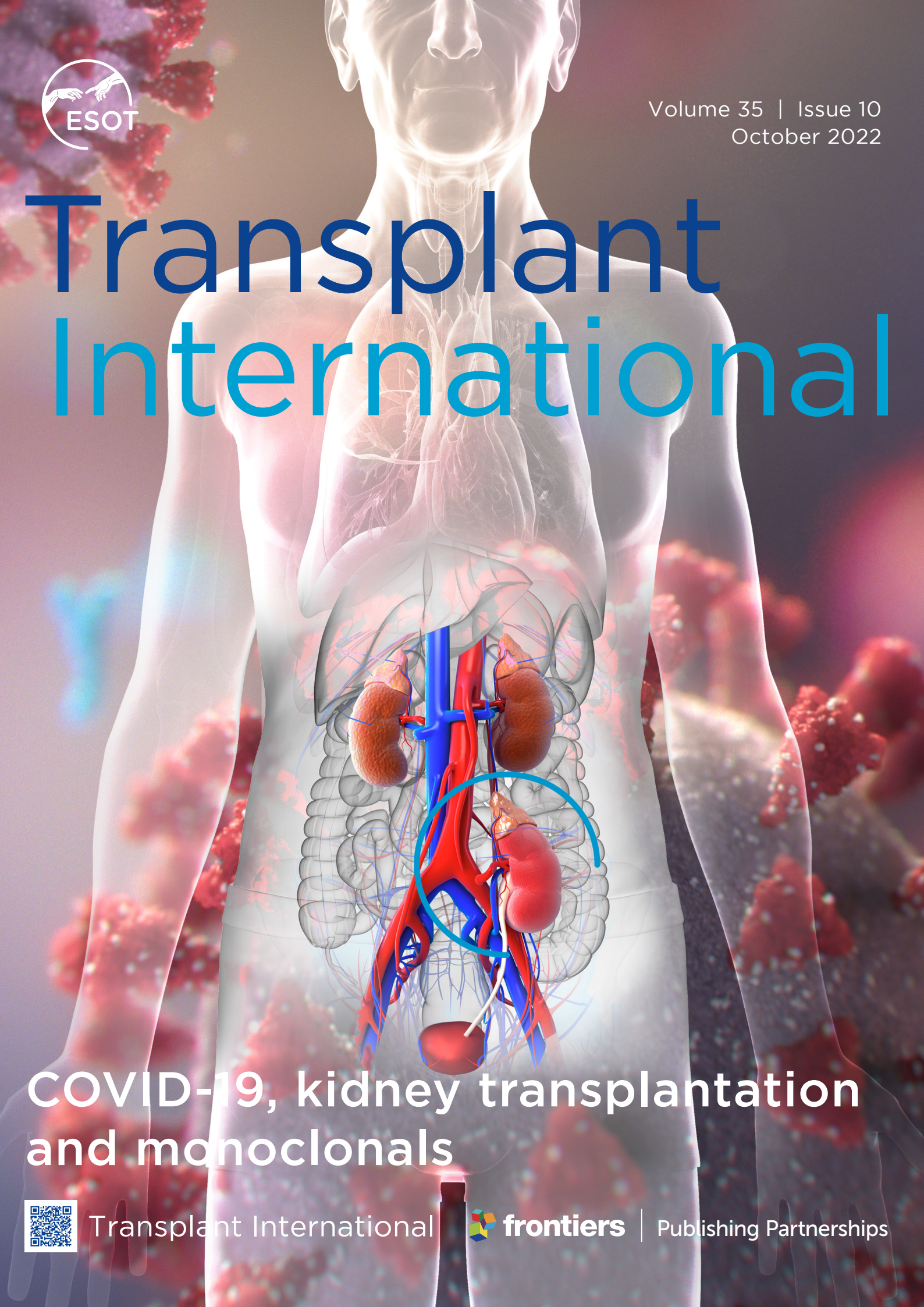
ESOT

Volume 35 | Issue 10
October 2022

Transplant International



COVID-19, kidney transplantation and monoclonals

Transplant International | frontiers | Publishing Partnerships

Transplant International

COVID-19, kidney transplantation and monoclonals

ISSN 1432-2277
ISBN 978-2-8325-5274-2
DOI 10.3389/978-2-8325-5274-2

Transplant International

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ESOT®
Leading the way in transplantation

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Legend

Area

- ORGAN SPECIFIC
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- EDUCATION
- PATIENT INCLUSION
- MACHINE PERFUSION

Audience

- SENIOR PROFESSIONALS
- YOUNG PROFESSIONALS
- PATIENT ADVOCATES

ACLF - Acute-on-Chronic Liver Failure
AIDPIT - Artificial Insulin Delivery, Pancreas and Islet Transplantation
EDTCO - The European Donation and Transplant Coordination Organisation
ELITA - The European Liver and Intestine Transplant Associationi
ELPAT - The European Platform on Ethical, Legal and Psychosocial Aspects of Organ Transplantation
EPITA - The European Pancreas and Islet Transplant Association
ESOT - European Society for Organ Transplantation
ITS - International Transplant Science

