 23(7):887–98. doi:10.1002/lt.24775
33. Heylen L, Pirenne J, Samuel U, Tieken I, Naesens M, Sprangers B, et al. The Impact of Anastomosis Time during Kidney Transplantation on Graft Loss: A Eurotransplant Cohort Study. *Am J Transpl* (2017) 17(3):724–32. doi:10.1111/ajt.14031
34. El-Sherbiny M, Abou-Elela A, Morsy A, Salah M, Foda A. The Use of the Inferior Epigastric Artery for Accessory Lower Polar Artery Revascularization in Live Donor Renal Transplantation. *Int Urol Nephrol* (2008) 40(2):283–7. doi:10.1007/s11255-007-9257-z
35. He B, Mitchell A. A Novel Technique for Reconstruction of Multiple Renal Arteries in Live Donor Kidney Transplantation: A Case Report and Literature Review. *Transplant Proc* (2012) 44(10):3055–8. doi:10.1016/j.transproceed.2012.03.054
36. Veeramani M, Jain V, Ganpule A, Sabnis RB, Desai MR. Donor Gonadal Vein Reconstruction for Extension of the Transected Renal Vessels in Living Renal Transplantation. *Indian J Urol* (2010) 26(2):314–6. doi:10.4103/0970-1591.65417
37. Uysal E, Yuzbasioglu MF, Ikidag MA, Dokur M, Gurer OA. Successful Elongation of a Short Graft Renal Artery by a Gonadal Vein. *Exp Clin Transpl* (2017) 15(4):467–9. doi:10.6002/ect.2015.0002
38. Barlas A, Moris D, Zavos G. Shortening a Carrel Patch in a Graft with Multiple Arteries: A Step-by-step Technical Analysis. *Exp Clin Transpl* (2016) 14(6):682–4. doi:10.6002/ect.2014.0202

ESOT | Transplant International

ORIGINAL RESEARCH
published: 12 April 2022
doi: 10.3389/ti.2022.10198

Hearing Impairments as an Overlooked Condition in Kidney Transplant Recipients

*Melis Simsir[1], Muhammed Gazi Yildiz[2], Murat Karatas[3], Abdullah Dalgic[4], Ilyas Ozturk[5], Erhan Tatar[6], Necmi Eren[7], Ertugrul Erken[5], Ozkan Gungor[5] and Orcun Altunoren[5]**

[1]Department of Internal Medicine, Faculty of Medicine, Kahramanmaras Sutcu Imam University, Kahramanmaras, Turkey, [2]Department of Otolaryngology Head and Neck Surgery, Faculty of Medicine, Kahramanmaras Sutcu Imam University, Kahramanmaras, Turkey, [3]Department of General Surgery, Izmir Bozyaka Education and Research Hospital, Izmir, Turkey, [4]Department of Otolaryngology Head and Neck Surgery, Izmir Bozyaka Education and Research Hospital, Izmir, Turkey, [5]Department of Nephrology, Faculty of Medicine, Kahramanmaras Sutcu Imam University, Kahramanmaras, Turkey, [6]Department of Nephrology, Izmir Bozyaka Education and Research Hospital, Izmir, Turkey, [7]Department of Nephrology, Faculty of Medicine, Kocaeli University, Kocaeli, Turkey*

It is not known whether hearing disorders improves with kidney transplantation. One of the neurotoxic effects of immunosuppressive drugs may be unrecognized hearing loss. In this study, our aim was to evaluate the hearing disorders in kidney transplant patients. Hearing problems in 46 kidney transplant patients [eGFR ≥ 60 ml/min/1.73 m^2 (30 Tacrolimus, 16 mTOR inhibitor users)], 23 hemodialysis patients, and 20 healthy controls were evaluated with a questionnaire and high-frequency audiometry. More than half (58.7%) of the transplant patients had at least one hearing problem. Hearing loss was observed in 50%, 60.9% and 76.1% of the transplant patients at 8,000, 16,000 and 20,000 Hz. Hearing thresholds of transplant and hemodialysis patients increased from 4,000 to 20,000 Hz and was higher than that of controls. Hearing thresholds were higher at 1,000–2,000 Hz in patients using tacrolimus and at 16,000–20,000 Hz in patients using mTOR inhibitor. No correlation was found between hearing threshold and blood tacrolimus or mTOR inhibitor levels. Most kidney transplant and hemodialysis patients have hearing loss at higher frequencies than medium frequencies. Hearing loss in chronic kidney patients is likely to be permanent and kidney transplantation may not improve hearing problems. Hearing problems may be more pronounced at medium frequencies in patients receiving tacrolimus but at higher frequencies in patients receiving mTOR inhibitors.

Keywords: kidney transplantation, extended high-frequency audiometry, hearing impairment, hemodialysis, immunsuppressants

OPEN ACCESS

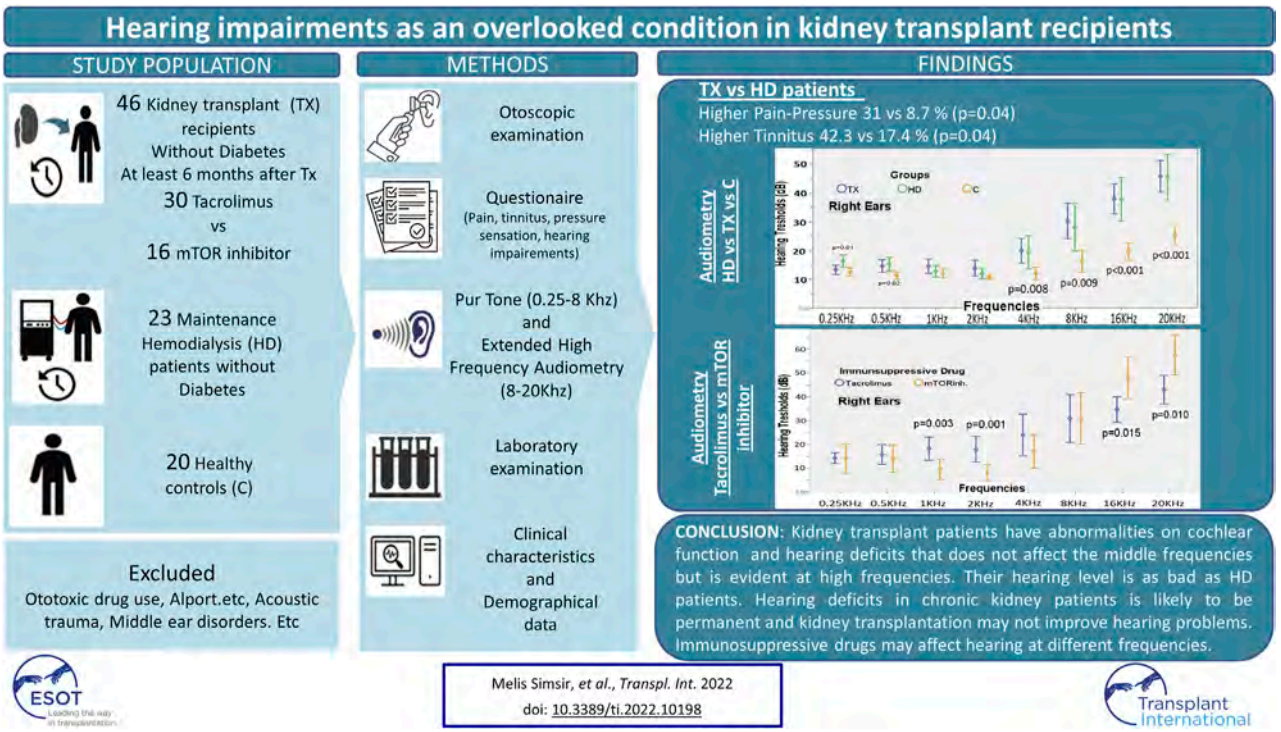
***Correspondence:**
Orcun Altunoren
orcunaltunoren@hotmail.com

***Received:** 07 November 2021*
***Accepted:** 17 March 2022*
***Published:** 12 April 2022*

Citation:
Simsir M, Yildiz MG, Karatas M, Dalgic A, Ozturk I, Tatar E, Eren N, Erken E, Gungor O and Altunoren O (2022) Hearing Impairments as an Overlooked Condition in Kidney Transplant Recipients. Transpl Int 35:10198. doi: 10.3389/ti.2022.10198

INTRODUCTION

Kidney transplantation is the most preferred treatment method for end-stage renal disease. According to the World Health Organization data, in 2019, 100,097 kidney transplants were performed all over the world (1). Kidney transplant patients take lifelong immunosuppressive drugs, which have many side effects. Currently, many transplant centres use tacrolimus as a calcineurin inhibitor (CNI) in their immunosuppressive regimen while mTOR inhibitors are used much less frequently (2,3). Calcineurin inhibitors generally have a similar side effect profile, with the most important one being neurotoxicity (4). Tacrolimus is slightly more neurotoxic



GRAPHICAL ABSTRACT |

than Cyclosporine (CsA) (4,5). Although neurotoxicity is most commonly seen in the form of tremors, more serious conditions such as epileptic seizures and confusion may also occur (6).

We have observed that some kidney transplant patients, albeit very few, experience hearing problems after transplantation. Advanced age, diabetes, ototoxic drug use, and uremia can partially explain this situation; it is possible that the immunosuppressive drugs used, especially tacrolimus, may also have ototoxic effects. It is accepted that mTOR inhibitors do not have neurotoxicity (7-9). There are only a few studies in the literature investigating hearing problems in kidney transplant patients. Moreover, there are no studies showing whether there is a relationship between the type of immunosuppressive drug used and hearing problems. Hearing tests are usually conducted in the 125–8000 Hz. The 9000–20000 Hz range is called Extended High-Frequency Audiometry (EHFA), and it is important tool in detecting hearing loss that starts at high frequencies and progresses to low frequencies, due to reasons such as aging and toxic causes (10).

In this study, our primary aim was to determine any hearing problems in kidney transplant patients in kidney transplant patients using questionnaire and EHFA. Our second goal was to determine whether there was a relationship between the hearing problem, if any, and the type of immunosuppressive drug used.

PATIENTS AND METHODS

This cross-sectional case-control study was conducted at the Department of Nephrology and Department of Ear-Nose, and Throat Clinics of Kahramanmaras Sutcu Imam University Medical Faculty Hospital, and Izmir Bozyaka Training and Research Hospital. A total of 89 patients; 46 kidney transplant recipients (TX group), 23 hemodialysis patients (HD group) and 20 healthy controls (C group) were included in the study. Ethical approval was obtained from the local ethics committee of Kahramanmaras Sutcu Imam University (date: September 09, 2020, session no.2020/17, decision no.18). Written informed consent was obtained from all patients.

Inclusion Criteria

Nondiabetic kidney transplant recipients with an estimated glomerular filtration rate (eGFR) > 60 ml/min/1.73 m^2 (calculated with the MDRD formula), between the ages of 18–50 years, that have passed at least 6 months after kidney transplant, and have been using tacrolimus or an mTOR inhibitor (Everolimus) in their immunosuppressive regimen were included in the TX group. Nondiabetic patients aged 18–50 years and that have received maintenance hemodialysis treatment three sessions/week for at least 6 months were included in the HD group. Healthy subjects who matched with kidney transplant patients for age and gender distribution were included in the control group.

Exclusion Criteria

Patients under the age of 18 and over 50, were diagnosed with Alport syndrome, had a known or newly developed diabetes mellitus (DM), have used ototoxic drugs within the last 3 months (furosemide, torsemide, aminoglycoside antibiotics,

TABLE 1 | Hearing problems in the HD and TX patients according to the survey results.

	TX *n* = 46	HD *n* = 23	*p*
Pain-Pressure sensation %	31.1	8.7	0.04
Tinnitus %	41.3	17.4	0.047
Dizziness %	19.6	26.1	0.53
Hearing loss before transplantation %	6.5	--	--
Hearing loss after transplantation %	10.9	--	--

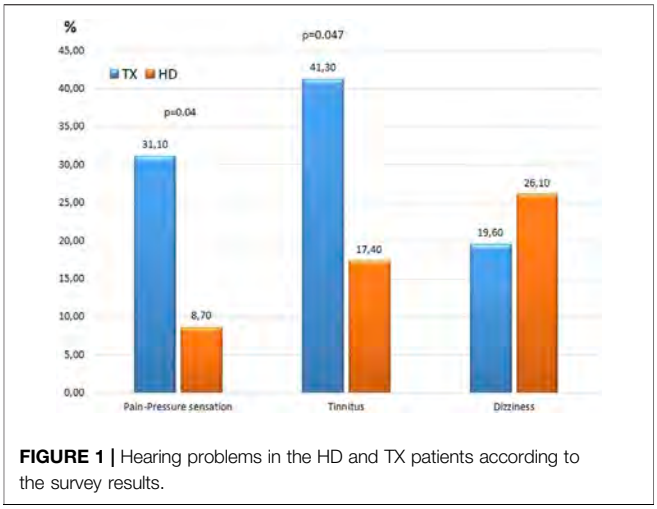


FIGURE 1 | Hearing problems in the HD and TX patients according to the survey results.

erythromycin, vancomycin, etc.), had a history of hereditary or acquired hearing loss problems due to several reasons (acoustic trauma, genetic syndromes with hearing loss, neurological-psychiatric problems, those with recurrent upper respiratory tract infection, tympanic membrane and middle ear pathology in otoscopic examination, Meniere's disease, Cogan Syndrome, Costen Syndrome, etc.), have had ear trauma or surgery, have intracranial pathology that may cause hearing loss, have malignancy and been receiving chemotherapy, and those in whom the time elapsed since the start of dialysis or after kidney transplant was less than 6 months, HD patients whose Kt/V value was less than 1.2 within the last 3 months, kidney transplant patients with eGFR < 60 ml/min/1.73 m^2, kidney transplant patients using regimens that do not contain tacrolimus or mTOR inhibitors were not included in the study.

Obtaining of Demographic and Laboratory Data

Data such as patients' age, gender, presence of comorbidity (hypertension or coronary artery disease,etc), the etiology of chronic kidney disease (CKD), the number of years they have been receiving HD treatment, duration of RRT (renal replacement therapy) before transplantation, the time elapsed after kidney transplant, and the immunosuppressive drugs used were obtained from their medical records. Systolic and diastolic blood pressures (SBP and DBP), height and weight were measured before the midweek dialysis session for HD patients and during the examination for TX and C groups. Body mass index (BMI) was calculated by dividing body weight in kilograms divided by square of height in meters.

Fasting blood glucose (FBG), blood urea nitrogen (BUN), creatinine (Cr), sodium (Na), potassium (K), calcium (Ca), phosphorus (P), LDL cholesterol, triglyceride (TG), uric acid (UA), serum albumin levels were measured with the standard methods. A blood sample of the HD patients was taken just before the midweek dialysis session. The patients' Kt/V values and urea reduction ratios (URR) were retrieved from their medical records, and the arithmetic average of the last 3 months was calculated. The blood of TX patients was taken during outpatient control and tacrolimus or mTOR inhibitor blood levels (C0) were measured.

Audiometric Measurements and Ear Examination

Audiometric measurements and the examinations of the external and middle ear and the throat were performed by a single otolaryngology specialist in each centre. Lavage and aspiration were performed on patients who required plug aspiration. All audiological evaluations were performed in a standard double-wall, soundproof booth (IAC Acoustics, Naperville, IL, United States). Airway hearing tresholds at 250, 500, 1,000, 2,000, 4,000 and 8,000 Hz frequencies using the Telephonics TDH 39P headphones (Telephonics Corp., Farmingdale, NY, United States) with the Interacoustics AC-40 audiometer (Interacoustics, Middelfart, Denmark), bone conduction hearing thresholds at the 500, 1,000, 2,000 and 4,000 Hz frequencies using the Rodioear B-71 bone transducer (RadioEar, Middelfart, Denmark), and high frequency airway hearing thresholds (>8,000 Hz) using Harward HR H903 headphones were determined. The mean and standard deviation of air and bone conduction thresholds were calculated for each frequency for all patients. The actual hearing levels were determined by masking in patients with a hearing level difference of more than 40 dB between both ears and an air-bone conduction difference of >10 dB. Apart from audiometric examinations, immitance measurements were made using Interacoustics AZ26 and AT235h clinical tympanometry devices. Middle ear pressur and ipsilateral and contralateral acoustic reflex thresholds of all participants were evaluated. In addition, Speech Reception Treshold (SRT), Speech Discrimination (SD) tests were performed on all patients.

Survey Data on Hearing

To define hearing problems, TX and HD patients were asked the following survey questions appropriate to the patient's group.

1. Did you have a hearing problem before the transplant?
2. Did your hearing decrease after the transplant?
3. Do you feel the need for a hearing aid?
4. Do you have any ear pain or a feeling of pressure in the ear?
5. Do you have ringing in the ear (Tinnitus)?
6. Do you have dizziness?
7. Did you experience sudden hearing loss after the transplant?

TABLE 2 | Comparison of demographic, laboratory and audiometric results of the TX, HD and C groups.

Kruskal Wallis Analysis		Tx *n* = 46	HD *n* = 23	C *n* = 20	*p*
		Mean ± SD	Mean ± SD	Mean ± SD	
Age (Year)		36.6 ± 11.9	35.4 ± 8.7	33.0 ± 10.0	0.13
Gender (Male %)		65.2	60.9	70.0	0.82
HT (%)		67.4	56.5	0	0.43
BMI (kg/m^2)		25.5 ± 4.7	23.5 ± 6.2	25.2 ± 5.0	0.13
SBP (mmHg)		126.0 ± 14.4	131.8 ± 21.6	114.2 ± 8.4[a]	<0.001
DBP (mmHg)		78.9 ± 10.2	79.3 ± 17.2	75.7 ± 7.1	0.33
BUN (mg/dl)		16.7 ± 7.8[b]	49.8 ± 24.8[b]	12.0 ± 2.9[b]	<0.001
sCr (mg/dl)		1.15 ± 0.26[b]	8.60 ± 3.49[b]	0.81 ± 0.13[b]	<0.001
eGFR ($ml/dk/1.73m^2$)		76.3 ± 16.6	-	112.2 ± 18.0	<0.001
FBG (mg/dl)		87.7 ± 10.6	90.7 ± 15.9	92.5 ± 12.1	0.29
Na (mEq/L)		139.9 ± 2.19	137.3 ± 1.91[a]	140.0 ± 2.59	<0.001
K (mEq/L)		4.35 ± 0.44	5.35 ± 0.82[a]	4.64 ± 0.67	<0.001
Ca (mg/dl)		9.70 ± 0.52	8.36 ± 0.88[a]	9.43 ± 0.33	<0.001
P (mg/dl)		3.16 ± 0.60	5.41 ± 0.94[a]	3.57 ± 0.50	<0.001
TG (mg/dl)		164.9 ± 71.0	166.9 ± 89.9	138.6 ± 72.8	0.33
LDL cholesterol (mg/dl)		118.3 ± 36.3	94.7 ± 28.7[a]	126.3 ± 39.8	0.01
UA (mg/dl)		5.9 ± 1.3	6.2 ± 1.1	5.2 ± 1.7	0.15
Albumin (gr/L)		43.3 ± 3.8[b]	37.2 ± 5.3[b]	47.6 ± 3.4[b]	<0.001
Hemoglobin (gr/dl)		14.1 ± 1.8	10.6 ± 1.4[a]	14.9 ± 1.8	<0.001
Odiometric data					
Right Ear	250 Hz (dB)	13.26 ± 5.49	16.30 ± 4.81[a]	12.50 ± 3.03	0.01
	500 Hz (dB)	14.56 ± 7.28	15.21 ± 5.53	11.25 ± 2.75[a]	0.02
	1,000 Hz (dB)	14.56 ± 8.42	12.82 ± 4.72	12.00 ± 3.40	0.60
	2,000 Hz (dB)	13.80 ± 9.32	11.95 ± 4.19	10.75 ± 1.83	0.69
	4,000 Hz (dB)	19.89 ± 14.43	19.34 ± 13.34	12.00 ± 4.70[a]	0.008
	8,000 Hz (dB)	30.21 ± 20.65	28.04 ± 19.05	16.25 ± 8.09[a]	0.009
	16,000 Hz (dB)	37.93 ± 17.49	37.82 ± 17.56	19.50 ± 6.26[a]	<0.001
	20,000 Hz (dB)	45.76 ± 18.70	45.43 ± 18.45	25.25 ± 6.78[a]	<0.001
Left Ear	250 Hz (dB)	13.04 ± 7.78	16.08 ± 6.56[a]	11.75 ± 3.72	0.02
	500 Hz (dB)	14.02 ± 8.07	15.86 ± 5.14	11.50 ± 2.85[a]	0.007
	1,000 Hz (dB)	14.13 ± 10.50	14.34 ± 5.70	11.50 ± 3.66	0.16
	2,000 Hz (dB)	14.02 ± 11.62	13.69 ± 4.81	11.00 ± 3.07	0.18
	4,000 Hz (dB)	20.43 ± 16.22	23.47 ± 18.79	12.50 ± 5.96[a]	0.007
	8,000 Hz (dB)	30.21 ± 20.24	30.86 ± 25.87	15.00 ± 6.48[a]	0.002
	16,000 Hz (dB)	39.56 ± 19.34	43.26 ± 23.81	23.50 ± 7.27[a]	0.002
	20,000 Hz (dB)	44.02 ± 19.79	48.26 ± 14.05	28.25 ± 6.34[a]	0.002
Right Ear SRT (dB)		14.13 ± 5.50	13.47 ± 4.37	11.25 ± 2.75[a]	0.04
Left Ear SRT (dB)		14.02 ± 7.19	14.34 ± 4.07	11.00 ± 2.05[a]	0.01
Right Ear SD (%)		95.28 ± 9.31	95.47 ± 3.90	99.00 ± 1.77[a]	0.005
Left Ear SD (%)		95.41 ± 9.24	95.47 ± 3.90	98.80 ± 2.28a	0.009

[a]*It represents the group whose value is different from the other two groups.*
[b]*It states that the values of each three groups are different from each other.*

8. Have you had ear surgery?
9. Have You had an Ear/Head Trauma?

Statistical Analysis

The power analysis of the study was performed with the G*Power 3.1.9.7 for Windows (11) software, and it was predicted that with the inclusion of 20 patients in each group, the alpha error would be 0.005 and the power of the study would be 99%. The SPSS v16.0 was used for statistical analysis. Data obtained by measurement were expressed as mean ± SD, and categorical data obtained by counting were expressed as percentages or ratios. The distribution characteristics of the data were evaluated with the Shapiro Wilk test. The Kruskal Wallis analysis of variance was used to compare the data obtained by measurements in the TX, HD and C groups. A p value of less than 0.05 was considered statistically significant. Bonferonni correction was applied to evaluate which group caused the difference; the groups were compared in pairs, and a p value of less than 0.016 was considered significant. The categorical variables were evaluated with the Chi-square test. The Mann-Whitney U and chi-square test were used to compare patients using tacrolimus or an mTOR inhibitor according to data type. A p value of less than 0.05 was considered significant.

RESULTS

Survey Results for Hearing Problems

More than half (58.7%) of the TX patients had evolved at least one hearing problem. A great majority (93.5%) said that they

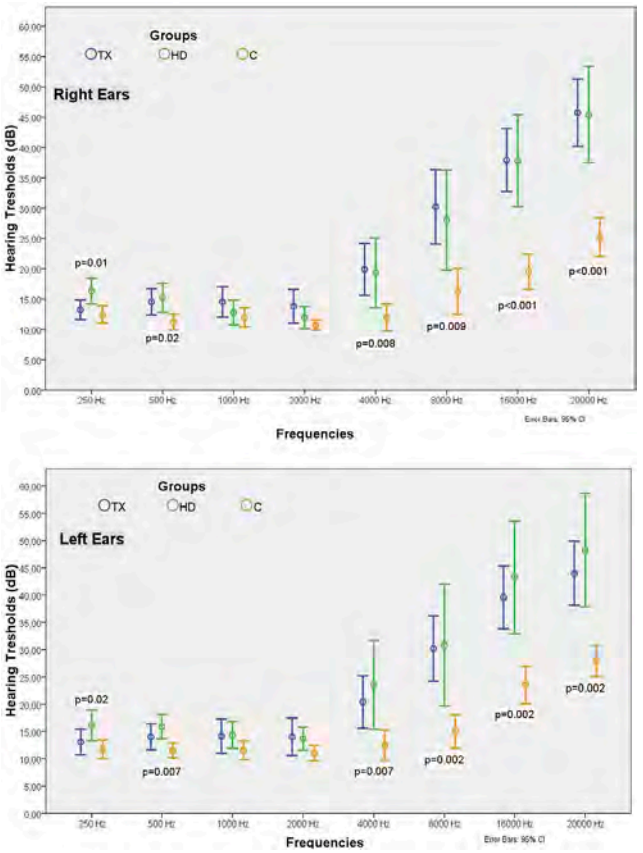


FIGURE 2 | Comparison of hearing tresholds of the TX, HD and C groups in different frequencies.

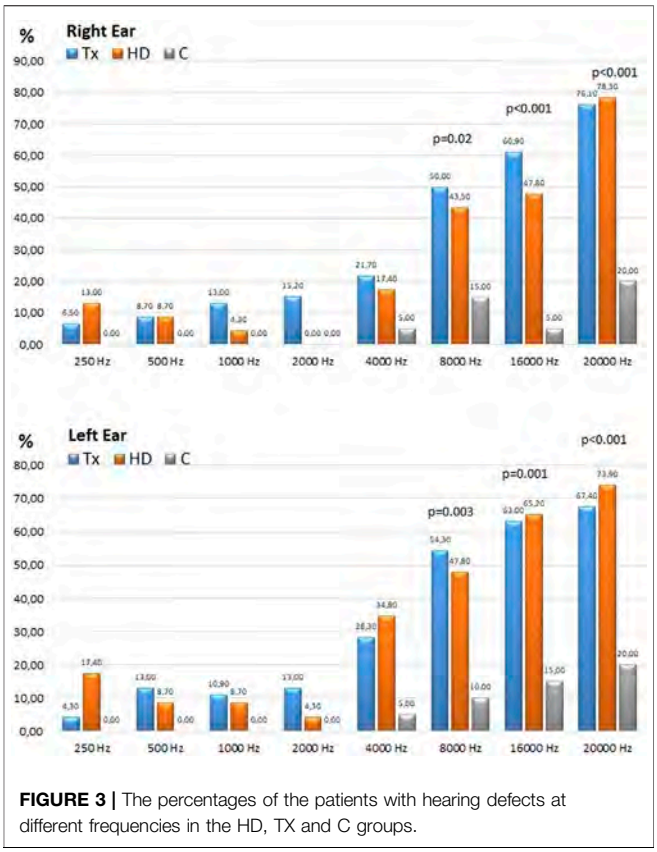


FIGURE 3 | The percentages of the patients with hearing defects at different frequencies in the HD, TX and C groups.

had no hearing problems before the transplantation. Among the TX patients, 31.1% had pressure sensation in the ear, 41.3% had tinnitus, and 19.6% had dizziness. While 97.8% of the patients said they did not need help for hearing, only 2.2% stated otherwise. None of the patients had sudden hearing loss after kidney transplantation. On the other hand, 8.7% of HD patients had pressure sensation in the ear, 17.4% had tinnitus and 26.1% had dizziness. Ear ache-pressure sensation and

TABLE 3 | The percentages of the patients with hearing defects at different frequencies in HD, TX and C groups.

	Hearing impairement treshold (dB)	Frequency (Hz)	Tx *n* = 46 (%)	HD *n* = 20 (%)	C *n* = 20 (%)	*p*
Right Ear	20	250	6.5	13	0	0.23
		500	8.7	8.7	0	0.39
		1,000	13	4.3	0	0.14
		2,000	15.2	0	0	0.03
		4,000	21.7	17.4	5	0.24
		8,000	50	43.5	15[a]	0.02
	30	16,000	60.9	47.8	5[a]	<0.001
		20,000	76.1	78.3	20[a]	<0.001
Left Ear	20	250	4.3	17.4	0	0.05
		500	13	8.7	0	0.23
		1,000	10.9	8.7	0	0.31
		2,000	13	4.3	0	0.14
		4,000	28.3	34.8	5	0.057
		8,000	54.3	47.8	10[a]	0.003
	30	16,000	63	65.2	15[a]	0.001
		20,000	67.4	73.9	20[a]	<0.001

[a]*It represents the group whose value is different from the other two groups.*

TABLE 4 | Comparison of the demographic, laboratory and audiometric findings of the TX patients using tacrolimus or an mTOR inhibitor.

Mann Whitney U		Non matched			Matched for age, eGFR and post Tx time		
		Tacrolimus *n* = 30	mTOR *n* = 16	*p*	Tacrolimus *n* = 20	mTOR *n* = 10	*p*
		Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD	
Age (Year)		33.70 ± 9.14	47.81 ± 11.47	<0.001	37.45 ± 8.01	41.6 ± 8.9	0.35
Gender (Male %)		63.3	68.8	0.71	65.0	70.0	1.00
PostTX time (months)		62.93 ± 35.14	98.50 ± 51.16	0.02	67.00 ± 32.37	93.50 ± 46.79	0.14
HT (%)		73.3	56.2	0.32	85.0	60.0	0.18
Pre TX RRT time		26.86 ± 51.57	55.06 ± 92.54	0.17	30.05 ± 60.37	72.20 ± 113.79	0.16
BMI (kg/m^2)		25.95 ± 4.99	24.71 ± 4.15	0.41	26.18 ± 4.39	23.95 ± 4.28	0.18
SBP(mmHg)		125.13 ± 15.16	127.87 ± 13.19	0.42	126 ± 16.93	126.81 ± 13.25	0.57
DBP(mmHg)		75.30 ± 8.87	85.87 ± 9.30	0.002	74.65 ± 9.64	86.70 ± 11.32	0.02
BUN (mg/dl)		13.10 ± 3.71	23.68 ± 9.05	<0.001	13.65 ± 3.92	23.60 ± 9.00	0.002
sCr (mg/dl)		1.07 ± 0.22	1.29 ± 0.27	0.012	1.12 ± 0.21	1.22 ± 0.28	0.28
eGFR ml/min/1.73m^2		79.96 ± 16.50	62.98 ± 20.20	0.001	72.35 ± 10.42	72.80 ± 18.63	0.27
FBG (mg/dl)		89.13 ± 11.58	85.12 ± 8.26	0.35	92.75 ± 11.76	85.50 ± 8.97	0.23
Na (mEq/L)		139.50 ± 1.83	140.66 ± 2.63	0.16	139.60 ± 1.60	140.07 ± 2.09	0.94
K (mEq/L)		4.36 ± 0.41	4.33 ± 0.50	0.90	4.36 ± 0.43	4.33 ± 0.41	0.96
Ca (mg/dl)		9.71 ± 0.46	9.68 ± 0.64	0.77	9.75 ± 0.44	9.80 ± 0.56	0.67
P (mg/dl)		3.17 ± 0.65	3.15 ± 0.51	0.65	3.04 ± 0.65	3.25 ± 0.62	0.58
TG (mg/dl)		166.00 ± 72.98	163.06 ± 69.68	0.86	172.70 ± 67.68	157.40 ± 74.55	0.45
LDL cholesterol (mg/dl)		105.13 ± 27.53	148.69 ± 36.79	<0.001	107.70 ± 27.04	147.77 ± 31.20	0.006
UA (mg/dl)		5.74 ± 1.19	6.38 ± 1.42	0.15	5.93 ± 1.16	6.57 ± 1.65	0.27
Albumin (gr/L)		44.62 ± 3.42	40.67 ± 3.24	0.001	44.59 ± 3.81	41.11 ± 3.50	0.017
Hemoglobin (gr/dl)		14.56 ± 1.95	13.48 ± 1.60	0.07	14.64 ± 2.00	13.44 ± 1.84	0.16
Tacrolimus C0 levels (mcg/L)		5.28	-	-	5.35 ± 1.77	-	-
Everolimus C0 levels (mcg/L)		-	3.49	-	-	3.94 ± 1.68	-
Odiometric data							
Right Ear	250Hz (dB)	13.33 ± 4.22	13.12 ± 7.50	0.72	14.25 ± 4.66	14.00 ± 8.43	0.71
	500Hz (dB)	14.83 ± 7.59	14.06 ± 6.88	0.98	15.75 ± 8.92	14.00 ± 8.09	0.76
	1000 Hz (dB)	16.33 ± 9.27	11.25 ± 5.32	0.046	18.25 ± 10.42	9.50 ± 5.98	0.003
	2000 Hz (dB)	15.50 ± 10.03	10.62 ± 7.04	0.016	18.00 ± 11.51	8.00 ± 4.83	0.001
	4000 Hz (dB)	20.33 ± 16.18	19.06 ± 10.83	0.81	24.00 ± 18.75	17.00 ± 9.77	0.39
	8000 Hz (dB)	27.83 ± 21.64	34.68 ± 18.48	0.11	31.00 ± 21.61	31.00 ± 15.23	0.62
	16000 Hz (dB)	32.00 ± 16.64	49.06 ± 13.44	0.001	34.75 ± 11.52	48.00 ± 12.29	0.015
	20000 Hz (dB)	39.00 ± 17.97	58.43 ± 12.74	<0.001	43.00 ± 12.60	57.50 ± 11.84	0.010
Left Ear	250Hz (dB)	12.66 ± 3.88	13.75 ± 12.31	0.37	13.25 ± 4.37	14.00 ± 15.23	0.14
	500Hz (dB)	13.83 ± 6.52	14.37 ± 10.62	0.95	14.50 ± 7.23	15.00 ± 13.12	0.56
	1000 Hz (dB)	15.83 ± 10.51	10.93 ± 10.03	0.004	17.75 ± 12.29	11.50 ± 12.25	0.017
	2000 Hz (dB)	16.00 ± 12.27	10.31 ± 9.56	0.004	18.25 ± 14.44	10.00 ± 11.54	0.004
	4000 Hz (dB)	20.66 ± 16.28	20.00 ± 16.63	0.68	23.75 ± 18.97	20.00 ± 17.75	0.44
	8000 Hz (dB)	28.66 ± 21.12	33.12 ± 18.78	0.26	32.25 ± 20.55	33.00 ± 18.73	0.61
	16000 Hz (dB)	32.83 ± 18.78	52.18 ± 13.41	<0.001	36.50 ± 16.47	52.00 ± 9.48	0.001
	20000 Hz (dB)	36.83 ± 18.40	57.50 ± 14.94	<0.001	40.25 ± 16.01	57.50 ± 10.60	0.001
Right Ear SRT (dB)		14.50 ± 5.92	13.43 ± 4.73	0.73	15.50 ± 6.66	13.00 ± 5.37	0.29
Left Ear SRT (dB)		13.83 ± 5.20	14.37 ± 10.14	0.50	14.50 ± 5.82	14.50 ± 12.79	0.13
Right Ear SD (%)		93.66 ± 11.06	98.31 ± 2.91	0.026	91.90 ± 13.11	98.10 ± 3.41	0.04
Left Ear SD (%)		93.80 ± 11.02	98.43 ± 2.55	0.022	92.10 ± 13.08	98.30 ± 2.90	0.03

tinnitus complaints were more common among the TX patients than HD patients (**Table 1**; **Figure 1**).

Laboratory Findings, Demographic and Audiometric Data

Chronic kidney disease etiologies observed in HD patients were hypertension (HT) in 21.7%, chronic glomerulonephritis (CGN) in 13.3%, unknown/other causes in 65%. In the TX group, 26.1% had HT, 6.5% had polycystic kidney disease (PKD), 10.9% had CGN, and 56.5% had unknown and other causes.

The TX, HD, and C groups were similar in terms of age (36.6 ± 11.9, 35.4 ± 8.7, and 33.0 ± 10.0 years, respectively; $p = 0.13$) and gender distribution (male gender: 65.2%, 60.9%, and 70.0%, respectively; $p = 0.82$). The SBP of TX and HD patients was higher than that of healthy controls (**Table 2**; **Figure 2**). As expected, the eGFR of the C group was significantly higher than the TX group, (112.2 ± 18.0 vs. 76.3 ± 16.6 ml/min/1.73 m^2, $p < 0.001$). Other laboratory parameters are summarised in **Table 2**.

The hearing thresholds at 250 Hz in both ears of HD patients were significantly higher than that of TX and C group patients (right ear measurements of the HD, TX, C groups were 16.30 ±

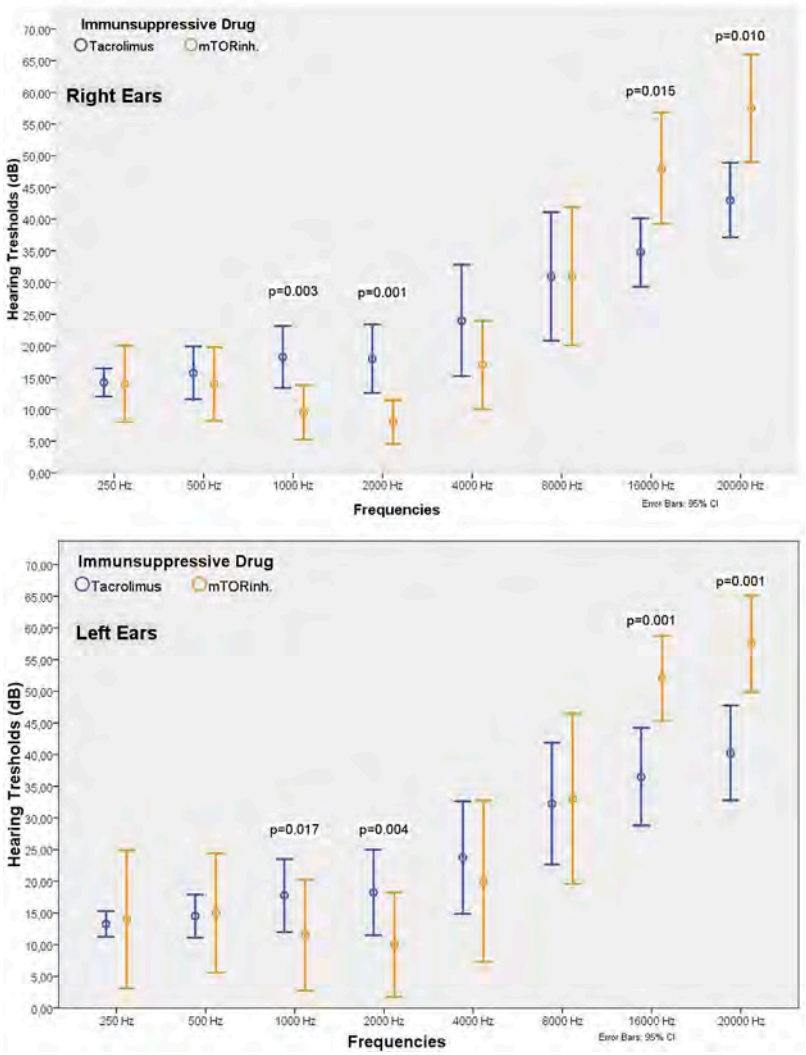


FIGURE 4 | Comparison of the hearing threshold values of patients receiving tacrolimus or an mTOR inhibitor (matched groups).

4.81 vs. 13.26 ± 5.49, 12.50 ± 3.03 dB, respectively; $p = 0.01$) (**Table 2**; **Figure 2**).

In both ears, the hearing thresholds in the TX and HD groups at 500 Hz were similar and significantly higher than that in the C group (right ear measurements of the HD, TX, C groups were 15.21 ± 5.53, 14.56 ± 7.28 vs. 11.25 ± 2.75 dB, respectively; $p = 0.02$) (**Table 2**; **Figure 2**).

Hearing thresholds in all groups were similar between the 1,000–2,000 Hz range ($p > 0.05$ for all).

At all frequencies between 4,000–20,000 Hz, the hearing thresholds of the HD and TX groups were similar in both ears and were significantly higher than that in the C group. As the frequency increased from 4,000 Hz to 20,000 Hz, the hearing thresholds of HD and TX patients also increased ($p < 0.01$ for all) (**Table 2**; **Figure 2**).

The first test of immittance testing was tympanometry, which returned normal values for middle ear pressure levels in all patients. The second test was the acoustic reflex test, which revealed that the stapedius reflexes were bilaterally normal for all participants.

For both ears, the SRT values of the HD and TX groups were significantly higher than that of the C group (right ear measurements of the TX, HD, C groups were 14.13 ± 5.50, 13.47 ± 4.37 and 11.25 ± 2.75 dB, respectively; $p = 0.04$) (**Table 2**). The SD values in the HD and TX groups were significantly lower in both ears than the C group (right ear measurements of the TX, HD, C groups were, 95.28 ± 9.31, 95.47 ± 3.90 and 99.00 ± 1.77, respectively; $p = 0.005$) (**Table 2**).

Hearing Loss Rates

The normal hearing thresholds was accepted as 20 dB for the 250–8,000 Hz frequency range and 30 dB for the 16,000 and 20,000 Hz frequency range. The percentage of patients with hearing defects in all are given in **Table 3**. As the frequency increased in the TX and HD groups, the proportion of patients with hearing impairment also increased, reaching 76% and 78% at

TABLE 5 | Correlation of hearing thresholds with clinical parameters (right ear data only shown).

	Frequency		Age	Post Tx time	eGFR	Ca	P	HD vintage	kt/v	URR	Tacrolimus levels C0 (*n* = 30)	mTOR inhibitor levels C0 (*n* = 16)
TX (n = 46)	Right 16,000 Hz	*p*	0.002	0.055	0.008	0.85	0.014	NA	NA	NA	0.50	0.98
		r	0.44	0.28	–0.38	0.02	–0.36	NA	NA	NA	–0.12	0.005
	Right 20,000 Hz	*p*	0.001	0.052	0.008	0.78	0.013	NA	NA	NA	0.50	0.98
		r	0.46	0.28	–0.38	0.04	–0.36	NA	NA	NA	–0.12	0.004
HD (n = 23)	Right 16,000 Hz	*p*	0.03	NA	NA	0.61	0.4	0.040	0.31	0.55	NA	NA
		r	0.45	NA	NA	0.11	0.18	0.44	0.22	0.13	NA	NA
	Right 20,000 Hz	*p*	0.05	NA	NA	0.51	0.45	0.043	0.35	0.61	NA	NA
		r	0.39	NA	NA	0.14	0.16	0.43	0.20	0.11	NA	NA
C (n = 20)	Right 16,000 Hz	*p*	0.048	NA	0.98	0.36	0.41	NA	NA	NA	NA	NA
		r	0.44	NA	0.006	0.21	–0.20	NA	NA	NA	NA	NA
	Right 20,000 Hz	*p*	0.30	NA	0.99	0.37	0.26	NA	NA	NA	NA	NA
		r	0.24	NA	0.001	0.21	–0.27	NA	NA	NA	NA	NA

NA, not applicable.

20,000 Hz. The percentage of patients with hearing loss at frequencies of 8,000 Hz and above was similar in the TX and HD groups and significantly higher than in the C group (**Figure 3**).