ABSTRACT SUBMISSION

ELITA
ESOT

The European
Liver and Intestine
Transplant Association

EF CLIF
EUROPEAN FOUNDATION
FOR THE STUDY OF
CHRONIC LIVER FAILURE

ELITA 30th Anniversary Meeting & ELITA-EF CLIF Monothematic Conference about ACLF, Alcohol and Liver Transplantation

9–11 March 2023, Madrid, Spain

#ESOT_ELITA

30th
ANNIVERSARY

ESOT | Transplant International | THE PETER MORRIS CENTRE FOR EVIDENCE IN TRANSPLANTATION

TRANSPLANT TRIAL WATCH
published: 03 October 2022
doi: 10.3389/ti.2022.10838

Transplant Trial Watch

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Keywords: heart transplantation, rejection, surveillance, hypothermic oxygenated perfusion, liver transplantation, extended criteria donor

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

Cardiovascular Magnetic Resonance for Rejection Surveillance After Cardiac Transplantation.
by Anthony, C., et al. Circulation 2022; 145(25): 1811–1824.

Aims
The aim of this study was to investigate the feasibility of cardiovascular magnetic resonance (CMR)-based monitoring for cardiac allograft rejection.

Interventions
Participants were randomised to receive either CMR-based or endomyocardial biopsy (EMB)-based rejection surveillance.

Participants
40 orthotopic heart transplant recipients.

Outcomes
The primary endpoint was frequency and cumulative freedom from significant (>grade 2R) rejection. The secondary endpoints included frequency and cumulative freedom from low-grade (grade 1R) rejection, kidney function, hospitalisation, duration of hospital stay, infection, myocardial function, death, immunosuppression exposure and the incidence of biopsy-related complications.

Follow-Up
1 year.

Transplant Trial Watch

OPEN ACCESS

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Received: *15 August 2022*
Accepted: *31 August 2022*
Published: *03 October 2022*

Citation:
Knight SR (2022) Transplant Trial Watch. Transpl Int 35:10838. doi: 10.3389/ti.2022.10838

CET Conclusion

This is an interesting and well-conducted study in cardiac allotransplantation. Cardiac magnetic resonance (CMR) imaging was compared to the standard protocol of surveillance for rejection with the invasive endomyocardial biopsy (EMB). The first part of the study was a cross-sectional analysis to understand cut-off values for acute rejection and the second part was a randomised study comparing the two surveillance methods using this information. The trial was set up as a noninferiority study and therefore the inclusion did not need to be large (20 in each arm), as per prior power calculation. A detailed analysis of the CMR validation is provided. The primary endpoint was frequency of significant rejection (grade 2R or higher). This was found to be similar in the two groups. In order not to miss high grade rejection, treating physicians could request EMB on any patient at their discretion. This option was taken 11 times in the CMR group and in 9 of these cases the EMB result was identical to the CMR result. In this single-centre study, a surveillance protocol using CMR instead of EMB in cardiac allograft recipients was safe, feasible and offers significant advantages over invasive cardiac biopsies.

Jadad Score

3.

Data Analysis

Strict intention-to-treat analysis.

Allocation Concealment

Yes.

Trial Registration

ACTRN12618000672257.

Funding Source

Non-industry funded.

RANDOMISED CONTROLLED TRIAL 2

Hypothermic Oxygenated Perfusion in Extended Criteria Donor Liver Transplantation—A Randomized Clinical Trial.

by Ravaioli, M., et al. American Journal of Transplantation [Online ahead of print].

Aims

The aim of this study was to compare the effect of hypothermic oxygenated perfusion (HOPE) vs. static cold storage (SCS) in extended criteria donor (ECD) liver transplantation.

Interventions

Participants undergoing transplantation of an ECD liver graft were randomly assigned to receive a liver after HOPE or after SCS alone.

Participants

135 potential ECD liver grafts were randomised, of which 110 were used for liver transplantation.

Outcomes

The primary outcome was the incidence of early allograft dysfunction (EAD). The secondary outcome were patient survival, graft survival, the early allograft failure simplified estimation (EASE) risk score, and the rate of graft or other graft-related complications.

Follow-Up

1 year.

CET Conclusion

This is an interesting and well-conducted trial in ECD liver transplantation. Livers were randomised to standard static cold storage (SCS) or to a period of Hypothermic Oxygenated Perfusion (HOPE), using the Vitasmart device (Bridge to Life, DG, United States). Organs in the HOPE group had a period of SCS of 4–5 h on average prior to starting HOPE for 2–3 h on average. No organ was discarded during perfusion. The study was single centre and designed with a prior power calculation to determine sample size. The primary endpoint was Early Allograft Dysfunction (EAD) using a well-established composite definition. There was a significant reduction in EAD with HOPE compared to SCS (13% vs. 35%) and also a significant reduction in re-transplantation (0% vs. 11%). This form of HOPE, using just portal vein perfusion in ECD liver transplantation, is associated with better early allograft function, which is very likely to impact on longer term function and graft survival.

Jadad Score

2.

Data Analysis

Per protocol analysis.

Allocation Concealment

No.

Trial Registration
ClinicalTrials.gov—NCT03837197.

Funding Source
Non-industry funded.

CLINICAL IMPACT SUMMARY

The shortage of suitable donors to meet demand has resulted in increasing use of extended criteria donor (ECD) organs to try to address the mismatch. ECD donor organs are known to be more at risk of adverse post-operative outcomes due to increased vulnerability to ischaemia-reperfusion injury. In attempts to counter this additional risk, there has been a great deal of interest in novel perfusion technologies to recondition, repair and assess grafts prior to transplant. Such technologies can be used in the donor (normothermic regional perfusion, NRP) or *ex-vivo* (hypothermic oxygenated perfusion, HOPE or normothermic machine perfusion, NMP). The technologies differ in their simplicity/ease of use, ability to assess organ viability and the duration of safe perfusion.

In a recent paper in the American Journal of Transplantation, Ravaioli and others report a single centre randomised controlled trial of HOPE after static cold storage (SCS) versus SCS alone in ECD liver grafts (1). 110 recipients were randomised and followed for a median of 473 days. The authors report a significant reduction in the risk of the primary endpoint of early allograft dysfunction with HOPE, from 35% to 13%. This reduction is similar in magnitude to that seen in previous studies of NMP (2) and HOPE in DCD livers (3). Unlike in these previous studies there was no difference in incidence of biliary complications, most likely as this study does not include DCD livers which are at higher risk for ischaemic-type biliary lesions.

Perhaps the most striking finding is that graft survival was significantly higher in the HOPE arm of the study, a finding not seen in the larger multicentre studies of HOPE or NMP. A detailed breakdown of causes and timings of graft loss is not provided, making the role of perfusion in this finding difficult to interpret. Another interesting finding is the numerically lower incidence of acute rejection in HOPE livers. This has been seen previously with use of HOPE in kidney transplantation (4), and may offer at least a partial explanation for the difference in graft survival seen.

Overall, these findings support previous studies in both liver and kidney transplantation that HOPE is a safe, simple and effective method of preservation which may be beneficial in marginal donor organs.

AUTHOR CONTRIBUTIONS

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

CONFLICT OF INTEREST

SK has received consultancy fees from OrganOx Ltd., for research design in the past.

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NEWS AND VIEWS
published: 25 October 2022
doi: 10.3389/ti.2022.10931

A Western World Perspective of Survival Benefit of Living Donor Liver Transplantation: A Commentary to the Article by Jackson et al. Published in JAMA Surgery

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Keywords: intention-to-treat, transplant oncology, survival benefit, hepatocellular cancer, colorectal metastases

Liver transplantation is the best treatment for several liver diseases causing acute or chronic hepatic failure, primary and secondary hepatobiliary tumors, and liver-based inborn metabolic errors (1). Unfortunately, many patients die on the list or are too sick and drop out, thus losing the opportunity to be transplanted (2). Consequently, every effort needs to be made to overcome the allograft shortage.

Recently, the deceased-donor pool has been substantially extended using technical variants like split and domino transplants (3–5), more aged or cardiac death donors, and machine perfusion technology (6, 7). However, all these measures remain insufficient to cover the actual needs.

Living donor liver transplantation (LDLT) represents the best, although ethically more complex, way to overcome allograft shortage. Recently, a study from the US by Jackson et al. published in JAMA Surgery has added relevance to the role of LDLT also in a Western setting (8).

LDLT has many significant advantages. First, LDLT allows transplanting a given patient without harming the patients inscribed on the waiting list (9). Secondly, LDLT consents to offer an "ideal" graft with minimal ischemia time (10). Thirdly, this procedure allows for an electively and timely transplant of a given recipient, therefore offering the best economic solution to cure given liver disease. All these advantages must be counterbalanced with the ethical justification of the procedure and the potential donor risk for morbidity and mortality (11, 12).

Live donation has flourished in Asian centers, mainly due to the historical shortage of deceased donor liver transplantation (DDLT) cases (13). In sharp contrast to the Eastern world, LDLT still represents a (too) limited activity in the Western world based on the challenging balance between the weight of the risks linked to the donor hepatectomy and the benefits to the recipient (14, 15).

This Western hesitation related to LDLT has been "fed" by teams embarked on such programs without having gathered enough experience in transplantation and advanced liver surgery. The too high morbidity rates and some donor mortalities hampered the evolution of LDLT in the Western world, leading in turn to the absence of adequately numbered studies allowing to identify the patient

Abbreviations: CRLM, colorectal liver metastases; DDLT, deceased donor liver transplantation; HCC, hepatocellular cancer; LDLT, living donor liver transplantation; MELD-Na, model for end-stage liver disease-sodium; RAPID, resection and partial liver segment 2-3 transplantation with delayed total hepatectomy.