Comparison of TX Patients Using Tacrolimus and mTOR Inhibitors

Among our cohort, 30 patients used tacrolimus and 16 used mTOR inhibitor. There were no differences in terms of hearing threshold between the two groups within 250–500 Hz frequency range. In both ears, the hearing threshold at 1,000–2,000 Hz in patients receiving tacrolimus was significantly higher than the value among the patients receiving an mTOR inhibitor (**Table 4**; **Figure 4**). The hearing thresholds at high frequencies such as 16,000 and 20,000 Hz in patients using an mTOR inhibitor were significantly higher for both ears than the patients using tacrolimus (49.06 ± 13.44 vs. 32.00 ± 16.64 dB at 16,000 Hz, $p = 0.001$; and 58.43 ± 12.74 dB vs. 39.00 ± 17.97 dB at 20,000 Hz for the right ear; $p < 0.001$) (**Table 4**; **Figure 4**). However, the mean age of the patients in the group receiving an mTOR inhibitor was higher than the group who received tacrolimus (47.81 ± 11.47 vs. 33.70 ± 9.14 years, $p < 0.001$), had passed a longer time after transplantation (98.50 ± 51.16 vs. 62.93 ± 35.14 months, $p = 0.02$) and had lower eGFR (62.98 ± 20.20 vs. 79.96 ± 16.50 ml/min/1.73 m^2, $p = 0.001$). Since these parameters are known to affect hearing, when the analysis was repeated after excluding some patients so that the two groups were matched in terms of age, eGFR and the time elapsed after kidney transplantation, it was observed that the hearing thresholds at 1,000–2,000 Hz in the patients receiving tacrolimus continued to be significantly higher than those receiving an mTOR inhibitor, and the hearing thresholds at 16,000–20,000 Hz in the patients who received an mTOR inhibitor were still higher than the patients who received tacrolimus (48.00 ± 12.29 vs. 34.75 ± 11.52 dB at 16,000 Hz, $p = 0.015$ and 57.50 ± 11.84 vs. 43.00 ± 12.60 dB at 20,000 Hz for the right ear, $p < 0.001$) (**Table 4**; **Figure 4**).

SRT values for both ears were not different between the mTOR inhibitor and tacrolimus-receiving groups ($p > 0.05$). However, patients who received an mTOR inhibitor had higher SD values in both ears than the patients who received tacrolimus (right ear measurements were 98.31 ± 2.91 and 93.66 ± 11.06 respectively, $p = 0.026$). When both groups were matched, the mean SD value of the patients receiving mTOR inh was still higher than those receiving tacrolimus (**Table 4**; **Figure 4**).

In the TX group, the hearing threshold was strongly correlated with age at frequencies of 8,000 Hz and above ($p = 0.002$ and, r = 0.44 at 16,000 Hz, and $p = 0.001$, r = 0.46 at 20,000 Hz). The hearing threshold was inversely correlated with eGFR and serum *p* values (**Table 5**). No correlation was found between blood tacrolimus or mTOR inhibitor levels and hearing thresholds. In the HD patients, the correlation between hearing thresholds and age started at 4,000 Hz and continued up to 20,000 Hz ($p = 0.03$, r = 0.45 for 16,000 Hz, and $p = 0.05$, r = 0.39 for 20,000 Hz) (**Table 5**).

DISCUSSION

This study is the first to evaluate the hearing function of kidney transplant patients with EHFA and evaluate the effect of immunosuppressive agents on hearing. Our main findings can be summarized as follows. The majority of kidney transplant patients have hearing-related abnormalities. Kidney transplant patients have hearing loss that does not affect the middle frequencies but is evident at high frequencies, and their hearing level is as bad as HD patients. Hearing loss in CKD patients is likely to be permanent and a kidney transplant may not improve their hearing problems. The SRT and SD values were

impaired with hearing loss. The use of tacrolimus seems to cause auditory deficit in the 1,000–2,000 Hz range, and the use of mTOR inhibitor mostly at high frequencies such as 16,000 and 20,000 Hz.

The frequency of sensorineural hearing loss in CKD patients ranges from 28 to 77%, and hearing function declines as the stage of CKD increases (12–14). The condition has a high prevalence in HD patients and is often bilateral (14). It has been reported that the frequency of hearing loss increases as the total duration of renal disease and HD duration increases with age advancement (14–16). Although many studies have shown that hearing loss is more pronounced at higher frequencies in HD patients (14, 15), few studies have suggested that it does not differ from low to high frequencies or is more pronounced at low frequencies (17, 18). In HD patients, there are many known risk factors to explain sensorineural hearing loss, such as the use of ototoxic drugs like furosemide, the presence of DM and advanced age. Apart from these classical risk factors, many pathogenetic mechanisms such as the direct effects of uremia itself, development of hydrops in the endolymph fluid of the inner ear, changes in endolymph composition and electrolyte imbalances, aluminium deposition and dialysis amyloid deposition have been blamed (12).

Unfortunately, there is not sufficient evidence that these abnormalities improve after kidney transplantation. In two studies conducted by Mitschke et al in 1975 and 1977, it was suggested that audiometric abnormalities returned to normal after kidney transplantation. The authors showed that 7 of 10 HD patients' hearing thresholds within the range of 256–8192 Hz returned to normal after kidney transplantation. The three patients in whom the hearing defect did not improve had hereditary nephritis (19). In the second study, it was shown that the hearing thresholds among 13 HD patients within the 2,000–8,000 Hz range returned to normal at an average of 21 months after kidney transplantation (from 29.3 to 7.7 for 8,000 Hz) (20). However, the pre-transplant serum BUN (102 mg/dl), creatinine (14 and 14.6 mg/dl) and albumin (2.7 g/dl) values of the patients in these two studies were below today's standards, indicating insufficient dialysis. In addition, the fact that the patients included in the study used ototoxic drugs such as digital and aluminium compounds and gentamicin necessitates a cautious approach to the results of these two studies. In our study, we showed that the proportion of transplant patients with hearing defects increased as the frequency went from 4,000 to 20,000 Hz, similar to HD patients, and the hearing thresholds increased as the frequency increased. In other words, the frequency of hearing defect and the hearing thresholds in kidney transplant patients were as bad as HD patients. De Los Santos et al. performed audiometric evaluation of 45 HD patients, 43 TX patients, and 40 healthy individuals, and showed that the prevalance of mild hearing loss at 3,000 Hz and was higher among the TX patients than the HD patients (21). However, there is no information about the immunosuppressive regimen and drug blood levels used in this study. Bains et al. evaluated the cochlear function abnormalities of stage 3–5 CKD patients and healthy controls with pure-tone audiometry and BERA [Brainstem Evoked Response Audiometry-BERA is an objective and non-invasive method for assessing the auditory pathways from the auditory nerve to the brainstem]. They showed that the hearing thresholds among the CKD patients were higher than healthy controls at all frequencies between 250–8,000 Hz, especially at higher frequencies (22). However, there was no significant improvement in the hearing thresholds of Stage 5 CKD patients 1 year after kidney transplantation compared to pre-transplant levels. When the same patients were evaluated with BERA, the researchers showed that CKD patients had more absolute and interpeak delays of waves I, III, and V than healthy controls. After kidney transplantation, there was only some improvement in the absolute delays of waves I, III, and V, but no significant improvement in interpeak delays. In the BERA test, absolute peak delay cannot distinguish the hearing losses from cochlea or post-cochlear auditory pathways (23). However, interpeak delays are not affected by cochlear function and reflect the defect between the central pathways of hearing (23). Lack of improvement in interpeak delays may indicate a problem with the auditory nerve. According to the results of these two studies as well as our study, it is possible to conclude that the majority of TX patients have hearing defects that cannot be noticed by patients, this defect is more prominent especially at high frequencies, and there is no significant improvement in hearing defect after kidney transplantation. There are two possible reasons for this. The first one is permanent damage to the cochlea from the CKD process: The data showing that the damage may be permanent in these patients come from a very old study conducted by Oda et al. In the pathological examination of the temporal bones of eight patients who died due to various reasons after kidney transplantation, the authors have shown that there was significant damage and even loss of the Corti organ, the petrification of the stria vascularis, especially in patients who had long-term dialysis treatment and had multiple kidney transplants (24). The second reason is the neurotoxic effects of immunosuppressive drugs on the auditory nerve. Calcineurin inhibitors are neurotoxic drugs. Ototoxicity could be a manifestation of neurotoxicity associated with CNI use and may not be noticed by the patient, but can be demonstrated by audiometric tests. Case reports of sudden hearing loss after kidney transplantation are available in the literature (25–28). Gulleroglu et al. (25) reported significant hearing loss at 4,000–8,000 Hz frequency in two pediatric kidney transplant patients, while tacrolimus levels were as high as 22 and 29 nmol/L in both patients. Even when drug level was reduced, the progression of the hearing loss stopped but did not improve. The same author later found hearing loss between 4,000 and 8,000 Hz by pure-tone audiometry in 17 of 27 pediatric kidney transplant patients and showed that patients with hearing loss had higher CsA levels than those without hearing loss (29). In our study, we could not demonstrate a relationship between blood tacrolimus or mTOR inhibitor levels and hearing thresholds. It is known that hearing problems occur in other patient groups who have not been exposed to uremia for a long time, or after organ transplantations other than kidney transplantation, or in other patient groups who have to use CNI due to glomerulonephritis. Rifai et al. reported that CNI levels were very high in five patients, who developed sudden

hearing loss after orthotopic liver transplantation, although there was no other risk factor, and hearing loss was permanent in four of these patients, even though the drug level was reduced to the normal range (30). It was also reported that 35% of the patients who used tacrolimus rather than cyclosporine had various hearing problems (31). In their 2012 study, Rifai et al. (32) detected hearing loss with pure tone audiometry in 53% of 70 liver transplant patients. Half of the patients who did not describe any hearing problems had audiometric abnormalities. Interestingly, in our study, patients receiving an mTOR inhibitor had worse hearing thresholds and SD values at 16,000 and 20,000 Hz compared to patients receiving tacrolimus. This finding may be difficult to explain, as no major neurotoxicity of mTOR inhibitors has been demonstrated (7–9). However, the mTOR pathway plays a role in axonal sprouting, astrocyte metabolism, mitochondrial functions, axonal regeneration and myelination, regulation of synaptic activity, and perhaps most importantly, the expression of some ion channels and receptors (9). In the realization of hearing, ion channels and receptors in hair cells play a key role in the conversion of mechanical energy into electrical messages by hair cells in the cochlea. mTOR inhibitors may be causing damage at the cochlea level. On the other hand, patients using tacrolimus had worse hearing thresholds at frequencies of 1,000–2,000 Hz than patients using an mTOR inhibitor. Tacrolimus is a neurotoxic drug and a defect at 1,000–2,000 Hz may be a sign of neurotoxicity.

The SRT values were higher while the SD values were lower for the study groups than the control group. These results are in close agreement with pure tone threshold results and confirm the validity of the pure tone thresholds. The immittance testing returned normal results in all groups. This may be expected since HD, kidney transplantation or the drugs used had no effects on the middle ear pressure and acoustic reflex.

Our study had some limitations. Since it was conducted in two different locations, the measurements made by two audiologists may partially affect the results. Its cross-sectional design rather than being a prospective one may be another limitation. However, there are only three studies in the literature evaluating hearing problems in kidney transplant patients with audiometry, while few others have evaluated the condition using questionnaires only. Our study is the first of its kind to make analyses with EHFA. In addition, there is no other study in the literature that evaluates the relationship between hearing problems and the immunosuppressive drug type used. The number of patients in our study was determined by power analysis, and the power of our study was over 80%.

In conclusion, there are defects in hearing and cochlear functions in kidney transplant patients due to permanent hearing defects because of CKD and the additive effects of immunosuppressive drugs. Hearing defects probably do not improve after a kidney transplant. This issue needs to be investigated with prospective studies.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved the local ethics committee of Kahramanmaras Sutcu Imam University (dated September 09, 2020, session No.2020/17, decision No.18). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

OA: mastermind of the study, designed research/study, analyzed data, wrote the paper, reviewed the literature, and collected scientific data. MS: collected patient data. MY, ET, AD, IO and MK: performed physical examinations and odiometric tests, and collected data. EE and NE: English translation and spell checking. OG: analyzed data.

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. Global Observatory on Donation and Transplantation. International Report on Organ Donation and Transplantation Activities. Executive Summary (2019). Available from: http://www.transplant-observatory.org/wp-content/uploads/2021/04/glorep2019.pdf (Accessed June 15, 2020).
2. Gaston RS. Current and Evolving Immunosuppressive Regimens in Kidney Transplantation. *Am J Kidney Dis* (2006) 47:S3–S21. doi:10.1053/j.ajkd.2005.12.047
3. Hart A, Smith JM, Skeans MA, Gustafson SK, Wilk AR, Castro S, et al. OPTN/SRTR 2018 Annual Data Report: Kidney. *Am J Transpl* (2020) 20(Suppl. s1): 20–130. doi:10.1111/ajt.15672
4. Farouk SS, Rein JL. The Many Faces of Calcineurin Inhibitor Toxicity-What the FK? *Adv Chronic Kidney Dis* (2020) 27:56–66. doi:10.1053/j.ackd.2019.08.006
5. Pirsch JD, Miller J, Deierhoi MH, Vincenti F, Filo RS. A Comparison of Tacrolimus (Fk506) and Cyclosporine for Immunosuppression after Cadaveric Renal Transplantation1. *Transplantation* (1997) 63:977–83. doi:10.1097/00007890-199704150-00013
6. Bechstein WO. Neurotoxicity of Calcineurin Inhibitors: Impact and Clinical Management. *Transpl Int* (2000) 13:313–26. doi:10.1111/j.1432-2277.2000.tb01004.x
7. van de Beek D, Kremers WK, Kushwaha SS, McGregor CGA, Wijdicks EFM. No Major Neurologic Complications with Sirolimus Use in Heart Transplant

Recipients. *Mayo Clinic Proc* (2009) 84:330–2. doi:10.1016/s0025-6196(11)60541-7
8. Kasner SE, Sheth KN, Wu GF, Messe SR, Wolf RL, Wijdicks E, et al. Sirolimus May Not Cause Neurotoxicity in Kidney and Liver Transplant Recipients. *Neurology* (2005) 65:337–8. doi:10.1212/wnl.65.2.337-a
9. Faravelli I, Velardo D, Podestà MA, Ponticelli C. Immunosuppression-related Neurological Disorders in Kidney Transplantation. *J Nephrol* (2021) 34:539–55. doi:10.1007/s40620-020-00956-1
10. Rodríguez Valiente A, Roldán Fidalgo A, Villarreal IM, García Berrocal JR. Extended High-Frequency Audiometry (9000-20000Hz). Usefulness in Audiological Diagnosis. *Acta Otorrinolaringologica (English Edition)* (2016) 67:40–4. doi:10.1016/j.otoeng.2015.02.001
11. Faul F, Erdfelder E, Lang A-G, Buchner A. G*Power 3: A Flexible Statistical Power Analysis Program for the Social, Behavioral, and Biomedical Sciences. *Behav Res Methods* (2007) 39:175–91. doi:10.3758/bf03193146
12. Krajewska J, Krajewski W, Zatoński T. Otorhinolaryngological Dysfunctions Induced by Chronic Kidney Disease in Pre- and post-transplant Stages. *Eur Arch Otorhinolaryngol* (2020) 277:1575–91. doi:10.1007/s00405-020-05925-9
13. Liu W, Meng Q, Wang Y, Yang C, Liu L, Wang H, et al. The Association between Reduced Kidney Function and Hearing Loss: a Cross-Sectional Study. *BMC Nephrol* (2020) 21:145. doi:10.1186/s12882-020-01810-z
14. Peyvandi A, Roozbahany NA. Hearing Loss in Chronic Renal Failure Patient Undergoing Hemodialysis. *Indian J Otolaryngol Head Neck Surg* (2013) 65:537–40. doi:10.1007/s12070-011-0454-9
15. Jakić M, Mihaljević D, Zibar L, Jakić M, Kotromanović Z, Roguljić H. Sensorineural Hearing Loss in Hemodialysis Patients. *Coll Antropol* (2010) 34(Suppl. 1):165–71.
16. Saeed HK, Al-Abbasi AM, Al-Maliki SK, Al-Asadi JN. Sensorineural Hearing Loss in Patients with Chronic Renal Failure on Hemodialysis in Basrah, Iraq. *Ci Ji Yi Xue Za Zhi* (2018) 30:216–20. doi:10.4103/tcmj.tcmj_149_17
17. Jamaldeen J, Basheer A, Sarma A, Kandasamy R. Prevalence and Patterns of Hearing Loss Among Chronic Kidney Disease Patients Undergoing Haemodialysis. *amj* (2015) 8:41–6. doi:10.4066/amj.2015.2258
18. Gatland D, Tucker B, Chalstrey S, Keene M, Baker L. Hearing Loss in Chronic Renal Failure-Hearing Threshold Changes Following Haemodialysis. *J R Soc Med* (1991) 84:587–9.
19. Mitschke H, Schmidt P, Kopsa H, Zazgornik J. Reversible Uremic Deafness after Successful Renal Transplantation. *N Engl J Med* (1975) 292:1062–3. doi:10.1056/nejm197505152922005
20. Mitschke H, Schmit P, Zazgornik J, Kopsa H, Pils P. Effect of Renal Transplantation on Uremic Deafness: a Long-Term Study. *Int J Audiol* (1977) 16:530–4. doi:10.3109/00206097709080024
21. Abaeté de los Santos C, Wiederkehr Baú A-R, d'Avila DO, Hausen de Souza EO, Moussalle S, Galvan Gomes NH. Audiometric Changes in Patients Undergoing Hemodialysis and Renal Transplantation. *Transplant Proc* (1999) 31:3011–2. doi:10.1016/s0041-1345(99)00646-6
22. Bains KS, Chopra H, Sandhu JS, Aulakh BS. Cochlear Function in Chronic Kidney Disease and Renal Transplantation: a Longitudinal Study. *Transplant Proc* (2007) 39:1465–8. doi:10.1016/j.transproceed.2007.02.085
23. Folsom RC, Diefendorf AO. Physiologic and Behavioral Approaches to Pediatric Hearing Assessment. *Pediatr Clin North America* (1999) 46:107–20. doi:10.1016/s0031-3955(05)70085-7
24. Oda M, Preciado MC, Quick CA, Paparella MM. Labyrinthine Pathology of Chronic Renal Failure Patients Treated with Hemodialysis and Kidney Transplantation. *The Laryngoscope* (1974) 84:1489–506. doi:10.1288/00005537-197409000-00005
25. Gulleroglu K, Baskin E, Baskin E, Bayrakci U, Aydin E, Ozluoglu L, et al. Sudden Hearing Loss Associated with Tacrolimus after Pediatric Renal Transplant. *Exp Clin Transpl* (2013) 11:562–4. doi:10.6002/ect.2012.0241
26. Lakshmi BS, Vidya B, Hari Krishna Reddy M, Venkata Kumar AC, Ram R, Siva Kumar V. Sensorineural Deafness Following Tacrolimus Use. *Exp Clin Transpl* (2020) 18:110–1. doi:10.6002/ect.2017.0114
27. Marioni G, Perin N, Tregnaghi A, Bellemo B, Staffieri A, de Filippis C. Progressive Bilateral Sensorineural Hearing Loss Probably Induced by Chronic Cyclosporin A Treatment after Renal Transplantation for Focal Glomerulosclerosis. *Acta Oto-Laryngologica* (2004) 124:603–7. doi:10.1080/00016480410016225
28. Arinsoy T, Akpolat T, Ataman M, Arik N, Sungur C, Yasavul Ü, et al. Sudden Hearing Loss in a Cyclosporin-Treated Renal Transplantation Patient. *Nephron* (1993) 63:116–7. doi:10.1159/000187158
29. Gulleroglu K, Baskin E, Aydin E, Ozluoglu L, Moray G, Haberal M. Hearing Status in Pediatric Renal Transplant Recipients. *Exp Clin Transpl* (2015) 13:324–8. doi:10.6002/ect.2014.0158
30. Rifai K, Bahr MJ, Cantz T, Klempnauer J, Manns MP, Strassburg CP. Severe Hearing Loss after Liver Transplantation. *Transplant Proc* (2005) 37:1918–9. doi:10.1016/j.transproceed.2005.02.102
31. Rifai K, Kirchner GI, Bahr MJ, Cantz T, Rosenau J, Nashan B, et al. A New Side Effect of Immunosuppression: High Incidence of Hearing Impairment after Liver Transplantation. *Liver Transpl* (2006) 12:411–5. doi:10.1002/lt.20610
32. Rifai K, Pischke S, Agne C, Rosenau J, Klempnauer JL, Manns MP. High Rate of Unperceived Hearing Loss in Patients after Liver Transplantation. *Clin Transpl* (2012) 26:577–80. doi:10.1111/j.1399-0012.2011.01592.x

ESOT | Transplant International

ORIGINAL RESEARCH
published: 14 April 2022
doi: 10.3389/ti.2022.10223

Patient Experience in Pancreas-Kidney Transplantation—A Methodological Approach Towards Innovation in an Established Program

OPEN ACCESS

***Correspondence:**
Beatriu Bayés-Genís
bebayes@clinic.cat

†ORCID:
Pedro Ventura-Aguiar
orcid.org/0000-0003-3381-7503
Beatriu Bayés-Genís
orcid.org/0000-0003-1233-7631
Antonio J. Amor
orcid.org/0000-0001-9237-0903
Miriam Cuatrecasas
orcid.org/0000-0003-3063-0110
Fritz Diekmann
orcid.org/0000-0001-6199-3016
Enric Esmatjes
orcid.org/0000-0002-6329-5059
Joana Ferrer-Fàbrega
orcid.org/0000-0002-5723-4209
Ángeles Garcia-Criado
orcid.org/0000-0003-3536-4288
Mireia Musquera
orcid.org/0000-0002-5915-9935
David Paredes
orcid.org/0000-0001-9740-4439
Esteban Poch
orcid.org/0000-0002-6492-024X
Joan Escarrabill
orcid.org/0000-0003-0492-9645

Received: 12 November 2021
Accepted: 15 February 2022
Published: 14 April 2022

Citation:
Ventura-Aguiar P, Bayés-Genís B, Amor AJ, Cuatrecasas M, Diekmann F, Esmatjes E, Ferrer-Fàbrega J, García-Criado Á, Musquera M, Olivella S, Palou E, Paredes D, Perea S, Perez A, Poch E, Romano B and Escarrabill J (2022) Patient Experience in Pancreas-Kidney Transplantation—A Methodological Approach Towards Innovation in an Established Program. Transpl Int 35:10223. doi: 10.3389/ti.2022.10223

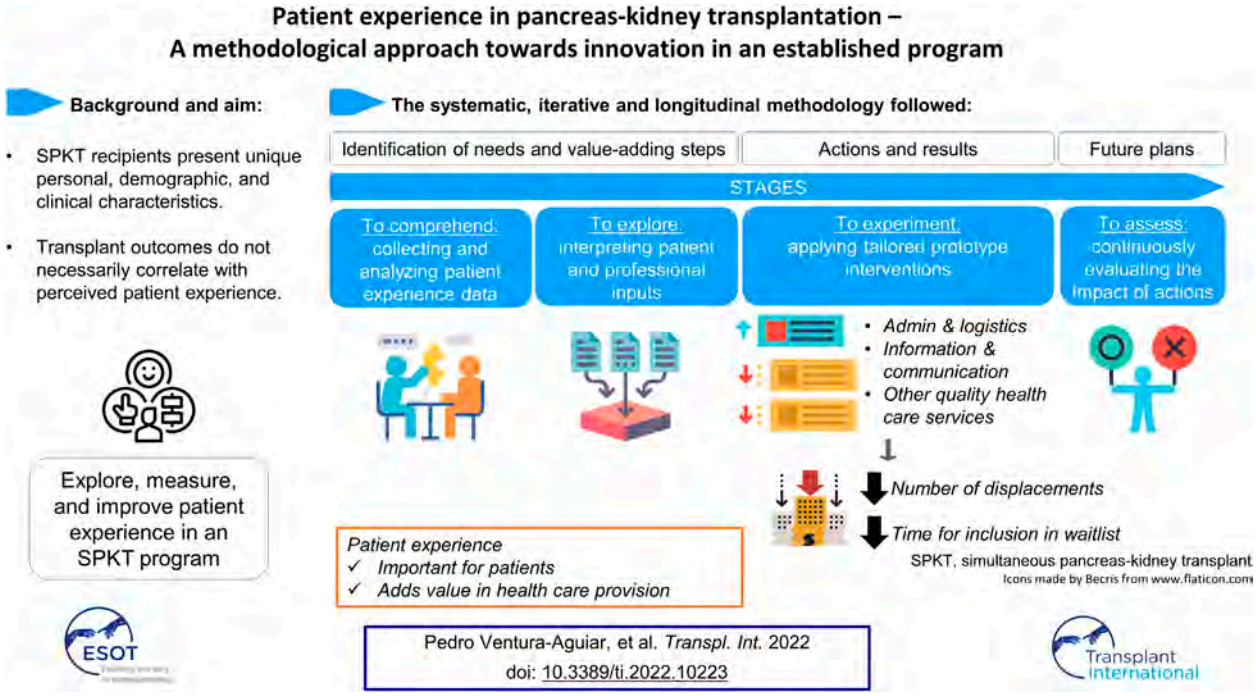
Pedro Ventura-Aguiar[1,2†], Beatriu Bayés-Genís[1,2†], Antonio J. Amor[3†], Miriam Cuatrecasas[4†], Fritz Diekmann[1,2,5†], Enric Esmatjes[3†], Joana Ferrer-Fàbrega[6†], Ángeles García-Criado[7†], Mireia Musquera[8†], Silvia Olivella[1], Eva Palou[9], David Paredes[10†], Sonia Perea[1], Anna Perez[1], Esteban Poch[1†], Barbara Romano[1] and Joan Escarrabill[9†]*

[1]Nephrology and Kidney Transplant Department, Hospital Clinic Barcelona, Barcelona, Spain, [2]Laboratori Experimental de Nefrologia i Trasplantament, Fundació Clínic, August Pi i Sunyer Biomedical Research Institute (IDIBAPS), Barcelona, Spain, [3]Endocrinology Department, Hospital Clinic Barcelona, Barcelona, Spain, [4]Pathology Department, Center for Biomedical Diagnosis, Hospital Clinic Barcelona, Barcelona, Spain, [5]Red de Investigación Renal (REDINREN), Madrid, Spain, [6]Hepato-Bilio-Pancreatic Surgery and Digestive Transplant Unit, Hospital Clinic Barcelona, Barcelona, Spain, [7]Radiology Department, Center for Imaging Diagnosis, Hospital Clinic Barcelona, Barcelona, Spain, [8]Urology Department, Hospital Clinic Barcelona, Barcelona, Spain, [9]Patient Experience, Hospital Clinic Barcelona, Barcelona, Spain, [10]Transplant Coordination Department, Hospital Clinic Barcelona, Barcelona, Spain

Simultaneous pancreas-kidney transplantation (SPKT) leads to increased survival and quality of life, and is an alternative treatment for insulin-dependent diabetes mellitus and end-stage kidney disease. Due to the particularities of this population (often with multiple comorbidities) and of the surgery (only performed in a few centers), a comprehensive analysis of patients' experience along the SPKT process is crucial to improve patient care and add value to this procedure. Therefore, we applied a systematic and iterative methodology with the participation of both patients and professional teams working together to explore and identify unmet needs and value-adding steps along the transplant patient journey at an established pancreas transplant program. Four main steps (to comprehend, to explore, to experiment and to assess) led to several interventions around three major areas: Administration and logistics, information and communication, and perceived quality of assistance. As a result, both displacements to the hospital for diagnostic purposes and the time delay involved in joining the patient waiting list for transplantation were reduced in parallel to the administrative procedures. In conclusion, the methodological implementation of key organizational changes has great impact on overall patient experience. Further quantitative analysis from the patient's perspective will consolidate our program and may add new prototype service design components.

Keywords: diabetes mellitus, simultaneous pancreas-kidney transplantation, chronic kidney failure, patient care, organizational innovation, focus groups

Abbreviations: A&E, Accident and Emergency; ANE, Anesthesiology; AQuAS, Catalan Agency for Health Quality and Evaluation; BMI, Body Mass Index; DALYs, disability-adjusted life years; DM, diabetes mellitus; ESRD, end-stage renal disease; HBP, Hepatobiliopancreatic surgery; HCB, Hospital Clinic Barcelona; NEF, Nephrology; ONCE, Spanish National Organization for the Blind; PREM, patient-reported experience measures; PROMs, patient-reported outcome measures; SPKT, simultaneous pancreas-kidney transplant; QoL, quality of life; T1DM, Type 1 diabetes mellitus; T2DM, Type 2 diabetes mellitus; URO, Urology.



GRAPHICAL ABSTRACT |

INTRODUCTION

In Type 1 diabetes mellitus (T1DM), the immune destruction of pancreatic beta cells leads to deficient production of insulin and renders patients dependent on life-long exogenous insulin therapy. Approximately 50% of diabetic patients develop serious complications, including chronic kidney disease (1), which was responsible for approximately 82,000 deaths worldwide and 3 million disability-adjusted life years (DALYs) in 2019 (2). Diabetic nephropathy is the leading cause of end-stage renal disease (ESRD) (3, 4). In these cases, simultaneous pancreas and kidney transplant (SPKT) is preferred over kidney transplant alone as it leads to increased patient and kidney graft survival rates (5–7). Moreover, since SPKT restores both organ functions in a single procedure, it overcomes the need for dialysis, insulin therapy, dietary restrictions and, most importantly, it minimizes diabetic complications (8, 9).

Concomitant improvement in quality of life (QoL) and other patient-reported outcome measures (PROMs) have also been extensively reported in cross-sectional studies including SPKT patients (10–14). However, none included patient reported experience measures (PREMs) throughout the transplant process. In this regard, several authors agree that prioritizing what patients value is key in quality healthcare provision. In the last years, patient's appraisal of their own experience with healthcare services has received much attention, with an ever-increasing number of studies that consider it in the design and upgrade of health systems (15–18). The major challenge lies in translating the heterogeneity of individual patient experience into measurables categories. For this, identifying the stakeholders involved in patient care and defining the patient journey map are useful to sort and characterize the added-value and non-added-value steps in the healthcare process (19). Qualitative data can subsequently be collected by methods such as interviews with patients, surveys and focus groups (19–22).

Herein we present a study aimed at integrating patient experience into qualitative healthcare assessment within the Pancreas-Kidney Transplant Program at the Hospital Clinic Barcelona (HCB). In order to achieve this, we followed a systematic, iterative and longitudinal research methodology to acquire data from patients and professionals while they interacted with each other.

PATIENTS AND METHODS

Study Design

We designed a systematic methodology to assess patient experience and improve the quality of the well-established Pancreas-Kidney Transplant Program at the HCB. This patient-centered project was developed in four phases that aimed to identify and validate current unmet needs and/or value-adding steps in our transplant process of care, as well as implementing specifically designed prototype proposals (**Figure 1**):

1) To comprehend—to collect and analyze data regarding the status of patient experience at the HCB (Pancreas-Kidney Transplant Program) from both professional and patient sources. Specifically, a team of professionals revised the relevant literature and were brought together at five co-

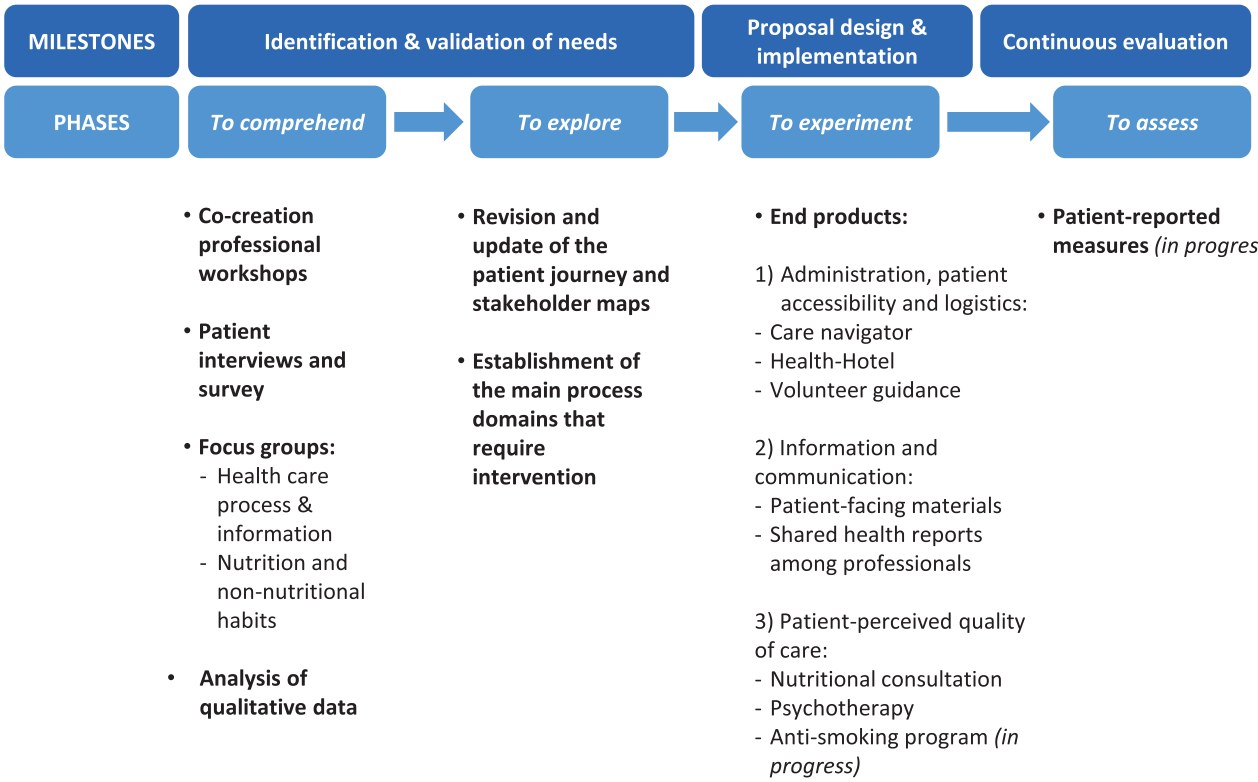


FIGURE 1 | In our project journey towards the improvement of the healthcare service delivered by the HCB's transplant unit, professionals and patients interacted with one another to provide feedback while engaging in different activities. These were organized in four phases to identify and confirm current unmet needs of our healthcare system (to comprehend and to explore) and to put solutions into practice (to experiment). Those proposals that have already been implemented are currently under assessment.

creation workshops. The activities involved mixed patient-professional teams taking part in three focus groups, a patient interview, an online survey and several open informative events for patients on social media (23–26).

2) To explore—to dissect and interpret the newly acquired information on uncovered or upgradable healthcare domains while checking to what extent they can be generalized.
3) To experiment—to design and implement new proposals according to the unveiled unmet needs.
4) To assess\to continuously evaluate the impact of the novel processes applying PREMs (currently in progress).

All these steps were carried out at the HCB, Spain, between October 2020 and February 2021. HCB performs an average of 20 pancreas transplants per year and is the main referral hospital for patients from five Spanish autonomous regions as well as Andorra (27, 28).

Study Participants

Healthcare Professionals

In 2019, the HCB established the Patient Experience Team, which is a living lab and multidisciplinary group of professionals (a sociologist, psychologist and physician) who work on the evaluation of the patient experience and on the design and analysis of PREMs following implementation of new protocols (29–31).

For this study, a total of 13 healthcare professionals from different disciplines and educational backgrounds were involved, including members from the HCB Patient Experience Team and others (physicians, nurses, administrative staff, a nutritionist and a participatory health care consultant). Professionals were involved in all co-creation workshops and focus groups.

Patients

A total of 12 patients worked together with the multidisciplinary professional team. Five patients participated in the focus group sessions, five responded to a logistics survey by email and two were interviewed online on World Diabetes Day 2020 (23). Patient selection was made according to clinical and demographic data and aimed to represent all patient archetypes that had been defined during the previous co-creation workshops.

Data Collection and Analysis

Focus Groups

Focus groups were carried out virtually and lasted between 60 and 90 min. Prior to the sessions, the focus group agenda was agreed on by the multidisciplinary professional team. Following contact, patients willing to participate received detailed information regarding the objective of the session, connection instructions as well as the consent form to participate and be recorded. Focus group sessions were moderated by two members from the patient

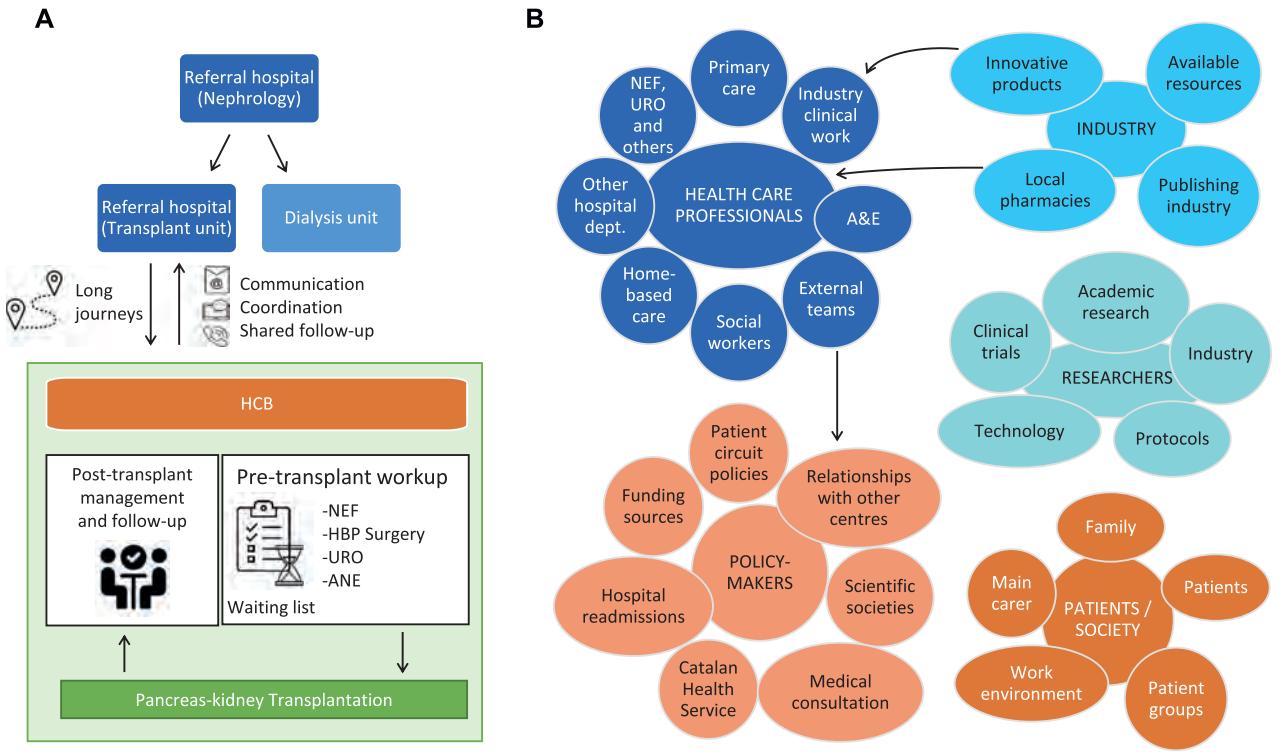


FIGURE 2 | (A) Patient journey and **(B)** stakeholder mapping helped to spot several pain points for patients undergoing a double pancreas-kidney transplant. ANE, Anesthesiology; A&E, Accident and Emergency; HCB, Hospital Clinic Barcelona; HBP, Hepatobiliopancreatic surgery; NEF, Nephrology; URO, Urology.

experience team. Of relevance, principal care physicians did not participate in these sessions, to avoid biasing patients' responses and interaction. In each session, the moderators introduced the purpose and aims of the study. Participants were also reminded that they would be recorded, and that all data collected during the session would be treated anonymously and confidentially. At the end of the session, patients were asked an open-end question in order to gather further feedback and/or suggestions.

During the first focus group, patients validated the general areas of improvement identified during the previous process mapping (**Table 1**; **Figure 2**), patient interview and survey (**Supplementary Tables S1, S2**). Afterwards, a formal script was prepared (**Supplementary Table S3**) for the second and main focus group about the healthcare process & information. Here, patients helped to identify the specific domains that needed to be addressed in the transplant unit and discussed them extensively (**Table 2**). The session was also useful for gaining aware of the emotions that were generated in each step of the care process (**Figure 2**).

MAXQDA software (VERBI GmbH, Germany) (32) was used to analyze the data from the verbatim transcriptions of the recorded focus group sessions. The analyses gave rise to the coding of meaning units (all expressions that have the same meaning) which were then combined into meta-categories. Further qualitative analyses (absolute frequency of meaning units) were performed according to the COREQ criteria for qualitative research (33, 34).

Patient Data

Patient data regarding the variables study time and number of displacements were collected from patients' electronic registries from 2019 to June 2021. Study time was defined as the total time since the first evaluation for pancreas transplantation until clinical decision regarding inclusion/exclusion of the patient in/from the waiting list. The number of displacements were defined by the number of visits to the HCB during the pre-transplant workup. Mean and standard deviation (SD) were used for these quantitative continuous data.

RESULTS

The methodology applied in this study led to the identification of key points and unmet needs as well as the implementation of novel protocols and circuits. To highlight the relevance of this stepwise systematic approach, the results obtained in each step will be described separately.

To Comprehend—Understanding Patient Experience

The Professional Viewpoint: Co-Creation Workshops and Literature Review

During the co-creation workshops, patient archetyping, stakeholder and patient journey mapping and categorization of the transplant

TABLE 1 | Pre-identified areas of interest for transplant patients according to professional opinion.

Key moments during the SPKT process	Areas of interest
At the time of referral to the HCB	The healthcare process that takes place at the HCB. This information must be given to the referral center.
	General information provided to each patient through HCB's Portal Clínic platform (42), QR code, etc.
	The details of the contact person before the first visit to the HCB.
	Information that should be provided by the patient: Medical report from their center of origin, diagnostic digital images.
	Legal information (especially relevant to foreigners).
	Access information for the first visit at the HCB.
	Available public services around the HCB such as the patient hotel.
During the candidate assessment for SPKT	Information to be given to the patient during the first visit to the HCB: All kinds of involved health professionals, the place, number and types of visits prior to the SPKT and the complementary and exploratory analyses.
	The duration of the assessment process.
	Overall information on the SPKT.
	Criteria for medical decisions.
	Contraindications of the SPKT (obesity, etc.).
	Patients at risk: Nutrition, smoking habit, alcohol, addictions, etc. Social acceptance.
During the waiting time and at the time of transplant surgery	Time management until the surgery date. Important topics to be addressed: Prioritization criteria and possible unexpected complications during the assessment and waiting period, given that they are fragile patients.
	Follow-up during the waiting period (analyses and periodic explorations) and contact channel for possible clinical incidents.
	Removal of the donor organ and viability assessment: Safety criteria and risk of donor incompatibility at the last moment (50% of patients cannot receive the organ after the first call).
	Informed consent before acceptance onto the patient waiting list for transplantation.
	Events that take place the day of the call (immediately getting to the HCB) and analyses that need to be carried out and/or repeated.
	Information for the caregiver.
At hospital discharge and follow-up	Pharmacological treatment: Lifelong prescriptions, adherence and secondary effects (vision, blood pressure, skin, tremor, etc.).
	Changes in nutritional habits (such as increased appetite) and food safety.
	Everyday life: Travelling, pets, vaccinations, and sexual and physical activity.
	The importance of smoking cessation.
	Follow-up information during outpatient care: First quarter, first year and thereafter.
	Benefits of shared follow-up with doctors and nurses and how this will take place.
	Contact details (email and phone).
	Warning signs and symptoms (infection and rejection).
	Asymptomatic hypoglycemia.
	Maintaining diabetes under control and possible complications (endocrinologic, cardiac, ophthalmologic, etc.).

HCB, Hospital Clinic Barcelona; SPKT, simultaneous pancreas-kidney transplant.

process were carried out by professionals to identify potential key steps for patients undergoing a double pancreas-kidney transplant.

Based on the literature review and professional experience, professionals classified pancreas transplant candidates into a number of archetypes, according to age (<45 or >45=years), residence zone (Barcelona, Catalonia or other autonomous regions), social and family support (good or dependent), Body Mass Index (BMI) (BMI > 27: High or BMI < 20: Low), vascular complications (micro or micro and macrovascular) and type of DM (T1DM or T2DM). Patient archetypes were used to select focus group participants to assure representation of all archetypes during the sessions.