News & Views

OPEN ACCESS

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Received: 26 September 2022
Accepted: 13 October 2022
Published: 25 October 2022

Citation:
Lai Q and Lerut J (2022) A Western World Perspective of Survival Benefit of Living Donor Liver Transplantation: A Commentary to the Article by Jackson et al. Published in JAMA Surgery. Transpl Int 35:10931. doi: 10.3389/ti.2022.10931

survival benefits. Even worse, the too rapid publication of studies, some of them also presenting methodological flaws, resulted in a negative attitude of the transplant community towards LDLT (16, 17). Thus, the initial enthusiasm was turned into a negative perception.

During the last decade, the safety of LDLT for both donor and recipient has been significantly improved by the Asian transplant centers, focusing on the importance of technical details and liver regenerative physiology (18). The donor risk has been markedly reduced by introducing the concept of technical versatility leading to the most appropriate use of left or right donor graft (19).

The recent US study by Jackson et al. in JAMA Surgery based on the data from the US Scientific Registry of Transplant Recipients (SRTR) refocused the view on the relevance of LDLT (8). Between January 2012 and September 2021, 119,275 liver transplant candidates were analyzed, and only 2,820 (2.4%) received a LDLT. The LDLT group had a significant survival benefit compared to patients remaining on the list. LDLT patients having a Model for End-stage Liver Disease-sodium (MELD-Na) ≥11 had an adjusted hazard ratio for the risk of 1-year mortality of 0.64 (95% CI = 0.47–0.88; p = 0.006). LDLT consented to gain 13–17 additional life years according to their different MELD-Na categories. The 13-year survival gain observed in low MELD-Na scores (values 6–10) was particularly appealing.

These results are not in line with previous experiences. A study from the US based on DDLT showed a survival benefit only when the MELD-Na was ≥15 (20). A study about 868 LDLT performed during the period 2002–2009 showed no benefit in patients with hepatocellular cancer (HCC) having a lab-MELD <15 (15).

The Jackson et al. study is the first Western world study confirming that LDLT has the most significant life-saving value with respect to any other curative procedure and that this beneficial effect is faithful also in patients with low MELD-Na, which are more often the patients harboring an HCC.

The field of transplant oncology, a term introduced in literature by our team in 2015, is the most promising field of LDLT (21).

Despite attributing bonus points to HCC patients, many cancer patients still do not get access to a potentially curative treatment in the Western world. Moreover, cholangiocellular cancer and secondary colorectal and neuro-endocrine tumors are not yet fully validated indications for LT (22–25).

This aspect is essential, as primary hepatobiliary cancers are becoming the main indications for LT in many countries. Two recent studies highlighted the importance of LDLT in treating HCC patients.

The monocentric Toronto study (N = 851, LDLT = 25.7%) showed that the 5-year intention-to-treat survival rates were 68% in LDLT vs. 57% in DDLT (p = 0.02), and that a potential live donation was a protective factor for death (hazard ratio = 0.67; 95% CI = 0.53–0.86) (26).

The Eastern-Western collaborative HCC-LT effort confirmed this evidence based on the analysis of 13 collaborative centers in Europe, Asia, and North America (N = 3958; LDLT = 31.7%) (27). After balancing the results with a propensity score, LDLT was an independent protective factor that reduced the risk of overall death by 33%–48% in both the international and external validation cohorts. These data indicate that LDLT minimizes the risk of death in HCC patients, mainly by reducing or completely zeroing the risk of drop-out on the waiting list. This effect is even more pronounced if more advanced tumors (i.e., Milan-Out criteria) receive a LDLT. A sub-analysis of this cohort showed that 5-year HCC-related deaths were similar after LDLT and DDLT (12% vs. 12%; p = 0.49). Conversely, 5-year HCC-unrelated death rates were markedly superior in the DDLT group (21 vs. 11%; $p < 0.001$), confirming the overall positive effect of LDLT performed in expert centers (28).

The role of LDLT is expected to be also relevant in terms of intention-to-treat survival benefit in the setting of well-selected secondary, colorectal, and neuroendocrine tumors (14, 24–29).

LDLT will allow for a modern oncologic approach in these well selected patients by electively being placed between neo-adjuvant and adjuvant chemotherapies. The Oslo experience with colorectal metastases showed that this approach is feasible and rewarding. The cross-fertilization between LDLT and advanced liver resection technologies has led to the development of the Resection And Partial liver segment 2-3 transplantation with Delayed total hepatectomy (RAPID) procedure, in which a left lobe from a live donor is used (30, 31).

This method may represent a way to substantially extend the number of transplantations for secondary liver tumors without interfering with the waiting list and using a safer approach for the donor. Recently, this technical variant has been successfully applied also in cirrhotic patients (32). The door to a significant extension of LDLT has been opened.

In conclusion, patients receiving a live donation have better survival rates when compared with patients remaining on the waiting list. Additional life-years have been obtained after LDLT in all the classes of MELD-Na severity and the lowest category (MELD-Na 6-10).

LDLT is a very efficacious therapy, especially for well-selected patients with primary and secondary hepatobiliary tumors. The superior intent-to-treat results are mainly due to the planning of elective surgery, thereby eliminating the risk of drop-out on the waiting list. Several technical innovations have been introduced to make live donation safe, and it is expected that this increased safety could lead to a significant role of LDLT in Europe and North America. The Western world should follow the path paved by Asian colleagues for almost four decades. The time has come that US and European centers should embrace LDLT as an option to adopt for curing liver diseases and hepatobiliary cancer patients.

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

QL and JL drafted the manuscript; QL and JL critically revised the manuscript; and all authors approved the final 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.

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FORUM
published: 06 October 2022
doi: 10.3389/ti.2022.10480

Quality of Life After Heart Transplantation for Congenital Heart Defect

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Keywords: heart transplantation, quality of life, congenital heart defect, impact of surgery, satisfaction

A Forum discussing:

Quality of Life and Patient Satisfaction With Outpatient Care After Heart Transplantation in Adult and Pediatric Patients - Room for Improvement?
by Schmithausen A, Tengler A, Birnbaum J, Haas NA, Rosenthal LL, Orban M, Hagl C, Dalla Pozza R, Jakob A, Fischer M, Ulrich SM (2021). Transpl Int 34:2578–88. doi: 10.1111/tri.14147

We read with great interest the article by colleagues Schmithausen et al. on quality of life (QoL) and satisfaction with outpatient follow-up of patients after heart transplantation [1]. The authors reported on 205 patients who underwent heart transplantation and are seen regularly on an outpatient basis.

Patients for whom neither corrective nor palliative surgical procedures are available are transplant candidates, as well as patients with end-stage heart failure, cardiomyopathies, and congenital heart diseases (CHD). There are currently around 300,000 CHD patients in Germany, and for 95% of them, their condition will persist into adulthood [2]. Despite great improvements in surgical techniques and peri-/postoperative care, these patients are still suffering from chronic illness. Heart transplantation can be indicated early or in the long-term course [3].

In our clinical setting at another German heart center (Muenster University Hospital), we also studied QoL after heart transplantation in patients with congenital heart defects (CHD). The first heart transplant took place in April 1990. Over the past 3 decades, 460 additional heart transplants have been performed.

4.6% of the patients studied ($n = 20$, 9 males and 11 females) suffered from CHD with heterogeneous diagnoses (**Figure 1A**). The mean age at the time of transplantation was 14.4 years, the youngest patient was 39 days, and the oldest was 42 years old. Most of the CHD patients (60%) were children. Only three patients (15%) had undergone no previous cardiac surgery. Fifteen (75%) patients had undergone a biventricular outflow tract, and five patients (25%) had undergone univentricular physiology. Seven patients (35%) underwent concurrent reconstructive procedures for concomitant malformations, and six (30%) received ventricular assist devices before transplantation. Surgical technique for congenital heart defects is complex and requires experience and careful perioperative management.

Four patients (20%) died within 30 days after heart transplantation; the high early mortality rate was mainly due to conversion from univentricular to biventricular physiology. Two additional patients died of non-cardiac causes in the long-term. In general, the survival rate in the early 1990s was lower than today, because both surgically and in terms of intensive care, the procedure was at the beginning of the learning curve. However, Schmithausen et al. report on comparable numbers [1].

When the follow-up of our study ended in October 2020, the remaining 14 patients were alive. 50% of the study population ($n = 7$) answered the Short-Form-12 Health Survey (SF-12) questionnaire—an instrument used to assess physical and mental function after transplantation.

Forum

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Received: *01 March 2022*
Accepted: *20 September 2022*
Published: *06 October 2022*

Citation:
Martens S, Tie H, Scheld HH, Martens S and Hoffmeier A (2022) Quality of Life After Heart Transplantation for Congenital Heart Defect. Transpl Int 35:10480. doi: 10.3389/ti.2022.10480

Although SF-12 is a very condensed query, the response rate was surprisingly low. The motivation to provide information was dampened, among other things, by the fact that some patients (especially those whose transplantation was a long time ago) were already participating in other clinical studies.

The SF-12 questionnaire includes fewer questions than the SF-36 questionnaire and is quick to answer in comparison. On the other hand, this means that not all of the eight subscales [4] that are illuminated by means of the SF-36 can be covered. It mainly focuses on general health (4 questions), physical health (4 questions), mental health (3 questions), and the impact on social contacts [5].

In addition to SF-36, Schmithausen et al. used the four-dimensional ZAP survey [6] and the German Federal Health Survey of 1998 [7] in addition to SF-36 to evaluate patients' satisfaction with outpatient care. This enabled a very comprehensive analysis; however, our interest was only in quality of life.

Separate summary scores of physical (PCS, focusing on physical functioning, physical role, bodily pain, and general health) as well as mental function scores (MCS, focusing on vitality, social functioning, emotional role, and mental health) were generated using an online calculator (https://orthotoolkit.com/sf-12/). For both PCS and MCS, higher scores indicate better QoL.

As shown in **Figure 1B**, two patients had a relatively low PCS (Patients #14 and #18), two others a relatively low MCS (Patients #4 and #5), and the other patients had neither low PCS nor low MCS. The results indicated that these patients live with a good QoL after transplantation, with an average MCS of 49.23 ± 13.49 and PCS of 46.35 ± 12.61.

Since the QoL in patients after heart transplantation has been addressed in many studies by now, we can confirm the assumption that patients after heart transplantation have an acceptable QoL [8].