The major stakeholders in our healthcare system were mapped as: Professionals from different medical specialties, from other disciplines and from public and private research and industry; policy-makers and society at large (including patients and caregivers). While defining the patient journey, three main dynamics were taken into consideration. Firstly, referral from multiple centers implies an administrative burden. Secondly,

TABLE 2 | Collected data during the focus group session on information and healthcare assistance.

Meta-category	Category	Results	Selected patient quotations
Contact and Communication	Contact via telephone	Most of the patients do not require any phone calls for urgent issues. Nevertheless, if that happened, they would like quick and effective telephone access.	*I think that, if I were involved in an urgent situation, I would try to call the hospital.*
	Contact via email	It was highly rated by those who used it, although they would appreciate a quicker reply (<48 h).	*I send emails to the Unit now and then when I have doubts. They don't reply immediately, they take a couple of days, but they usually reply*.
	Displacements to the hospital (pre- and post-transplant)	The pre-transplant phase does not require many displacements. After the procedure, they go through check-ups every 4–5 months, which usually require less than a day. Also, due to the COVID-19 pandemic, patients try to avoid public transportation.	*I have scheduled visits every 6 months or so with the endocrinologist at the HCB, but I see my private ophthalmologist and the rest of the specialties here in San Sebastián*.
	Communication between hospital of origin and HCB	Inter-hospital miscommunication was mentioned and some patients experienced issues with the transfer of their files. This caused longer waiting times and more anxiety. A shared digital platform for medical histories was suggested to ease data access and increase health system efficiency.	*My endocrinologist recommended medical consultation with the HCB for this type of intervention. I underwent several tests for 2 years and when my file was ready to be transferred, it got lost and had to be redone. It was such a long process [. . .].*
Information	Previous information	Most patients agreed that the information they had received about the SPKT was clear and honest but probably not enough, especially for highly vulnerable patients.	*I mean receiving more information such as what a double transplant is, etc. [. . .] As you can well imagine, when they tell you that [the need for a double organ transplant] you have no other choice than to adapt and make plans for a new life. In my case, I needed much more information. . ..*
	Information on waiting time	There is room for improvement here too. Patients would like to have more knowledge of the waiting time. Even rough estimates would be useful to be psychologically more prepared and better organize their everyday lives.	*I didn't feel anxious while waiting, but I would have preferred a bit more extra time to conclude some matters or to better plan them. For example, the week before the transplant I bought a car and right before getting to the HCB I had to deal with some paperwork. If I had known a month in advance about the possibility of an imminent organ donor, I would have postponed my purchase. You have your own life and events continue to unfold, but the moment you receive the call you're certain that it will all change [. . .].*
Waiting time	Waiting time	There was a great variety of opinions. Those who had added health complications or came from far away recalled a long wait.	*In my case, I received the first transplant very quickly, but then I rejected it and had to wait over 5 years for the second one.*
	Consequences of waiting time	The majority of patients were convinced that longer waiting times have physical consequences. Some of them have experienced it. As a result, they stressed the importance of receiving the new organs as soon as possible.	*People tend to associate diabetes with a different lifestyle, but they forget about all the problems that may suddenly arise. In my case, one of my feet burst, my vision got worse and I don't know what else I could have had. Maybe, if the waiting time had been shorter, we would have avoided or minimized such events. On the other hand, I understand that other surgeries are going on at the same time. . ..*
Impact on patients' everyday life	Family and social awareness	Having a serious illness and going through such a delicate procedure helps increase awareness.	*I have experienced it in my family too. They now see organ transplantation very differently. My friends from the swimming club now give blood. People are more conscious if they know of someone who is going through that*.
	Improvements in working life	SPKT improves patients' professional life too. They were able to work afterwards.	*I started working for ONCE as a lottery ticket seller. I became blind in 2008, I started dialysis in 2010, I was transplanted in 2013 and then, 4 years later, I found this job. I am entitled to a disability pension, but I can work and honestly, this makes a tremendous difference*.
	Transplants that are finally not performed	The fact that sometimes pre-scheduled transplants cannot be performed cause a great deal of distress to patients. Still, they are sympathetic towards medical decisions.	*This is hard. I had reached an impasse right before the second transplant, but I was on the reserve list and nonetheless I had to go home. "We will call you back," they say. Another year. . ..*
	Psychological support	Patients agreed to receiving emotional support, especially during (but not limited to) the waiting time and after the transplant in order to adjust to new living and working conditions. Psychological aid may be appropriate.	*I finally relaxed, but you pay for all the stress that you have suffered during the previous months. Then I was alone, and it took me a while before I realized I was depressed*.

(Continued on following page)

TABLE 2 | (*Continued*) Collected data during the focus group session on information and healthcare assistance.

Meta-category	Category	Results	Selected patient quotations
	Improvements in everyday life	Everyone agreed that there is a substantial improvement in their daily life after the transplant.	*You feel so much better after the transplant. The rest of your activities improve. The freedom you get to move around is of great importance to me.*

HCB, Hospital Clinic Barcelona; ONCE, Spanish National Organization for the Blind; SPKT, simultaneous pancreas-kidney transplant.

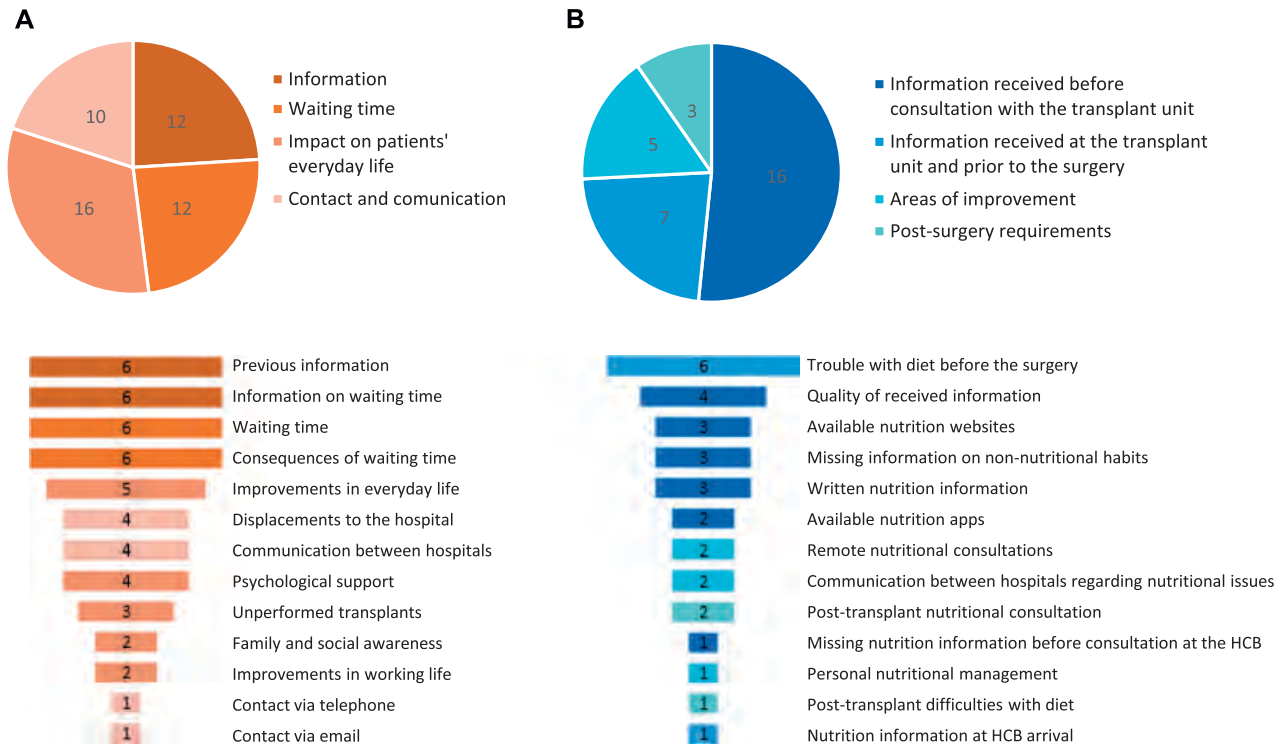


FIGURE 3 | Meta-categories (upper graphs) and categories (lower graphs) of patient preferences and needs that were identified during the focus group sessions: **(A)** Healthcare process & information (*N* = 50 preferences/needs) and **(B)** nutrition and non-nutritional habits (*N* = 31 preferences/needs). Absolute frequencies are shown. HCB, Hospital Clinic Barcelona.

there is a high number of patients travelling long distances from other cities within the same region (30%) or from other autonomous regions (40%–50%). Finally, the pre-transplant workup before a clinical decision regarding inclusion in/exclusion from the patient waiting list for transplantation is a complex procedure (**Figure 2**).

Professionals further characterized the pancreas transplant process into four steps which were of potential interest for intervention. These were defined as: 1) Referral to pancreas transplantation, 2) workup and candidate assessment for SPKT, 3) wait listing and transplant day, and 4) hospital discharge and follow-up (**Table 1**).

The Patient Viewpoint: Individual Interviews and Survey

To explore individual patients' perspectives, a live online interview with two pancreas-kidney transplant recipients was broadcasted on World Diabetes Day (23). During this interview (**Supplementary Table S1**), questions were raised concerning five relevant areas: Challenges in everyday life (work, education, leisure and others), treatment (management, compliance, medical check-ups, complications and hospitalizations, adverse events, etc.), required information (pre- and post-transplant), emotional impact (due to the physical change after the transplant, anxiety, fear, feeling of insecurity, etc.) and overall impact on the family and social environment. Data from the interviewees as well as comments and questions raised by the audience were recorded for further analysis.

Additionally, five patients responded to a survey on logistics requirements for patients coming from other regions during the COVID-19 pandemic. According to them, the areas that needed improvement were the limited visiting hours and comfort currently offered by the hospital as well as other affordable alternatives to lengthy daily travelling. Patients' response to the survey questions and their suggestions for improvement are shown in **Supplementary Table S2**.

TABLE 3 | Collected data during the focus group session on nutrition and other non-nutritional habits.

Meta-category	Category	Results	Selected patient quotations
Information received before consultation with the transplant unit	Missing nutrition information before consultation at the HCB	Only a minority reported not having received any kind of nutritional guidance before contacting the HCB.	*I was unlucky with this [nutritional consultation]. My doctor retired around the time they called me regarding the transplant. I didn't have any nutritionist during the first transplant either*.
	Written nutrition information	Patients confirmed they had received such information on paper.	*I was given plenty of written dietary information such as home recipes and books. I had already decreased the amount of salt and given up smoking*.
	Available nutrition apps	Some patients received the names of apps to help them design appropriate dietary patterns.	*They encouraged us to download an app with preestablished meals and cooking tips during the time I was on dialysis, to make it easier to bear*.
	Available nutrition websites	Internet was also an option for some of them to find dietary patterns which, in most cases, led to successful search results.	*I had access to the internet and could get information on the protein and potassium content of certain foods. I also checked different activities that I could do. I felt this was necessary*.
	Missing information on non-nutritional habits.	Despite available nutritional guidance, they had not been informed about other healthy habits like exercising and quitting smoking. However, they were already aware and tried to follow them.	*I wasn't told but I've always exercised and never smoked. That was a personal choice. I used to go to the gym, cycle, run, etc., even looking after the elderly, everything I could physically do except swimming to avoid infection of the peritoneal tubes*.
	Quality of received information	In general, nutritional recommendations before arriving at the HCB were considered adequate.	*At the Hospital Complex of Navarre, we had nutrition services that I received at the pre- and post-transplant stages and during dialysis while working together with the nurses. I also saw a personal nutritionist through the Renal Disease Association for a year and a half*.
Information received at the transplant unit and prior to the surgery	Nutrition information at HCB arrival	Some patients did not receive further instructions or recommendations as they already had them in abundance.	*Not in my case. Apart from the visits with my regular doctor, I didn't have any with nutrition specialists. I may have got some advice, but it was minor. Lately, I've visited the endocrinologist, but only a couple of times*.
	The trouble with diet before the surgery	This was one of the most popular and anxiety-inducing topics. There was unanimity among patients on fluid intake (and not food) as the most troublesome dietary issue before the transplant.	*Water becomes an obsession. When I had to be treated intravenously, I remained obsessed with liquids 24/7. The drinking situation is overwhelming*.
Areas of improvement	Personal nutritional management	Overall healthcare assistance could be improved if personal and individualized nutrition therapy was offered.	*I think that we need more nutrition treatment, and this should be more personalized. I received a lot of information about diets. However, I miss having professional support, someone to talk to and who follows up on you*.
	Remote nutritional consultations	Telemedicine could be applied, whenever possible, for those who live far away from the HCB.	*Regardless of your location, I reckon that videoconferences are a good communication channel*.
	Communication between hospitals regarding nutritional issues	Patients agreed that this should be improved towards a shared information system.	*In my experience, the nephrologist I was seeing in Alicante was not communicating with the HCB. Once, when I was having an organ rejection, I had to drive by myself to the A&E service in Barcelona even though my blood sugar was already at 600*.
Post-surgery requirements	Post-transplant difficulties with diet.	Although patients have some diet restrictions, it is not a major problem for them.	*After the second transplant, I was told I could eat normally, although all this food contained sugar, even fruit. Sugar in excess is not good for a non-transplant person either and it forces the pancreas (which is not yours) to release insulin*.
	Post-transplant nutritional consultation	This is not a major concern either since they usually have enough information on dietary patterns to follow.	*In my case, I don't require any nutritionist support anymore because I've been a diabetic person all my life and I'm more than used to dietary restrictions. To be honest, I've never changed my food habits except when I was on dialysis and had to watch the levels of potassium and phosphorus. I've always been in good shape and fit too*.

A&E, Accident and Emergency; HCB, Hospital Clinic Barcelona.

TABLE 4 | Study time and number of displacements for joining the patient waiting list for transplantation.

	2019	2020	2021[a]
Study time, months Mean (SD)	7.5 (3.1)	5.3 (3.2)	2.0 (1.0)
Displacements to and from hospital Mean (SD)	7.3 (3.2)	5.9 (2.6)	4.0 (2.7)

[a]*January to June.*
SD, standard deviation.

The Patient Viewpoint: Focus Groups

The most important categories reported by patients during the focus groups were receiving sufficient information prior to the intervention and the waiting time for transplantation and its consequences (**Figure 3A**). The latter may correspond to patients at the most severe clinical stage and for whom transplantation could imply more serious complications. Patients also highlighted the importance of having rough estimates for the transplantation date to better organize their personal and work life and to decrease anxiety during this period (**Table 2**).

In addition to this, the emotional impact caused by the SPKT was also discussed. Although they all agreed that their quality of life had improved, emotional support would have been appreciated too, for instance in terms of psychological follow-up (**Figure 3A**). This was especially relevant during the adaptation process after the transplant and throughout the waiting period. The need to better manage the distress caused by last-minute cancellation of their surgery was also highlighted (**Table 2**).

Other concerns raised by patients were those related to their displacements to and from the HCB and to communication between the hospitals, especially for patients that had been treated in more than one center (**Figure 3A**; **Table 2**). According to patients, administrative barriers such as the delayed transfer of medical records between hospitals usually increase the waiting period and trigger anxiety. A full description of focus group results is given in **Table 2**.

To Explore—Interpreting Patient and Professional Input

Following the input obtained from the interviews, survey and focus group sessions, the patient journey and stakeholder maps were reviewed and updated (**Figure 2**).

The analysis of qualitative data from the main focus group yielded 50 unmet needs, which were grouped into 13 categories and 4 meta-categories: The information received throughout the process; the waiting time; the impact of the SPKT on patients' day-to-day life; and the contact and communication with the HCB before, during and after the transplant (**Figure 3A**). Finally, the third nutrition-oriented focus group (**Supplementary Table S4**) spotted 31 categories that were grouped into 4 meta-categories. The main ones were those related to the amount and quality of nutrition information received before the intervention, especially regarding fluid intake restrictions (**Figure 3B**; **Table 3**).

To sum up, these results led to the understanding that there were three major domains encompassing the main meta-categories identified (**Figure 3**): 1) Administration, patient accessibility and logistics; 2) patient-facing information and shared health reports between professionals and 3) patient-perceived quality of care throughout the transplant process regarding emotional impact, nutritional support and other non-nutritional habits (**Figure 1**).

To Experiment—Designing and Applying Tailored Prototype Proposals

Following the establishment of the main domains requiring interventions to improve patient experience, a set of protocols and proposals were co-designed between professionals and patients. Protocols were further categorized regarding three major considerations for their implementation, such as pertinence, opportunity and available resources.

From an administrative point of view, the circuit of care was optimized by creating a new care navigator role, of which the main duties are to centralize and coordinate patient visits to the outpatient clinic to perform diagnostic and other complementary tests. In consequence, we observed that both patient eligibility assessment time and the number of displacements to the HCB before acceptance onto the patient waiting list for transplantation were reduced. During 2020 and in the first 6 months of 2021, and despite being an atypical period due to the coronavirus pandemic, the study time decreased by 29.3% and 73.3% and the number of displacements, by 19.2% and 45.2% compared to 2019, respectively (**Table 4**).

To overcome the patient-reported unease surrounding the first hospital visit and the tight schedule of the pre-transplant workup, a transplant patient welcome protocol was introduced, which included the use of a patient hotel (Health-Hotel) and the volunteer guidance. On the one hand, the Health-Hotel was set up near the HCB as a result of a joint public-private partnership between the HCB and the hotel sector. Besides offering more comfortable stays to patients and accompanying adults, this project was intended to alleviate their travelling and/or accommodation expenses (as it implies no direct cost for them), avoid hospital admissions during diagnosis and shorten the post-discharge phase. On the other hand, volunteer guides offered useful first-hand information and personal accompaniment to medical appointments, depending on the patient's comorbidities and/or impairments (visual, motor, etc.) (25, 26).

At the time of acceptance onto the patient waiting list for transplantation, patients often require a large amount of information on their procedure, treatment options, clinical benefits, etc. (**Figure 3**; **Table 2**). For this reason, we increased the printed and online resources available and organized informative patient workshops. For instance, educational videos on SPKT were posted online after receiving the approval of patients, medical societies and the Catalan Agency

for Health Quality and Evaluation (AQuAS). The aim of this animated plain-language tool is to aid shared transplant decision-making (24). In addition, at the professional level, we established a quarterly and annual report system to share patient records between the HCB and other centers, therefore speeding up the data flow.

Finally, long and uncertain waiting periods, bureaucracy hurdles and the post-transplant adaptation period impact patient's emotional wellbeing (**Table 2**). Hence, we allocated funding resources towards more affective support and closer follow-up through routine psychological visits. Additionally, other medical services were designed to improve the quality of care, namely pre- and post-transplant nutritional consultation at the unit and the medium-to-long-term implementation of an anti-smoking program.

DISCUSSION

We used a systematic strategy based on professional-patient interaction that translated into a package of potentially long-term interventions to improve the health system performance of the Pancreas Transplant Program of the HCB while upgrading patient experience.

SPKT improves clinical and non-clinical outcomes in eligible diabetic patients (5–7), (10–14). To further improve them, several authors have suggested that patient input is of utmost importance, but they do not specify how this can be put into practice. Usually, patient-reported outcomes measure QoL, psychological status or other domains with generic or specific questionnaires such as the 36-Item Short Form Survey (SF-36) or the Psychosocial Assessment of Candidates for Transplantation (PACT), respectively (10, 12–14,35). Recently, Gibbons et al. observed improvement of several PROMs while comparing post-transplant patients with those on the patient waiting list for transplantation as a surrogate of pre-transplant information. Their research was also based on qualitative interviews, which were used to better understand the impact of diabetes and kidney diseases and the transplant procedure on their QoL. Of note, diabetes-specific QoL had not improved after the surgery at least because of persistent diabetic complications, anxiety and self-imposed uninformed nutritional restrictions (13), which is in line with the emotional and nutritional support needs that were identified during the focus groups herein reported.

In contrast to these exploratory reports, and for the first time, we used patient experience assessment as a robust tool to co-design long-lasting improvement strategies and measure SPKT outcomes. Moreover, we added the focus group qualitative method analysis. Unlike individual interviews and questionnaires, these collective interviews rely on communication among participants to create and contrast data on how the system is perceived by the group in an interactive and dynamic way. Also, since group discussion is usually more stimulating than one-on-one interviews, it can give rise to more clues, insights and criticism (20,21,36).

Upon integration of focus data, several end products were implemented. Regarding logistics, the benefits of alternatives to conventional hospitalization have long been discussed (37). Among them, patient hotels, with the support from Home-Hospital units, are facilities that have been partially transformed to provide healthcare assistance and, therefore, alleviate the high demand for acute care hospital beds and other overcrowding-related problems such as nosocomial infections (38,39). By providing a Health-Hotel for patients being studied for the kidney-pancreas waiting list, we were able to concentrate outpatient visits and pre-transplant workup, which reduces the travel burden and its associated costs, and improves comfort during their stay.

Centralization of specialized care and minor procedures is common practice in healthcare organizations. This centralization may, nonetheless, lead to inequity of access to certain treatments and varying disease outcomes. In kidney transplantation, receiving dialysis more than 100 km away from a transplant center has been reported to reduce the likelihood of being referred for a transplant (40). On the other hand, pancreas transplantation is a procedure that is performed in a few centers nationwide, with patients' referral from rural areas often implying long travelling time and costs. Therefore, minimizing the displacement requirements and costs is of the utmost importance to reduce inequity in healthcare access (41). This topic was also highlighted by patients during both the interview and focus group sessions. The introduction of a care navigator to schedule visits on the same or consecutive days, among other tasks, and the Health-Hotel protocol led to considerable savings in time and money. Conversely, the busy outpatient visits and pre-transplant workup schedule might increase patients' already reported anxiety associated with the first contact with the Hospital. In this sense, the supporting role of HCB's volunteers will hopefully translate into a reduction of patient uneasiness.

We prioritized actions based on their prompt implementation, which depended on readily available resources, coordination of identified gaps among hospital services and/or the need to previously shape certain professional skills and competencies. Other identified needs were not deployed immediately due to a lack of resources. Nonetheless, this methodology enabled them to be flagged as patients' priorities and therefore they warrant adequate response in the near future.

Our work has some limitations. First, the results presented here are limited to the patient cohort, which has disease-specific requirements and several particular constraints imposed by the hospital logistics. Hence, end solutions cannot be directly extrapolated to other hospital environments without the corresponding customized variations. Secondly, the highly specific patient archetyping led to a rather small sample size. Finally, the prototype proposals are still subject to patient-based auditing to fine-tune them and hence ensure their continuity. New ones may

be also designed based on the present report. In this regard, we envision future challenges such as persistent professional and patient engagement and adaptation to new protocols despite being time- and effort-consuming tasks. Furthermore, the sustained provision of organizational structures and funding will be necessary to support these interventions within a resistant healthcare culture.

In conclusion, we have shown that value in healthcare provision is ultimately revealed by taking action to improve it. In this sense, our action plan was concentrated around the areas of administration, patient accessibility and logistics (care navigator role, Health-Hotel and volunteer guidance), information and communication (patient-facing materials and shared health reports) and patient-perceived quality of assistance (nutritionist and psychologist) with promising preliminary outcomes regarding a reduced number of displacements to the hospital and reduced delay before joining the patient waiting list for transplantation. Our work also highlights the use of focus groups as a well-suited methodology to work with and for patients towards a better care system, fostering similar initiatives in other hospital units and centers.

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 were informed about the voluntary and noninterventional nature of their participation in the focus group sessions, interview and survey, their right to withdraw at any time without consequence, as well as the anonymity, confidentiality, and publication of the results. Focus group participants and interviewed patients gave their formal consent to take part in them and be recorded. We also complied with the General Data Protection Regulation 2016/679 of the European Parliament and of the Council of 27 April 2016, and with the Spanish Organic Law 3/2018 of 5 December 2018 on Data Protection and Guarantee of Digital Rights.

AUTHOR CONTRIBUTIONS

PV-A, BB, and JE: contributed to the conception and design of the study. PV-A, BB, AJA, MC, FD, EE, JF-F, AG-C, MM, SO, EvP, DP, SP, AP, EsP, BR, and JE: contributed to the acquisition and analysis of data for the work. PV-A, BB, and JE: contributed to data analysis. PV-A, BB, FD, EsP, BR, FD, and JE: contributed to the interpretation of data for the work. PV-A, BB, and JE: wrote the first draft of the manuscript. All authors contributed to the manuscript revision, read and approved the submitted version.

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

We acknowledge Neus Cantariño (Trialance SCCL, Spain) for providing Medical Writing services and Marta Cabezali (Living Lab, HCB, Spain) for the logistic support during the focus group sessions.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10223/full#supplementary-material

REFERENCES

1. DiMeglio LA, Evans-Molina C, Oram RA. Type 1 Diabetes. *The Lancet* (2018) 391:2449–62. doi:10.1016/S0140-6736(18)31320-5
2. Institute for Health Metrics and Evaluation (IHME). The Global Burden of Disease Study (GBD). Global Health Metrics. *Chronic kidney Dis due Diabetes mellitus type 1—Level 4 cause* (2019).
3. Finne P, Reunanen A, Stenman S, Groop P-H, Grönhagen-Riska C. Incidence of End-Stage Renal Disease in Patients with Type 1 Diabetes. *JAMA* (2005) 294:1782–7. doi:10.1001/JAMA.294.14.1782
4. Thurlow JS, Joshi M, Yan G, Norris KC, Agodoa LY, Yuan CM, et al. Global Epidemiology of End-Stage Kidney Disease and Disparities in Kidney Replacement Therapy. *Am J Nephrol* (2021) 52:98–107. doi:10.1159/000514550
5. Barlow AD, Saeb-Parsy K, Watson CJE. 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) 30:884–92. doi:10.1111/TRI.12957
6. Sung RS, Zhang M, Schaubel DE, Shu X, Magee JC. A Reassessment of the Survival Advantage of Simultaneous Kidney-Pancreas versus Kidney-Alone Transplantation. *Transplantation* (2015) 99:1900–6. doi:10.1097/TP.0000000000000663
7. Mohan P, Safi K, Little DM, Donohoe J, Conlon P, Walshe JJ, et al. Improved Patient Survival in Recipients of Simultaneous Pancreas-Kidney Transplant Compared with Kidney Transplant Alone in Patients with Type 1 Diabetes Mellitus and End-Stage Renal Disease. *Br J Surg* (2003) 90:1137–41. doi:10.1002/bjs.4208
8. Kobayashi T, Gruessner AC, Wakai T, Sutherland DER. *Transplant. Proc.*, 46. Elsevier USA (2014). p. 948–53. doi:10.1016/j.transproceed.2013.11.058Three Types of Simultaneous Pancreas and Kidney Transplantation*Transplant Proc*
9. Jiang AT, Rowe N, Sener A, Luke P. Simultaneous Pancreas-Kidney Transplantation: The Role in the Treatment of Type 1 Diabetes and End-Stage Renal Disease. *Cuaj* (2014) 8:135–8. doi:10.5489/cuaj.1597
10. Adang EMM, Engel GL, Van Hooff JP, Kootstra G. Comparison before and after Transplantation of Pancreas-Kidney and Pancreas-Kidney with Loss of Pancreas-A Prospective Controlled Quality of Life Study1,2. *Transplantation* (1996) 62:754–8. doi:10.1097/00007890-199609270-00009

11. Sudan D, Sudan R, Stratta R. Long-term Outcome of Simultaneous Kidney-Pancreas Transplantation. *Transplantation* (2000) 69:550–5. doi:10.1097/00007890-200002270-00015
12. Smith GC, Trauer T, Kerr PG, Chadban SJ. Prospective Quality-Of-Life Monitoring of Simultaneous Pancreas and Kidney Transplant Recipients Using the 36-Item Short Form Health Survey. *Am J Kidney Dis* (2010) 55:698–707. doi:10.1053/j.ajkd.2009.12.025
13. Gibbons A, Cinnirella M, Bayfield J, Watson CJE, Oniscu GC, Draper H, et al. Changes in Quality of Life, Health Status and Other Patient-reported Outcomes Following Simultaneous Pancreas and Kidney Transplantation (SPKT): a Quantitative and Qualitative Analysis within a UK-wide Programme. *Transpl Int* (2020) 33:1230–43. doi:10.1111/tri.13677
14. Posegger KR, Linhares MM, Mucci S, Romano TM, Gonzalez AM, Salzedas Netto AA, et al. The Quality of Life in Type I Diabetic Patients with End-Stage Kidney Disease Before and After Simultaneous Pancreas-Kidney Transplantation: A Single-Center Prospective Study. *Transpl Int* (2020) 33:330–9. doi:10.1111/tri.13562
15. Doyle C, Lennox L, Bell D. A Systematic Review of Evidence on the Links between Patient Experience and Clinical Safety and Effectiveness. *BMJ Open* (2013) 3:1570. doi:10.1136/bmjopen-2012-001570
16. Porter ME. What Is Value in Health Care? *N Engl J Med* (2010) 363:2477–81. doi:10.1056/NEJMp1011024
17. Manary MP, Boulding W, Staelin R, Glickman SW. The Patient Experience and Health Outcomes. *N Engl J Med* (2013) 368:201–3. doi:10.1056/nejmp1211775
18. OECD/European Union. *Health at a Glance: Europe 2018: State of Health in the EU Cycle. Paris/European Union*. Brussels: OECD Publishing (2018).
19. Trebble TM, Hansi N, Hydes T, Smith MA, Baker M. Process Mapping the Patient Journey: an Introduction. *BMJ* (2010) 341:c4078–7. doi:10.1136/BMJ.C4078
20. Kitzinger J. Qualitative Research: Introducing Focus Groups. *BMJ* (1995) 311:299–302. doi:10.1136/bmj.311.7000.299
21. Barbour RS. *Doing Focus Groups. Second*. Los Angeles: Sage (2018).
22. Sheard L, Marsh C, Mills T, Peacock R, Langley J, Partridge R, et al. Using Patient Experience Data to Develop a Patient Experience Toolkit to Improve Hospital Care: a Mixed-Methods Study. *Health Serv Deliv Res* (2019) 7:1–104. doi:10.3310/hsdr07360
23. Aula Clinic - Hospital Clinic Barcelona. Living with... A Kidney-Pancreas Transplant (2020). YouTube. Available at: https://www.youtube.com/watch?v=SvWNYatVLJw (Accessed June 15, 2021).
24. Ventura-Aguiar P. Infographics on the Pancreas Transplant (2021). Available at: https://www.youtube.com/watch?v=rQ7CETBVHa0&list=PLpKd5Z54LopmX9BhAmEJypS_kmRyOkDWj (Accessed January 7, 2022).
25. Shared Patient eXperience. SPX Award - Patient Experience in Pancreas Transplantation (2021). Available at: https://www.youtube.com/watch?v=gFL05Vccy-A (Accessed November 5, 2021).
26. Hospital Clinic Barcelona. Volunteer Guide Service for Transplants (2021). Available at: https://www.youtube.com/watch?v=cdGwh3WghWk (Accessed November 5, 2021).
27. Fernández-Cruz L. Pancreas Transplantation. *Med Clin* (1983) 80:723–8.
28. Ventura-Aguiar P, Ferrer J, Paredes D, Rodriguez-Villar C, Ruiz A, Fuster J, et al. Outcomes from Brain Death Donors with Previous Cardiac Arrest Accepted for Pancreas Transplantation. *Ann Surg* (2021) 273:e230–e238. doi:10.1097/SLA.0000000000003218
29. Quirós C, Jansà M, Viñals C, Giménez M, Roca D, Escarrabill J, et al. Experiences and Real Life Management of Insulin Pump Therapy in Adults with Type 1 Diabetes. *Endocrinología, Diabetes y Nutrición* (2019) 66:117–23. doi:10.1016/j.endinu.2018.05.017
30. Clèries X, Solà M, Chiner E, Escarrabill J. Defining Patient and Caregivers' Experience of Home Oxygen Therapy. *Archivos de Bronconeumología (English Edition)* (2016) 52:131–7. doi:10.1016/j.arbres.2015.02.00910.1016/j.arbr.2016.01.005
31. Escarrabill J, Almazán C, Barrionuevo-Rosas L, Moharra M, Fité A, Jiménez J. *Patients Reported Experience Measurements*. Barcelona (2020).
32. VERBI GmbH. MAXQDA. All-In-One Tool for Qualitative Data Analysis & Mixed Methods (2021). Available at: https://www.maxqda.com/ (Accessed June 17, 2021).
33. Tong A, Sainsbury P, Craig J. Consolidated Criteria for Reporting Qualitative Research (COREQ): a 32-item Checklist for Interviews and Focus Groups. *Int J Qual Health Care* (2007) 19:349–57. doi:10.1093/INTQHC/MZM042
34. Caneiras C, Jácome C, Mayoralas-Alises S, Ramon Calvo J, Almeida Fonseca J, Escarrabill J, et al. Patient Experience in Home Respiratory Therapies: Where We Are and where to Go. *Jcm* (2019) 8:555. doi:10.3390/jcm8040555
35. Joseph JT, Baines LS, Morris MC, Jindal RM. Quality of Life after Kidney and Pancreas Transplantation: A Review. *Am J Kidney Dis* (2003) 42:431–45. doi:10.1016/S0272-6386(03)00740-6
36. Leung FH, Savithiri R. Spotlight on Focus Groups. *Can Fam Physician* (2009) 55:218–9.
37. Hensher M, Fulop N, Coast J, Jefferys E. The Hospital of the Future: Better Out Than in? Alternatives to Acute Hospital Care. *BMJ* (1999) 319:1127–30. doi:10.1136/bmj.319.7217.1127
38. Chesterton L, Stephens M, Clark A, Ahmed A. A Systematic Literature Review of the Patient Hotel Model. *Disabil Rehabil* (2021) 43:317–23. doi:10.1080/09638288.2019.1628314
39. Salazar A, Corbella X, Sanchez JL, Argimon JM, Escarrabill J. How to Manage the ED Crisis when Hospital And/or ED Capacity Is Reaching its Limits. Report about the Implementation of Particular Interventions during the Christmas Crisis. *Eur J Emerg Med* (2002) 9:79–80. doi:10.1097/00063110-200203000-00019
40. Kim SJ, Gill JS, Knoll G, Campbell P, Cantarovich M, Cole E, et al. Referral for Kidney Transplantation in Canadian Provinces. *Jasn* (2019) 30:1708–21. doi:10.1681/asn.2019020127
41. Irvine L, Elliott L, Wallace H. Closing the Health Inequalities Gap: An International Perspective. 2005.
42. Hospital Clinic Barcelona. PortalCLÍNIC (2021). Available at: https://www.clinicbarcelona.org/en/portalclinic (Accessed June 18, 2021).

ESOT | Transplant International

ORIGINAL RESEARCH
published: 22 April 2022
doi: 10.3389/ti.2022.10390

Normothermic Regional Perfusion and Hypothermic Oxygenated Machine Perfusion for Livers Donated After Controlled Circulatory Death With Prolonged Warm Ischemia Time: A Matched Comparison With Livers From Brain-Dead Donors

*Damiano Patrono[1], Marinella Zanierato[2], Marco Vergano[3], Chiara Magaton[1], Enrico Diale[1], Giorgia Rizza[1], Silvia Catalano[1], Stefano Mirabella[1], Donatella Cocchis[1], Raffaele Potenza[4], Sergio Livigni[3], Roberto Balagna[5] and Renato Romagnoli[1]**

[1]General Surgery 2U–Liver Transplant Unit, A.O.U. Città della Salute e della Scienza di Torino, University of Turin, Turin, Italy, [2]Department of Anesthesia and Critical Care, Azienda Ospedaliera Universitaria Città della Salute e della Scienza di Torino, Turin, Italy, [3]Department of Anesthesia and Intensive Care, San Giovanni Bosco Hospital, Turin, Italy, [4]Regional Procurement Organization, Azienda Ospedaliera Universitaria Città della Salute e della Scienza di Torino, Turin, Italy, [5]Anesthesia Department 2, A.O.U. Città Della Salute e Della Scienza di Torino, Turin, Italy

OPEN ACCESS

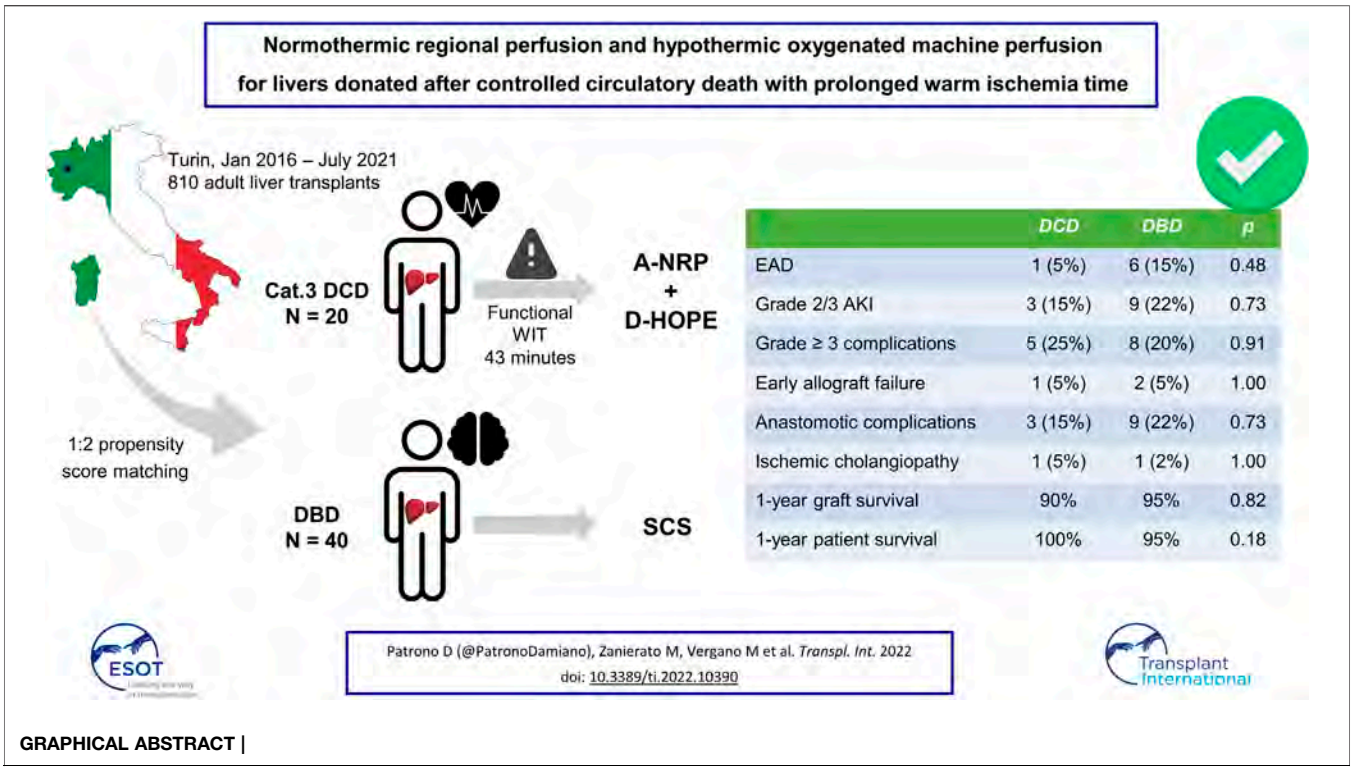
***Correspondence:**
Renato Romagnoli
renato.romagnoli@unito.it

Received: 28 January 2022
Accepted: 31 March 2022
Published: 22 April 2022

Citation:
Patrono D, Zanierato M, Vergano M, Magaton C, Diale E, Rizza G, Catalano S, Mirabella S, Cocchis D, Potenza R, Livigni S, Balagna R and Romagnoli R (2022) Normothermic Regional Perfusion and Hypothermic Oxygenated Machine Perfusion for Livers Donated After Controlled Circulatory Death With Prolonged Warm Ischemia Time: A Matched Comparison With Livers From Brain-Dead Donors. Transpl Int 35:10390. doi: 10.3389/ti.2022.10390

Prolonged warm ischemia time (WIT) has a negative prognostic value in liver transplantation (LT) using grafts procured after circulatory death (DCD). To assess the value of abdominal normothermic regional perfusion (A-NRP) associated with dual hypothermic oxygenated machine perfusion (D-HOPE) in controlled DCD LT, prospectively collected data on LTs performed between January 2016 and July 2021 were analyzed. Outcome of controlled DCD LTs performed using A-NRP + D-HOPE (n = 20) were compared to those performed with grafts procured after brain death (DBD) (n = 40), selected using propensity-score matching. DCD utilization rate was 59.5%. In the DCD group, median functional WIT, A-NRP and D-HOPE time was 43, 246, and 205 min, respectively. Early outcomes of DCD grafts recipients were comparable to those of matched DBD LTs. In DCD and DBD group, incidence of anastomotic biliary complications and ischemic cholangiopathy was 15% versus 22% (p = 0.73) and 5% versus 2% (p = 1), respectively. One-year patient and graft survival was 100% versus 95% (p = 0.18) and 90% versus 95% (p = 0.82). In conclusion, the association of A-NRP + D-HOPE in DCD LT with prolonged WIT allows achieving comparable outcomes to DBD LT.

Keywords: donation after circulatory death, abdominal normothermic regional perfusion, hypothermic oxygenated machine perfusion, warm ischemia time, ischemic cholangiopathy, liver transplantation outcome



GRAPHICAL ABSTRACT |

INTRODUCTION

In liver transplantation (LT) using grafts from donors whose death has been determined by circulatory criteria (DCD), warm ischemia time (WIT) has a major impact on the outcome. Prolonged WIT has consistently been associated with an increased risk of primary non-function, ischemic cholangiopathy (IC) and inferior graft survival (1–5). In contrast with most countries with active DCD transplant programs, Italian law requires a 20-min period of absent cardiac electrical activity for death declaration (6), which significantly increases the risks associated with the use of these grafts and has slowed down implementation of DCD LT in Italy (7).

However, mainly prompted by the favourable Spanish experience with the use of abdominal normothermic regional perfusion (A-NRP) to recover DCD liver grafts from Maastricht category 2 donors (8), DCD LT was introduced in Italy in 2015 (9, 10). Given the unique characteristics of the Italian setting, use of A-NRP has been established as mandatory, while subsequent ex-situ machine perfusion (MP) has been encouraged and adopted by most centres.

A growing body of literature supports the benefits of A-NRP for livers procured from Maastricht category 3 (controlled) DCD donors (11–17). In the same setting, use of end-ischemic (dual)-hypothermic oxygenated machine perfusion (HOPE/D-HOPE) has been consistently associated with better liver graft function and lower incidence of IC as compared to static cold storage (SCS) (18–21). However, these studies reported shorter WIT compared to what can possibly be achieved in Italy.

In the Italian setting, previous studies have shown that the association of A-NRP with ex-situ machine perfusion for controlled DCD liver grafts allows achieving good LT outcomes (22–24), which appear to be comparable to those of DCD livers preserved by ultra-rapid recovery and preserved by SCS (25). However, a formal comparison with LT using livers from donors after neurologic determination of death (DBD) accounting for potential confounders and demonstrating comparable outcomes is still lacking.

Thus, the aim of the study was to report our results with the use of A-NRP + D-HOPE for controlled DCD liver grafts with prolonged WIT. To assess the effectiveness of this approach, outcomes of DCD grafts recipients were compared to those of a matched cohort of DBD LTs, selected using propensity score matching.

MATERIALS AND METHODS

Prospectively collected data on adult (≥18-year-old) patients who underwent LT at our centre from January 2016 to July 2021 were retrospectively analyzed. Collected data included donor and recipient baseline characteristics, operational details, and prognostic scores (26, 27). The UK-DCD risk score (4), a prognostic score for DCD LT based on 4 donor and 3 recipient variables, was used to grade the risk profile associated with each case. Recipients of a combined transplant, retransplant, partial graft or suffering from on-table death were excluded. To limit confounding, recipients of a DBD graft treated with any type of machine perfusion were also excluded, as well as recipients of Maastricht category 2 DCD grafts and of Maastricht category 3

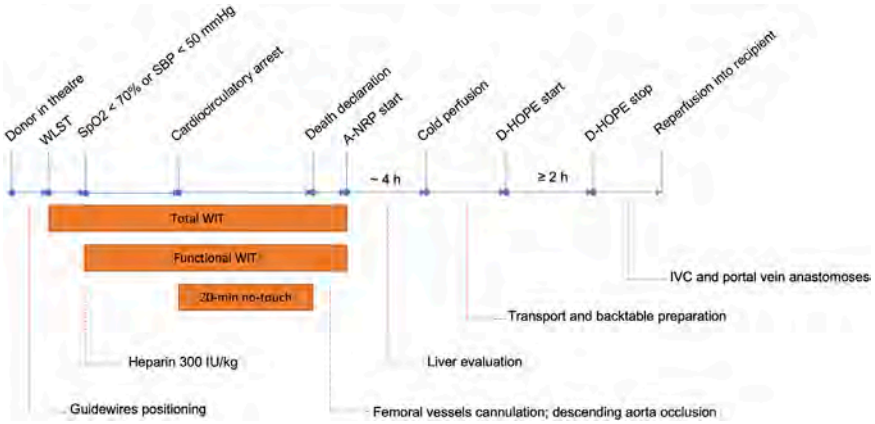


FIGURE 1 | DCD procurement protocol. Abbreviations: WLST, withdrawal of life-sustaining treatment; SBP, systemic blood pressure; A-NRP, abdominal normothermic regional perfusion; D-HOPE, dual hypothermic oxygenated machine perfusion; WIT, warm ischemia time; IVC, inferior vena cava.

DCD grafts treated with a machine perfusion modality other than D-HOPE. To control selection bias, two comparable cohorts of DBD and controlled DCD LTs were selected using 1:2 propensity score matching. Minimal patient follow-up was 6 months. The study was conducted according to the principles of the Istanbul and Helsinki declarations and was approved by the ethics committee of our Institution (protocol 506/2021).

Our procurement and machine perfusion protocols are depicted in **Figure 1**. Briefly, withdrawal of life-sustaining treatment (WLST) took place in the operating theatre, after guidewires for subsequent femoral vessels cannulation had been placed under ultrasound guidance (pre-mortem cannulation is not allowed in Italy). At the onset of functional warm ischemia (peripheral O_2 saturation ≤70% or systolic blood pressure ≤50 mmHg, whichever occurred first) 300 IU/kg heparin was administered. After 20-min electrical asystole, death was declared, femoral vessels were cannulated and descending aorta was occluded by an endovascular balloon or a surgical clamp, depending on theatre logistic, after which A-NRP was started. During A-NRP, pump flow was maintained ≥1.7 L/min/m^2 and temperature at 35–36°C (28). Target perfusion pressure was 55–70 mmHg, which was sustained using low dose vasopressin or norepinephrine when necessary, in addition to flow settings and fluid replacement. The circuit sweep gas levels (FiO_2 and air flow) were adjusted to maintain $PaCO_2$ between 35 and 45 mmHg, SaO_2 about 96–98%, and SvO2 > 60%. If needed, packed red blood cells were transfused to maintain haematocrit ≥20%. Heparin boluses were administered based on activated clotting time values. Blood samples were obtained prior to A-NRP start, at 30 min and then hourly to adjust A-NRP parameters (gas flow, blood flow, FiO_2, pump speed) and to assess liver injury and function. Target A-NRP duration was 4 h and it was never less than 2 h or more than 6 h. During A-NRP, liver viability assessment was based on a modified version of the criteria proposed by De Carlis et al. (29), including pump flow >1.7 lt/min/m^2, transaminase level <1,000 IU/L, downward lactate trend, absence of significant (≥15%) macrovesicular steatosis or Ishak >1 fibrosis, good liver and abdominal viscera perfusion, and evidence of bile production. A liver biopsy was systematically obtained to rule out significant necrosis or macrovesicular steatosis. At the end of A-NRP, the liver graft was cold flushed with Celsior (IGL, Lissieu, France) solution through the arterial cannula and trough a portal vein tributary. Liver was prepared on the backtable immediately upon arrival at our transplant centre and subsequently underwent a minimum of 2 h of D-HOPE using the LiverAssist device (XVivo, Groningen, Netherlands), primed with 3 lt of Belzer MP solution (BridgeToLife, Northbrook, IL). Temperature, portal vein and hepatic artery pressure were set at 8–10°C, 3–5 mmHg and 25 mmHg, respectively. D-HOPE was not used with the purpose of viability assessment and all grafts treated by D-HOPE were subsequently transplanted. At the end of recipient hepatectomy, the liver was disconnected from the device and brought to the operating table for implantation.

In DBD group, the liver was flushed with Celsior and preserved by static cold storage until implantation into the recipient. In both groups, the liver was flushed with chilled 5% albumin solution before implantation.

As a rule, liver transplant was performed by the piggyback technique with portal reperfusion first. Following hepatic artery anastomosis, an end-to-end biliary anastomosis was performed using a 2.5 mm T-tube. In all patients graft histology was assessed on time-0 biopsies, which were systematically obtained at the end of transplant operation. Standard immunosuppression included basiliximab, tacrolimus, steroids and mycophenolate mofetil, and was not modified according to treatment group.

Early outcome endpoints included rate of post-reperfusion syndrome (30, 31), transaminase peak, early allograft dysfunction (32), rate and severity of acute kidney injury (AKI) (33), requirement for renal replacement therapy, hospital and intensive care unit (ICU) stay, postoperative complications (34, 35), and the rate of early graft failure (EAF), defined as death of relisting for LT withing 90 days from transplant.

Post-reperfusion syndrome was defined as a drop in mean arterial pression ≥30% from the baseline for at least 1 min and within 5 min from reperfusion (30), whereas severe post-reperfusion syndrome was defined as the onset of severe hemodynamic instability, persistent hypotension, cardiac arrest

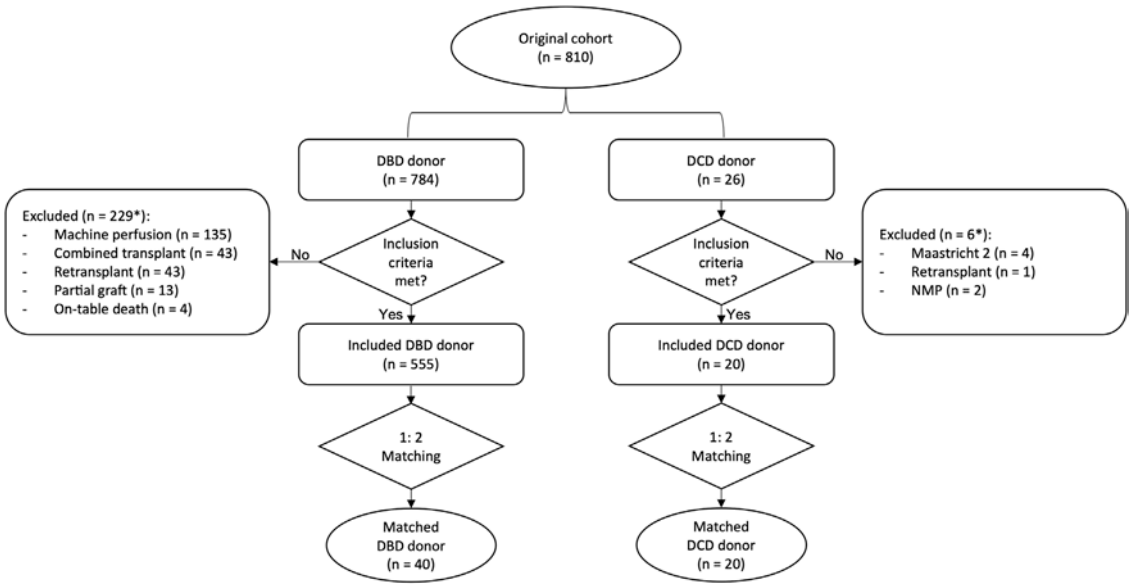


FIGURE 2 | Patient selection flowchart. *some patients met more than one exclusion criterium. NMP, normothermic machine perfusion.

or hemodynamically significant arrhythmias (31). EAD and AKI were defined according to Olthoff et al. (32) and KDIGO guidelines (33). Postoperative complications were graded according to Clavien-Dindo classification (34), which was also used to calculate comprehensive complication index (CCI) (35).

Biliary complications (36) were diagnosed based on the 3-month cholangiogram obtained before removing the T-tube, or by magnetic resonance cholangiopancreatography (MRCP), which was performed if clinically indicated. Recipients of a DCD graft underwent systematic 6-month and 12-month MRCP.

Variables are presented as number (percentage) of median (interquartile range), as appropriate, and compared using Fisher's, Chi-square and Mann-Whitney tests. To control selection bias, 1:2 propensity score matching without replacement and using the nearest method was used to select two patient cohorts with comparable characteristics. Variables included in the model were recipient age, body mass index (BMI) and model for end-stage liver disease (MELD) score, hepatocellular carcinoma (HCC) as an indication for LT, donor age and BMI, percentage of macrovesicular steatosis and presence of macrovesicular steatosis ≥15%. Standardized mean differences were used to assess balance obtained by propensity score matching. Patient and graft survival was analyzed using Kaplan-Meier curves. Statistical analysis was performed using R: a language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

During study period, 810 adult LTs were performed, of which 26 using organs proceeding from a DCD donor (cat. 3, $n = 22$; cat. 2, $n = 4$). A total of 37 category 3 DCD donors were signalled in our region during study period, of which 22 were transplanted by our centre. As per Italian regulations, livers from regional DCD donors were allocated locally to our centre, which is the only liver transplant centre in our region, and referred elsewhere only upon refusal by our unit. Four livers were refused based on donor characteristics and the organs were reallocated to other centres. Three of these grafts were successfully transplanted, whereas one was discarded during A-NRP due to elevated transaminases and lack of lactate clearance. Of the remaining 11 livers, 6 were discarded by our and all other Italian centres based on donor features, whereas of 5 offers initially accepted by our centre, 2 were subsequently discarded due to excessive functional WIT, and 3 during A-NRP. The reason to discard the liver during A-NRP was mainly elevated transaminases, which was associated to persistently elevated lactate levels in one case and evidence of gallbladder and bile duct necrosis in another. No liver was discarded based on histological findings. Overall utilization rate of livers from category 3 DCD donors was 25/37 (67.6%), whereas it was 22/37 (59.5%) if we consider only those transplanted at our centre.

Based on exclusion criteria, 229 and 6 patients were excluded from DBD and DCD group, respectively (**Figure 2**). In the DCD group, besides 4 recipients of livers from category 2 DCD donors, 2 further cases, including one retransplant, were excluded due to the use of normothermic machine perfusion instead of D-HOPE. Thus, 555 DBD and 20 DCD liver transplants were included for analysis. Finally, outcomes of the 20 DCD LTs were compared to those of 40 recipients of a DBD graft, selected by 1:2 propensity score matching.

Baseline patient and donor characteristics and operational details are summarized in **Table 1**. In the DCD group, median donor age and BMI were 60.1 (55.1, 61.5) and 25.0 [23.0, 26.1], and only one liver had 15% macrovesicular steatosis, reflecting our policy of avoiding overlap of additional donor risk factors in this high-risk cohort, characterized by a functional WIT of 43 (35, 46) min. A-NRP and D-HOPE times were 246 (221, 269) and 205

TABLE 1 | Baseline covariates balance.

	Whole cohort				Matched cohort		
	DBD	**DCD**	**p**	**SMD**	**DBD**	**DCD**	**SMD**
n	555	20			40	20	
Rec. age	57.5 [52.4, 62.1]	60.7 [57.4, 66.7]	0.02	0.64	60.6 [56.2, 65.6]	60.7 [57.4, 66.7]	0.04
Gender (male)	404 (73)	16 (80)	0.65	0.17	30 (75)	16 (80)	0.12
Rec. BMI	25.0 [22.7, 27.7]	25.3 [22.6, 27.3]	0.90	0.05	25.2 [22.5, 27.8]	25.3 [22.6, 27.3]	0.01
Indication			0.28	0.65			0.76
Viral hepatitis	276 (50)	9 (45)			27 (68)	9 (45)	
Alcoholic cirrhosis	98 (18)	6 (30)			7 (18)	6 (30)	
Cholestatic disease	39 (7)	2 (10)			0 (0)	2 (10)	
NASH	17 (3)	2 (10)			1 (2)	2 (10)	
Autoimmune	16 (3)	0 (0)			0 (0)	0 (0)	
Acute liver failure	3 (1)	0 (0)			0 (0)	0 (0)	
Other	106 (19)	1 (5)			5 (12)	1 (5)	
MELD	13.0 [9.0, 18.0]	10.5 [8.8, 14.5]	0.17	0.21	11.5 [8.0, 17.2]	10.5 [8.8, 14.5]	0.10
Creatinine (mg/dl)	0.8 [0.7, 1.1]	0.8 [0.7, 1.0]	0.95	0.02	0.9 [0.7, 1.2]	0.8 [0.7, 1.0]	0.20
Dialysis pre-LT	11 (2)	0 (0)	1.00	0.20	1 (2)	0 (0)	0.23
Prev. abdo. surgery	206 (37)	10 (50)	0.35	0.26	21 (52)	10 (50)	0.05
Life support	17 (3)	1 (5)	1.00	0.10	1 (2)	1 (5)	0.13
Ascites	211 (38)	7 (35)	0.96	0.06	14 (35)	7 (35)	<0.01
Encephalopathy	114 (21)	2 (10)	0.38	0.30	7 (18)	2 (10)	0.22
HCC	296 (53)	16 (80)	0.03	0.59	33 (82)	16 (80)	0.06
Donor age	65.4 [52.4, 74.4]	60.1 [55.1, 61.5]	0.13	0.30	63.1 [44.8, 71.7]	60.1 [55.1, 61.5]	0.04
Donor BMI	25.3 [22.9, 27.7]	25.0 [23.0, 26.1]	0.57	0.17	25.3 [23.3, 27.6]	25.0 [23.0, 26.1]	0.14
Macrosteatosis (%)	1.0 [0.0, 5.0]	0.0 [0.0, 1.2]	0.05	0.35	0.0 [0.0, 3.5]	0.0 [0.0, 1.2]	0.02
Macrosteatosis ≥15%	64 (12)	1 (5)	0.57	0.24	2 (5)	1 (5)	<0.01
Microsteatosis (%)	10.0 [1.0, 25.0]	5.0 [0.0, 10.0]	0.04	0.53	10.0 [4.5, 20.0]	5.0 [0.0, 10.0]	0.36
D-MELD	800 [573, 1117]	542 [488, 1014]	0.05	0.33	699 [533, 977]	542 [488, 1014]	0.12
BAR	5.0 [3.0, 19.0]	5.0 [3.0, 8.0]	0.99	0.18	5.0 [3.0, 17.0]	5.0 [3.0, 8.0]	0.09
WIT (min)		43 [40, 48]				43 [40, 48]	
Functional WIT (min)		43 [35, 46]				43 [35, 46]	
A-NRP time (min)		246 [221, 269]				246 [221, 269]	
CIT (min)	431 [379, 482]	261 [229, 295]	<0.01	2.06	418 [375, 510]	261 [229, 295]	1.86
D-HOPE time (min)		205 [146, 277]				205 [146, 277]	
Total pres. time (min)	431 [379, 482]	492 [426, 531]	0.01	0.65	418 [375, 510]	492 [426, 531]	0.58
Portal rep. time (min)	23.0 [21.0, 27.0]	22.0 [20.5, 26.2]	0.47	0.19	23.0 [21.0, 26.2]	22.0 [20.5, 26.2]	0.01
Total rep. time (min)	38.0 [24.0, 50.2]	48.5 [42.0, 59.5]	0.01	0.51	41.0 [24.0, 55.2]	48.5 [42.0, 59.5]	0.41
PRBC units (n)	3.0 [0.0, 8.0]	2.5 [0.0, 7.2]	0.70	0.04	5.0 [0.8, 9.2]	2.5 [0.0, 7.2]	0.01
Graft weight (gr)	1490 [1290, 1720]	1455 [1222, 1610]	0.39	0.19	1475 [1295, 1692]	1455 [1222, 1610]	0.09

*Abbreviations: SMD, standardized mean difference; BMI, body mass index; NASH, non-alcoholic steatohepatitis; MELD, model for end-stage liver disease; prev, previous; HCC, hepatocellular carcinoma; D-MELD, donor age * MELD score; BAR, balance of risk score; WIT, warm ischemia time; A-NRP, abdominal normothermic regional perfusion; CIT, cold ischemia time; D-HOPE, dual hypothermic oxygenated machine perfusion; pres, preservation; rep, reperfusion; PRBC, packed red blood cells.*

(146, 277) min, respectively. DCD livers were preferentially allocated to low-MELD (10.5 [8.8, 14.5]) patients, with HCC being the indication for LT in 80% of cases. However, with increasing experience, livers from elderly donors were also accepted and procured organs were more frequently allocated to higher-MELD recipients (**Figure 3**). Despite donor and recipient selection, median UK-DCD risk score (4) was 13 (11, 14) with 17 cases being classified as "futile" and 3 as "high-risk".

Patient cohorts selected by propensity score matching showed good comparability, as reflected by a standardized mean difference ≤0.10 for all major confounders, including recipient age, BMI and MELD score, HCC as the indication for LT, donor age, graft macrovesicular steatosis, balance of risk (BAR) score and portal reperfusion time (**Table 1**).

Outcomes in the unmatched and matched cohort are reported in **Table 2**. Overall, early outcomes in the DCD group were comparable to those observed in the DBD group.

In the DCD and DBD group, EAD and grade 2/3 AKI rates were 5% versus 15% and 15% versus 22%, respectively, with no patient requiring renal replacement therapy after LT. Five (25%) and 8 (20%) recipients of a DCD or DBD liver, respectively, developed grade ≥3 complications and median comprehensive complication index was 16.5 (0.0, 33.9) versus 21.8 (8.7, 35.4). Intensive care unit and hospital length of stay was 4 (2, 5) versus 4 (2, 6) and 10 (8, 19.5) versus 12 (9, 19) days, respectively. Two grafts were lost in the DCD group, which were the first and the second in our series. The first graft loss resulted from a hepatic artery injury that occurred during an attempt at performing hepaticojejunostomy for a late biliary fistula 97 days after LT. The vascular injury resulted from the severe inflammatory reaction caused by the biloma involving the porta hepatis and was deemed not amenable to repair. The second graft loss was caused by hepatic artery thrombosis occurring on postoperative day 2. Despite the graft was showing good function, large necrotic areas were apparent at computed tomography scan, so a decision was made

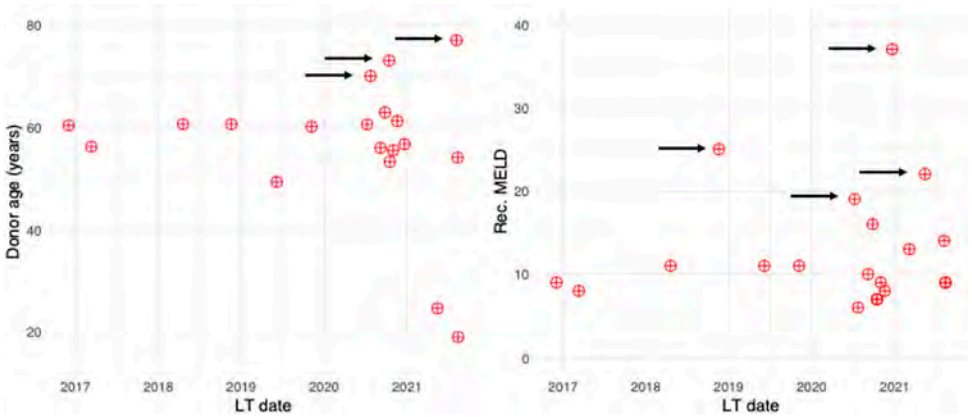


FIGURE 3 | Scatter plot depicting donor age and recipient MELD as a function of study period. During study period, donors of increasing age were considered, and DCD grafts were more frequently allocated to higher-MELD recipients (arrows).

TABLE 2 | Outcome.

	Whole cohort			Matched cohort		
	DBD	**DCD**	**p**	**DBD**	**DCD**	**p**
n	555	20		40	20	
Severe PRS	77 (14)	3 (15)	1.00	4 (10)	3 (15)	0.89
End-LT lactate (mmol/l)	2.0 [1.4, 2.8]	1.6 [1.0, 2.4]	0.13	2.0 [1.4, 2.9]	1.6 [1.0, 2.4]	0.26
AST peak (IU/L)	1111 [692, 1752]	761 [589, 1345]	0.13	937 [663, 1438]	761 [589, 1345]	0.63
ALT peak (IU/L)	702 [448, 1126]	461 [385, 608]	0.01	632 [360, 835]	461 [385, 608]	0.18
EAD	157 (28)	1 (5)	0.04	6 (15)	1 (5)	0.48
AKI stage			0.53			0.27
0	178 (32)	8 (40)		10 (25)	8 (40)	
1	226 (41)	9 (45)		21 (52)	9 (45)	
2	107 (19)	3 (15)		4 (10)	3 (15)	
3	44 (8)	0 (0)		5 (12)	0 (0)	
Grade 2/3 AKI	151 (27)	3 (15)	0.34	9 (22)	3 (15)	0.73
Renal replacement therapy	13 (2)	0 (0)	1.00	0 (0)	0 (0)	NA
Early rejection	46 (8)	1 (5)	0.91	3 (8)	1 (5)	1.00
Grade ≥3 complications	126 (23)	5 (25)	1.00	8 (20)	5 (25)	0.91
ICU stay (days)	3.0 [2.0, 5.0]	4.0 [2.0, 5.0]	0.92	4.0 [2.0, 6.0]	4.0 [2.0, 5.0]	0.55
Hospital stay (days)	12.0 [9.0, 17.0]	10.0 [8.0, 19.5]	0.59	12.0 [9.0, 19.0]	10.0 [8.0, 19.5]	0.35
Hospital CCI	22.6 [12.0, 33.7]	16.5 [0.0, 33.9]	0.10	21.8 [8.7, 35.4]	16.5 [0.0, 33.9]	0.26
Early allograft failure	28 (5)	1 (5)	1.00	2 (5)	1 (5)	1.00
Biliary complications						
Anastomotic	85 (15)	3 (15)	1.00	9 (22)	3 (15)	0.73
Fistula	10 (2)	1 (5)	0.85	2 (5)	1 (5)	1.00
Stricture	75 (14)	2 (10)	0.91	7 (18)	2 (10)	0.70
IC	28 (5)	1 (5)	1.00	1 (2)	1 (5)	1.00
Treatment			0.06			0.15
Operational	69 (71)	1 (33)		7 (78)	1 (33)	
Surgery	24 (25)	1 (33)		2 (22)	1 (33)	
Retransplant	4 (4)	1 (33)		0 (0)	1 (33)	
N° of treatments	2.0 [1.0, 3.0]	3.0 [2.5, 4.5]	0.33	2.0 [2.0, 3.0]	3.0 [2.5, 4.5]	0.43
Determining graft loss	5 (1)	1 (5)	0.51	0 (0)	1 (5)	0.72
Determining patient death	1 (0)	0 (0)	1.00	0 (0)	0 (0)	NA

Abbreviations: PRS, post-reperfusion syndrome; LT, liver transplant; EAD, early allograft dysfunction; AKI, acute kidney injury; ICU, intensive care unit; CCI, comprehensive complication index; IC, ischemic cholangiopathy.

to relist the recipient for urgent retransplantation. Both patients were successfully retransplanted.

The rate of anastomotic biliary complications and ischemic cholangiopathy was comparable between groups (**Table 2**). In particular, only one case of IC was observed in the DCD group. This patient had a percutaneous biliary drain inserted before undergoing hepaticojejunostomy for a tight anastomotic stricture. Cholangiogram showed an isolated posterior duct

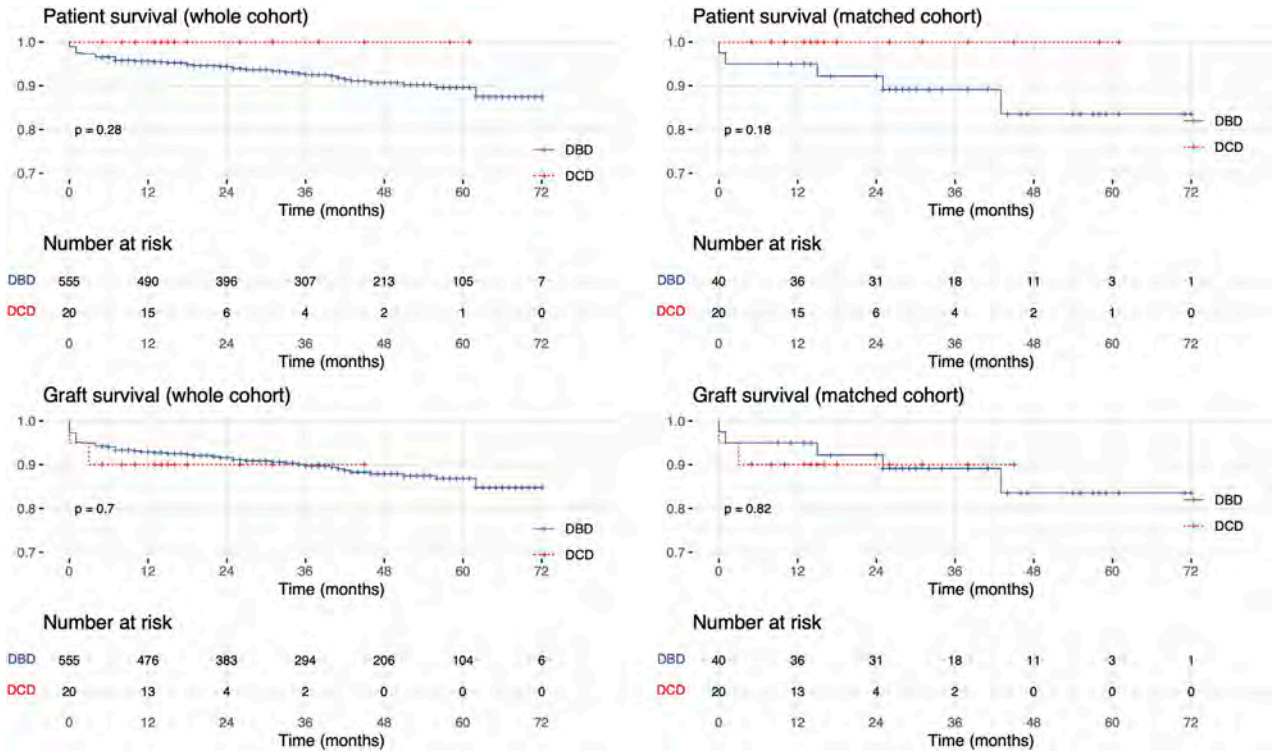


FIGURE 4 | Kaplan-Meier patient and graft survival curves in the unmatched and matched cohorts.

stricture, likely representing an incidental finding. Patient was treated with a single balloon dilatation and has neither clinical nor radiological evidence of recurrence 8 months after the procedure.

Median follow-up was 40 (21, 56) and 15.5 (12, 27) months in the DBD and DCD group, respectively. Graft and patient survival was comparable between groups (**Figure 4**). In the matched cohort, 1-year patient survival in the DCD and DBD group was 100% (confidence interval [CI] = 100%, 100%) and 95% (CI = 88.5%, 100%), respectively, whereas 1-year graft survival was 90% (CI = 77.8%, 100%) and 95% (CI = 88.5%, 100%).

DISCUSSION

This study shows that a combination of A-NRP followed by D-HOPE is effective in preserving grafts from controlled DCD donors with prolonged WIT and allows obtaining comparable outcomes to DBD LT. These results appear to be even more remarkable if some peculiarities of the Italian setting are considered. Besides the 20-min no-touch time, which is unique among countries with active DCD programs (6), pre-mortem cannulation is not allowed in Italy, which further prolongs WIT due to the time necessary to cannulate femoral vessels and occlude the descending aorta (**Figure 1**). Furthermore, as the required 20 min of flat EKG recording are preceded by a variable time of pulseless electric activity, procured organs are exposed to a no-flow time that is frequently much longer than the 20-min no-touch time. If these livers were procured by ultra-rapid recovery and preserved by static cold storage, a poor outcome would be expected (1–4). In contrast, reconditioning and preservation by A-NRP + D-HOPE appears to allow obtaining good results, which are not different from those observed after DBD LT. It is worth noting that, despite initial concerns and logistic obstacles, our ~60% utilization rate compares favourably with that observed in other realities (37, 38).

Overall, our results confirm the benefits of both A-NRP and D-HOPE in controlled DCD LT. As compared to ultra-rapid recovery followed by static cold storage, use of A-NRP has been associated with better graft function, lower rate of overall biliary complications and ischemic cholangiopathy, and improved graft survival (11–13, 15–17, 39). A recent large Spanish study has shown that use of A-NRP alone in DCD LT allows achieving comparable outcome to DBD LT (13). Additionally, use of A-NRP appears to positively impact on utilization rate and post-transplant function of other abdominal organs, especially kidneys (40, 41). On the other hand, DCD LT is the setting in which the advantages of end-ischemic D-HOPE have been more convincingly demonstrated (18, 19, 21, 42–44), with a recent randomized controlled trial showing that use of D-HOPE in this context is associated with a significant reduction of symptomatic non-anastomotic biliary stricture incidence from 18% to 6% (19). However, these data come from countries where local regulations allow usually limiting WIT to 10–15 min, which is much shorter than what is currently observed in Italy. Therefore, Italian centres have frequently considered to combine these two approaches. In

Italy, successful use of controlled DCD donors by combining A-NRP and D-HOPE or normothermic machine perfusion has already been reported (9, 10, 22–24, 29), with a recent study by De Carlis et al.(25) showing that, despite longer WIT, outcome of liver grafts procured by this approach is comparable to those of DCD liver grafts procured by ultra-rapid recovery and SCS. To our knowledge, the present study is the first suggesting that the outcome of controlled DCD LT performed by combining A-NRP and D-HOPE, despite a functional WIT almost invariably exceeding 40 min, is not inferior to that of matched DBD LT.

Undoubtedly, these favourable results also issue from accurate donor selection and liver function assessment during A-NRP. In our experience, four (12.9%) initially accepted grafts were discarded based on parameters obtained during A-NRP. Different criteria for liver viability assessment during A-NRP have been proposed in different countries (8, 16, 17, 45, 46). Given the expected long WIT, we chose to adopt a modified version of the rather unrestrictive criteria proposed by De Carlis et al.(29). These criteria were not modified during study period and are still currently adopted at our centre. The good outcome observed in our series seems to confirm their validity. However, these data must be considered preliminary and future larger studies should investigate whether these criteria could be safely expanded further.

As LT outcomes are also influenced by recipient condition (26, 27), it is likely that recipient selection also played a role in achieving the good results observed in this series. This is the reason why, in order to allow a meaningful comparison, recipient characteristics were accounted for in the matching process. However, although initially DCD livers were preferentially allocated to low-MELD patients undergoing LT for HCC, the good results observed during the initial phases of this study fostered an increased confidence with DCD grafts utilization, which led to consider donor of progressively increasing age and to allocate DCD grafts also to patients with severe hepatic disease (**Figure 3**), without observing any detrimental effect on outcomes. This was also associated with an increasing number of DCD LTs per year (**Figure 3**). Overall, these findings are in keeping with the good outcome achieved and reflect how utilization of DCD liver grafts has become standard practice.

Limitations of our study include retrospective single-centre design and limited numerosity. Given the exploratory nature of this analysis, formal sample size calculation was not made. Also, as the majority of DCD LTs were performed in 2020–2021, follow-up was shorter in DCD group. Although 6-months minimal follow-up should have allowed capturing the majority of biliary complications, late-onset complications could have been missed. We are aware that an updated definition of functional WIT has been recently introduced (47). However, all cases included in this study were antecedent to its introduction and a retrospective recalculation of functional WIT was not possible. Finally, as all grafts included in this study were treated with D-HOPE, we could not evaluate the additional value of D-HOPE after A-NRP. It could be argued that use of machine perfusion could be omitted in selected cases, whereas additional viability assessment by normothermic machine perfusion could be indicated in others (48). In our experience, use of D-HOPE has been systematic for grafts meeting all viability criteria during A-NRP, which are those included in this series. So far, use of normothermic machine perfusion has been limited to cases characterized by doubtful evaluation during A-NRP (24), or in which logistics constraints imposed prolonging preservation time. Well designed and appropriately powered randomized studies are needed to define when and by which modality machine perfusion after A-NRP is indicated in DCD LT.

In conclusion, despite apparently prohibitive WIT, outcome of LT using livers from controlled DCD donors treated by a combination of A-NRP and D-HOPE is comparable to that of DBD LT, suggesting that a wider implementation of this approach could contribute improving the results of DCD LT and expand donor pool. Larger studies are required to confirm these findings, refine our evaluation process, and establish when and by which modality machine perfusion is indicated in this setting.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comitato Etico Interaziendale A.O.U. Città della Salute e della Scienza di Torino–A.O. Ordine Mauriziano–A.S.L. Città di Torino. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

DP designed the study, collected and analysed data, and drafted the paper; MZ, MV, and RP contributed to data collection, data analysis and paper drafting; CM and ED collected data and critically revised the paper; GR, SC, SM, and DC contributed to data collection and critically revised the manuscript; SL, RB, and RR critically revised 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.

ACKNOWLEDGMENTS

The authors are deeply thankful to all healthcare professionals who, with astonishing dedication and despite the disruption caused by Covid-19 pandemic in recent years, have supported and keep supporting transplant activity in our region.

REFERENCES

1. Blok JJ, Detry O, Putter H, Rogiers X, Porte RJ, van Hoek B, et al. Longterm Results of Liver Transplantation from Donation after Circulatory Death. *Liver Transpl* (2016) 22:1107–14. doi:10.1002/lt.24449
2. Coffey JC, Wanis KN, Monbaliu D, Gilbo N, Selzner M, Vachharajani N, et al. The Influence of Functional Warm Ischemia Time on DCD Liver Transplant Recipients' Outcomes. *Clin Transpl* (2017) 31:31. doi:10.1111/ctr.13068
3. Mateo R, Cho Y, Singh G, Stapfer M, Donovan J, Kahn J, et al. Risk Factors for Graft Survival after Liver Transplantation from Donation after Cardiac Death Donors: an Analysis of OPTN/UNOS Data. *Am J Transpl* (2006) 6:791–6. doi:10.1111/j.1600-6143.2006.01243.x
4. Schlegel A, Kalisvaart M, Scalera I, Laing RW, Mergental H, Mirza DF, et al. The UK DCD Risk Score: A New Proposal to Define Futility in Donation-After-Circulatory-Death Liver Transplantation. *J Hepatol* (2018) 68:456–64. doi:10.1016/j.jhep.2017.10.034
5. Kalisvaart M, Schlegel A, Umbro I, de Haan JE, Scalera I, Polak WG, et al. The Impact of Combined Warm Ischemia Time on Development of Acute Kidney Injury in Donation after Circulatory Death Liver Transplantation. *Transplantation* (2018) 102:783–93. doi:10.1097/tp.0000000000002085
6. 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) 33:76–88. doi:10.1111/tri.13506
7. Vergano M, Magavern E, Baroncelli F, Frisenda V, Fonsato A, Artusio D, et al. Making a Case for Controlled Organ Donation after Cardiac Death: the story of Italy's First Experience. *J Crit Care* (2017) 38:129–31. doi:10.1016/j.jcrc.2016.10.028
8. Fondevila C, Hessheimer AJ, Ruiz A, Calatayud D, Ferrer J, Charco R, et al. Liver Transplant Using Donors after Unexpected Cardiac Death: Novel Preservation Protocol and Acceptance Criteria. *Am J Transpl* (2007) 7:1849–55. doi:10.1111/j.1600-6143.2007.01846.x
9. De Carlis L, De Carlis R, Lauterio A, Di Sandro S, Ferla F, Zanierato M. Sequential Use of Normothermic Regional Perfusion and Hypothermic Machine Perfusion in Donation after Cardiac Death Liver Transplantation with Extended Warm Ischemia Time. *Transplantation* (2016) 100:e101–e102. doi:10.1097/tp.0000000000001419
10. De Carlis L, Lauterio A, De Carlis R, Ferla F, Di Sandro S. Donation after Cardiac Death Liver Transplantation after More Than 20 minutes of Circulatory Arrest and Normothermic Regional Perfusion. *Transplantation* (2016) 100:e21–e22. doi:10.1097/tp.0000000000001136
11. Hessheimer AJ, Coll E, Torres F, Ruíz P, Gastaca M, Rivas JI, et al. Normothermic Regional Perfusion vs. Super-rapid Recovery in Controlled Donation after Circulatory Death Liver Transplantation. *J Hepatol* (2019) 70:658–65. doi:10.1016/j.jhep.2018.12.013
12. Ruiz P, Gastaca M, Bustamante FJ, Ventoso A, Palomares I, Prieto M, et al. Favorable Outcomes after Liver Transplantation with Normothermic Regional Perfusion from Donors after Circulatory Death: A Single-center Experience. *Transplantation* (2019) 103:938–43. doi:10.1097/tp.0000000000002391
13. Ruiz P, Valdivieso A, Palomares I, Prieto M, Ventoso A, Salvador P, et al. Similar Results in Liver Transplantation from Controlled Donation after Circulatory Death Donors with Normothermic Regional Perfusion and Donation after Brain Death Donors: A Case-Matched Single-Center Study. *Liver Transpl* (2021) 27:1747–57. doi:10.1002/lt.26281
14. 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) 104:1943–51. doi:10.1097/tp.0000000000003372
15. Oniscu GC, Randle LV, Muiesan P, Butler AJ, Currie IS, Perera MTPR, et al. In SituNormothermic Regional Perfusion for Controlled Donation after Circulatory Death-The United Kingdom Experience. *Am J Transpl* (2014) 14:2846–54. doi:10.1111/ajt.12927
16. Watson CJE, Hunt F, Messer S, Currie I, Large S, Sutherland A, et al. *In Situ* normothermic Perfusion of Livers in Controlled Circulatory Death Donation May Prevent Ischemic Cholangiopathy and Improve Graft Survival. *Am J Transpl* (2019) 19:1745–58. doi:10.1111/ajt.15241
17. Hessheimer AJ, de la Rosa G, Gastaca M, Ruíz P, Otero A, Gómez M, et al. Abdominal Normothermic Regional Perfusion in Controlled Donation after Circulatory Determination of Death Liver Transplantation: Outcomes and Risk Factors for Graft Loss. *Am J Transpl* (2021) 22:1169–81. doi:10.1111/ajt.16899
18. van Rijn R, Karimian N, Matton APM, Burlage LC, Westerkamp AC, van den Berg AP, et al. Dual Hypothermic Oxygenated Machine Perfusion in Liver Transplants Donated after Circulatory Death. *Br J Surg* (2017) 104:907–17. doi:10.1002/bjs.10515
19. van Rijn R, Schurink IJ, de Vries Y, van den Berg AP, Cortes Cerisuelo M, Darwish Murad S, et al. Hypothermic Machine Perfusion in Liver Transplantation - A Randomized Trial. *N Engl J Med* (2021) 384:1391–401. doi:10.1056/nejmoa2031532
20. van Rijn R, van Leeuwen OB, Matton APM, Burlage LC, Wiersema-Buist J, van den Heuvel MC, et al. Hypothermic Oxygenated Machine Perfusion Reduces Bile Duct Reperfusion Injury after Transplantation of Donation after Circulatory Death Livers. *Liver Transpl* (2018) 24:655–64. doi:10.1002/lt.25023
21. Schlegel A, Muller X, Kalisvaart M, Muellhaupt B, Perera MTPR, Isaac JR, et al. Outcomes of DCD Liver Transplantation Using Organs Treated by Hypothermic Oxygenated Perfusion before Implantation. *J Hepatol* (2019) 70:50–7. doi:10.1016/j.jhep.2018.10.005
22. De Carlis R, Di Sandro S, Lauterio A, Botta F, Ferla F, Andorno E, et al. Liver Grafts from Donors after Circulatory Death on Regional Perfusion with Extended Warm Ischemia Compared with Donors after Brain Death. *Liver Transpl* (2018) 24:1523–35. doi:10.1002/lt.25312
23. Dondossola D, Ravaioli M, Lonati C, Maroni L, Pini A, Accardo C, et al. The Role of *Ex Situ* Hypothermic Oxygenated Machine Perfusion and Cold Preservation Time in Extended Criteria Donation after Circulatory Death and Donation after Brain Death. *Liver Transpl* (2021) 27:1130–43. doi:10.1002/lt.26067
24. Ghinolfi D, Dondossola D, Rreka E, Lonati C, Pezzati D, Cacciatoinsilla A, et al. Sequential Use of Normothermic Regional and *Ex Situ* Machine Perfusion in Donation after Circulatory Death Liver Transplant. *Liver Transpl* (2021) 27:385–402. doi:10.1002/lt.25899
25. De Carlis R, Schlegel A, Frassoni S, Olivieri T, Ravaioli M, Camagni S, et al. How to Preserve Liver Grafts from Circulatory Death with Long Warm Ischemia? A Retrospective Italian Cohort Study with Normothermic Regional Perfusion and Hypothermic Oxygenated Perfusion. *Transplantation* (2021) 105:2385–96. doi:10.1097/tp.0000000000003595
26. Dutkowski P, Oberkofler CE, Slankamenac K, Puhan MA, Schadde E, Müllhaupt B, et al. Are There Better Guidelines for Allocation in Liver Transplantation? *Ann Surg* (2011) 254:745–54. doi:10.1097/sla.0b013e3182365081
27. Halldorson JB, Bakthavatsalam R, Fix O, Reyes JD, Perkins JD. D-MELD, a Simple Predictor of Post Liver Transplant Mortality for Optimization of Donor/Recipient Matching. *Am J Transpl* (2009) 9:318–26. doi:10.1111/j.1600-6143.2008.02491.x
28. Zanierato M, Dondossola D, Palleschi A, Zanella A. Donation after Circulatory Death: Possible Strategies for *In-Situ* Organ Preservation. *Minerva Anestesiol* (2020) 86:984–91. doi:10.23736/S0375-9393.20.14262-7
29. De Carlis R, Di Sandro S, Lauterio A, Ferla F, Dell'Acqua A, Zanierato M, et al. Successful Donation after Cardiac Death Liver Transplants with Prolonged Warm Ischemia Time Using Normothermic Regional Perfusion. *Liver Transpl* (2017) 23:166–73. doi:10.1002/lt.24666
30. Aggarwal S, Kang Y, Freeman JA, Fortunato FL, Pinsky MR. Postreperfusion Syndrome: Cardiovascular Collapse Following Hepatic Reperfusion during Liver Transplantation. *Transpl Proc* (1987) 19:54–5.
31. Hilmi I, Horton CN, Planinsic RM, Sakai T, Nicolau-Raducu R, Damian D, et al. The Impact of Postreperfusion Syndrome on Short-Term Patient and Liver Allograft Outcome in Patients Undergoing Orthotopic Liver Transplantation. *Liver Transpl* (2008) 14:504–8. doi:10.1002/lt.21381
32. Olthoff KM, Kulik L, Samstein B, Kaminski M, Abecassis M, Emond J, et al. Validation of a Current Definition of Early Allograft Dysfunction in Liver Transplant Recipients and Analysis of Risk Factors. *Liver Transpl* (2010) 16:943–9. doi:10.1002/lt.22091
33. Khwaja A. KDIGO Clinical Practice Guidelines for Acute Kidney Injury. *Nephron* (2012) 120:c179–c184. doi:10.1159/000339789

34. Dindo D, Demartines N, Clavien P-A. Classification of Surgical Complications. *Ann Surg* (2004) 240:205–13. doi:10.1097/01.sla.0000133083.54934.ae
35. Slankamenac K, Graf R, Barkun J, Puhan MA, Clavien P-A. The Comprehensive Complication Index. *Ann Surg* (2013) 258:1–7. doi:10.1097/sla.0b013e318296c732
36. de Vries Y, von Meijenfeldt FA, Porte RJ. Post-transplant Cholangiopathy: Classification, Pathogenesis, and Preventive Strategies. *Biochim Biophys Acta (Bba) - Mol Basis Dis* (2018) 1864:1507–15. doi:10.1016/j.bbadis.2017.06.013
37. Haque O, Yuan Q, Uygun K, Markmann JF. Evolving Utilization of Donation after Circulatory Death Livers in Liver Transplantation: The Day of DCD Has Come. *Clin Transpl* (2021) 35:e14211. doi:10.1111/ctr.14211
38. Research NBaT-SaC. *Organ and Tissue Donation and Transplantation Activity Report 2020/21. NHS Blood and Transplant* (2021). Available from: https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/24053/activity-report-2020-2021.pdf (Accessed on January 10, 2022).
39. Jochmans I, Hessheimer AJ, Neyrinck AP, Paredes D, Bellini MI, Dark JH, et al. Consensus Statement on Normothermic Regional Perfusion in Donation after Circulatory Death: Report from the European Society for Organ Transplantation's Transplant Learning Journey. *Transpl Int* (2021) 34:2019–30. doi:10.1111/tri.13951
40. De Beule J, Vandendriessche K, Pengel LHM, Bellini MI, Dark JH, Hessheimer AJ, et al. A Systematic Review and Meta-analyses of Regional Perfusion in Donation after Circulatory Death Solid Organ Transplantation. *Transpl Int* (2021) 34:2046–60. doi:10.1111/tri.14121
41. Gregorini M, Ticozzelli E, Abelli M, Grignano MA, Pattonieri EF, Giacomoni A, et al. Kidney Transplants from Donors on Extracorporeal Membrane Oxygenation Prior to Death Are Associated with Better Long-Term Renal Function Compared to Donors after Circulatory Death. *Transpl Int* (2021) 35:10179. doi:10.3389/ti.2021.10179
42. Dutkowski P, Polak WG, Muiesan P, Schlegel A, Verhoeven CJ, Scalera I, et al. First Comparison of Hypothermic Oxygenated PErfusion versus Static Cold Storage of Human Donation after Cardiac Death Liver Transplants. *Ann Surg* (2015) 262:764–71. doi:10.1097/sla.0000000000001473
43. Dutkowski P, Schlegel A, de Oliveira M, Müllhaupt B, Neff F, Clavien P-A. HOPE for Human Liver Grafts Obtained from Donors after Cardiac Death. *J Hepatol* (2014) 60:765–72. doi:10.1016/j.jhep.2013.11.023
44. Op den Dries S, Sutton ME, Karimian N, de Boer MT, Wiersema-Buist J, Gouw ASH, et al. Hypothermic Oxygenated Machine Perfusion Prevents Arteriolonecrosis of the Peribiliary Plexus in Pig Livers Donated after Circulatory Death. *PLoS One* (2014) 9:e88521. doi:10.1371/journal.pone.0088521
45. Justo I, Nutu A, García-Conde M, Marcacuzco A, Manrique A, Calvo J, et al. Incidence and Risk Factors of Primary Non-function after Liver Transplantation Using Grafts from Uncontrolled Donors after Circulatory Death. *Clin Transpl* (2021) 35:e14134. doi:10.1111/ctr.14134
46. Valero R, García-Valdecasas JC, Tabet J, Taurá P, Rull Rn., Beltran J, et al. Hepatic Blood Flow and Oxygen Extraction Ratio during Normothermic Recirculation and Total Body Cooling as Viability Predictors in Non-heart-beating Donor Pigs1. *Transplantation* (1998) 66:170–6. doi:10.1097/00007890-199807270-00005
47. Kalisvaart M, Croome KP, Hernandez-Alejandro R, Pirenne J, Cortés-Cerisuelo M, Miñambres E, et al. Donor Warm Ischemia Time in DCD Liver Transplantation-Working Group Report from the ILTS DCD, Liver Preservation, and Machine Perfusion Consensus Conference. *Transplantation* (2021) 105:1156–64. doi:10.1097/tp.0000000000003819
48. Bruggenwirth IMA, van Leeuwen OB, Porte RJ, Martins PN. The Emerging Role of Viability Testing during Liver Machine Perfusion. *Liver Transpl* (2021) 2021. doi:10.1002/lt.26092

ESOT | Transplant International

ORIGINAL RESEARCH
published: 13 April 2022
doi: 10.3389/ti.2022.10454

Treatment With Diflunisal in Domino Liver Transplant Recipients With Acquired Amyloid Neuropathy

*Velina Nedkova-Hristova[1,2], Carmen Baliellas[2,3], José González-Costello[2,4], Laura Lladó[2,3], Emma González-Vilatarsana[2,3], Valentina Vélez-Santamaría[1,2,5] and Carlos Casasnovas[1,2,5,6]**

[1]Neuromuscular Unit, Neurology Department, Bellvitge University Hospital-IDIBELL, Barcelona, Spain, [2]Multidisciplinary Unit of Familial Amyloidosis, Bellvitge University Hospital-IDIBELL, Barcelona, Spain, [3]Liver Transplantation Unit, Bellvitge University Hospital-IDIBELL, Barcelona, Spain, [4]Advanced Heart Failure and Transplantation Unit, Cardiology Department, Bellvitge University Hospital-IDIBELL, Barcelona, Spain, [5]Neurometabolic Diseases Group, Bellvitge Biomedical Research Institute (IDIBELL), Barcelona, Spain, [6]Biomedical Research Network Center in Rare Diseases (CIBERER), Valencia, Spain

Objectives: To analyze the efficacy and tolerability of diflunisal for the treatment of acquired amyloid neuropathy in domino liver transplant recipients.