Schmidthausen et al. even concluded that QoL after pediatric heart transplantation is comparable to a standardized reference population in our country [1].

It is notable that QoL is significantly increased after heart transplantation and continuously improves over time [9]. Our

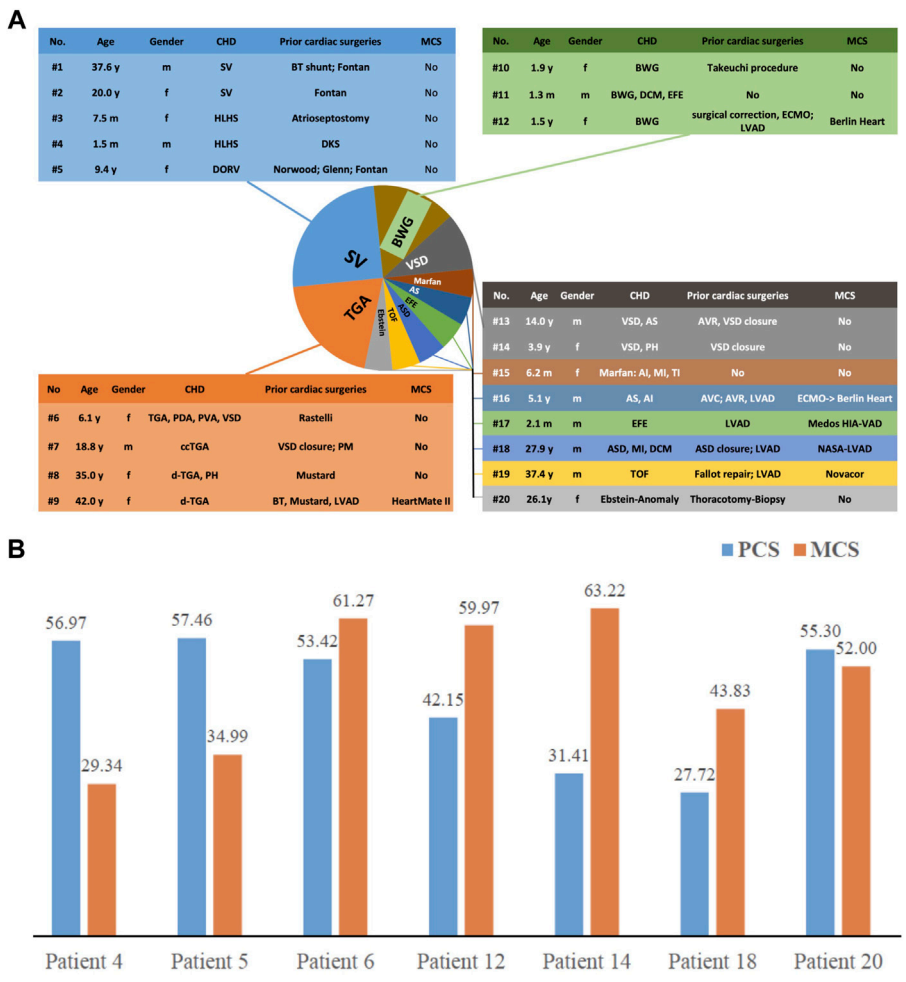

FIGURE 1 | (A) Baseline characteristics of the 20 patients with CHD. **(B)** Quality of life of patients after heart transplantation—presented as summary scores of physical (PCS) and mental function (MCS).

results are consistent with previous studies where the PCS at 3 months and 1 year after heart transplantation was 42.6 and 47.7, while the MCS at 3 months was 48.0 and remained stable [10].

However, QoL can be affected by demographic characteristics, clinical issues, time after transplantation, and individual lifestyle. In spite of great clinical heterogeneity and diverse assessment points after heart transplantation in our cohort, MCS and PCS results revealed a good QoL in CHD patients.

When compared to adult patients who undergo heart transplantation, QoL even seems to be superior [1,11]. However, Cavalli et al. discovered marked sensitivity due to the chronic underlying disease. Pediatric patients are at high risk for repeated hospitalizations, and this psychological stress, in turn, can negatively impact their adherence to treatment [11].

The group of pediatric patients includes those who are operated on in early childhood and patients who are just reaching adulthood. General statements about this heterogeneous group of patients are therefore difficult and subgroup analyses, adapted to the respective age, are desirable.

In summary, recent studies have produced encouraging results in terms of quality of life and treatment options should be continuously improved to achieve the best possible outcomes for patients and all practitioners.

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 Ethikkommission der Ärztekammer Westfalen-Lippe und der Westfälischen Wilhelms-Universität; AZ 2018-687-f-S. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

SaM: Conceptual design, data acquisition, writing; HT: Statistical analysis; HS: Review; SvM: Review; AH: Conceptual design, supervision.