Methods: We performed a retrospective longitudinal study of prospectively collected data for all domino liver transplant recipients with acquired amyloid neuropathy who received diflunisal at our hospital. Neurological deterioration was defined as an score increase of ≥2 points from baseline on the Neurological Impairment Scale/Neurological Impairment Scale-Lower Limbs.

Results: Twelve patients who had received compassionate use treatment with diflunisal were identified, of whom seven had follow-up data for ≥12 months. Five patients (71.4%) presented with neurological deterioration on the Neurological Impairment Scale after 12 months (p = 0.0382). The main adverse effects were cardiovascular and renal, leading to diflunisal being stopped in five patients and the dose being reduced in two patients.

Conclusion: Our study suggests that most domino liver transplant recipients with acquired amyloid neuropathy will develop neurological deterioration by 12 months of treatment with diflunisal. This therapy was also associated with a high incidence of adverse effects and low treatment retention. The low efficacy and low tolerability of diflunisal treatment encourage the search for new therapeutic options.

Keywords: diflunisal, transthyretin, amyloidosis, domino liver transplant, neuropathy

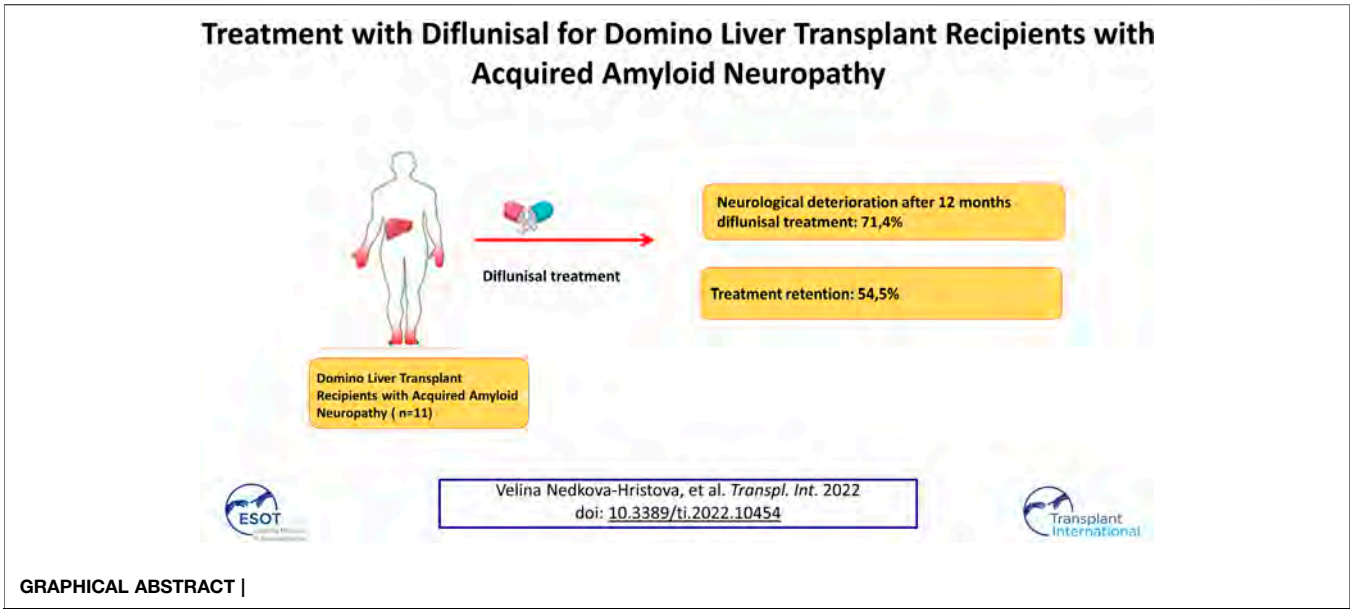
OPEN ACCESS

***Correspondence:**
Carlos Casasnovas
carloscasasnovas@bellvitgehospital.cat

Received: *22 February 2022*
Accepted: *10 March 2022*
Published: *13 April 2022*

Citation:
Nedkova-Hristova V, Baliellas C, González-Costello J, Lladó L, González-Vilatarsana E, Vélez-Santamaría V and Casasnovas C (2022) Treatment With Diflunisal in Domino Liver Transplant Recipients With Acquired Amyloid Neuropathy. Transpl Int 35:10454. doi: 10.3389/ti.2022.10454

Abbreviations: hTTRA, hereditary transthyretin-mediated amyloidosis; V30M, Val30Met; TTR, transthyretin; DLT, domino liver transplantation; FAP, familial amyloid neuropathy; AAN, acquired amyloid neuropathy; UMAF, Familial Amyloidosis Multidisciplinary Unit; NIS, Neurological Impairment Scale; NIS-LL, Neurological Impairment Scale-Lower Limbs; PND, Polyneuropathy Disability score; HCV, hepatitis C virus; HBV, hepatitis B virus; LC, liver cirrhosis; DLT, domino liver transplant; LT, liver transplant; DM, diabetes mellitus; M, male; UL, upper limbs; LL, lower limb.



GRAPHICAL ABSTRACT |

INTRODUCTION

Hereditary transthyretin amyloidosis (hATTR) is an autosomal dominant hereditary disease caused by a mutation in the transthyretin gene, which codes for the protein of the same name [1]. Transthyretin (TTR) is dissociated into dimers and monomers that precipitate to form amyloid aggregates that are deposited in various organs [2]. One of the main manifestations is length-dependent axonal polyneuropathy that initially affects small fibers and causes painful dysesthesias and numbness [3].

Given that TTR production mainly occurs in the liver, orthotopic liver transplant has been the main treatment strategy for years. Recently, nonsurgical options have emerged to treat familial amyloid polyneuropathy (FAP), including stabilizer therapies (tafamidis and diflunisal) and transthyretin silencers (inotersen and patisiran) [4]. Diflunisal is a nonsteroidal anti-inflammatory drug and a nonspecific tetramer stabilizer that has been used off-label to treat hATTR. Tafamidis, which binds to the unoccupied thyroxine binding sites of tetrameric TTR and prevents its dissociation into monomers [5, 4], has been approved in Europe for the treatment of hATTR amyloidosis in adults with early-stage symptomatic polyneuropathy [4]. Inotersen and patisiran reduce TTR protein by degrading nuclear TTR messenger RNA (inotersen) and forming a cytoplasmic RNA-induced silencing complex (patisiran) [4–8]. Patisiran and inotersen have received authorization for the treatment of neuropathy in patients with both early and late disease [4].

When orthotopic liver transplant is performed, the removed liver is functionally healthy and can be donated to another patient with liver failure in domino liver transplantation (DLT) [9–12, 13, 14–20]. The graft gradually produces mutated TTR in the recipient, and over time, this can result in iatrogenic acquired amyloid neuropathy (AAN). As of December 2017, there had been 1,234 DLTs worldwide from donors with FAP [21]. However, the first cases of AAN began to be reported in these patients from 2005 [11, 12, 22, 23]. When DLT recipients develop neuropathies, few treatment options exist. Liver re-transplantation, which can stabilize or even improve symptoms [11, 12, 24], may be considered but is often limited by the patient's age or comorbidities. Regarding medical treatment, case reports have suggested that treatment with TTR stabilizers (diflunisal or tafamidis) can produce clinical stabilization in some cases [25–27]. To date, there have been no data from case series with long-term follow-up of the effects of diflunisal or other treatments in these patients.

In this report, we aimed to describe our experience in our tertiary care center of the efficacy and tolerability of diflunisal for neurological symptoms in DLT recipients with AAN.

MATERIALS AND METHODS

Study Design and Variables

In this retrospective longitudinal study, data were collected from the electronic medical records of patients who developed AAN after DLT and treated with compassionate-use diflunisal between 2014 and 2019 at our hospital. All DLT recipients underwent prospective routine annual neurological evaluations for early AAN diagnosis in the Familial Amyloidosis Multidisciplinary Unit (UMAF). Patients without medical contraindications (e.g., severe renal failure, uncontrolled cardiac failure, or arterial hypertension) started on treatment with diflunisal. We collected data from serial neurological assessment at baseline (before starting treatment), at 6 months of treatment, and annually thereafter (12 ± 2, 24 ± 2, and 36 ± 2 months). Assessment involved full neurological examination, with patients given Neurological Impairment Scale (NIS) and Neurological Impairment Scale-Lower Limbs (NIS-LL) scores

TABLE 1 | Demographic and clinical data for domino liver transplant recipients who developed acquired amyloid neuropathy.

Demographic and Clinical Data (n = 12)	
Gender (male), No. (%)	8 (66.6)
Personal history	
- arterial hypertension No. (%)	11 (91.6)
- Dyslipidemia No. (%)	9 (75)
- Diabetes mellitus No. (%)	6 (50)
- Insulin-dependent diabetes. No. (%)	5 (41.6)
Initial transplant indication	
- HCV LC, No. (%)	6 (50)
- HBV LC, No. (%)	2 (16.7)
- Alcoholic LC, No. (%)	1 (8.3)
- HBV and alcoholic LC, No. (%)	1 (8.3)
- HCV and alcoholic LC, No. (%)	1 (8.3)
- Autoimmune hepatitis LC, No. (%)	1 (8.3)
Age at the time of receiving DLT, mean (rang), years	57.7 (52, 65)
Age at onset of neurological symptoms, mean (rang), years	66.7 (57; 76)
Time between transplant and onset of symptoms, mean (rang), years	8.5 (5; 15)

DLT, domino liver transplant; HBV, hepatitis B virus; HCV, hepatitis C virus; LC, liver cirrhosis.

and undergoing sensory and motor neurography. Neurological deterioration was defined as an increase in the NIS or NIS-LL of ≥2 points from baseline.

We also collected data on diflunisal side effects, focusing on new onset or worsening hypertension (need to start or adjust antihypertensives) or worsening of renal function (reduction in the estimated glomerular filtration rate of >10 ml/min from baseline on two consecutive measurements, or any value < 30 ml/min during treatment). Cardiac assessments were based on the New York Heart Association (NYHA) Functional Classification scale, NT-proBNP levels, echocardiography, and 99 mTc-DPD scintigraphy at baseline and during follow-up. Finally, we recorded any dose changes or therapy cessation.

Ethical approval was obtained by Ethics Committee for Drug Research of our center (reference number: EPA015/20; CCP-DIF-2020-01). The Ethics Committee for Drug Research of our center waived the need for written informed consent. We obtained verbal consent for data collection and noted this in patients' electronic medical records. All methods were performed in accordance with the relevant guidelines and regulations.

Statistical Analysis

Changes in NIS and NIS-LL scores were analyzed by two-tailed Student t-tests for paired data, after confirming distribution normality. p-values of <0.05 were considered statistically significant.

TABLE 2 | Demographic and clinical characteristics, plus neurological changes.

	DLT Indication	Time DLT—symptoms (years)	Other causes of polyneuropathy	IS treatment	Follow-up (months)	NIS baseline	Neurological deterioration
Patient 1 (M)	Recurrence of HCV after first LT (HCV)	15	DM on insulin therapy,HbA1c:6.6%	Mycophenolate,1,000 mg/24 h	12	8	No
Patient 2 (M)	HCV LC	10	Vitamin B12 deficiency (normal B12 levels)	Mycophenolate,2,000 mg/24 h	64	8	Yes (12 months FU)
Patient 3 (F)	Alcoholic and HCV LC	7	DM on insulin therapy,HbA1c: 7.6–7.9%	Everolimus,1 mg/24 h	12	4	Yes (12 months FU)
Patient 4 (M)	HCV LC	9	—	Mycophenolate,1,000 mg/24 h Everolimus,1.5 mg/24 h	12	2	Yes (12 months FU)
Patient 5 (F)	Alcoholic LC	7	DM on insulin therapy,HbA1c: <6%	Mycophenolic acid 1,080 mg/24 h	12	12	Yes (12 months FU)
Patient 6 (F)	HCV LC	13	—	Mycophenolate,1,000 mg/24 h	12	14	Yes (12 months FU)
Patient 7 (M)	HCV LC	13	—	Mycophenolic acid 1080mg/24 h	36	14	Yes (36 months FU)
Patient 8 (M)	Ischemic cholangitis after first LT (HCV LC)	9	—	Everolimus,1.5 mg/24 h	—	3	—
Patient 9 (M)	Thrombosis and rejection following LT (alcoholic and HBV LC)	6	DM on insulin therapy,HbA1c: 6.3%–6.6%	Mycophenolate,1,000 mg/24 h Everolimus,2 mg/24 h	—	0	—
Patient 10 (M)	Chronic rejection after LT (HBV LC)	5	DM on insulin therapy,HbA1c: 6%–6.1%	Tacrolimus 1 mg/24 h Azathioprine 100 mg/24 h	--	6	--
Patient 11 (F)	Chronic rejection after LT	12	—	Tacrolimus 1 mg/24 h	—	12	—
Patient 12 (M)	HBV LC	11	DM on insulin therapy,HbA1c: 5.6%	Tacrolimus 0.5 mg/48 h	—	—	—

DLT, domino liver transplant; DM, diabetes mellitus; F, female; FU, follow-up; HbA1c, Glycated hemoglobin; HBV, hepatitis B virus; HCV, hepatitis C virus; IS, Immunosuppressive therapy; LC, liver cirrhosis; LT, liver transplant; M, male.

TABLE 3 | Clinical findings at diagnosis of acquired amyloid neuropathy.

Patient	Weakness	Sensibility Disturbance	Autonomic Symptoms (*)	Neurological Examination
1	No	Dysesthesia and numbness in distal LL	No	Hypoesthesia in distal LL
2	No	Numbness in distal LL	Asthenia and weight loss	Tactile and thermal hypoesthesia in distal LL Tactile hypoesthesia in distal UL Absent Achilles reflex
3	No	Painful dysesthesia in distal LL	No	Hypopallesthesia in distal LL. Decreased Achilles reflex
4	No	Painful dysesthesia in distal LL	Erectile dysfunction	Thermal hypoesthesia in distal LL
5	No	Painful dysesthesia in distal LL	No	Thermo-algesic hypoesthesia and hypopallesthesia in distal LL Absent Achilles reflex
6	No	Painful dysesthesia in distal LL	Diarrhea	Tactile and thermo-algesic hypoesthesia in distal LL Hypopallesthesia in distal LL Absent patellar and Achilles's reflex
7	No	Painful dysesthesia in distal LL	No	Tactile and thermo-algesic hypoesthesia in distal UL and LL Hypopallesthesia in distal LL Decreased Achilles reflex
8	No	Painful dysesthesia in distal LL	Erectile dysfunction, weight loss, diarrhea	Thermo-algesic hypoesthesia and hypopallesthesia in distal LL
9	No	Numbness in distal LL	Erectile dysfunction	Thermo-algesic hypoesthesia in distal UL and LL. Hypopallesthesia in distal LL
10	Yes	Painful dysesthesia in distal LL	No	Thermal hypoesthesia in distal LL
11	No	Painful dysesthesia in distal LL	Erectile dysfunction	Normal
12	Yes	Dysesthesia and numbness in UL and LL	Orthostatism, diarrhea	Thermo-algesic hypoesthesia and hypopallesthesia in UL and LL. Distal weakness in UL and LL. Absent patellar and Achilles's reflex

(*) Excludes erectile dysfunction prior to domino liver transplant.
LL, lower limb; UL, upper limbs.

TABLE 4 | Diflunisal-related complications and dose changes.

	Renal function Worsening	Worsening or *de novo* AH	Discontinuation or Dose Reduction of diflunisal	Adverse Events after Therapy Modification
Patient 1 (M)	Yes (-12 ml/min, + 4 months)	No	Dose reduction to 250 mg/24 h due to renal function impairment (+5 months)	Mild improvement in renal function after dose reduction
Patient 2 (M)	Yes (-10 ml/min, +36 months)	Yes	Dose reduction to 250 mg/24 h (+59 months) due to renal function impairment Discontinued due to heart failure (+64 months)	Mild improvement in renal function after dose reduction Heart failure recovery after discontinuation
Patient 3 (F)	No	No	No	—
Patient 4 (M)	No	No	No	—
Patient 5 (F)	No	No	No	—
Patient 6 (F)	No	No	No	—
Patient 7 (M)	No	No	No	—
Patient 8 (M)	Yes, acute renal failure in patient with chronic renal failure (EGFR<30 ml/min)	Yes	Discontinued due to acute renal failure (+13 days)	Mild improvement in renal function after discontinuation
Patient 9 (M)	—	—	No follow –up Liver re-transplantation	— —
Patient 10 (M)	—	—	Discontinued after acute cholestasis (+3 days)	—
Patient 11 (F)	—	—	Discontinued due to high hemorrhagic risk following anticoagulant therapy	—
Patient 12 (M)	Yes, acute renal failure in patient with chronic renal failure (EGFR<30 ml/min)	No	Discontinued due to acute renal failure (+35 days)	Mild improvement in renal function after discontinuation

AH, arterial hypertension; EGFR, Estimated Glomerular Filtration Rate; F, female; M, male.

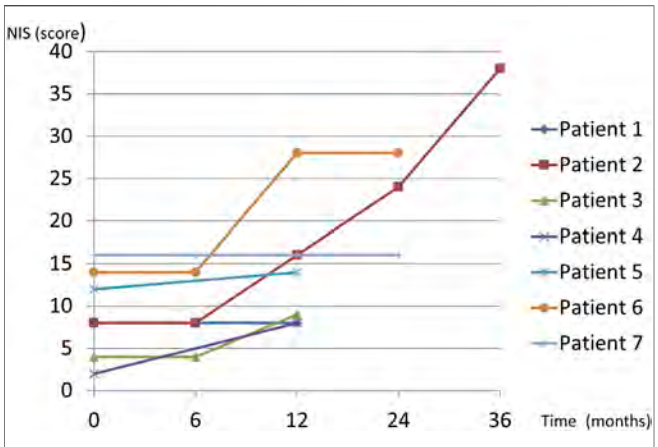


FIGURE 1 | Diflunisal treatment in domino liver transplant recipients with acquired amyloid neuropathy.

RESULTS

Demographic and Clinical Data

We identified 12 DLT recipients who developed AAN in whom treatment with diflunisal was started as a compassionate use stabilising treatment, at a dose of 250 mg twice daily.

Six patients (50%) were diabetic, but all of them had excellent glycemic control (**Tables 1**, **2**). Those with a history of alcohol use had been abstinent from alcohol before transplantation and at all follow-ups. Most recipients were graded as in NYHA class I (83.3%) two (16.7%) were class II, and none had evidence of amyloid deposits on cardiac scintigraphy with 99 mTc-DPD before receiving diflunisal. One patient developed heart failure, whereas all others remained stable, and none developed amyloid deposits on follow-up cardiac scintigraphy (Supplementary material).

All liver donors had V30M genotypes and early-onset hATTR with neuropathic phenotypes. The mean time between transplant and symptom onset was 8.5 years (range, 5–15 years) (**Tables 1**, **2**). The first manifestations of polyneuropathy were sensory, including painful dysesthesias and numbness in the feet (**Table 3**).

The median pretreatment scores were 10.8 (range, 0–46.5) for the NIS and 9.3 (range, 0–34.5) for the NIS-LL. All patients were Stage I–II of the Polyneuropathy Disability stage (PND) scale before starting diflunisal. Initial conventional neurophysiological study was normal in 2 patients (16.7%), but all patients developed a sensory-motor axonal polyneuropathy during the disease course. AAN was confirmed in all patients by the presence of amyloid deposition on sural nerve biopsy.

Tolerability and Adverse Effects of Diflunisal

Diflunisal was started for compassionate use in all cases at a dosage of 250 mg twice daily as a stabilizing treatment. One patient received treatment for <6 months because he underwent re-transplantation. Among the remaining patients, five (45.5%) stopped treatment due to side effects (**Table 4**). Seven patients did persist with diflunisal for >12 months, but two of these (28.6%) required a dose reduction due to worsening renal function and one (14.3%) required that the drug be stopped due to heart failure. Two patients (28.6%) developed new-onset or worsening hypertension (**Table 4**), which was managed by adjusting antihypertensive therapy in all cases. All patients who developed impaired renal function showed a mild improvement in glomerular filtration rate after dose adjustment or stopping diflunisal, but none recovered to baseline levels.

Treatment Efficacy

Neurological follow-up data for at least 12 months after starting diflunisal were available for seven patients. The mean follow-up duration was 22.8 months (range, 12–36). No patient with assessment data at 6 months (4 patients, 57%) experienced neurological deterioration based on the NIS and NIS-L (**Figure 1**). However, five patients (71.4%) met the criteria for neurological deterioration at 12 months (**Figure 1**). Changes in the NIS from before treatment to 12 months of follow-up were statistically significant ($p = 0.0382$) whereas those in the NIS-LL were not ($p = 0.09$).

DISCUSSION

In the series presented by Misumi et al., the prevalence of symptomatic AAN among DLT recipients was 23% [20], whereas in our center, Lladó et al. reported that all patients had developed AAN at 90 months of follow-up [19]. Although liver re-transplantation is a viable treatment option, most patients are ineligible due to age or comorbidities [24, 28, 29], necessitating that we consider other treatment options. The generic nonsteroidal anti-inflammatory drug diflunisal is a nonspecific tetramer stabilizer of TTR that may prevent misfolding monomers and dimers from forming amyloid deposits in the heart and peripheral nerves [30].

A clinical trial has shown positive results on neurological progression when giving diflunisal to patients with hATTR [31], but evidence of its efficacy in DLT recipients with AAN is scarce. To date, there has only been one reported case of a patient with these features, which showed that neurological symptoms improved after 18 months of treatment [25]. Another patient who was given a trial of diflunisal needed their treatment to be stopped because of worsening heart failure [26]. Compassionate treatment with tafamidis was initiated in another patient with AAN, who remained stable for 2 years [27]. Prophylactic use of diflunisal or tafamidis has also been proposed in DLT recipients [32].

The efficacy of diflunisal in cardiac amyloidosis due to mutant or wild-type TTR has been analyzed in several studies [30, 33]. In our study we found that no patient had evidence of cardiac amyloidosis on cardiac scintigraphy before or during treatment in this case series. Only one patient developed heart failure at 36 months treatment, but this was without demonstrating cardiac amyloidosis (Supplementary material).

Our study is the first to analyze the effects of diflunisal in a series of seven patients with at least 12 months' follow-up data. Before starting treatment, all patients were in stage I–II on the PND scale and stage I on Coutinho's FAP scale. Most patients (71.4%) showed neurological deterioration by 1 year while only 28.6% remained stable on the NIS and NIS-LL. These results are similar to those reported in the clinical trial by Berk et al., in which 29.7% of patients with hATTR presented neurological stability (increase <2 points on the NIS +7 scale) after 2 years of treatment with diflunisal versus only 9.4% in the placebo group. It may be that a subgroup of patients responds to treatment and remains stable during the first years of treatment, as Bourque et al. [25] and Matsushima et al. [27] described. Analyzing the predictive factors of long-term response to diflunisal may be of benefit. Although we found that the change in NIS score at 12 months was statistically significant, whereas that for the NIS-LL score was not, it should be noted that these scales do not account for the proximal progression of sensory deficits and may underestimate deterioration.

In addition to the low efficacy we found high percentages of renal function impairment (36%), heart failure (9%), and treatment discontinuation (45%) in the medium/long-term course in our series that contrast with data in other studies of diflunisal for patients with hATTR or wild-type amyloidosis in which less renal function impairment was reported and diflunisal discontinuation occurred less often (0%–13%) [31, 30, 34]. This may be because DLT recipients are frail and have underlying comorbidities, with adverse effects being not only more common but also more likely to require drug cessation. Special attention must also be ensured for patients with chronic renal failure, poorly controlled hypertension, or receiving anticoagulants, ensuring close follow-up for possible complications. This data encourage the search for new therapeutic options.

Whether other treatment options for FAP, such as tafamidis [5], patisiran [6], or inotersen [7, 8], could be used with similar or better efficacy and fewer side effects in DLT recipients with AAN remains to be evaluated in prospective clinical trials.

Limitations

The present series was limited by its retrospective nature, lack of a control group, small sample size, and inability to include follow-up data beyond 1 year for all patients. Nevertheless, sample size is an inherent problem of diseases with a low prevalence and a low rate of treatment continuation (54.5%).

Follow-up assessment of DLT recipients may be improved by using more sensitive scales such as the modified NIS + 7 together with a full neurological examination and the inclusion of functional scales.

CONCLUSION

Our study suggests most of DLT recipients with AAN develop neurological deterioration after 12 months diflunisal treatment, and throughout, the high incidence of adverse effects frequently necessitates the drug being stopped. The low efficacy and the unfavorable side effect profile of diflunisal indicate that we need to identify new therapeutic options for patients who develop AAN after DLT.

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 Ethics Committee for Drug Research at Bellvitge University Hospital-IDIBELL. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

VN-H; Design and conceptualized study, analyzed the data; drafted the manuscript for intellectual content. CB; Role in the acquisition of data, revised the manuscript for intellectual content. JG-C; Role in the acquisition of data, revised the manuscript for intellectual content. LL; Role in the acquisition of data, revised the manuscript for intellectual content. EG-V; Role in the acquisition of data, revised the manuscript for intellectual content. VV-S; Design and conceptualized study, analyzed the data, drafted the manuscript for intellectual content. CC; Major role in the acquisition of data, design and conceptualized study, analyzed the data, drafted the manuscript for intellectual content.

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.2022.10454/full#supplementary-material

REFERENCES

1. Coelho T, Inês M, Conceição I, Soares M, de Carvalho M, Costa J. Natural History and Survival in Stage 1 Val30Met Transthyretin Familial Amyloid Polyneuropathy. *Neurology* (2018) 91:e1999–11. doi:10.1212/WNL.0000000000006543
2. Park GY, Jamerlan A, Shim KH, An SSA. Diagnostic and Treatment Approaches Involving Transthyretin in Amyloidogenic Diseases. *Int J Mol Sci* (2019) 20(12):2982. doi:10.3390/ijms20122982
3. Benson MD, Kincaid JC. The Molecular Biology and Clinical Features of Amyloid Neuropathy. *Muscle Nerve* (2007) 36(4):411–23. doi:10.1002/mus.20821
4. Gertz MA, Mauermann ML, Grogan M, Coelho T. Advances in the Treatment of Hereditary Transthyretin Amyloidosis: A Review. *Brain Behav* (2019) 9(9): e01371. doi:10.1002/brb3.1371
5. Coelho T, Maia LF, Martins da Silva A, Waddington Cruz M, Planté-Bordeneuve V, Lozeron P, et al. Tafamidis for Transthyretin Familial Amyloid Polyneuropathy: a Randomized, Controlled Trial. *Neurology* (2012) 79(8):785–92. doi:10.1212/WNL.0b013e3182661eb1
6. Adams D, Gonzalez-Duarte A, O'Riordan WD, Yang CC, Ueda M, Kristen AV, et al. Patisiran, an RNAi Therapeutic, for Hereditary Transthyretin Amyloidosis. *N Engl J Med* (2018) 379(1):11–21. doi:10.1056/NEJMoa1716153
7. Benson MD, Waddington-Cruz M, Berk JL, Polydefkis M, Dyck PJ, Wang AK, et al. Inotersen Treatment for Patients with Hereditary Transthyretin Amyloidosis. *N Engl J Med* (2018) 379(1):22–31. doi:10.1056/NEJMoa1716793
8. Moshe-Lilie O, Dimitrova D, Heitner S, Brannagan T, III, Zivkovic S, Hanna M, et al. TTR Gene Silencing Therapy in post Liver Transplant Hereditary ATTR Amyloidosis Patients. *Amyloid* (2020) 27(4):250–3. doi:10.1080/13506129.2020.1784134
9. Ando Y, Coelho T, Berk JL, Cruz MW, Ericzon B-G, Ikeda S-i., et al. Guideline of Transthyretin-Related Hereditary Amyloidosis for Clinicians. *Orphanet J Rare Dis* (2013) 208:31. doi:10.1186/1750-1172-8-31
10. Adams D, Suhr OB, Hund E, Obici L, Tournev I, Campistol JM, et al. First European Consensus for Diagnosis, Management, and Treatment of Transthyretin Familial Amyloid Polyneuropathy. *Curr Opin Neurol* (2016) 29(1):S14–26. doi:10.1097/WCO.0000000000000289
11. Mnatsakanova D, Živković SA. Iatrogenic Amyloid Polyneuropathy after Domino Liver Transplantation. *World J Hepatol* (2017) 9(3):126–30. doi:10.4254/wjh.v9.i3.126
12. Abdelfatah MM, Hayman SR, Gertz MA. Domino Liver Transplantation as a Cause of Acquired Familial Amyloid Polyneuropathy. *Amyloid* (2014) 21: 136–7. doi:10.3109/13506129.2014.885894
13. Kapoor M, Rossor AM, Laura M, Reilly MM. Clinical Presentation, Diagnosis and Treatment of TTR Amyloidosis. *J Neuromuscul Dis* (2019) 6(2):189–99. doi:10.3233/jnd-180371
14. Ericzon B-G. Domino Transplantation Using Livers from Patients with Familial Amyloidotic Polyneuropathy: Should We Halt. *Liver Transpl* (2007) 13(2):185–7. doi:10.1002/lt.21073
15. Takei Y-i., Gono T, Yazaki M, Ikeda S-i., Ikegami T, Hashikura Y, et al. Transthyretin-derived Amyloid Deposition on the Gastric Mucosa in Domino Recipients of Familial Amyloid Polyneuropathy Liver. *Liver Transpl* (2007) 13(2):215–8. doi:10.1002/lt.20954
16. Yamamoto S, Wilczek HE, Iwata T, Larsson M, Gjertsen H, Söderdahl G, et al. Long-term Consequences of Domino Liver Transplantation Using Familial Amyloidotic Polyneuropathy Grafts. *Transpl Int* (2007) 20:926–33. doi:10.1111/j.1432-2277.2007.00516.x
17. Adams D, Lacroix C, Antonini T, Lozeron P, Denier C, Epelbaum S, et al. Risk of Developing De Novo Amyloid Deposits and Induced Polyneuropathy in FAP Domino Liver Recipients [abstract]. *Eur J Neurol* (2009) 16(Suppl. 13): 16–54. https://www.researchgate.net/publication/295156252_Risk_of_developing_de_novo_amyloid_deposits_and_induced_polyneuropathy_in_FAP_domino_liver_recipients/stats.
18. Barreiros AP, Geber C, Birklein F, Galle PR, Otto G. Clinical Symptomatic De Novo Systemic Transthyretin Amyloidosis 9 Years after Domino Liver Transplantation. *Liver Transpl* (2010) 16:109. doi:10.1002/lt.21928
19. Lladó L, Baliellas C, Casasnovas C, Ferrer I, Fabregat J, Ramos E, et al. Risk of Transmission of Systemic Transthyretin Amyloidosis after Domino Liver Transplantation. *Liver Transpl* (2010) 16:1386–92. doi:10.1002/lt.22174
20. Misumi Y, Ueda M, Masuda T, Tsuda Y, Nomura T, Okada M, et al. Characteristics of Acquired Transthyretin Amyloidosis. *Neurology* (2019) 93(17):e1587–e1596. doi:10.1212/wnl.0000000000008360
21. FAPWTR. Familial Amyloidotic Polyneuropathy World Transplant Registry and Domino Liver Transplant Registry (2021). Available from www.fapwtr.org. (Accesed MAR. 14 2021).
22. Stangou AJ, Heaton ND, Hawkins PN. Transmission of Systemic Transthyretin Amyloidosis by Means of Domino Liver Transplantation. *N Engl J Med* (2005) 352(22):2356. doi:10.1056/nejm200506023522219
23. Obayashi K, Yamashita T, Tasaki M, Ueda M, Shono M, Jono H, et al. Amyloid Neuropathy in a Younger Domino Liver Transplanted Recipient. *Muscle Nerve* (2011) 43:449–50. doi:10.1002/mus.21941
24. Antonini TM, Lozeron P, Lacroix C, Mincheva Z, Durrbach A, Slama M, et al. Reversibility of Acquired Amyloid Polyneuropathy after Liver Retransplantation. *Am J Transpl* (2013) 13:2734–8. doi:10.1111/ajt.12378
25. Bourque PR, Shafi S, Jansen GH, McCurdy A, Warman Chardon J. Amyloid Neuropathy Following Domino Liver Transplantation. *JAMA Neurol* (2016) 73(4):477–8. doi:10.1001/jamaneurol.2015.4715
26. Dixit N, Castano A, Farr MJ, Traub R, Lentzsch S, Brown RS, et al. Rapidly Progressive Transthyretin-Mediated Amyloidosis in a Domino Liver Transplant Recipient of a Ser23Asn Donor. *J Clin Neuromuscul Dis* (2016) 17:142–5. doi:10.1097/cnd.0000000000000110
27. Matsushima M, Yabe I, Tsuda M, Sakakibara M, Shimamura T, Sasaki H. Amyloid Polyneuropathy and Myocardial Amyloidosis 10 Years after Domino Liver Transplantation from a Patient with a Transthyretin Ser50Arg Mutation. *Intern Med* (2017) 56:3231–5. doi:10.2169/internalmedicine.8434-16
28. Vieira H, Rodrigues C, Pereira L, Jesus J, Bento C, Seco C, et al. Liver Retransplantation in Patients with Acquired Familial Amyloid Polyneuropathy: a Portuguese center Experience. *Transplant Proc* (2015) 47:1012–5. doi:10.1016/j.transproceed.2015.04.003
29. Zarrinpar A, Hong JC. What Is the Prognosis after Retransplantation of the Liver. *Adv Surg* (2012) 46:87–100. doi:10.1016/j.yasu.2012.03.005
30. Castaño A, Helmke S, Alvarez J, Delisle S, Maurer MS. Diflunisal for ATTR Cardiac Amyloidosis. *Congest Heart Fail* (2012) 18(6):315–9. doi:10.1111/j.1751-7133.2012.00303.x
31. Berk JL, Suhr OB, Obici L, Sekijima Y, Zeldenrust SR, Yamashita T, et al. Repurposing Diflunisal for Familial Amyloid Polyneuropathy. *JAMA* (2013) 310(24):2658–67. doi:10.1001/jama.2013.283815
32. Yoshinaga T, Yazaki M, Sekijima Y, Kametani F, Miyashita K, Hachiya N, et al. The Pathological and Biochemical Identification of Possible Seed-lesions of Transmitted Transthyretin Amyloidosis after Domino Liver Transplantation. *J Path: Clin Res* (2016) 2:72–9. doi:10.1002/cjp2.36
33. Lohrmann G, Pipilas A, Mussinelli R, Gopal DM, Berk JL, Connors LH, et al. Stabilization of Cardiac Function with Diflunisal in Transthyretin (ATTR) Cardiac Amyloidosis. *J Card Fail* (2020) 26(9):753–9. doi:10.1016/j.cardfail.2019.11.024
34. Ikram A, Donnelly JP, Sperry BW, Samaras C, Valent J, Hanna M. Diflunisal Tolerability in Transthyretin Cardiac Amyloidosis: a Single center's Experience. *Amyloid* (2018) 25(3):197–202. doi:10.1080/13506129.2018.1519507

ESOT | Transplant International

ORIGINAL RESEARCH
published: 14 April 2022
doi: 10.3389/ti.2022.10256

The Main Thing is to be Alive—Exploring Patients' Experiences With Weight Gain After Liver Transplantation: A Qualitative Study

Sonja Beckmann[1,2]*, *Patrizia Künzler-Heule*[1,3,4], *Kajetan Kabut*[5] and *Oliver Mauthner*[1,6]

[1]Nursing Science, University of Basel, Basel, Switzerland, [2]Center of Clinical Nursing Science, University Hospital Zurich, Zurich, Switzerland, [3]Department of Gastroenterology/Hepatology, Cantonal Hospital St. Gallen, St. Gallen, Switzerland, [4]Department of Nursing, Cantonal Hospital St. Gallen, St. Gallen, Switzerland, [5]Zentrum für NeuroRehabilitation, Beatmungs- und Intensivmedizin, BDH-Klinik Elzach, Elzach, Germany, [6]University Department of Geriatric Medicine Felix Platter, Basel, Switzerland

Weight gain after liver transplantation (LTx) contributes to new-onset obesity. We explored patients' experiences with gaining weight after LTx. Individual interviews were guided by open-ended questions. We analyzed transcripts with the reflexive thematic analysis approach by Braun and Clarke. The 12 participants gained 11.5 kg weight (median) over a median of 23 months after LTx. The constitutive theme "The main thing is to be alive" was a recurrent insight, captured in three facets: "The arduous path back to living" was the emotional expression of the ups and downs during a life-threatening illness to finally being grateful for the new life. "A pleasurable new phase of life" was the legitimation, reflecting the appreciation of gaining weight and returning to a healthy appearance. "I am allowed to look like this now" was the consoling facet after a time of burden due to the increased weight and frustration of being unsuccessful in losing weight. Finally, the awareness of being a LTx survivor outplayed the burden of the excess weight. Early interventions are crucial because the comforting insight "I am allowed to look like this now" may hinder further engagement in weight loss activities. Our recommendations on education and self-management support may guide clinical practice.

Keywords: education, obesity, self-management, behavior change, communication, thematic analysis

Correspondence:
Sonja Beckmann
sonja.beckmann@unibas.ch

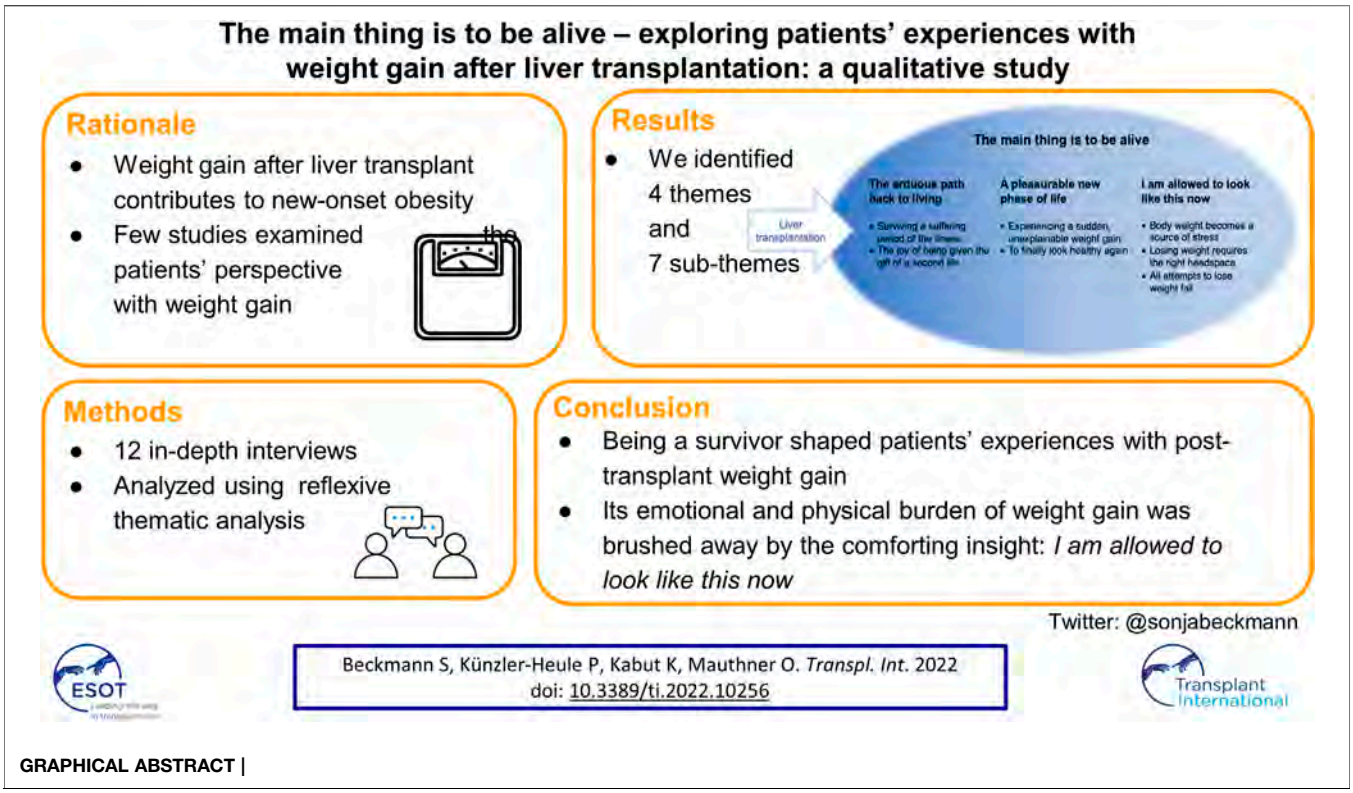
Received: *21 November 2021*
Accepted: *08 March 2022*
Published: *14 April 2022*

Citation:
Beckmann S, Künzler-Heule P, Kabut K and Mauthner O (2022) The Main Thing is to be Alive—Exploring Patients' Experiences With Weight Gain After Liver Transplantation: A Qualitative Study. Transpl Int 35:10256. doi: 10.3389/ti.2022.10256

INTRODUCTION

Weight gain after liver transplantation (LTx) has been a research focus for over 30 years (1). Studies report a mean weight gain between 2 and 9 kg within the first year post-LTx (2-4). At 2- and 3-year following transplantation, continuous weight gain contributes to new-onset obesity in 22%–38% of patients (2–4). Post-LTx weight gain and new-onset obesity contribute to increased long-term mortality (5) and comorbidities such as metabolic syndrome (6), non-alcoholic fatty liver disease (7) and cardiovascular events (8). These significant weight gains and risks prompt the question of how patients cope with this situation. Unfortunately, few studies examined the patients' perspective (1, 9, 10).

Abbreviations: BMI, body mass index; COREQ, Consolidated Criteria for Reporting Qualitative Health Research; LTx, liver transplantation; TTM, transtheoretical model.



GRAPHICAL ABSTRACT |

The etiology of weight gain is complex and depends on a multitude of individual and interconnected factors (11). Previous studies suggested that post-LTx weight gain was related to higher weight or body mass index (BMI) pre-LTx, being a former smoker, older age at LTx (2), alcoholic liver disease as reason for LTx (2, 8) and genetic factors (12). Interestingly, one study found no association between energy intake or daily physical activity and overweight or obesity after LTx (13). This contrasts two quantitative studies that added unstructured qualitative questions to their data collection, asking patients about the causes of their post-LTx weight gain (1, 9). Constant hunger with increased food intake and reduced daily physical activity were among the most common answers. Although those results should be interpreted cautiously due to methodological weaknesses, they are supported by a study exploring the perceptions of 20 patients on weight gain after LTx (10). That analysis revealed several reasons for weight gain including behavioral factors (e.g., diet, improved health/appetite, sedentary behavior) and other factors such as medication, weight regain, older age and addiction. To prevent weight gain, patients emphasized the need for supportive group programs, consultation with a dietician and advice for future recipients. Delivering supportive advice only is, however, not necessarily effective. A study examined the impact of lifestyle advice among overweight or obese patients after LTx (14). Despite being advised to lose weight, 62% gained weight (median 4.0 kg) during the study period. The authors concluded that simply reiterating the importance of following guidelines was ineffective for LTx recipients. Indeed, evidence suggests that effective weight loss interventions should understand predictors of behavior such as motivation, opportunity and capability (15) and also account for a person's readiness to change this behavior. A well-known framework to explain the stages of changing a behavior is the transtheoretical model (TTM) (16, 17). Each stage of the TTM requires a distinct intervention approach and it has been frequently used in dietary or physical activity weight loss interventions (18).

Given the research on the evolution and impact of post-LTx weight gain, there is a surprising lack of high-quality evidence on the content, delivery, timing and efficacy of weight management interventions (19, 20). This study therefore explored how patients experienced weight gain after LTx. *In-depth* knowledge of the lived experience, beliefs and motivations provides information to improve patient care and for developing interventions after LTx.

METHODS

Sample

Participants were recruited at the University Hospital Zurich with following inclusion criteria: ≥18 years, german speaking, ≥5 kg weight gain between LTx until recruitment, and ≥12 months since LTx. Participants were purposefully selected on gender, age and time since LTx to ensure a heterogeneous study group allowing diverse perspectives to be explored. The Cantonal Ethics Committee Zurich approved the study (BASEC 2017-01429).

The first author (SB) worked in the LTx nurse counseling service and identified possible participants from the hospital's electronic patient charts. Measuring weight is a routine procedure in the hospital's follow-up visits. Patients who gained at least 5 kg

from LTx until the latest follow-up visit were approached *via* telephone or face-to-face to provide oral and written study information. The contact information of interested persons was transferred to another author (KK), who conducted the interviews and was not involved in caring for the patients.

Fourteen people were asked to participate; two people declined. After providing a written informed consent, participants were interviewed in a place convenient for them, either in hospital or at home. The interviewer followed a guideline with open-ended questions to encourage the participants to share their experiences. The interview started with the question: "People report that their lives changed after LTx. Could you please tell me how your daily life and routine have changed since the LTx?" The subsequent questions explored more specific experiences with gaining weight, eating or activity, such as "What effects did the weight gain have on your everyday life?"

Data Collection and Analysis

Individual interviews were conducted between September 2017 and June 2018, lasting between 29 and 84 min (mean: 47 min). They were conducted in German, digitally recorded, transcribed and pseudonymized. Field notes were made during and after the interview. The research group consisted of a junior researcher and three senior researchers with expertise in qualitative research and clinical care of transplant patients. SB and KK listened to all interviews and read all transcripts. The other members listened to and read selected interviews or text passages for trustworthiness. Codes and themes were discussed in the group throughout the analysis, and final results were discussed with two interviewees for feedback. Sociodemographic and clinical data were self-reported by the participants before the interviews.

Data analysis followed the six phases of Braun and Clarke's reflexive thematic analysis (21, 22), which identifies, analyses and reports patterns (themes) of shared meaning: 1) Familiarizing with the data by transcribing the interviews, re-reading and taking notes of thoughts and interesting characteristics. 2) Generating codes by identifying meaningful text passages. 3) Constructing themes by grouping and naming the coded data that were related to each other. 4) Revising themes to clarify the scope and avoid confusion or overlap. Checking the fit of the themes against each other and the dataset. 5) Final definition and naming of themes to ensure clarity and comprehensiveness. 6) Writing the article as a final check if the themes made sense and would answer the research question by telling a coherent story. Data were managed using the computer software MAXQDA, Release 20.0.8 (Verbi GmbH, Berlin, Germany). The reporting followed the Consolidated Criteria for Reporting Qualitative Health Research (COREQ) Checklist (**Supplementary Material S1**) (23).

RESULTS

Twelve participants shared their experiences of post-LTx gaining weight. Median time since LTx was 23 months (range 17–58 months), individual weight gain ranged between 5 kg and 24 kg (median 11.5 kg). The participants were equally distributed in three groups: normal weight, overweight or obese at time of interview. Characteristics are shown in **Table 1**. We identified four themes and seven sub-themes, depicted in **Figure 1**. Representative quotes are in the text and **Table 2**.

The Main Thing is to be Alive

This theme was identified as constitutive as it represented the most definitive and recurring insight into weight gain post-LTx: A recurring insight because it was captured in each of three other themes. Shortly after the LTx, it exemplified the manifold emotions generated by the theme *The arduous path back to living*. This was followed by a period in which those affected felt justified in indulging themselves, the theme *A pleasurable new phase of life* began. Then, after experiencing burden due to the increased weight and when all attempts to lose weight were unsuccessful, came the consoling theme *I am allowed to look like this now*. The three themes followed one another chronologically, although not all participants contributed to the final theme. The transition between themes was individual, depending on the recovery process and post-LTx weight attained.

The arduous Path Back to Living

Surviving a Suffering Period of the Illness

The participants dramatically described surviving a severe liver disease, the life-saving LTx and subsequent recovery. Some experienced concomitant complications such as multiple organ failure or organ rejection. Nearly all described the regeneration process as arduous, with daily life marked by physical limitations, difficulty concentrating and extreme, ongoing fatigue. The participants' physical ailments were compounded by emotional distress. They felt anxiety and uncertainty regarding transplantation success and were confronted with their own mortality. This long, energy-sapping phase left its mark psychologically. In Lara's case it led to psychological trauma, for which she was still in treatment.

While the participants experienced emotional and physical improvement over time and reported a «slow return to living» (Lara), this did not always succeed to the extent expected or wished for. At the time of the interviews, almost no participants had regained the same level of stamina or strength they had had previously. In order to not overtax themselves, individuals had to be mindful of their own energy reserves and to use them carefully to best deal with the «arduous daily life» (Valérie).

The Joy of Being Given the Gift of a Second Life

Although, for some, ongoing physical problems and experiences continued to be emotionally stressful, the participants all looked back positively on this period. Any enduring limitations were accepted with equanimity, including several participants concluding that their bodies simply were «no longer what they had once been» (Lara). Generally, a feeling of joy at still being alive predominated, with many participants describing the great happiness they had experienced. Margret's transplantation was due to hepatic cancer. She had been symptom free and «had been rather lucky» that her cancer was discovered in time. Angelo felt it was an «immense luck» that he had received a donor organ. The

TABLE 1 | Sociodemographic and clinical data.

Pseudonym	Gender, age at interview	Months after LTx	Reason for LTx	Weight category (BMI) in 1st follow-up after LTx	Weight gain from 1st follow-up after LTx until interview (kg)	Weight category (BMI) at time of interview
Joe	Male, 70	18	Hepatocellular carcinoma	Obesity (34.5 kg/m^2)	8	Obesity (37.1 kg/m^2)
Marlene	Female, 55	31	Alcoholic liver cirrhosis	Normal weight (24.5 kg/m^2)	24	Obesity (32.9 kg/m^2)
Norbert	Male, 72	17	Primary sclerosing cholangitis	Normal weight (20.4 kg/m^2)	11	Normal weight (23.9 kg/m^2)
Kitty	Female, 23	22	Re–LTx, acute on chronic liver failure	Normal weight (19 kg/m^2)	9	Normal weight (22.0 kg/m^2)
Elisabeth	Female, 55	29	Liver cirrhosis after autoimmune hepatitis	Normal weight (18.8 kg/m^2)	8	Normal weight (22.2 kg/m^2)
Lara	Female, 58	71	Primary biliary cirrhosis	Normal weight (21.6 kg/m^2)	18	Overweight (27.7 kg/m^2)
Margret	Female, 67	51	Hepatocellular carcinoma	Obesity (33.3 kg/m^2)	11	Obesity (37.3 kg/m^2)
Rudi	Male, 62	58	Alcoholic liver cirrhosis	Normal weight (20.5 kg/m^2)	20	Overweight (27.6 kg/m^2)
Angelo	Male, 68	21	Alcoholic liver cirrhosis	Normal weight (20.1 kg/m^2)	12	Normal weight (24.4 kg/m^2)
Valérie	Female, 45	24	Autoimmune hepatitis	Normal weight (22.7 kg/m^2)	24	Obesity (30.7 kg/m^2)
Katja	Female, 43	16	Acute liver failure	Overweight (21.6 kg/m^2)	14	Overweight (26.6 kg/m^2)
Hans	Male, 64	22	Hepatocellular carcinoma	Overweight (24.8 kg/m^2)	8	Overweight (27.2 kg/m^2)

LTx, liver transplant; BMI, body mass index.

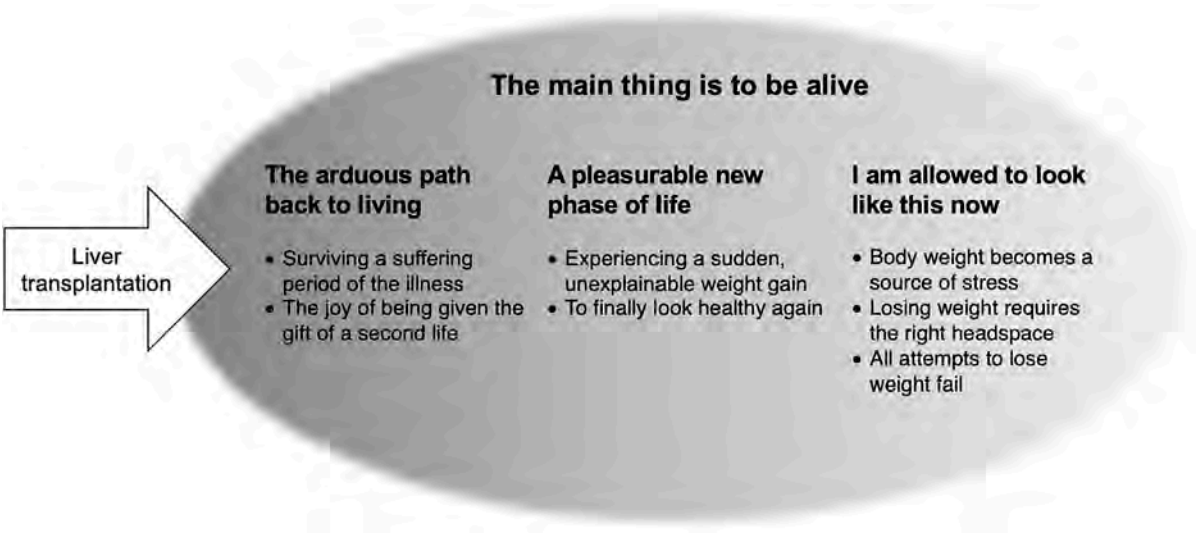


FIGURE 1 | Overview over the four themes and the seven subthemes.

euphoria and gratitude for this «gift of a second life» (Elisabeth) accompanied the participants afterwards.

A Pleasurable new Phase of Life

The participants conveyed a foundational positivity as they spoke of their joie de vivre and their desire to make the most of this phase of life. The enjoyment also manifested itself at mealtimes. After the lack of appetite and the deprivations experienced earlier, many participants reported feeling intense hunger. They had the feeling that food was a means of making up for something. Going grocery shopping, the preparation and enjoyment of meals once again became an integral part of daily life. Nutrition was often mentioned in connection with the belief that, having overcome the illness, they were justified in indulging themselves and were entitled to enjoy something pleasurable. Angelo, for example, consumed up to 1.5 L of sugar-sweetened soft drinks daily and took the view that «I didn't undergo an operation just so that I could restrict myself to water.»

Experiencing a Sudden, Unexplainable Weight Gain

Along with pleasurable indulgence came weight gain. Only Norbert, Kitty und Elisabeth were all consciously aware of the process of weight gain. Upon reaching their pre-LTx weight, they reminded themselves «to having to exercise self-discipline» (Norbert) to avoid further weight gain. In contrast, most participants first became aware of having

TABLE 2 | Additional representative quotes within the themes.

Theme	Representative quotes
The arduous path back to living	«I was so exhausted after showering that I could barely towel myself dry. It's hard to imagine, but that's how it was.» (Rudi)
Surviving a suffering period of the illness	«I was traumatized as well. [. . .] These 4 weeks in a coma left me very weak physically. [. . .] And then came the period after the transplantation when I was in an induced coma [. . .] When you are informed of that afterwards when you're conscious again, that really is something that takes some time to work through. It took me a long time to regain my strength, it went on for 2 years for sure.» (Lara)
The joy of being given the gift of a second life	«Even now there are limits to what I can do because I just don't have the stamina or the strength.» (Katja) «I have to plan ahead if I want to go somewhere: What will I be doing the following day? If I do whatever it is, will I be too tired to manage to go to work the next day?» (Marlene)
A pleasurable new phase of life	«After the transplantation things slowly got better, but it took a while for hunger pangs to return to normal. But after that I was really ravenously hungry [. . .] I had the feeling that I had to make up for everything I had missed in the previous months.» (Marlene)
Experiencing a sudden, unexplainable weight gain	«Eating should be a pleasure» (Hans)
To finally look healthy again	«Before my transplantation, I had to take my temperature every day, weigh myself, and measure my blood pressure. I'm still a bit traumatized. I'm done with scales.» (Lara) «Ate too much? Lounged around too much? No idea, I don't know. [. . .] All of a sudden it was more. There's no accounting for it.» (Margret) «I haven't felt this good in ages.» (Angelo) «They (family) accept me the way I am and are glad that I'm healthy. That's what's most important to them.» (Marlene)
I am allowed to look like this now	«I get too little exercise, it's true. Sometimes because I also have a nap in the afternoon, even in nice weather. I'm not making excuses, that's just how it is.» (Joe)
Body weight becomes a source of stress	«I feel better and everything is back to working the way it should.» (Katja)
Losing weight requires the right headspace	«I'm at a standstill with my weight, basically 10 kg overweight. It's apparent to me that it puts a great strain on me. And it enrages me because there's nothing I can do about it. [. . .] But I can't change anything about it. And that's what makes me kind of sad and crazy at the same time.» (Lara)
All attempts to lose weight fail	«Whenever I was at the hospital (for a follow-up), they said: Oh, you've put on weight, that's good. And then suddenly it was: Stop, no more! You really have to watch your weight now! Oh, okay. Now what am I supposed to do: eat or not eat? Yeah, that was pretty stupid.» (Valérie) «(It doesn't bother me that I) am a bit overweight. That's just how it is. No, I have to look like this. [. . .] They (the family) see me as an individual and not as an overweight person. They say: You're still here. Never mind.» (Valérie)

gained weight when their clothes grew tighter. Almost no-one had weighed themselves regularly, regarding it as unnecessarily stressful or as reminding them too much of when they were ill. In the absence of regular weighing, they were taken aback once they stood on scales; it was just so «sudden» (Marlene) and happened «at lightning speed» (Rudi).

In retrospect, the participants self-identified potential reasons for their weight gain: menopause, hormones, age, giving up smoking, and some spoke of a voracious appetite due to cortisone therapy. In terms of eating and exercise, one opinion was nearly universally held: «I don't think it's from eating but rather from a lack of exercise» (Valérie). Participants did not perceive any change in their eating habits over time. Only one person thought he ate larger portions than before, while all others made the point that «really, I just eat normally» (Rudi). However, almost everybody estimated their own level of exercise as being too low. Only a few took part in sporting activities and everyday activity was restricted, in particular, because pain or fatigue kept them at home, they had become unemployed or socialized less often. Looking back, no-one had a clear explanation for what exactly had caused the weight gain.

To Finally Look Healthy Again

Overall, weight gain was welcome and positively assessed by all participants because everybody had lost weight pre- and post-LTx. Some had undergone extreme weight loss, greatly impacting their physical appearance, at times amounting to little more than «skin and bones» (Katja), which was «not a pretty sight» (Lara). To be thin and ill-looking was stressful, and all participants were glad when this phase had ended. Many noted that their muscles were regaining strength, they had more energy and a better quality of life. They emphasized that they felt better overall, regardless of whether they had reached their normal weight or had become overweight or obese. The participants' perceptions of their reinvigorated and well-functioning bodies reinforced this positive attitude. A severe illness had been overcome and weight gain was a visible and tangible representation of a return to health. They were back in the fullness of life. Family and friends reinforced this positive attitude towards weight gain. In this instance also, it did not matter whether the weight gain led to overweight or obesity. The most important thing was that the individual affected had survived the disease and was healthy once again.

I am Allowed to Look Like This Now

Body Weight Becomes a Source of Stress

For those who did not halt their weight gain deliberately and in a timely manner, what had been an emotionally reassuring and positive experience turned into a stressful one. Those affected felt uncomfortable with their weight, experienced it as unpleasant or

were «ashamed» (Lara). The weight category they fell into made no difference. Angelo was normal weight and dissatisfied after gaining five kilos in 4 months. Marlene was particularly adversely affected by her new-onset obesity: «It bothers me a lot the way it looks. [. . .] Sometimes I have bad days where everything tends to go wrong. [. . .] You get home in the evening totally wiped out, and then I look at myself in the mirror and think: Man, you look like complete crap!».

The (over-)weight was also a problem physically. Many reported limited flexibility, having become cumbersome, or even experiencing pain. Considering the emotional and physical burdens, the participants struggled with their weight and engaged in losing weight. However, the energy and motivation with which they approached the topic varied greatly and showed itself in two groups.

Losing Weight Requires the Right Headspace

Several participants had given some thought to what they could do to lose weight: Pay attention during meals, reduce portion size or carbohydrates, buy fitness equipment or join a gym. Although these ideas were clearly stated, the narratives remained vague: «I'm trying now to get back to that weight. With food, maybe eat a little less [. . .] and maybe being more active.» (Katja).

Some tried to transform an idea into action but were often frustrated in daily life. Either the individual was unable to resist the temptation of delicious foods or the need for quiet and recuperation was greater than the urge to be active. Added to this was a certain lethargy due to various physical conditions such as pain or lack of stamina. Because the plans to lose weight were undertaken so half-heartedly, if at all, they met with little or no success. Many realized in retrospect that their plans to lose weight were not progressing because losing weight requires the right headspace for which you have to «overcome your "inner laziness"» (Margret). For some, their ambition was constrained by, as they explained, them having struggled with their weight even pre-LTx. Ultimately, those affected concluded for themselves that, given all that they had lived through, their present situation was acceptable. The awareness of being alive lessened the motivation to invest more energy to lose the bothersome extra weight.

All Attempts to Lose Weight Fail

Some participants attempted to lose weight by means of strict dieting and self-discipline. While everyone in this group lost a few kilos, they were either unable to reach their self-identified goal weight or, if they did, it was only a short time before they experienced a yo-yo effect, something they were unable to account for. In their own perception, they had tried to lose weight as best as they knew how and with deep commitment. The inexplicability of this failure and the realization that all of their efforts were in vain led to frustration and anger. They felt that they were at the mercy of a situation they were powerless to affect. Many felt that they had been abandoned in this situation—including by the LTx center's healthcare professionals. Participants regarded the discussions at their follow-up appointments at the hospital as confusing and deemed the nutritional recommendations unhelpful: «You can forget it, I already know all of that and have done it for years» (Lara).

The original intention to lose weight began to fall by the wayside as failure and disappointment took a toll. The participants chose to confront the situation in various ways. Some adapted their goal to the new circumstances: at least do not gain more weight. Others, like Marlene, questioned the basic concept of weight loss: «What is the good of all of this to me?».

Memories of their illness, the transplantation, their recovery and the awareness of their own survival have been burned into their memory. The issue of excess weight lost its magnitude in comparison to what they had experienced. Rather, it became something they were able to come to terms with, borne out in the statements of their family members and social contacts. Physical and emotional burdens were brushed aside and a space was created for an awareness of having gotten through a difficult time. This insight not only offered consolation for the failure to lose weight; it also curbed the impulse to engage further with the topic. Having survived the severe illness served as a welcome justification and enabled a more forgiving relationship to their own overweight body: «You have to make the best of these sorts of things. But I always say to myself: It could be worse, at least I'm still around.» (Marlene).

DISCUSSION

Our findings highlight how patients put into perspective their lived experience of being an LTx survivor with the perception of post-LTx weight gain. *The Main Thing is to be Alive* Section was captured as a recurrent yet multifaceted conclusion in the three other themes, thereby shaping the patients' perceptions of weight gain and coping mechanisms. Professionals should be aware of the dynamics to support patients in weight management. Based on the participants' perceptions about a lack of support from healthcare professionals, we also provide clinical implications and suggestions for education and self-management support, based on the TTM in **Table 3** (16, 17).

The Arduous Path Back to Living Section characterized the emotional course of the main theme. Our participants' illness and recovery trajectory matched with previous descriptions. Life pre-LTx was dominated by distressing complications associated with a decreased quality of life and frequent hospitalizations, turning it into an unpredictable roller-coaster (24-26). Post-Tx, patients experienced increased physical functioning, emotional health and quality of life, contributing to the perception that the LTx was a salvation, miracle or gift (27-29). Those strong analogies emphasize the meaning of LTx for those affected. This meaning was also highlighted in our study, and the intense experiences framed the patients' subsequent perception of their weight.

A Pleasurable New Phase of Life Section characterized the joyful part of the main theme, which was accompanied by weight gain. Participants in previous studies named increased food

TABLE 3 | Education and self-management support by healthcare professionals based on the Transtheoretical Model (TTM). The recommendations are based on the authors' clinical experiences and the TTM, which describes stages of change: precontemplation, contemplation, preparation, action, maintenance and termination (16, 17). The stages represent a time dimension, although people may advance through the stages non-linearly. Progressing through the stages is accompanied by (overt or covert) activities that are described as processes of change (e.g., consciousness raising, self-reevaluation, environmental evaluation, stimulus control). Based on these core constructs, each stage requires a distinct intervention approach.

Theme	Transtheoretical model			Education and self-management support
	Stage of change	Process of change	Aim	
The arduous path back to living	Not applicable	Not applicable		• Priority is the physical and emotional recovery after LTx. Management of unplanned weight gain is most probably less important • Focus on relationship building during the frequent follow up appointments in the LTx center
A pleasurable new phase of life	Precontemplation: A person does not intend to take any action to prevent weight gain in the near future (usually described as 6 months)	Consciousness raising	Increase awareness on causes, consequences and potential treatment	Provide information on • Short- and long-term evolution of weight after LTx • Factors associated with weight gain in general • Body composition: offer repeated measurements to assess and specify the evolution of weight gain (e.g., increasing muscle mass or fat) • Risk of developing new-onset obesity and its associated outcomes after LTx (e.g., cardiovascular and metabolic comorbidities) • Concept of energy balance (calory consumption and expenditure) • Physical activity and healthy eating • Importance of self-monitoring of weight • The advantage of preventing excessive weight gain instead of losing weight afterwards Provide feedback • It may be important to acknowledge the patient's healthy appearance with the regained weight. However, healthcare professionals should also critically question this development • Focus the communication on empowerment and self-management to intensify relationship building
I am allowed to look like this now	Contemplation: A person intends to take action within the next 6 months	Self-reevaluation	Facilitate the person's assessment that behavior change is part of the own identity	• Assess the perception of weight gain and a potential burden during clinical follow-ups • Be aware of and listen to patient's talking about pro and con arguments for changing their behavior • Identify the motivation, barriers and facilitators for behavior change
	Preparation: A person intends to take action within the next 30 days or has taken some behavioral steps already			• Define individual goals regarding the patients' behavior (e.g., eating or activity) or weight loss (e.g., target weight) • Make sure that goals are specific, measurable, achievable, relevant, and time bound. Pay special attention to feasible goals regarding activity in case of functional impairment • Identify strategies to achieve the goals • Plan timely follow-up appointments • Evaluate the involvement of a nutritionist and physiotherapist
	Action stage: A person has changed the behavior for less than 6 months	Self-liberation	Support the persons commitment to change	• Provide feedback on achievement and celebrate the success • Strengthen the patient's self-efficacy and self-consciousness

intake and improved appetite as main reasons for weight gain (9, 10). This finding contrasts to those in our narratives, where almost everyone insisted that their eating habits had returned to those pre-LTx. Nonetheless, this effect may have contributed to weight gain due to the concurrent decrease in physical activity, resulting in an energy imbalance between calory consumption and expenditure (30). Reduced activity is indeed common after LTx. Recipients have worse physical functioning compared to the general population (27), and only 45% meet the recommended physical activity levels (31). Although our participants were well aware of their inactivity, they did not prominently mention the idea of adapting their food intake accordingly. Moreover, as they

did not regularly weigh themselves, the increased weight came as a surprise to most of them. This behavior should be targeted in interventions because self-monitoring of weight is crucial in successful weight management (32).

Another remarkable aspect was the participants' appreciation of weight gain. As a body composition measurement was not available, the participants' weight gain could not be specified in muscle mass, which would be positive and desired, or increasing fat mass, which would be associated with negative health outcomes such as cardiovascular or metabolic comorbidities (5-8). But in general, weight gain has been associated with decreased physical health-related quality of life (33), while obesity has been consistently associated with depression (34). None of our participants mentioned overweight or obesity in connection with potential negative health outcomes. In contrast, even if weight gain had led to overweight or obesity, the gain was equalized with looking healthy. Health seemed to be defined as the absence of liver disease, which remains an assumption as we did not further explore this topic. Nonetheless, weight gain was a visible sign of having survived the severe illness. Increased energy and wellbeing were indicators of recovery and normality. The importance of those reference points should not be underestimated. Patients in another study described the return to a self-defined normality as a milestone after LTx (28). Patients' appreciation of the increased body weight combined with, 1) having no coherent explanation for weight gain, 2) the lack of awareness about intervening appropriately, and 3) not linking the excessive weight to potential negative outcomes present an opportunity for early and preventive interventions (**Table 3**).

I am Allowed to Look Like This Now Section characterized the consoling part of the main theme, which was visible in both groups, who differed with regard to the motivation to tackle weight gain. The vague wording by participants in the "Losing weight requires the right headspace" group indicated ambivalence. Although they felt physically and emotionally uncomfortable, they were not sufficiently triggered to engage in effective weight loss behaviors (e.g., reduce calorie intake, increase activity). Participants in the "All attempts to lose weight fail" group tried hard to lose weight or maintain weight loss, unfortunately unsuccessfully. Although our participants felt frustrated, angry and deserted by the LTx team, no-one mentioned in the interview to have actively sought additional support. However, weight loss and its maintenance are hard to achieve due to physiological compensation mechanisms (35), even with professional support. A telehealth-delivered lifestyle program combined Mediterranean diet with aerobic and resistance exercise after LTx (36). Although the intervention group lost weight over 12 weeks and the controls did not (mean −1.8 kg vs. +0.1 kg), the results show that weight loss comes in small steps and takes time. Although guidelines consistently advise to prevent weight gain instead of trying to lose weight afterwards (37), studies examining preventive weight interventions after LTx are lacking. Participants who contributed experiences to this theme expressed stress and burden due to the increased weight and unsuccessful weight loss attempts. Yet our analysis left us wondering if the participants' negative narratives were really part of their daily life or if they were rather nudged by our interview. Our reservations arose because it seemed as if participants in both groups did not feel enough pressure to more rigorously lose weight by finding alternatives to the previous failed attempts. Instead, the theme *I am allowed to look like this now* emerged as an insight. This may have comforted participants but it bares the potential of a killer argument because it may stop further engagement in weight loss. Professionals should proactively assess the TTM's stages of change to provide targeted support (**Table 3**).

Our study findings should be interpreted in light of some limitations. In qualitative studies results are not generalizable to all patients. The categorization of the weight category at time of the interview relied on a self-reported weight measure and was not verified by an objective measurement performed by a healthcare professional. The weight category at LTx was not considered as inclusion criteria, which contributed to the heterogeneity of the group. The analysis did not consider disease etiologies to account for various perspectives on weight gain. Future studies in distinct subgroups are needed to explore potential differences in experiences and coping strategies. As we only included German-speaking patients, we lack understanding of how people with other ethnic or cultural backgrounds experience post-LTx weight gain. They may have different perceptions resulting in a need for other supportive interventions diverse.

CONCLUSION

Exploring patients' experiences with weight gain after LTx revealed the importance of having survived the severe illness, which shaped perceptions of and coping with weight gain. After suffering during the course of LTx, the weight increase was initially appreciated and equated with health. For some participants, ongoing weight gain led to an emotional and physical burden, which was brushed away by the comforting insight *I am allowed to look like this now.* As this argument might hinder further engagement in weight loss interventions, professionals should be aware of the need for early interventions that address patients' specific needs related to weight gain after LTx.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

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

AUTHOR CONTRIBUTIONS

SB, KK, and OM designed the research; SB, KK, and OM conducted the research; SB, PK-H, KK, and OM analyzed the data; SB, PK-H, KK, and OM wrote 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.

ACKNOWLEDGMENTS

A wholehearted thank you goes to all participants for sharing their experiences and especially to Ines and Irène who volunteered to review and discuss the findings. We thank Jane White for translating the qualitative results into English and Anne Wegner for the editing.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10256/full#supplementary-material

REFERENCES

1. Palmer M, Schaffner F, Thung SN. Excessive Weight Gain after Liver Transplantation. *Transplantation* (1991) 51(4):797–800. doi:10.1097/00007890-199104000-00012
2. Rezende Anastácio L, García Ferreira L, Costa Liboredo J, de Sena Ribeiro H, Soares Lima A, García Vilela E, et al. Overweight, Obesity and Weight Gain up to Three Years after Liver Transplantation. *Nutr Hosp* (2012) 27(4):1351–6. doi:10.3305/nh.2012.27.4.5768
3. Richards J, Gunson B, Johnson J, Neuberger J. Weight Gain and Obesity after Liver Transplantation. *Transpl Int* (2005) 18(4):461–6. doi:10.1111/j.1432-2277.2004.00067.x
4. Beckmann S, Nikolic N, Denhaerynck K, Binet I, Koller M, Boely E, et al. Evolution of Body Weight Parameters up to 3 Years after Solid Organ Transplantation: The Prospective Swiss Transplant Cohort Study. *Clin Transpl* (2017) 31(3). doi:10.1111/ctr.12896
5. van Son J, Stam SP, Gomes-Neto AW, Osté MCJ, Blokzijl H, van den Berg AP, et al. Post-transplant Obesity Impacts Long-Term Survival after Liver Transplantation. *Metabolism* (2020) 106:154204. doi:10.1016/j.metabol.2020.154204
6. Jiménez-Pérez M, González-Grande R, Omonte Guzmán E, Amo Trillo V, Rodrigo López JM. Metabolic Complications in Liver Transplant Recipients. *Wjg* (2016) 22(28):6416–23. doi:10.3748/wjg.v22.i28.6416
7. Shaker M, Tabbaa A, Albeldawi M, Alkhouri N. Liver Transplantation for Nonalcoholic Fatty Liver Disease: New Challenges and New Opportunities. *Wjg* (2014) 20(18):5320–30. doi:10.3748/wjg.v20.i18.5320
8. Beckmann S, Denhaerynck K, Stampf S, Saigi-Morgui N, Binet I, Koller M, et al. New-onset Obesity after Liver Transplantation-Outcomes and Risk Factors: the Swiss Transplant Cohort Study. *Transpl Int* (2018) 31(11):1254–67. doi:10.1111/tri.13308
9. Anastácio LR, Ferreira LG, de Sena Ribeiro H, Liboredo JC, Lima AS, Toulson Davisson Correia MI. Metabolic Syndrome after Liver Transplantation: Prevalence and Predictive Factors. *Nutrition* (2011) 27(9):931–7. doi:10.1016/j.nut.2010.12.017
10. Bolton LA, Walsh SM, O'Sullivan NB, Barnes E, McCormick AP, Corish CA. Patient Perspectives of Weight Gain Following Orthotopic Liver Transplantation: A Qualitative Study. *Liver Transpl* (2020) 26(4):591–7. doi:10.1002/lt.25698
11. Butland B, Jebb S, Kopelman P, McPherson K, Thomas S, Mardell J, et al. *Foresight. Tackling Obesities: Future Choices – Project Report*. London: Government Office for Science (2007). Available at: http://www.bis.gov.uk/foresight/our-work/projects/published-projects/tackling-obesities/reports-and-publications (Accessed October 3, 2021).
12. Saigi-Morgui N, Quteineh L, Bochud P-Y, Crettol S, Kutalik Z, Wojtowicz A, et al. Weighted Genetic Risk Scores and Prediction of Weight Gain in Solid Organ Transplant Populations. *PLoS One* (2016) 11(10):e0164443. doi:10.1371/journal.pone.0164443
13. Anastácio LR, Ferreira LG, de Sena Ribeiro H, Lima AS, Garcia Vilela E, Toulson Davisson Correia MIMI. Body Composition and Overweight of Liver Transplant Recipients. *Transplantation* (2011) 92(8):947–51. doi:10.1097/tp.0b013e31822e0bee
14. Patel SS, Siddiqui MB, Chadrakumaran A, Faridnia M, Lin F-P, Hernandez Roman J, et al. Office-Based Weight Loss Counseling Is Ineffective in Liver Transplant Recipients. *Dig Dis Sci* (2020) 65(2):639–46. doi:10.1007/s10620-019-05800-6
15. Michie S, van Stralen MM, West R. The Behaviour Change Wheel: a New Method for Characterising and Designing Behaviour Change Interventions. *Implementation Sci* (2011) 6:42. doi:10.1186/1748-5908-6-42
16. Prochaska JO, Velicer WF. The Transtheoretical Model of Health Behavior Change. *Am J Health Promot* (1997) 12(1):38–48. doi:10.4278/0890-1171-12.1.38
17. Prochaska JO, Redding CA, Evers KE. The Transtheoretical Model and Stages of Change. In: Glanz K, Rimer B, Viswanath K, editors. *Health Behavior and Health Education: Theory, Research, and Practice*. 4 ed. San Francisco: Jossey-Bass (2008). p. 501–92.
18. Mastellos N, Gunn LH, Felix LM, Car J, Majeed A. Transtheoretical Model Stages of Change for Dietary and Physical Exercise Modification in Weight Loss Management for Overweight and Obese Adults. *Cochrane Database Syst Rev* (2014)(2) CD008066. doi:10.1002/14651858.CD008066.pub3
19. European Association for the Study of the Liver, European Association for the Study of Diabetes, European Association for the Study of Obesity. EASL-EASD-EASO Clinical Practice Guidelines for the Management of Non-alcoholic Fatty Liver Disease. *J Hepatol*. 2016;64(6):1388–402.
20. Hickman IJ, Coran D, Wallen MP, Kelly J, Barnett A, Gallegos D, et al. 'Back to Life'-Using Knowledge Exchange Processes to Enhance Lifestyle Interventions for Liver Transplant Recipients: A Qualitative Study. *Nutr Diet* (2019) 76(4):399–406. doi:10.1111/1747-0080.12548
21. Braun V, Clarke V, Hayfield N, Terry G. Thematic Analysis. In: Liamputtong P, editor. *Handbook of Research Methods in Health Social Sciences*. Singapore: Springer (2019). doi:10.1007/978-981-10-5251-4_103
22. Braun V, Clarke V. Using Thematic Analysis in Psychology. *Qual Res Psychol* (2006) 3(2):77–101. doi:10.1191/1478088706qp063oa
23. Tong A, Sainsbury P, Craig J. Consolidated Criteria for Reporting Qualitative Research (COREQ): a 32-item Checklist for Interviews and Focus Groups. *Int J Qual Health Care* (2007) 19(6):349–57. doi:10.1093/intqhc/mzm042
24. Peng J-K, Hepgul N, Higginson IJ, Gao W. Symptom Prevalence and Quality of Life of Patients with End-Stage Liver Disease: A Systematic Review and Meta-Analysis. *Palliat Med* (2019) 33(1):24–36. doi:10.1177/0269216318807051
25. Asrani SK, Hall L, Hagan M, Sharma S, Yeramaneni S, Trotter J, et al. Trends in Chronic Liver Disease-Related Hospitalizations: A Population-Based Study. *Am J Gastroenterol* (2019) 114(1):98–106. doi:10.1038/s41395-018-0365-4
26. Hjorth M, Svanberg A, Sjöberg D, Rorsman F, Kaminsky E. Liver Cirrhosis Turns Life into an Unpredictable Roller Coaster: A Qualitative Interview Study. *J Clin Nurs* (2020) 29(23-24):4532–43. doi:10.1111/jocn.15478
27. Yang LS, Shan LL, Saxena A, Morris DL. Liver Transplantation: a Systematic Review of Long-Term Quality of Life. *Liver Int* (2014) 34(9):1298–313. doi:10.1111/liv.12553
28. Beckmann S, Künzler-Heule P, Biotti B, Spirig R. Mastering Together the Highs and Lows. *Prog Transpl* (2016) 26(3):215–23. doi:10.1177/1526924816654769
29. McKie P, Webzell I, Tavabie O, Loewenthal D, Heaton N. An Exploratory Study of the Experiences of Deceased-Donor Liver Transplant Recipients and

Their Need for Psychotherapeutic Support. *J Clin Nurs* (2020) 29(15-16): 2991–8. doi:10.1111/jocn.15309
30. Hall KD, Heymsfield SB, Kemnitz JW, Klein S, Schoeller DA, Speakman JR. Energy Balance and its Components: Implications for Body Weight Regulation. *Am J Clin Nutr* (2012) 95(4):989–94. doi:10.3945/ajcn.112.036350
31. van Adrichem EJ, Dekker R, Krijnen WP, Verschuuren EAM, Dijkstra PU, van der Schans CP. Physical Activity, Sedentary Time, and Associated Factors in Recipients of Solid-Organ Transplantation. *Phys Ther* (2018) 98(8):646–57. doi:10.1093/ptj/pzy055
32. Burke LE, Wang J, Sevick MA. Self-monitoring in Weight Loss: a Systematic Review of the Literature. *J Am Diet Assoc* (2011) 111(1):92–102. doi:10.1016/j.jada.2010.10.008
33. Hayes M, Baxter H, Müller-Nordhorn J, Hohls JK, Muckelbauer R. The Longitudinal Association between Weight Change and Health-Related Quality of Life in Adults and Children: a Systematic Review. *Obes Rev* (2017) 18(12):1398–411. doi:10.1111/obr.12595
34. Preiss K, Brennan L, Clarke D. A Systematic Review of Variables Associated with the Relationship between Obesity and Depression. *Obes Rev* (2013) 14(11):906–18. doi:10.1111/obr.12052
35. Greenway FL. Physiological Adaptations to Weight Loss and Factors Favouring Weight Regain. *Int J Obes* (2015) 39(8):1188–96. doi:10.1038/ijo.2015.59
36. Hickman IJ, Hannigan AK, Johnston HE, Elvin-Walsh L, Mayr HL, Staudacher HM, et al. Telehealth-delivered, Cardioprotective Diet and Exercise Program for Liver Transplant Recipients: A Randomized Feasibility Study. *Transpl Direct* (2021) 7(3):e667. doi:10.1097/txd.0000000000001118
37. National Institute for Health and Care Excellence. *Maintaining a Healthy Weight and Preventing Excess Weight Gain Among Adults and Children*. London: NICE guideline (2015).