CONFLICT OF INTEREST

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

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ESOT | Transplant International

EXECUTIVE SUMMARY
published: 24 October 2022
doi: 10.3389/ti.2022.10915

ESOT Consensus Platform for Organ Transplantation: Setting the Stage for a Rigorous, Regularly Updated Development Process

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Received: 07 October 2022
Accepted: 10 October 2022
Published: 24 October 2022

Citation:
Cillo U, Weissenbacher A, Pengel L, Jochmans I, Roppolo D, Amarelli C, Belli LS, Berenguer M, De Vries A, Ferrer J, Friedewald J, Furian L, Greenwood S, Monbaliu D, Nadalin S, Neyrinck A, Strazzabosco M, Toso C, Zaza G, Thuraisingham R, Berney T, Potena L, Montserrat N and Selzner N (2022) ESOT Consensus Platform for Organ Transplantation: Setting the Stage for a Rigorous, Regularly Updated Development Process. Transpl Int 35:10915. doi: 10.3389/ti.2022.10915

Abbreviations: AGREE, Appraisal of Guidelines for REsearch & Evaluation; CET, Centre for Evidence in Transplantation; ECTTA, European Cardio Thoracic Transplant Association; EKITA, European Kidney Transplant Association; ELITA, European Liver and Intestine Transplant Association; EPITA, European Pancreas and Islet Transplant Association; ESOT, European Society for Organ Transplantation; ETAHP, European Transplant Allied Healthcare Professionals; GRADE, Grading of Recommendations, Assessment, Development and Evaluation; GT, Guideline Taskforce; ILTS, International Liver Transplantation Society; NIH, National Institute of Health; PICO, Population, Intervention, Comparator and Outcome; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; SC, Steering committee; TJL, Transplant Learning Journey; VBHC, Value-based health care; YPT, Young Professionals in Transplantation.

The European Society for Organ Transplantation (ESOT) has created a platform for the development of rigorous and regularly updated evidence based guidelines for clinical practice in the transplantation field. A dedicated Guideline Taskforce, including ESOT-council members, a representative from the Centre for Evidence in Transplantation, editors of the journal Transplant International has developed transparent procedures to guide the development of guidelines, recommendations, and consensus statements. During ESOT's first Consensus Conference in November 2022, leading experts will present in-depth evidence based reviews of nine themes and will propose recommendations aimed at reaching a consensus after public discussion and assessment by an independent jury. All recommendations and consensus statements produced for the nine selected topics will be published including the entire evidence-based consensus-finding process. An extensive literature review of each topic was conducted to provide final evidence and/or expert opinion.

Keywords: organ transplantation, methodology, guidelines, consensus conference, platform

INTRODUCTION

High-quality, evidence-based clinical practice guidance documents to support best practice in solid organ transplantation along with improving the quality of life are increasingly needed. These are statements that include recommendations intended to optimize patient care, lead to better clinical outcomes, and improve cost effectiveness. Furthermore, they provide the opportunity to identify areas requiring further research and serve an educational scope. Clinical Practice Guideline statements are informed by a systematic review of evidence and an assessment of the benefits of alternative care options. The multidisciplinary and multiprocedural nature of organ transplantation, the intrinsic difficulty in designing and carrying out numerically and methodologically sound comparative studies, and the ever-changing landscape of knowledge and therapeutics, challenge the realization of a solid evidence framework in some crucial areas of the field. Solid organ transplants, therefore, more than other clinical areas, need implementation of a systematic, continuous expert work dedicated to guideline and consensus production to help clinicians with framing evidence and expert opinions into clinical practical approaches (1–3).

The European Society of Organ Transplantation (ESOT) is recently giving high priority to the development of clinical practice guidelines launching a structured and continuous dedicated action plan. In January 2022, ESOT created a guideline taskforce (GT) composed of ESOT leadership and Transplant International editorial board members. The GT has the fundamental commitment to promote methodologically homogeneous guideline and consensus activities and to warrant trustworthiness, transparency and continuity of the processes. Furthermore, the GT selects cutting edge topics, initiates and realizes consensus processes among experts, draws guidelines and promotes dissemination of the compiled products.

Guideline and consensus related material will undergo widespread dissemination within the transplant community through publications in Transplant International, ESOT congresses, and platforms as well as through networking *via* social media. Patients and their representatives will play an active role in the consensus development processes and will be targets of the dissemination activities according to the principles and concepts of value-based health care (VBHC). When appropriate, the GT will involve stakeholders including those in health care management and economics, organ sharing organizations, and health care policy makers.

Besides drafting a uniform methodology for ESOT guidance/guideline production and promoting topic selection, the GT created a platform for the development of methodologically solid and up-to-date evidence-based guidelines for clinical practice in the transplantation field. This platform guarantees procedural and logistical continuity to ESOT activities in the field of consensus processes and guideline production.

The first edition of the Transplant Learning Journey (TLJ) 3.0, after several months of preparatory work, is there to produce systematic reviews of evidence and to grade evidence followed by drafting and sharing recommendations. During TLJ 3.0 in Prague 13th–15th November 2022, the 3-day consensus conference, a series of consensus-based clinical guidance documents comprising research topics considered as cutting-edge will be established.

AIMS

The main purpose of the TLJ 3.0 ESOT GT and the consensus conference is to provide methodologically solid evidence-based and best-practice recommendations reflecting the latest knowledge.