ESOT | Transplant International

ORIGINAL RESEARCH
published: 22 April 2022
doi: 10.3389/ti.2022.10044

Optimal Intervention for Initial Treatment of Anastomotic Biliary Complications After Right Lobe Living Donor Liver Transplantation

Min Seob Kim, Suk Kyun Hong, Hye Young Woo, Jae-Hyung Cho, Jeong-Moo Lee, Kyung Chul Yoon, YoungRok Choi, Nam-Joon Yi, Kwang-Woong Lee and Kyung-Suk Suh*

Department of Surgery, Seoul National University College of Medicine, Seoul, South Korea

Background: This study evaluated endoscopic retrograde cholangiopancreatography (ERCP) and percutaneous transhepatic biliary drainage (PTBD) as interventions for patients with anastomotic biliary complications (ABC) after living donor liver transplantation (LDLT).

Methods: Prospectively collected data of patients who were diagnosed with ABC after LDLT between January 2013 and June 2017 were retrospectively reviewed.

Results: There were 57 patients who underwent LDLT with a right liver graft using duct-to-duct biliary reconstruction and experienced ABC. Among the patients with RAD involvement, there were no significant differences in the intervention success (p = 0.271) and patency rates (p = 0.267) between ERCP and PTBD. Similarly, among the patients with RPD involvement, there were no significant differences in the intervention success (p = 0.148) and patency rates (p = 0.754) between the two procedures. Graft bile duct variation (p = 0.013) and a large angle between the recipient and graft bile duct (R-G angle) (p = 0.012) significantly increased the likelihood of failure of ERCP in the RAD. When the R-G angle was greater than 47.5°, the likelihood of ERCP failure increased.

Conclusion: We recommend PTBD when graft bile duct variation is presented in patients with RAD involvement and/or when the R-G angle is greater than 47.5°.

Keywords: living donor liver transplantation, anastomotic biliary complications, endoscopic retrograde cholangiography, percutaneous transhepatic biliary drainage, right anterior hepatic duct, right posterior hepatic duct

OPEN ACCESS

Correspondence:
Suk Kyun Hong
nobel1210@naver.com

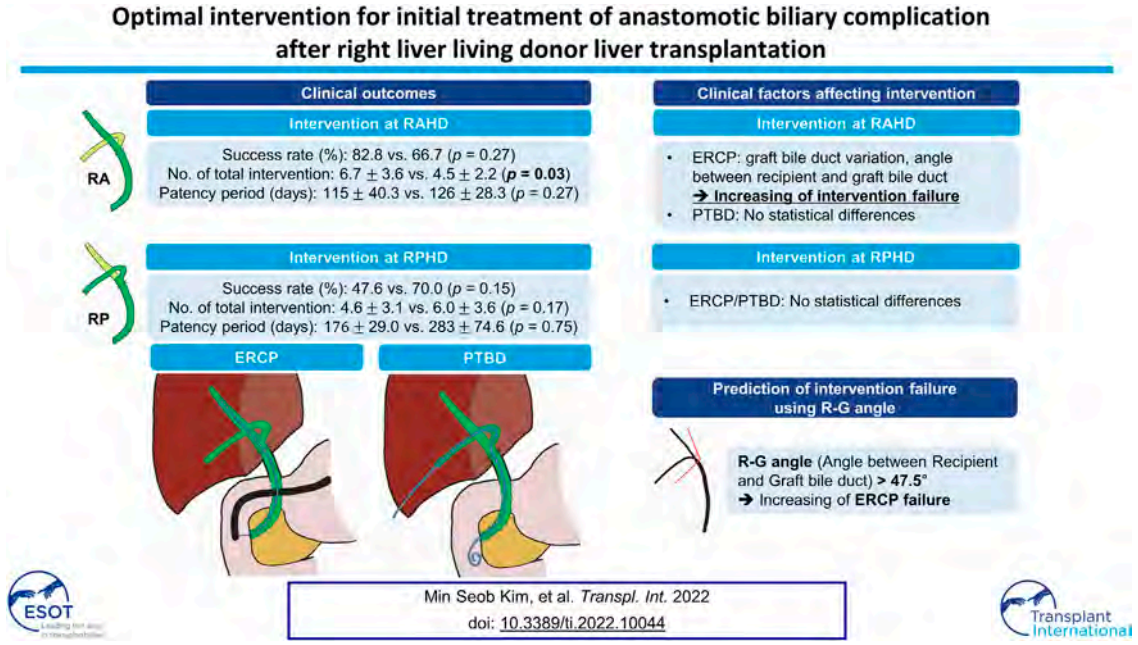
Received: *21 September 2021*
Accepted: *23 March 2022*
Published: *22 April 2022*

Citation:
Kim MS, Hong SK, Woo HY, Cho J-H, Lee J-M, Yoon KC, Choi Y, Yi N-J, Lee K-W and Suh K-S (2022) Optimal Intervention for Initial Treatment of Anastomotic Biliary Complications After Right Lobe Living Donor Liver Transplantation. Transpl Int 35:10044. doi: 10.3389/ti.2022.10044

INTRODUCTION

Liver transplantation (LT) with duct-to-duct (DD) biliary reconstruction has several physiologic advances and is a lifesaving treatment for patients with end-stage liver disease and hepatocellular carcinoma (1). In Asia, living donor liver transplantation (LDLT) is performed more often than deceased liver transplantation due to a shortage of cadaveric organ donors (2–4). Biliary

Abbreviations: ABC, Anastomotic biliary complications; ERCP, Endoscopic retrograde cholangiopancreatography; LDLT, Living donor liver transplantation; PTBD, Percutaneous transhepatic biliary drainage; RAD, Right anterior hepatic duct; R-G angle, Angle between recipient bile duct and graft bile duct; RL, Right hemi-liver; RPD, Right posterior hepatic duct.



Graphical Abstract |

anastomotic strictures or leakage are the most common complications following LT (5). LDLT is more susceptible to anastomotic biliary complications (ABC) compared to deceased liver transplantation (4, 6), because the right hemi-liver (RL) graft bile duct is short, arises at an acute angle, and has multiple openings that are prone to peribiliary vascular plexus damage. Interventional treatment is recommended for patients with ABC following LDLT with DD biliary reconstruction, because it is effective, non-invasive, and more convenient than surgery (7, 8). Endoscopic retrograde cholangiopancreatography (ERCP) is the first-line treatment, and percutaneous transhepatic biliary drainage (PTBD) may be performed as a rescue treatment if endoscopic treatment is unsuccessful (1, 3, 8, 9).

Anatomical variations of the RL graft bile duct influence the outcomes of DD biliary reconstruction (1, 4–6). The RL bile duct may have one or two duct openings, and a recent study by You *et al.* (4) recommended bilateral drainage for each of the right anterior and posterior hepatic ducts (RAD and RPD, respectively) of patients with ABC after LDLT with a RL graft, to improve final outcomes. There are limited studies examining which intervention (ERCP or PTBD) is more superior for each duct (RAD or RPD), with several factors affecting the success of ERCP or PTBD in either duct. Selection of the first-line treatment is particularly important because these patients have to undergo multiple consecutive procedures; therefore, the first-line treatment must be safe and convenient.

This study compared the efficacy of ERCP and PTBD in patients with ABC in the RAD or RPD after LDLT with a RL graft. We examined the factors that should be considered when selecting an intervention as a first-line treatment.

MATERIALS AND METHODS

Study Design and Population

This study included 418 patients who underwent LDLT at Seoul National University Hospital (SNUH) between January 2013 and June 2017. Sixty-nine patients (69/418, 16.5%) were newly diagnosed with ABC, such as anastomotic biliary stricture, anastomotic leakage, and anastomotic stricture with leakage, after LDLT, and these patients underwent either the ERCP or PTBD intervention initially. Among the patients with ABC, 12 were excluded because of hepaticojejunostomy biliary reconstruction (n = 6), a left liver graft (n = 5), and lack of data (n = 1). The demographic and baseline characteristics of the remaining 57 patients were analyzed. Among the 57 patients who were diagnosed with biliary complications, six patients underwent intervention only for the RAD, 13 patients underwent intervention only for the RPD, and 28 patients underwent intervention for both the RAD and RPD. Overall, 44 RAD interventions and 51 RPD interventions were performed, including both ERCP and PTBD procedures (**Figure 1**).

This study was approved by the Institutional Review Board of SNUH (approval no. 2101-132-1190). The requirement for informed consent was waived because of the study's retrospective design. Data were retrospectively collected from medical records and reviewed. No organs from executed prisoners were used.

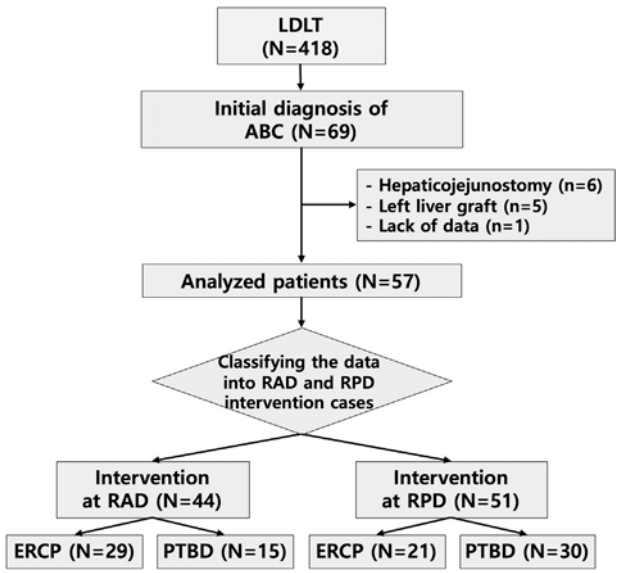


FIGURE 1 | The employed process for patient exclusion and data classification. LDLT, living donor liver transplantation; ABC, anastomotic biliary complications; RAD, right anterior hepatic duct; RPD, right posterior hepatic duct; ERCP, endoscopic retrograde cholangiopancreatography; PTBD, percutaneous transhepatic biliary drainage.

Biliary Anastomosis

When performing DD biliary anastomosis, anastomoses between the graft hepatic duct and recipient bile duct were performed in an end-to-end fashion. A mixed interrupted and continuous suturing technique was performed using 6–0 absorbable suture material. The tailored telescopic reconstruction method (TTR) (10) was selected and performed intraoperatively according to the operator. In the case of TTR, the graft hepatic duct was anastomosed to the inner layer of the recipient bile duct with good vascularity. The shape of the anastomosis was similar to that of a telescope. The posterior and anterior walls were sutured continuously with 6–0 non-absorbable suture material. During anastomosis, if the graft bile duct opening was in the form of binoculars or the distance across the bile duct opening was short, one biliary anastomosis was performed according to the operator.

Diagnosis of Anastomotic Biliary Complications

All patients received routine postoperative care according to the SNUH protocol. Inpatients and outpatients were assessed periodically, and liver computed tomography (CT) or magnetic resonance imaging (MRI) was performed when clinical symptoms, such as jaundice, itching, and abdominal pain, or abnormal laboratory findings, such as liver enzyme elevation and hyperbilirubinemia, were elicited. ABC was diagnosed in the presence of upstream bile duct dilatation or bile leakage in the anastomosis site.

Management of Anastomotic Biliary Complications

Patients with ABC after LDLT were initially managed with supportive medical care. The intervention was selected with a multidisciplinary approach based on the patient's history and clinical and laboratory findings. For patients diagnosed with biliary complications after LDLT, a multidisciplinary team, including the transplant and radiology teams, discussed the treatment plan together. If CT or MRI was performed in cases where biliary complications were suspected, the more appropriate intervention was determined based on the imaging findings. The findings we considered included the size of peripheral bile duct dilatation, angulation of the anastomosis site, and the possibility of percutaneous access.

Endoscopic Retrograde Cholangiopancreatography

ERCP was performed under sedation. The side-view endoscope was inserted into the duodenum to check the ampulla of Vater, then cannulation was performed. If cannulation failed several times, papillotomy was performed using a needle knife. After cannulation, a guidewire was inserted and passed through the ABC. If anastomotic stricture was found through fluoroscopy, 4–10 mm sized balloon dilatation was performed. Plastic stents were inserted, with sizes 7, 8.5, and 10 F, and lengths between 5 and 15 cm, along the guidewire that had been passed through the ABC. In some cases, either a straight or pig tail catheter was selected according to the interventionist.

If the stent insertion through ERCP was successful, recurrence of biliary complications and procedure-related complications were not expected; thus, outpatient follow-up was performed after three to 6 months. After follow-up, it was decided whether to perform planned internal stent removal or revision. Further stricture site dilatation was optionally performed when there was no improvement in the patient's biliary complications after initial stent insertion.

Percutaneous Transhepatic Biliary Drainage

PTBD was performed by radiologic interventionists under local anesthesia. When a biliary stricture site was confirmed through fluoroscopy, 4–6 mm balloon dilatation was carried out after passage of the guidewire. After that, an 8.5 F pigtail catheter was inserted initially. For planned gradual dilatation, the catheter was extended from size 10 up to 14 F every 2–3 days while maintaining external PTBD. When the catheter was expanded to its maximum size, external PTBD was maintained for approximately 1 month and follow-up was performed at the outpatient clinic. Finally, when the patient's symptoms remained stable, replacement with an internal stent was performed. The size and diameter of the internal stent were similar to those of the ERCP plastic stent.

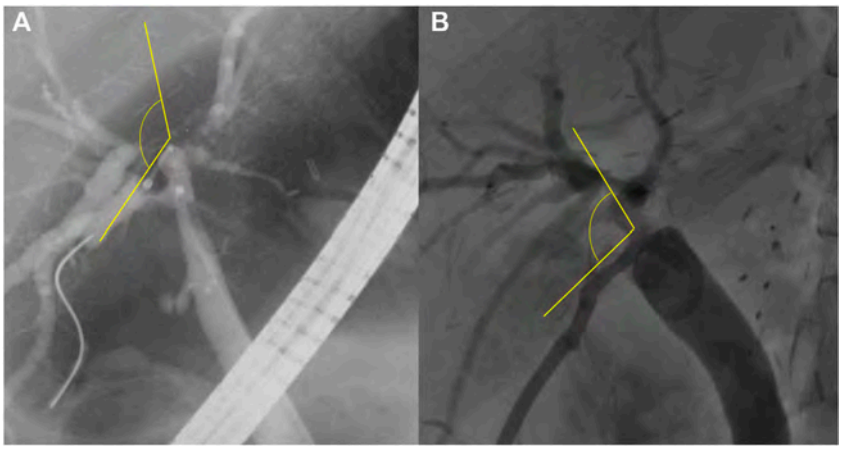


FIGURE 2 | The angle between the recipient and graft bile ducts (R-G angle). The R-G angle is measured as the angle between the two straight yellow lines, shown on fluoroscopic imaging **(A)** during endoscopic retrograde cholangiopancreatography and **(B)** during percutaneous transhepatic biliary drainage.

The rendezvous method was also considered when the angle of the anastomosis site was acute or twisted, making it difficult to insert an internal stent through PTBD. If replacement with an internal stent was not possible due to tight biliary stricture, ERCP was re-tried while maintaining external biliary drainage. In addition, if it was determined that the biliary stricture could not be replaced by internal drainage, hepaticojejunostomy was performed in consideration of the patient's quality of life. Similarly, an 8.5 or 10 F PTBD catheter was inserted in a place with biliary leakage to cover the site. External drainage was continued until the patient's symptoms and radiologic findings improved.

Definition

ABC was classified as stricture only, leakage only, and both stricture and leakage. Procedural success in strictures was defined by the ability to pass a catheter or stent through the anastomotic stricture site, which resulted in the improvement of clinical symptoms and/or laboratory findings during the hospitalization period. Procedural success in leakages was defined by the ability to cover the anastomotic leakage site with a catheter or stent. The overall success rate was defined as the ratio between the number of successful interventions and total number of interventions.

Patency was defined as the period from the first intervention performed for initial biliary complications to the second intervention performed to treat recurred biliary complications. If the first intervention was performed over several days, the patency rate was defined as the period from the last day of the planned first intervention until the recurrence of complications. The angle between the recipient and graft bile ducts (R-G angle) was defined as the angle formed by the passage of the catheter or stent between the recipient and graft bile ducts on fluoroscopic imaging during ERCP or PTBD (**Figure 2**).

Statistical Analysis

Categorical variables were presented as numbers and percentages, whereas continuous variables were presented as mean ± standard deviation. Categorical variables were compared using the χ^2 test, Fisher's exact test, and linear-by-linear association, and continuous variables were compared using the Student's *t*-test. The patency rates were estimated with Kaplan-Meier survival analysis, and the groups were compared using the log-rank test. Data were analyzed using the Statistical Package for the Social Sciences software version 25.0 (IBM Corp, Armonk, New York, NY, United States). A p value of <0.05 was considered statistically significant.

RESULTS

Baseline Characteristics

The baseline characteristics of the patients with ABC after LDLT using an RL graft are summarized in **Table 1**. The mean age of the patients was 55.2 ± 8.6 years, and 77.2% were male. The most common etiology of liver cirrhosis was hepatitis B virus (34/57, 59.6%), followed by alcoholic liver cirrhosis (8/57, 14%) and hepatitis C virus (7/57, 12.3%). The average Model for End-stage Liver Disease score was 16.2 ± 6.8, and the average Child-Pugh score was 7.8 ± 2.6. The number of ABO-compatible donors and recipients was 82.5% (47/57). The mean follow-up duration was 44.2 ± 1.7 months. The total number of biliary interventions performed during follow-up was 5.2 ± 3.4.

The mean age of the liver donors was 35.5 ± 12.2 years, with 33 (57.9%) laparoscopic donor hepatectomies and 24 (42.1%) open donor hepatectomies performed. There were 17 (29.8%) cases with graft bile duct variation. The average number of bile duct openings was 1.7 ± 0.7, and the average bile duct diameter was 4.8 ± 2.1 mm. Thirty-six (63.2%) patients underwent DD biliary reconstruction using the TTR method (10), and seven (12.3%) patients underwent intraoperative biliary drainage. Intraoperative hepatic artery complications, postoperative hepatic artery occlusion, and postoperative bleeding were noted in six (10.5%), three (5.3%), and eight (14%) cases, respectively. The mean period from the LDLT to the first

TABLE 1 | Demographic and baseline characteristics of the study population.

Variables	(*n* = 57)
Age, mean ± SD, years	55.2 ± 8.6
Sex, *n* (%)	
Male	44 (77.2%)
Female	13 (22.8%)
Etiology of liver cirrhosis, *n* (%)	
Hepatitis B virus	34 (59.6%)
Alcoholic liver cirrhosis	8 (14.0%)
Hepatitis C virus	7 (12.3%)
Non-B and non-C hepatitis	3 (5.3%)
Autoimmune hepatitis	2 (3.5%)
Hepatitis B virus with alcoholic liver cirrhosis	1 (1.8%)
Primary biliary cirrhosis	1 (1.8%)
BMI, mean ± SD, kg/m^2	23.4 ± 3.3
MELD score, mean ± SD	16.2 ± 6.8
Child-Pugh score, mean ± SD	7.8 ± 2.6
ABO compatibility between donor and recipient, *n* (%)	
Compatible pairs	47 (82.5%)
Incompatible pairs	10 (17.5%)
Follow-up duration, mean ± SD, month	44.2 ± 1.7
Total interventions during the follow-up period, mean ± SD	5.2 ± 3.4
Donor age, mean ± SD, years	35.5 ± 12.2
Donor hepatectomy type, *n* (%)	
Laparoscopic method	33 (57.9%)
Open method	24 (42.1%)
Graft bile duct	
Number of variations, *n* (%)	17 (29.8%)
Number of openings, mean ± SD	1.7 ± 0.7
Size, mean ± SD, mm	4.8 ± 2.1
Bile duct anastomosis—TTR method, *n* (%)	36 (63.2%)
Intraoperative biliary drainage, *n* (%)	7 (12.3%)
Intraoperative hepatic artery problem, *n* (%)	6 (10.5%)
Postoperative hepatic artery occlusion, *n* (%)	3 (5.3%)
Postoperative bleeding, *n* (%)	8 (14.0%)
Duration to initial intervention, mean ± SD, month	
All interventions	9.0 ± 8.6
RAD intervention	10.6 ± 9.0
RPD intervention	10.9 ± 9.2
Clinical manifestation, *n* (%)	
LFT abnormality	52 (91.2%)
Itching	14 (24.6%)
Jaundice	9 (15.8%)
Fever	5 (8.8%)
Abdominal pain	5 (8.8%)
Laboratory findings before the initial intervention, mean ± SD	
WBC, 10^3/μL	5.8 ± 2.7
CRP, mg/dL	1.7 ± 4.0
Total bilirubin, mg/dL	2.3 ± 2.7
Direct bilirubin, mg/dL	1.6 ± 2.2
ALP, IU/L	305.0 ± 198.2
GGT, IU/L	569.8 ± 593.3
AST, IU/L	95.7 ± 77.6
ALT, IU/L	153.1 ± 170.8

SD, standard deviation; BMI, body mass index; MELD, model for end-stage liver disease; TTR, tailored telescopic reconstruction; RAD, right anterior hepatic duct; RPD, right posterior hepatic duct; LFT, liver function test; WBC, white blood cell; CRP, C-reactive protein; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; AST, aspartate aminotransferase.

intervention performed for ABC was 9 ± 8.6 months. Among patient diagnosed with ABC, the most common clinical manifestation was abnormal blood liver function test results (52/77, 91.2%), followed by itching (14/57, 24.6%), jaundice (9/57, 15.8%), fever (5/57, 8.8%), and abdominal pain (5/57, 8.8%). The mean total bilirubin and C-reactive protein levels prior to the first intervention for ABC were 2.3 ± 2.7 mg/dl and 1.7 ± 4.0 mg/dl, respectively.

Clinical Outcomes

The clinical outcomes of both interventions are summarized in **Table 2**. These results were analyzed according to whether the interventions were performed on the RAD or RPD.

Among the patients with RAD involvement, ERCP and PTBD were attempted in 29 and 15 cases, respectively. Among the patients who underwent ERCP, 28 (96.6%) patients had anastomosis site stricture, and 1 (3.4%) patient had stricture with leakage. Among the patients who underwent PTBD, 11 (73.3%), 1 (6.7%), and 3 (20.0%) patients had anastomosis stricture, leakage, and stricture with leakage, respectively. The success rates of ERCP and PTBD were 82.8% and 66.7%, respectively. There was no significant difference in the success rate between the two groups ($p = 0.27$); however, the ERCP group underwent significantly more interventions during the follow-up period than the PTBD group (6.7 ± 3.6 vs. 4.5 ± 2.2, $p = 0.03$). Among the patients who underwent ERCP, the patency rates at 6, 12, and 24 months were 27.6%, 10.3%, and 3.4%, respectively. In contrast, the patency rates at 6, 12, and 24 months in the PTBD group were 40%, 33.3%, and 13.3%, respectively. There was no significant difference in the median patency period (115 ± 40.3 vs. 126 ± 28.3 days; $p = 0.27$) between the two groups.

Among the patients with RPD involvement, ERCP was performed for 17 (81%), 3 (14.3%), and 1 (4.7%) cases of anastomosis site stricture, leakage, and stricture with leakage, respectively, whereas PTBD was performed for 23 (76.7%), 3 (10%), and 4 (3.3%) cases of anastomosis site stricture, leakage, and stricture with leakage, respectively. The success rates of ERCP and PTBD were 47.6% and 70%, respectively; however, the difference was not statistically significant ($p = 0.15$). There was no significant difference in the total number of interventions during the follow-up period between both groups (4.6 ± 3.1 vs. 6 ± 3.6; $p = 0.17$). The patency rates of ERCP at 6, 12, and 24 months were 47.6%, 28.6%, and 28.6%, respectively, whereas the patency rates of PTBD at the same timepoints were 60%, 40%, and 19.4%, respectively. There was no significant difference in the median patency period between the two groups (176 ± 29 vs. 283 ± 74.6 days; $p = 0.75$).

Comparison of Variables Affecting Intervention Success

We analyzed the variables that affected the success of ERCP and PTBD in the RAD and RPD involvement groups. Among the patients with RAD involvement, ERCP was significantly more likely to fail in patients with graft bile duct variations than in patients without variations (80% vs. 16.7%, $p = 0.013$). The R-G angle was also significantly greater in the group where ERCP failed than in the group where ERCP was successful (54.8 ± 24.2° vs. 28.8 ± 18.6°; $p = 0.012$). In comparison, there were no significant differences in the above variables when PTBD was performed for ABC of the RAD (**Table 3**). However, the success rate of PTBD tended to decrease as the number of

TABLE 2 | Clinical outcomes of biliary interventions in the study population.

	RAD Involvement (*n* = 44)			RPD Involvement (*n* = 51)		
	ERCP (*n* = 29)	PTBD (*n* = 15)	*p* value	ERCP (*n* = 21)	PTBD (*n* = 30)	*p* value
Type of biliary complication, *n* (%)						
Stricture	28 (96.6%)	11 (73.3%)		17 (81%)	23 (76.7%)	
Leakage	0 (0%)	1 (6.7%)		3 (14.3%)	3 (10%)	
Stricture with leakage	1 (3.4%)	3 (20%)		1 (4.7%)	4 (13.3%)	
Success rate, *n* (%)	24 (82.8%)	10 (66.7%)	0.27	10 (47.6%)	21 (70%)	0.15
Total interventions during the follow-up period, mean ± SD	6.7 ± 3.6	4.5 ± 2.2	0.03	4.6 ± 3.1	6 ± 3.6	0.17
Patency period, mean ± SD, days	115 ± 40.3	126 ± 28.3	0.27	176 ± 29.0	283 ± 74.6	0.75

RAD, right anterior hepatic duct; RPD, right posterior hepatic duct; ERCP, endoscopic retrograde cholangiopancreatography; PTBD, percutaneous transhepatic biliary drainage; SD, standard deviation.

TABLE 3 | Comparison of clinical variables with the intervention results across the RAD and RPD involvement groups.

	RAD Involvement (*n* = 44)						RPD Involvement (*n* = 51)					
	ERCP in RAD (N = 29)			PTBD in RAD (*n* = 15)			ERCP in RPD (*n* = 21)			PTBD in RPD (*n* = 30)		
	Success (*n* = 24)	Failure (*n* = 5)	*p* value	Success (*n* = 10)	Failure (*n* = 5)	*p* value	Success (*n* = 10)	Failure (*n* = 11)	*p* value	Success (*n* = 21)	Failure (*n* = 9)	*p* value
Graft bile duct variation, *n* (%)	4 (16.7%)	4 (80%)	0.013	3 (30%)	3 (60%)	0.33	2 (20%)	2 (18.2%)	1.00	8 (38.1%)	3 (33.3%)	1.00
Hepatic artery complications, *n* (%)	2 (8.3%)	0 (0%)	1.00	3 (30%)	0 (0%)	0.51	0 (0%)	2 (18.2%)	0.48	3 (14.3%)	1 (11.1%)	1.00
Bile duct anastomosis—TTR method, *n* (%)	15 (62.5%)	4 (80%)	0.63	6 (60%)	4 (80%)	0.60	7 (70%)	4 (36.3%)	0.20	16 (76.2%)	5 (55.6%)	0.39
Donor surgical approach, *n* (%)						1.00			0.18			0.43
Laparoscopic method	9 (37.5%)	3 (60%)	0.62	7 (70%)	3 (60%)		5 (50%)	9 (81.8%)		9 (42.9%)	6 (66.7%)	
Open method	15 (62.5%)	2 (40%)		3 (30%)	2 (40%)		5 (50%)	2 (18.2%)		12 (57.1%)	3 (33.3%)	
Intraoperative drainage, *n* (%)	2 (8.3%)	0 (0%)	1.00	2 (20%)	2 (40%)	0.56	1 (10%)	1 (9.1%)	1.00	3 (14.3%)	1 (11.1%)	1.00
Number of bile ducts, *n* (%)			0.72			1.00			1.00			0.57
One	10 (41.7%)	2 (40%)		2 (20%)	2 (40%)		3 (30%)	6 (54.5%)		6 (28.6%)	4 (44.4%)	
Two	12 (50%)	2 (40%)		6 (60%)	1 (20%)		7 (70%)	3 (27.3%)		11 (52.4%)	4 (44.4%)	
Three	2 (8.3%)	1 (20%)		2 (20%)	2 (40%)		0 (0%)	2 (18.2%)		4 (19%)	1 (11.1%)	
Number of bile duct anastomoses, *n* (%)			0.553			0.083			0.635			0.477
One	21 (87.5%)	4 (80.0%)		8 (80.0%)	2 (40.0%)		8 (80.0%)	7 (63.6%)		18 (85.7%)	6 (66.7%)	
Two	3 (12.5%)	1 (20.0%)		2 (20.0%)	2 (40.0%)		2 (20.0%)	4 (36.4%)		2 (9.5%)	3 (33.3%)	
Three	0	0		0	1 (20.0%)		0	0		1 (4.8%)	0	
Bile duct size, mean ± SD, mm	4.7 ± 1.8	6.6 ± 4.1	0.37	4.5 ± 1.8	2.8 ± 1.5	0.13	5.7 ± 1.7	5.45 ± 2.1	0.76	3.74 ± 1.26	4.56 ± 2.02	0.20
Angle between graft and recipient bile ducts, mean ± SD, °	28.8 ± 18.6	54.8 ± 24.2	0.012	47.5 ± 25.8	44.7 ± 26.8	0.85	90.8 ± 41.1	100.13 ± 25.7	0.55	99.62 ± 23.20	99.79 ± 15.01	0.98

RAD, right anterior hepatic duct; RPD, right posterior hepatic duct; ERCP, endoscopic retrograde cholangiopancreatography; PTBD, percutaneous transhepatic biliary drainage; TTR, tailored telescopic reconstruction; SD, standard deviation.

bile duct anastomoses increased ($p = 0.083$). There were also no statistically significant differences in the variables when both ERCP and PTBD were performed for ABC of the RPD (**Table 3**).

Prediction of Intervention Failure Using the R-G Angle

To predict the likelihood of intervention failure, we analyzed the R-G angles of the RAD and RPD groups for each intervention with the receiver operating characteristic (ROC) curve. An optimal cut-off point was calculated using Youden's index.(11) The ROC curve analysis in **Figure 3** demonstrates that when the R-G angle was greater than 47.5°, ERCP was more likely to fail (sensitivity, 93.8%; specificity, 67.6%). The ROC curve analysis for PTBD showed no statistically significant R-G angle cut-off value.

DISCUSSION

Our study demonstrated two novel features. First, we compared the clinical outcomes between ERCP and PTBD

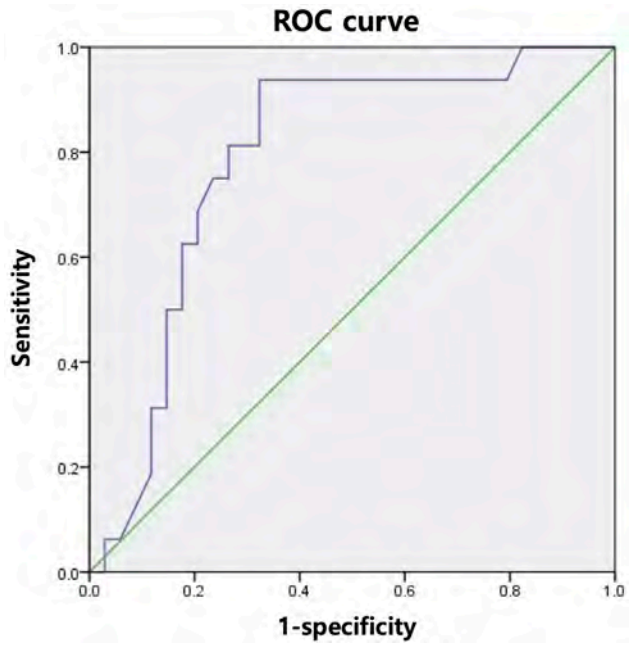


FIGURE 3 | A receiver operating characteristic curve analysis for the angle between the graft and recipient bile ducts and likelihood of endoscopic retrograde cholangiopancreatography failure. ROC, receiver operating characteristic.

as the primary treatment for ABC after LDLT. Despite the advancements in biliary reconstruction techniques, biliary complications after LT are major and unavoidable complications. Non-surgical alternatives, such as endoscopic and radiologic interventional treatment, are increasingly becoming the treatment of choice for biliary complications after LT. Endoscopic treatment is generally performed as the first-line treatment, and PTBD is performed as a rescue treatment for when endoscopic interventions fail. Most studies consider ERCP as the safer and more convenient alternative, and ERCP is associated with fewer complications than PTBD. Only one study has compared the clinical outcomes of ERCP and PTBD (5, 12). This study demonstrated that the success and patency rates of ERCP and PTBD were similar, but the number of repeated procedures was higher in PTBD. The study also presented several disadvantages associated with PTBD, such as incidental PTBD removal, catheter associated pain, bile leakage around the catheter, and infection. The clinical outcomes in our study correlated well with the results of previous studies. The success rate, total number of interventions, and patency rate were similar between the ERCP and PTBD groups with RPD involvement. However, concerning RAD involvement, the total number of interventions and short-term patency rates were superior in the PTBD group than in the ERCP group. Therefore, when biliary intervention was attempted under specific patient conditions, including patients with RAD involvement who desired fewer interventions or a shorter follow-up period, our data demonstrated that PTBD was a good first-line option for ABC.

Second, our study highlighted several clinical criteria that should be considered when selecting between ERCP and PTBD for the treatment of ABC in the RAD or RPD. LDLT is currently performed in countries with low deceased donor availability, which are mainly comprised of Asian countries, such as South Korea (13–15). LDLT typically uses an RL graft, but this can have multiple bile duct openings, which is a risk factor for ABC (1, 16, 17). Performing simultaneous bilateral bile duct drainage of multiple openings with either ERCP or PTBD may provide more effective long-term benefits in patients with ABC after LDLT than unilateral biliary drainage (4). We further analyzed the factors affecting the success rates of ERCP and PTBD for ABC with RAD and RPD involvement. Previous studies proposed that hepatic artery stenosis and stricture morphology affect the success of endoscopic management (3, 18, 19). We examined several intraoperative technical factors associated with stricture morphology and intraoperative hepatic artery complications, such as whether TTR of the bile duct or laparoscopic donor hepatectomy was the superior method (10, 15).

Among the patients with RAD involvement, the presence or absence of graft bile duct variation affected the success of ERCP. Variations in the right hepatic duct are determined by the location of the RPD, and RPD variation of the RL graft increases the likelihood for ABC (6, 20). Our results contrasted with published literature, as our study demonstrated that RAD involvement was more associated with ABC than RPD involvement. In single-centers, a multidisciplinary approach with surgeons, radiologists, and interventionists should be considered when postoperative complications are expected in LT recipients. In particular, when anatomic complications are likely due to RPD involvement, PTBD is preferred over ERCP. Our study followed a retrospective design and examined a small sample size. Therefore, selection bias was possible, and the presence or absence of graft bile duct variation in RPD involvement may not significantly affect the success of ERCP. While our results suggested that PTBD was more effective for patients with RAD involvement, further large-scale studies are needed to confirm the clinical significance of this result.

Our study also indicated that large R-G angles increase the likelihood of failure of ERCP with RAD involvement. The mean R-G angles for successful and unsuccessful procedures were 28.8 ± 18.6° and 54.8 ± 24.2°, respectively. Our fluoroscopic findings were consistent with the results of previous studies, which demonstrated that acute angulation increased the likelihood of failure of ERCP (4, 21). Our ROC analysis (**Figure 3**) suggested that an R-G angle (in either the RAD or RPD) greater than 47.5° was significantly associated with ERCP failure (sensitivity, 93.8%; specificity, 67.6%). As shown in **Table 2**, while there was no statistically significant difference in the success rates between ERCP and PTBD, the success rate of ERCP with RAD involvement was 82.8%, which

was higher than that of PTBD. In contrast, the success rate of ERCP with RPD involvement fell to 47.6%, which was lower than that of PTBD. Therefore, when bile duct angulation is considered alone (in either the RAD or RPD), PTBD may be the superior first-line treatment of choice for biliary drainage in ABC compared to ERCP when the R-G angle is greater than 47.5°.

Of the 57 patients in our study, 15 underwent biliary interventions for concomitant RAD and RPD involvement. Three, six, one, and five patients underwent bilateral ERCP (E/E group), ERCP and PTBD (E/P group), PTBD and ERCP (P/E group), and bilateral PTBD (P/P group), respectively. The success rates in the RAD and RPD were both 100% in the E/E group, 100% and 50% in the E/P group, both 0% in the P/E group, and both 80% in the P/P group. While it was difficult to determine whether there was a statistically significant difference among these results, the success rate seemed to be higher when the same intervention was performed for both the RAD and RPD. Further studies are needed to accurately evaluate the suitability of combining ERCP and PTBD.

This study has several limitations. First, our study design may have been prone to selection bias, because it was a retrospective, single-center cohort with a small sample size. Second, while we analyzed clinical outcomes and influential factors based on RAD and RPD involvement, performing multiple procedures in a clinical setting may affect the results. Third, ABC was diagnosed based on radiologic findings, which might have been influenced by the researcher's subjectivity. ABC can be difficult to differentiate from non-ABC. Fourth, several interventionists performed the ERCP and PTBD procedures, and differences in operative technique might have affected the final outcomes. Fifth, we limited our study participants to patients newly diagnosed with ABC following LDLT, but previous studies have shown that 12–35.6% of patients who undergo LDLT develop biliary complications, and biliary complications recur in approximately 20% (5, 9). In addition, non-anastomotic biliary complications (non-ABC) are diagnosed in 5–15% of patients, and non-ABC are associated with high recurrence rates and poor graft prognosis (22). Therefore, it is necessary to conduct further studies with a larger sample size including patients with recurrent biliary complications and non-ABC, as these account for a large proportion of patients with biliary complications.

Despite these limitations, our study was significant, clearly demonstrating that either ERCP or PTBD may be used as first-line treatment options for ABC after LDLT. Our study was also the first to attempt classification and evaluation of these interventions based on RAD and RPD involvement.

In conclusion, both ERCP and PTBD were appropriate first-line treatments for ABC after LDLT. Several factors must be considered when determining the optimal treatment for ABC, and the success of ERCP and PTBD may be influenced by whether the RAD and/or RPD are involved. Specifically, PTBD is recommended in patients with RAD involvement when there is graft bile duct variation and in patients with either RAD or RPD involvement where the angle between the recipient and graft bile ducts is greater than 47.5°.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board of Seoul National University Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

MSK and SKH participated in research design. MSK, SKH, HYW, J-HC, J-ML, KCY, YC, N-JY, K-WL and K-SS performed the research. MSK and SKH performed data analysis and wrote the article.

CONFLICT OF INTEREST

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

REFERENCES

1. Ishiko T, Egawa H, Kasahara M, Nakamura T, Oike F, Kaihara S, et al. Duct-to-duct Biliary Reconstruction in Living Donor Liver Transplantation Utilizing Right Lobe Graft. *Ann Surg* (2002) 236:235–40. doi:10.1097/00000658-200208000-00012
2. Rela M, Reddy MS. Living Donor Liver Transplant (LDLT) Is the Way Forward in Asia. *Hepatol Int* (2017) 11:148–51. doi:10.1007/s12072-016-9780-z
3. Tsujino T, Isayama H, Kogure H, Sato T, Nakai Y, Koike K. Endoscopic Management of Biliary Strictures after Living Donor Liver Transplantation. *Clin J Gastroenterol* (2017) 10:297–311. doi:10.1007/s12328-017-0754-z
4. You MS, Paik WH, Choi YH, Shin BS., Lee SH, Ryu JK, et al. Optimal Biliary Drainage for Patients with Biliary Anastomotic Strictures after Right Lobe Living Donor Liver Transplantation. *Liver Transpl* (2019) 25:8. doi:10.1002/lt.25472
5. Wadhawan M, Kumar A. Management Issues in post Living Donor Liver Transplant Biliary Strictures. *World J Hepatol* (2016) 8:461. doi:10.4254/wjh.v8.i10.461
6. Jeon YM, Lee K-W, Yi N-J, Lee JM, Hong G, Choi Y, et al. The Right Posterior Bile Duct Anatomy of the Donor Is Important in Biliary Complications of the

Recipients after Living-Donor Liver Transplantation. *Ann Surg* (2013) 257: 702–7. doi:10.1097/SLA.0b013e318268a5d5
7. Hong SY, Hu X-G, Lee HY, Won JH, Kim JW, Shen X-Y, et al. Longterm Analysis of Biliary Complications after Duct-To-Duct Biliary Reconstruction in Living Donor Liver Transplantations. *Liver Transpl* (2018) 24:1050–61. doi:10.1002/lt.25074
8. Na GH, Kim DG, Choi HJ, Han JH, Hong TH, You YK. Interventional Treatment of a Biliary Stricture after Adult Right-Lobe Living-Donor Liver Transplantation with Duct-To-Duct Anastomosis. *Hpb* (2014) 16:312–9. doi:10.1111/hpb.12151
9. Park JK, Yang J-I, Lee JK, Park JK, Lee KH, Lee KT, et al. Long-term Outcome of Endoscopic Retrograde Biliary Drainage of Biliary Stricture Following Living Donor Liver Transplantation. *Gut and Liver* (2020) 14:125–34. doi:10.5009/gnl18387
10. Kim SH, Lee K-W, Kim Y-K, Cho SY, Han S-S, Park S-J. Tailored Telescopic Reconstruction of the Bile Duct in Living Donor Liver Transplantation. *Liver Transpl* (2010) 16:1069–74. doi:10.1002/lt.22116
11. Ruopp MD, Perkins NJ, Whitcomb BW, Schisterman EF. Youden Index and Optimal Cut-point Estimated from Observations Affected by a Lower Limit of Detection. *Biom J* (2008) 50:419–30. doi:10.1002/bimj.200710415
12. Lee SH, Ryu JK, Woo SM, Park JK, Yoo JW, Kim Y-T, et al. Optimal Interventional Treatment and Long-Term Outcomes for Biliary Stricture after Liver Transplantation. *Clin Transpl* (2008) 22:484–93. doi:10.1111/j.1399-0012.2008.00813.x
13. IRODaT. IRODaT: International Registry in Organ Donation and Transplantation. World Liver Transplant from Living Donors (2019) Available at: https://www.irodat.org [Accessed September 20, 2021].
14. Cherqui D, Ciria R, Kwon CHD, Kim K-H, Broering D, Wakabayashi G, et al. Expert Consensus Guidelines on Minimally Invasive Donor Hepatectomy for Living Donor Liver Transplantation from Innovation to Implementation. *Ann Surg* (2021) 273:96–108. doi:10.1097/SLA.0000000000004475
15. Hong SK, Choi GS, Han J, Cho HD, Kim JM, Han YS, et al. Pure Laparoscopic Donor Hepatectomy: A Multicenter Experience. *Liver Transpl* (2021) 27: 67–76. doi:10.1002/lt.25848
16. Jung D-H, Ikegami T, Balci D, Bhangui P. Biliary Reconstruction and Complications in Living Donor Liver Transplantation. *Int J Surg* (2020) 82: 138–44. doi:10.1016/j.ijsu.2020.04.069
17. Seo JK, Ryu JK, Lee SH, Park JK, Yang KY, Kim Y-T, et al. Endoscopic Treatment for Biliary Stricture after Adult Living Donor Liver Transplantation. *Liver Transpl* (2009) 15:369–80. doi:10.1002/lt.21700
18. Chang JH, Lee IS, Choi JY, Yoon SK, Kim DG, You YK, et al. Biliary Stricture after Adult Right-Lobe Living-Donor Liver Transplantation with Duct-To-Duct Anastomosis: Long-Term Outcome and its Related Factors after Endoscopic Treatment. *Gut Liver* (2010) 4:226–33. doi:10.5009/gnl.2010.4.2.226
19. Chok KSH, Chan SC, Cheung TT, Sharr WW, Chan ACY, Fan ST, et al. A Retrospective Study on Risk Factors Associated with Failed Endoscopic Treatment of Biliary Anastomotic Stricture after Right-Lobe Living Donor Liver Transplantation with Duct-To-Duct Anastomosis. *Ann Surg* (2014) 259: 767–72. doi:10.1097/SLA.0b013e318294d0ce
20. Varotti G, Gondolesi GE, Goldman J, Wayne M, Florman SS, Schwartz ME, et al. Anatomic Variations in Right Liver Living Donors1 1No Competing Interests Declared. *J Am Coll Surgeons* (2004) 198:577–82. doi:10.1016/j.jamcollsurg.2003.11.014
21. Lee YY, Gwak G-Y, Lee KH, Lee JK, Lee KT, David Kwon CH, et al. Predictors of the Feasibility of Primary Endoscopic Management of Biliary Strictures after Adult Living Donor Liver Transplantation. *Liver Transpl* (2011) 17:1467–73. doi:10.1002/lt.22432
22. Lee ES, Han JK, Baek J-H, Suh S-W, Joo I, Yi N-J, et al. Long-Term Efficacy of Percutaneous Internal Plastic Stent Placement for Non-anastomotic Biliary Stenosis after Liver Transplantation. *Cardiovasc Intervent Radiol* (2016) 39: 909–15. doi:10.1007/s00270-016-1297-2

ESOT | Transplant International

PROTOCOL
published: 07 April 2022
doi: 10.3389/ti.2022.10167

Quantification of Unmethylated Insulin DNA Using Methylation Sensitive Restriction Enzyme Digital Polymerase Chain Reaction

Fenna E. M. van de Leemkolk[1,2], Rogier J. Nell[3], Mieke Versluis[3], Eelco J. P. de Koning[1,4], Volkert A. L. Huurman[1,2], Ian P. J. Alwayn[1,2], Rutger J. Ploeg[1,5], Pieter A. van der Velden[3] and Marten A. Engelse[1,4]**

[1]LUMC Transplant Center, Leiden University Medical Center, Leiden, Netherlands, [2]Department of Surgery, Leiden University Medical Center, Leiden, Netherlands, [3]Department of Ophthalmology, Leiden University Medical Center, Leiden, Netherlands, [4]Department of Internal Medicine, Leiden University Medical Center, Leiden, Netherlands, [5]Nuffield Department of Surgical Sciences, University of Oxford, Oxford, United Kingdom

Assessment of specific β-cell death can be used to determine the quality and viability of pancreatic islets prior to transplantation and hence predict the suitability of the pancreas for isolation. Recently, several groups have demonstrated that unmethylated insulin (*INS*)-DNA is correlated to β-cell death in type 1 diabetes patients and during clinical islet isolation and subsequent transplantation. Here, we present a step-by-step protocol of our novel developed method for quantification of the relative amount of unmethylated *INS*-DNA using methylation sensitive restriction enzyme digital polymerase chain reaction This method provides a novel and sensitive way to quantify the relative amount of β-cell derived unmethylated *INS*-DNA in cellular lysate. We therefore suggest that this technique can be of value to reliably determine the purity of an islet preparation and may also serve as a measure of the quality of islets prior to transplantation measuring unmethylated *INS*-DNA as a reflection of the relative amount of lysed β-cells.

Keywords: transplantation, biomarker, unmethylated insulin DNA, methylation sensitive restriction enzyme, digital PCR, β-cell, islets

OPEN ACCESS

Correspondence:
Fenna E. M. van de Leemkolk
f.e.m.van_de_leemkolk@lumc.nl
Marten A. Engelse
m.a.engelse@lumc.nl

Received: *31 October 2021*
Accepted: *15 March 2022*
Published: *07 April 2022*

Citation:
van de Leemkolk FEM, Nell RJ, Versluis M, de Koning EJP, Huurman VAL, Alwayn IPJ, Ploeg RJ, van der Velden PA and Engelse MA (2022) Quantification of Unmethylated Insulin DNA Using Methylation Sensitive Restriction Enzyme Digital Polymerase Chain Reaction. Transpl Int 35:10167. doi: 10.3389/ti.2022.10167

INTRODUCTION

β-cell replacement therapy has been established as a therapy for patients with complex Type 1 Diabetes (T1D) not amenable to optimal conventional diabetes management (1). One example of β-cell replacement therapy is the transplantation of deceased donor derived pancreatic islets that has proven its long-term efficacy during the past 20 years (2, 3). In order to aim for optimal post-transplant outcomes, the use of high-quality pancreatic islets is essential. Reliable assays are needed to assess the quality and viability of islets prior to transplantation. Soluble β-cell specific biomarkers may serve as a relevant diagnostic target to determine the quality and viability of islets at an early

Abbreviations: ddPCR, Digital Droplet polymerase Chain Reaction; DNA, DeoxyriboNucleic Acid; DTZ, Dithizone Staining; IEQ, Islet Equivalent; INS, Insulin; MSRE, Methylation Sensitive Restriction Enzyme; PCR polymerase Chain Reaction; T1D, Type 1 Diabetes; TTC5, Tetratricopeptide Repeat Domain 5.

stage as they can be used to assess the amount of β-cell loss during islet isolation and subsequent transplantation.

Recently, several groups have reported unmethylated Insulin (*INS*)-DNA as a specific β-cell death marker during the early development of T1D. During the progression of the disease, autoimmune destruction of β-cells occurs and unmethylated *INS*-DNA is released in the bloodstream that can be identified (4-11). As the concentration of this marker is extremely low, digital polymerase chain reaction (PCR) is often used to detect the amount of β-cell death in a quantitative manner. Recent studies using digital PCR to analyze unmethylated *INS*-DNA were based on a sodium-bisulfite conversion method that chemically converts unmethylated cytosine into uracil (6, 8-10, 12). However, this method comprises an insurmountable problem as regards heterogeneity since it depends on the completeness of the chemical conversion. Overshooting or incomplete bisulfite conversion can lead to reduced sensitivity and may hamper quantitative and qualitative interpretation (13).

To avoid bisulfite conversion whilst still allowing the possibility to specifically quantify the methylation fraction of a specific allele, we recently published a methylation sensitive restriction enzyme (MSRE) digital PCR assay (14). MSREs are used to differentiate between methylated and unmethylated alleles and in combination with digital PCR it provides the opportunity to determine specific allele quantification.

Based on this methodology we now describe here the step-by-step approach how to quantify the unmethylated *INS*-DNA fraction using a MSRE and digital PCR assay. In this proof-of-concept study, we aim to demonstrate that this novel assay can be used as a helpful method to determine the purity of an islet preparation by measuring the amount of β-cells specific genomic DNA in an islet suspension. The subsequent step to then test this particular assay as a clinically quality marker of islets prior to transplantation by measuring the relative amount of lysed β-cells was beyond the scope of this proof-of-concept study. .

METHOD

Sample Collection and DNA Isolation

Human insulinoma EndoC-βH1 cells (Univercell-Biosolutions (15), Toulouse, France) and human monocytic THP-1 cells (Invivogen, Toulouse, France) were used as a positive and negative control, respectively. Isolated human pancreatic islets with different purities were obtained from seven individual pancreases (Leiden University Medical Center, Netherlands). Human donor pancreases were used that were declined for clinical purposes according to national criteria. Written informed consent for research of pancreatic tissue from donors was present, according to local guidelines of the medical ethical committee (Leiden University Medical Center, Netherlands) and of the Dutch Transplantation Foundation as the competent authority for organ donation in Netherlands. Regarding the culture of the EndoC-βH1 and THP-1 cells and isolation and maintenance of human islets, please find further details in the Supplemental document.

1) Stored pellets of $2.5 \times 10\hat{6}$ EndoC-βH1 cells, $2.5 \times 10\hat{6}$ THP-1 cells and 10 µL tissue of different purities from human islets were resuspended with phosphate buffer up to a final volume of 200 µL.

From these samples genomic DNA was extracted using a QIAamp DNA Mini Kit (Qiagen, Venlo, Netherlands) according to the manufacturer's instructions.

2) DNA concentrations were measured using NanoDrop TM 1000 Spectrophotometer (Thermo Fisher Scientific, Landsmeer, Netherlands).

Treatment With Methylation Sensitive Restriction Enzyme

The restriction enzyme, HpaII (Thermo Fisher Scientific), was used according to manufacturer's instructions. The restriction enzyme was added for the *INS target DNA* (**Figure 1A**) as it cleaves the unmethylated *INS*-DNA and leaves the methylated *INS*-DNA intact. Each sample was either left untreated or treated with HpaII.

1) Take two separate units of 100 ng genomic DNA from each sample and add each of these units to a separate PCR tube (8-strip PCR tubes). Mark the first strip as "with MSRE" and the second strip as "without MSRE". Include at least one sample in each strip containing only nuclease-free H_2O (negative control).
2) Add 2 units/reaction of HpaII, 1.0 µL CutSmart Buffer (Bioké, Leiden, Netherlands), and nuclease-free H_2O up to a total volume of 10 µL to the strip marked as "with MSRE".
3) Add 1.0 µL CutSmart Buffer (Bioké, Leiden, Netherlands), and nuclease-free H_2O up to a total volume of 10 µL to the strip marked as "without MSRE".
4) Incubate both strips at 37°C for 1 hour.

Duplex Analysis Using Digital PCR

Primers and FAM-labelled hydrolysis probes (both Sigma-Aldrich) were designed to be 1) gene specific, 2) to contain an MSRE specific CpG site and 3) to possess optimal melting temperature (±55°C) based on the region identified previously (**Supplementary Figure S1**) (11, 16). Probes directed to the *INS target DNA* were labelled with FAM (**Supplementary Table S1**). The probe directed to the reference TTC5 (tetratricopeptide repeat domain 5) gene was labelled with HEX (BioRad, Veenendaal, Netherlands).

1) To prepare the PCR mastermix, add 11 µL per reaction of Droplet PCR Supermix™ (No dUTP) (BioRad) (e.g., 110 µL per 10 samples), 0.5 µL per reaction 36uM forward *INS* primer (e.g., 5 µL per 10 samples), 0.5 µL per reaction 36uM *INS* reverse primer (e.g., 5 µL per 10 samples), 0.5 µL per reaction 10uM *INS* FAM probe (e.g., 5 µL per 10 samples), 1 µL per reaction 20x TTC5 HEX assay (e.g., 10 µL per 10 samples) and 6.5 µL per reaction nuclease-free H_2O (e.g., 65 µL per 10 samples).

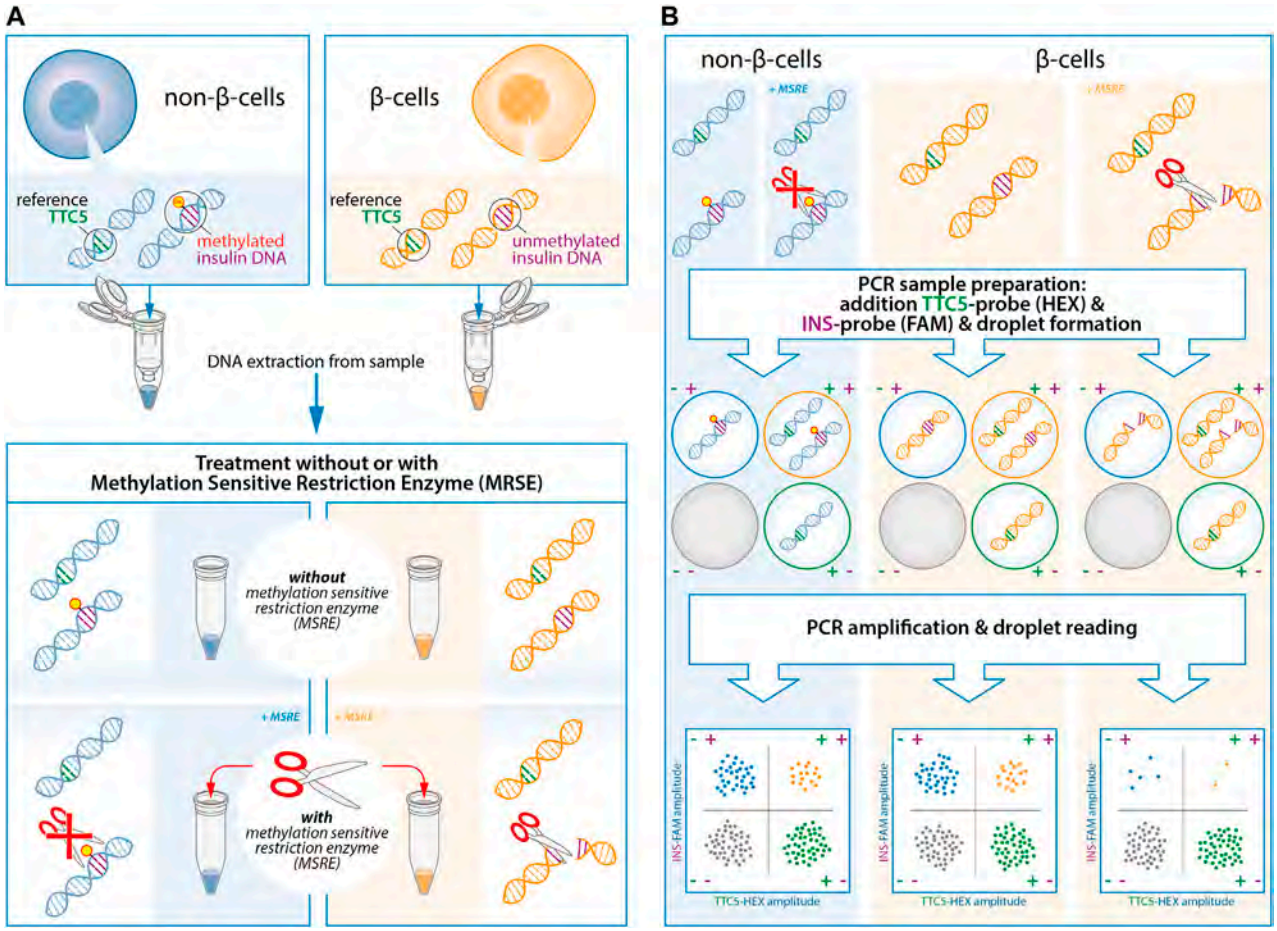


FIGURE 1 | Experimental design of the quantification of unmethylated insulin (*INS*)-DNA using methylation sensitive restriction enzyme (MSRE) digital droplet polymerase chain reaction (ddPCR). **(A)** DNA is isolated from the samples and subsequently split in two and treated with or without the MSRE. The MSRE cleaves the unmethylated *INS*-DNA and leaves the methylated *INS*-DNA intact. **(B)** The DNA sample is partitioned into thousands of droplets followed by PCR amplification with FAM-labelled hydrolysis probes directed to the *INS target DNA* and probes directed to a reference gene (HEX-labelled). Droplet reading takes place after amplification. Droplets that are positive or negative for the *INS target DNA* and/or reference gene are counted to calculate the fraction of unmethylated *INS*-DNA in the sample. Abbreviations: ddPCR, Digital Droplet polymerase Chain Reaction; INS, Insulin; MSRE, Methylation Sensitive Restriction Enzyme; PCR, polymerase Chain Reaction.

2) In order to set up a PCR reaction in a 96-well plate, first, add 20 µL mastermix to each well. Add 2 µL of cleaved unmethylated *INS*-DNA (from the "with MSRE" PCR-strip) or uncleaved unmethylated *INS*-DNA (from the "without MSRE" PCR-strip) to each appropriate well. Mix wells by pipetting up-and-down several times.

All eight wells in a column must contain cleaved unmethylated *INS*-DNA (from the "with MSRE" PCR-strip) or uncleaved unmethylated *INS*-DNA (from the "without MSRE" PCR-strip).

3) Seal the 96-well PCR plate with foil and centrifuge shortly to remove liquid from the sides of the wells.
4) Digital PCR is performed using the digital droplet PCR (ddPCR) method described below (**Figure 1B**).
   4.1) Use the Automated Droplet Generator (BioRad) to generate droplets according to manufacturer's instructions.
   4.2) In order to prevent evaporation of the newly formed droplets, the droplets should be collected in a second 96-well PCR plate placed into a properly frozen cooling block.
   4.3) When finished, remove the 96-well PCR plate including the newly formed droplets and use a Plate Sealer (BioRad) in order to cover the 96-well PCR plate with a heat-sealed foil.

NOTE: Careful handling is strongly advised as the newly formed droplets are fragile in this stage.

5) Perform a PCR reaction in a T100 Thermal Cycler (BioRad) using the following protocol:
   - 10 min of activation at 95°C
   - 30s at 94°C denaturation and 60s at 60°C for 40 cycles
   - 10 min inactivation at 98°C
   - Cooling at 12°C until droplet reading

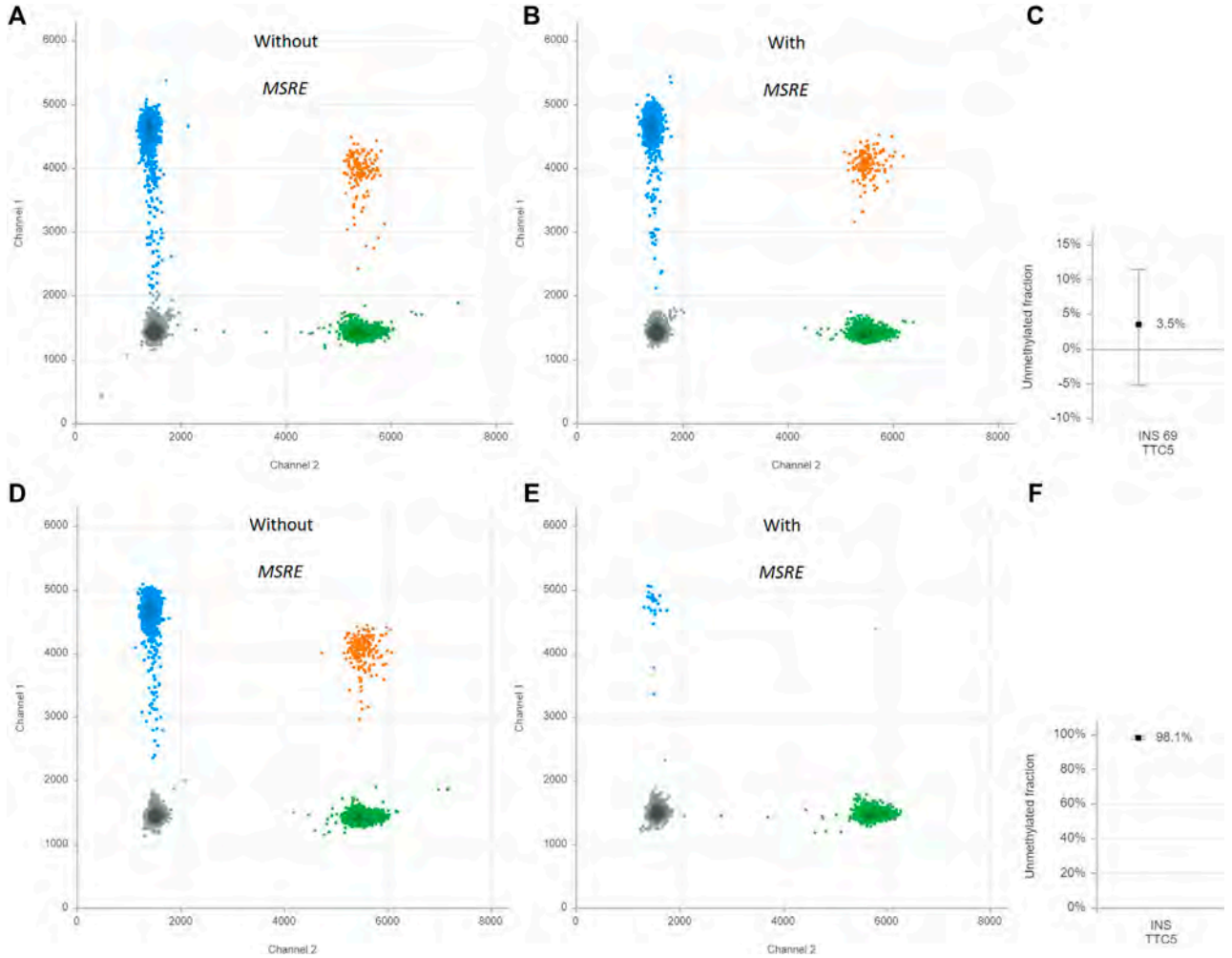


FIGURE 2 | Unmethylated *INS*-DNA fraction in EndoC-βH-1 cells **(A–C)** and THP-1 cells **(D–F)** as positive and negative control samples, respectively. The two-dimensional plots from the digital droplet polymerase chain reaction (ddPCR) demonstrate four different clusters each representing different DNA containing droplets: Gray cluster: FAM- HEX-, containing no *INS target DNA* and no reference copies. Green cluster: FAM- HEX+, containing no *INS target DNA* but contains reference copies. Blue cluster: FAM+ HEX-, containing *INS target DNA* but no reference copies. Orange cluster: FAM+ HEX+ containing both *INS target DNA* and reference copies. The control samples are both split in two and either treated with or without methylation sensitive restriction enzyme (MSRE). The unmethylated *INS*-DNA fraction is calculated: $1 - \frac{\frac{[INS]}{[TTC5]}_{with\ MSRE}}{\frac{[INS]}{[TTC5]}_{without\ MSRE}} * 100\%$. Abbreviations: ddPCR, Digital Droplet polymerase Chain Reaction; INS, Insulin; MSRE, Methylation Sensitive Restriction Enzyme.

6) Analyze the DNA content of the droplets using the QuantaSoft™ software with the QX200 Droplet Reader (BioRad) according to manufacturer's protocol.
7) Calculate for each sample the unmethylated *INS*-DNA fraction as follows:
   - Unmethylation fraction = $1 - \frac{\frac{[INS]}{[TTC5]}_{with\ MSRE}}{\frac{[INS]}{[TTC5]}_{without\ MSRE}} * 100\%$

RESULTS

With attention to previous studies (11, 16) on target areas of DNA methylation in the human *INS* gene, we designed a methylation sensitive restriction enzyme (MSRE) duplex digital PCR assay to determine the relative amount of unmethylated *INS*-DNA fraction in our DNA samples of interest.

First, the assay was validated in cell line models. DNA was isolated from EndoC-βH1 cells, a cell line that was derived from human β-cells(17). The MSRE duplex digital PCR assay was performed. This results in two-dimensional plots that demonstrate four different clusters each of them representing different DNA containing droplets (**Figure 2**). The green cluster contains no *INS target DNA* but only TTC5 copies; the blue cluster contains only *INS target DNA* but no TTC5 copies; the orange cluster contains both *INS target DNA* and TTC5 copies; the gray cluster includes the empty droplets. Without treatment of the MSRE (**Figure 2A**), the *INS target DNA* reflects the quantification of both unmethylated and

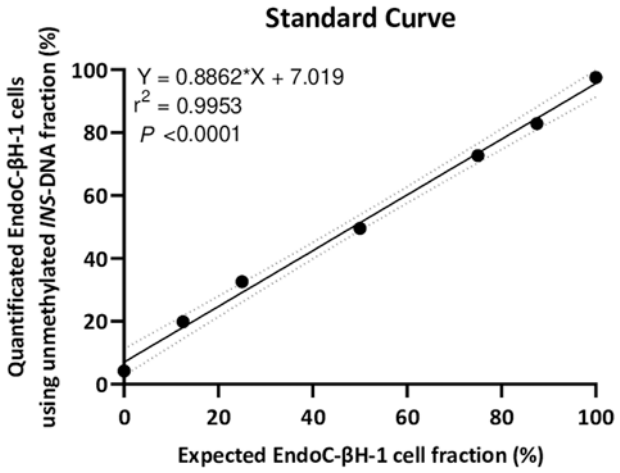


FIGURE 3 | A seven point standard curve demonstrates the relation between input percentage of EndoC-βH-1 cells DNA (diluted in a background of THP-1 cells DNA) that could be expected and EndoC-βH-1 cells DNA quantified as unmethylated *INS*-DNA was measured using digital PCR. (Y = 0.8862 *X + 7.019, r^2 = 0.9953, $p < 0.0001$). Abbreviations: PCR; polymerase Chain Reaction.

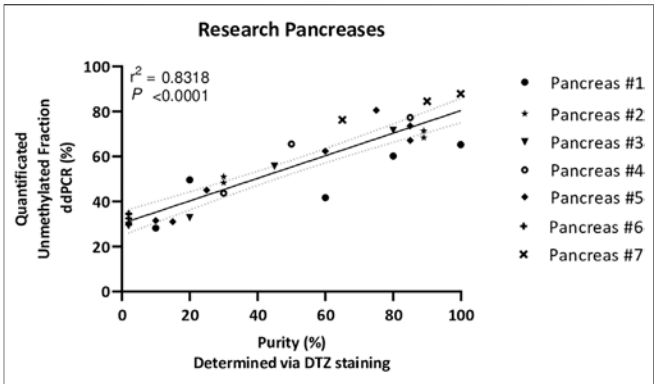


FIGURE 4 | Unmethylated *INS*-DNA fraction quantified by digital PCR in different purities of islets, determined via DTZ staining, after isolation from seven donor research pancreases. (r^2 = 0.8318, $p < 0.0001$). Abbreviations: DTZ; Dithizone Staining, PCR; polymerase Chain Reaction.

methylated *INS target DNA*. After treatment with the MSRE HpaII (**Figure 2B**), the unmethylated *INS target DNA* is digested, resulting in less blue and orange droplets. For both, with and without treatment of MSRE, a stable independent reference, TTC5, was used to correct for input differences as it is not digested by the MSRE. When using both ratios from *INS target DNA* and reference TTC5 in the samples with and without treatment with MSRE, an unmethylated *INS*-DNA fraction of 98.1% (95% CI 97.3–98.8) was determined (**Figure 2C**). With regards to DNA isolated from THP-1 cells, both ratios from *INS target DNA* and reference TTC5, when treated with (**Figure 2E**) or without (**Figure 2D**) the MSRE HpaII, were calculated and this resulted in an unmethylated *INS*-DNA fraction of 3.5% (95% CI -5.2–11.5) (**Figure 2F**).

As isolated DNA from EndoC-βH1 cells was essentially unmethylated for the *INS target DNA* whilst isolated DNA from THP-1 cells was mainly methylated for the *INS target DNA*, a 7-points standard curve was generated to technically validate the quantitative experimental setup. Isolated DNA from EndoC-βH1 cells diluted in the background of isolated DNA from THP-1 cells resulted in a strong linear correlation (r^2 = 0.9953, Y = 0.8862*X + 7.019, $p < 0.0001$) (**Figure 3**).

Next, the unmethylated *INS*-DNA fraction was determined in 24 human islets preparations which were isolated from seven different human donor pancreases obtained for research. For each sample, islet purity was determined, varying from <5 to 99%, via dithizone staining which is currently used by most centers to estimate the fraction of pancreatic islets in an isolated islet preparation (18, 19). In the case of a sample with <5% purity, the sample was categorized as islet depleted tissue (i.e., pancreatic tissue left over from islet isolation). After using this MSRE duplex digital PCR assay on DNA isolated from all the different purities of the islets, the unmethylated *INS*-DNA fraction was quantified (**Figure 4**). When comparing the purity of the pancreatic islets a significant linear correlation was observed (R squared = 0.8318, $p < 0.0001$). In the samples containing islet depleted tissue an unmethylated *INS*-DNA fraction of 29.4%–34.5% was observed.

DISCUSSION

Previous studies have demonstrated that the human *INS* gene is controlled epigenetically by methylation as it is unmethylated in β-cells and methylated in most other cell types (4, 20-22). When cells are dying or lysed - either *in vivo* or for experimentation purposes - their genomic DNA is released into the milieu. This makes unmethylated-*INS* DNA a highly interesting marker to detect the death of β-cells. Several research groups have developed assays to measure the circulating fraction of unmethylated *INS*-DNA in humans, often aiming to be used in the context of early detection of β-cell death in type 1 Diabetes. In 2020 Speake et al. (23) assessed the performance of three different methodologies (5, 9 11) to quantify circulating levels of unmethylated *INS*-DNA in patients undergoing total pancreatectomy and subsequent islet auto-transplantation. This was considered a reliable model as damage or cell death of β-cells is known to occur during transplantation. Not only did the group measure a different CpG site or sites in the human *INS* gene in these three assays, they also applied different sample collection methods and measurement techniques (e.g., next generation sequencing or digital PCR). We agree with Speake's group that to further develop these assays, optimization of the three different techniques might be beneficial. A similarity between all three assays was that DNA was treated with sodium bisulfite. This technique, which was first described by Frommer et al. (24), is still regarded as the gold standard to analyze DNA methylation. To prevent partial conversion and subsequent misinterpretation, the chemical conversion is performed at high concentrations. As a result, however, fragmentation and degradation of DNA will occur that may lead to an incorrect quantitative interpretation

(13, 25). In addition, with regard to the bisulfite conversion kits used in these studies focusing on unmethylated-*INS* DNA, it remains a relatively time consuming technique e.g., as approximately 12–16 h are needed for the incubation period.

To circumvent or even avoid these limitations, we report in this protocol a proof-of-concept study where we have combined the MSRE with digital PCR techniques to measure unmethylated-*INS* DNA. As an MSRE can differentiate between methylated and unmethylated alleles, MSRE treatment for only 1 hour results in digestion of unmethylated DNA, with the methylated DNA remaining intact. This allows for the rapid calculation of the fraction of unmethylated alleles in our target of interest (*INS target DNA*). When using two different cell lines, a strong correlation was observed (**Figure 3**) demonstrating a high sensitivity and specificity of this assay.

Next, we extended the use of this assay to measure the unmethylated *INS*-DNA fraction in different purities of islets obtained after pancreas isolation (**Figure 4**). Interestingly, the purity of the samples was not directly proportional to the quantified unmethylated *INS*-DNA fraction as was found in the standard curve obtained from the 2 cell lines (**Figure 3**). When using the MSRE duplex digital PCR in islet depleted tissue (i.e. containing <5% islets) an unmethylated *INS*-DNA fraction of 29.4–34.5% was observed. Of note is that this observed fraction is likely not a limitation of the assay itself but an indication that the biological variability in methylation of the human *INS* gene promotor in non β-cells may play an important role. Our result is in line with the study by Kuroda et al. (22) who investigated nine CpG sequences in the human *INS* gene promotor and compared the methylation pattern in this region in the 'islet cell fraction' and in the 'non-islet cell fraction'. In their study they demonstrated that the human *INS* gene promotor was mainly unmethylated in the islet cell fraction and predominantly methylated in the non-islet cell fraction (i.e., 13 of 15 clones (86%) in the non-islet cell fraction exhibited at least one unmethylated CpG out of the nine CpG sequences investigated).

With regard to the samples including high purity of islets, the quantified unmethylated *INS*-DNA fraction did not reach 100% which could be explained as the ratio of β-cells versus non-β-cells (e.g., alpha and delta) in human islets is generally assumed to be 50–70% (26). This is in line with the ±70% unmethylated *INS*-DNA fraction we have found (**Figure 4**).

A limitation of this proof-of-concept study is that our protocol was performed in cell lines and in different purities of human islet preparations obtained after isolation. Further validation experiments of this assay during islet isolation, islet culture and subsequent islet transplantation are necessary. During these next steps of the process an unknown amount of β-cell destruction occurs. To be able to specifically quantify the amount of β-cell loss using this promising assay could be helpful to differentiate between low or high quality and viability of islet preparations (12, 27). In clinical islet transplantation the accurate determination of the number of (viable) β-cells in a pancreatic islet preparation is essential. Not only assessment of the islet depleted tissue fraction, but more important the total number of isolated islets in the preparation is key for a successful transplant (28). In islet transplantation, the islet yield has previously been determined using various methods such as size-dependent islet counting by visualizing islets under a microscope and subsequent measurement of their volume (19), calculating both islet purity and graft volume or specific β-cell counting (28-31). To date, in most centers the estimation of the fraction of pancreatic islets in an isolated islet preparation is based on a method that uses dithizone staining (DTZ) (18, 19). Dithizone is a zinc chelating agent that, when added to an islet prep, results in a rapidly and reversibly red staining of islets which can therefore be distinguished from exocrine tissue. Importantly, this method cannot be used to determine the total number of β-cells in an isolated islet preparation. In addition, in case of β-cell degranulation, the red staining will not take place. Therefore, due to the human error that is intrinsic to this subjective method, an over- or under-estimation of the islet equivalent (IEQ) may easily occur. As such, determination of IEQ by eye or by digital image analysis has proven difficult within and between different centers (32).

Based on these notions, we suggest in this preliminary study that our newly developed MSRE duplex digital PCR assay using unmethylated *INS*-DNA may be a fast and easy method to specifically quantify β-cells. As shown previously, the combination of MSRE with digital PCR provides both specificity and sensitivity by quantitative assessment of target alleles (14). By measuring the concentration of the targeted unmethylated *INS*-DNA and therefore the number of lysed β-cells, this combined technique may be a promising tool to determine the fraction of β-cells immediately after islet isolation, during culture and immediately prior to islet transplantation. Pending further validation trials, the MSRE duplex digital PCR assay using unmethylated INS-DNA may therefore help decision making on islet quality (through the measurement of β-cell death) and islet quantity in islet transplantation centers.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Leiden University Medical Center, Leiden, Netherlands. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

FL, RN, PV and ME participated in conception and research design. The acquisition of data was performed by FL and MV. FL, RN, MV, Ed, VH, IA, RP, PV and ME participated in data analysis and interpretation. FL and ME drafted the manuscript. FL, RN, MV, Ed, VH, IA, RP, PV and ME participated in critical revision and final approval of the manuscript. PV and ME participated in study supervision. FL and ME are the guarantors of this work and, as such, had full access to all of

the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

FUNDING

RN is supported by the European Union's Horizon 2020 research and innovation program under grant agreement No 667787 (UM Cure 2020 project).

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

We would like to thank our colleagues from the islet lab, for their help in islet isolation and providing sample collection and our colleagues from the digital droplet PCR group, for their expertise regarding digital droplet PCR and helpful discussions. We also would like to thank M.S. Zuurmond for the graphic design of **Figure 1**.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10167/full#supplementary-material

REFERENCES

1. Shapiro AMJ, Pokrywczynska M, Ricordi C. Clinical Pancreatic Islet Transplantation. *Nat Rev Endocrinol* (2017) 13(5):268–77. doi:10.1038/nrendo.2016.178
2. Farney AC, Sutherland DER, Opara EC. Evolution of Islet Transplantation for the Last 30 Years. *Pancreas* (2016) 45(1):8–20. doi:10.1097/mpa.0000000000000391
3. Vantyghem M-C, de Koning EJP, Pattou F, Rickels MR. Advances in β-cell Replacement Therapy for the Treatment of Type 1 Diabetes. *The Lancet* (2019) 394(10205):1274–85. doi:10.1016/s0140-6736(19)31334-0
4. Akirav EM, Lebastchi J, Galvan EM, Henegariu O, Akirav M, Ablamunits V, et al. Detection of β Cell Death in Diabetes Using Differentially Methylated Circulating DNA. *Proc Natl Acad Sci U.S.A* (2011) 108(47):19018–23. doi:10.1073/pnas.1111008108
5. Lehmann-Werman R, Neiman D, Zemmour H, Moss J, Magenheim J, Vaknin-Dembinsky A, et al. Identification of Tissue-specific Cell Death Using Methylation Patterns of Circulating DNA. *Proc Natl Acad Sci U S A* (2016) 113(13):E1826–34. doi:10.1073/pnas.1519286113
6. Tersey SA, Nelson JB, Fisher MM, Mirmira RG. Measurement of Differentially Methylated INS DNA Species in Human Serum Samples as a Biomarker of Islet β Cell Death. *J Vis Exp* (2016)(118) 54838. doi:10.3791/54838
7. Zhang K, Lin G, Han Y, Xie J, Li J. Circulating Unmethylated Insulin DNA as a Potential Non-invasive Biomarker of Beta Cell Death in Type 1 Diabetes: a Review and Future prospect. *Clin Epigenet* (2017) 9:44. doi:10.1186/s13148-017-0343-5
8. Usmani-Brown S, Lebastchi J, Steck AK, Beam C, Herold KC, Ledizet M. Analysis of β-Cell Death in Type 1 Diabetes by Droplet Digital PCR. *Endocrinology* (2014) 155(9):3694–8. doi:10.1210/en.2014-1150
9. Herold KC, Usmani-Brown S, Ghazi T, Lebastchi J, Beam CA, Bellin MD, et al. β Cell Death and Dysfunction during Type 1 Diabetes Development in At-Risk Individuals. *J Clin Invest* (2015) 125(3):1163–73. doi:10.1172/jci78142
10. Simmons KM, Fouts A, Pyle L, Clark P, Dong F, Yu L, et al. Unmethylated Insulin as an Adjunctive Marker of Beta Cell Death and Progression to Type 1 Diabetes in Participants at Risk for Diabetes. *Int J Mol Sci* (2019) 20(16):3857. doi:10.3390/ijms20163857
11. Fisher MM, Watkins RA, Blum J, Evans-Molina C, Chalasani N, DiMeglio LA, et al. Elevations in Circulating Methylated and Unmethylated Preproinsulin DNA in New-Onset Type 1 Diabetes. *Diabetes* (2015) 64(11):3867–72. doi:10.2337/db15-0430
12. Bellin MD, Clark P, Usmani-Brown S, Dunn TB, Beilman GJ, Chinnakotla S, et al. Unmethylated Insulin DNA Is Elevated after Total Pancreatectomy with Islet Autotransplantation: Assessment of a Novel Beta Cell Marker. *Am J Transpl* (2017) 17(4):1112–8. doi:10.1111/ajt.14054
13. Warnecke PM, Stirzaker C, Melki JR, Millar DS, Paul CL, Clark SJ. Detection and Measurement of PCR Bias in Quantitative Methylation Analysis of Bisulphite-Treated DNA. *Nucleic Acids Res* (1997) 25(21):4422–6. doi:10.1093/nar/25.21.4422
14. Nell RJ, Steenderen D, Menger NV, Weitering TJ, Versluis M, Velden PA. Quantification of DNA Methylation Independent of Sodium Bisulfite Conversion Using Methylation-sensitive Restriction Enzymes and Digital PCR. *Hum Mutat* (2020) 41(12):2205–16. doi:10.1002/humu.24111
15. Ravassard P, Hazhouz Y, Pechberty S, Bricout-Neveu E, Armanet M, Czernichow P, et al. A Genetically Engineered Human Pancreatic β Cell Line Exhibiting Glucose-Inducible Insulin Secretion. *J Clin Invest* (2011) 121(9):3589–97. doi:10.1172/jci58447
16. Yang BT, Dayeh TA, Kirkpatrick CL, Taneera J, Kumar R, Groop L, et al. Insulin Promoter DNA Methylation Correlates Negatively with Insulin Gene Expression and Positively with HbA1c Levels in Human Pancreatic Islets. *Diabetologia* (2011) 54(2):360–7. doi:10.1007/s00125-010-1967-6
17. Tsonkova VG, Sand FW, Wolf XA, Grunnet LG, Kirstine Ringgaard A, Ingvorsen C, et al. The EndoC-Bh1 Cell Line Is a Valid Model of Human Beta Cells and Applicable for Screenings to Identify Novel Drug Target Candidates. *Mol Metab* (2018) 8:144–57. doi:10.1016/j.molmet.2017.12.007
18. Latif ZA, Noel J, Alejandro R. A Simple Method of Staining Fresh and Cultured Islets. *Transplantation* (1988) 45(4):827–30.
19. Ricordi C, Lacy PE, Finke EH, Olack BJ, Scharp DW. Automated Method for Isolation of Human Pancreatic Islets. *Diabetes* (1988) 37(4):413–20. doi:10.2337/diabetes.37.4.413
20. Cate RL, Chick W, Gilbert W. Comparison of the Methylation Patterns of the Two Rat Insulin Genes. *J Biol Chem* (1983) 258(10):6645–52. doi:10.1016/s0021-9258(18)32461-x
21. Husseiny MI, Kaye A, Zebadua E, Kandeel F, Ferreri K. Tissue-specific Methylation of Human Insulin Gene and PCR Assay for Monitoring Beta Cell Death. *PloS one* (2014) 9(4):e94591. doi:10.1371/journal.pone.0094591
22. Kuroda A, Rauch TA, Todorov I, Ku HT, Al-Abdullah IH, Kandeel F, et al. Insulin Gene Expression Is Regulated by DNA Methylation. *PloS one* (2009) 4(9):e6953. doi:10.1371/journal.pone.0006953
23. Speake C, Ylescupidez A, Neiman D, Shemer R, Glaser B, Tersey SA, et al. Circulating Unmethylated Insulin DNA as a Biomarker of Human Beta Cell Death: A Multi-Laboratory Assay Comparison. *J Clin Endocrinol Metab* (2020) 105(3):781–91. doi:10.1210/clinem/dgaa008
24. Frommer M, McDonald LE, Millar DS, Collis CM, Watt F, Grigg GW, et al. A Genomic Sequencing Protocol that Yields a Positive Display of 5-methylcytosine Residues in Individual DNA Strands. *Proc Natl Acad Sci U.S.A* (1992) 89(5):1827–31. doi:10.1073/pnas.89.5.1827
25. Tanaka K, Okamoto A. Degradation of DNA by Bisulfite Treatment. *Bioorg Med Chem Lett* (2007) 17(7):1912–5. doi:10.1016/j.bmcl.2007.01.040
26. Dolenšek J, Rupnik MS, Stožer A. Structural Similarities and Differences between the Human and the Mouse Pancreas. *Islets* (2015) 7(1):e1024405. doi:10.1080/19382014.2015.1024405

27. Bellin MD, Abu-El-Haija M, Morgan K, Adams D, Beilman GJ, Chinnakotla S, et al. A Multicenter Study of Total Pancreatectomy with Islet Autotransplantation (TPIAT): POST (Prospective Observational Study of TPIAT). *Pancreatology* (2018) 18(3):286–90. doi:10.1016/j.pan.2018.02.001
28. Papas KK, Colton CK, Qipo A, Wu H, Nelson RA, Hering BJ, et al. Prediction of Marginal Mass Required for Successful Islet Transplantation. *J Invest Surg* (2010) 23(1):28–34. doi:10.3109/08941930903410825
29. Pisania A, Weir GC, O'Neil JJ, Omer A, Tchipashvili V, Lei J, et al. Quantitative Analysis of Cell Composition and Purity of Human Pancreatic Islet Preparations. *Lab Invest* (2010) 90(11):1661–75. doi:10.1038/labinvest.2010.124
30. Lehmann R, Fernandez LA, Bottino R, Szabo S, Ricordi C, Alejandro R, et al. Evaluation of Islet Isolation by a New Automated Method (Coulter Multisizer Ile) and Manual Counting. *Transplant Proc* (1998) 30(2):373–4. doi:10.1016/s0041-1345(97)01314-6
31. Huang H-H, Harrington S, Stehno-Bittel L. The Flaws and Future of Islet Volume Measurements. *Cel Transpl* (2018) 27(7):1017–26. doi:10.1177/0963689718779898
32. Kissler HJ, Niland JC, Olack B, Ricordi C, Hering BJ, Naji A, et al. Validation of Methodologies for Quantifying Isolated Human Islets: an Islet Cell Resources Study. *Clin Transplant* (2010) 24(2):236–42. doi:10.1111/j.1399-0012.2009.01052.x

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