While creating clinical guidance through expertise and knowledge from all stakeholders involved in organ transplantation within the ESOT community and beyond, a further goal is to provide resources in the form of reference databases on an available platform maintained and updated continuously to lead the way in organ transplantation.

The present report is intentionally submitted for publication and it will be freely available prior to TLJ 3.0 event, to make publicly available and report fully with trustworthiness and transparency (1, 2) the new course of ESOT guideline and consensus processes in organ transplantation. The aim is to disclose the methodology of the ESOT consensus platform from its conception to its development, in line with the principles of openness and transparency (1, 2), which are fundamental where relevant potential policy changes are expected. In that light, this report was submitted to Transplant International prior to the event.

METHODS

A dedicated ESOT GT established a methodologic action plan in January 2022 and elaborated a handbook formalizing the processes associated with the preparation of ESOT Clinical Practice Guidelines, including selection of topics for new guidelines, writing, reviewing, approval, dissemination, and update. The document also defines the governance of the process and the roles of the various committees. This handbook has been open to be consulted on the ESOT website since the end of September 2022.

In line with the established action plan, the ESOT GT launched the event "Transplant Learning Journey (TLJ) 3.0" as an in-person consensus conference, designed as a modified NIH (National Institute of Health) model consensus development conference (1–6). Such a consensus development process was organized in collaboration with ESOT sections ELITA, EKITA, EPITA, ECTTA, ETAHP, the Education Committee, and YPT. The ILTS collaborated as well for some specific topics.

The platform, and its future developments, will represent ESOT's permanent operative tool to regularly elaborate and deliver rigorous and homogenous consensus statements and publications. Due to the known limitations related to face-to-face consensus conferences, particular attention has been given to methods for topic selection, selection and number of steering committee members, and review of evidence.

The Delphi method will be applied to arrive at a group opinion by surveying the expert panels including SC, conference attendees and jury members. The final result will reflect a solid consensus of experts in the field (7, 8).

In the setting of the ESOT TLJ consensus conferences, the Delphi method is an appropriate technique as it can help to come to a conclusion under several circumstances which have been described in the late 1970s already (9). When a topic, or facing a challenge, in transplantation is not perfectly suitable for precise objective analytical techniques but benefit from subjective experts' opinions, Delphi rounds can be particularly useful to find consensus. This technique is also helpful and supportive to draw a conclusion when discussion participants cannot be brought together to have direct, face-to-face interactions and discussions for a variety of reasons (timing, costs, pandemic, etc.) and remote ± anonymous voting is needed (9). In the particular setting of TLJ 3.0, a public appraisal of the results the Delphi conducted study "ENGAGE" (European Giudelines for the Management of Graft Recipient Consensus Project) will be realized.

The Delphi method will also be applied to rediscuss and modify crucial recommendations if consensus will not be reached at TLJ 3.0.

Topic Selection for the 2022 European Society for Organ Transplantation Consensus Conference

An open call for topic proposals was issued to ESOT Sections and Committees in January 2022. Overall, 25 topic proposals were received and sent out to all members of the GT who rated them individually at a first step according to following criteria: 1) rating the proposal from 1 to 10; 2) recommending the topic yes/no; 3) marking the proposed group members 1) good proposition, 2) good but unbalanced, i3) needs to be discussed.

In a joint meeting, the GT reviewed and prioritized all submitted proposals and selected nine that met the following criteria: 1) cutting edge topics for which a consensus would have an impact on healthcare; 2) lack of similar guidelines or recommendations for this topic or an urgent need for an update of a previous version; 3) identification of barriers or data gaps requiring consensus recommendations to progress the field; 4) feasibility in the context of TLJ 3.0 meeting including minimal availability of published evidence; 5) completion of previous activated ESOT consensus processes; 6) collaborative forum of European and international leaders to exchange experience and knowledge.

Figure 1 shows the nine topics selected by the GT and validated by the ESOT Executive Committee for the ESOT consensus conference during the TLJ 3.0 in Prague on November 13th–15th (10).

Steering Committee Member Selection

For each of the selected topics, a specific steering committee (SC) was composed. The SC consists of a chair and co-chair, expert-members in the topic field, the Centre for Evidence in Transplantation (CET) (11), a YPT-representative working with the SC to collect and analyze the available topic-relevant literature, and a GT member to liaise with ESOT.

The GT had the final responsibility to nominate the SC members for each topic, though it did invite the topic proposers to suggest expert members. Depending on the balance of the proposed group representatives (expertise, gender, nationality etc., see below), the GT did either accept or request a modification of the member composition.

Each SC is led by a chair and a co-chair to warrant independency between topic proposers and guideline developers and to avoid bias and imbalances (12); selection of chair and co-chair followed a collaborative decision making process (GT and topic proposers) after exclusion of conflict of interests. The SC comprises of 8–14 members with a range of backgrounds to warrant a multidisciplinary expert discussion. In one case (Biomarker prediction in solid organ transplantation) the wide range of subtopics required a larger SC of 23 experts. When selecting

Topic	Subject
Machine perfusion in cardiothoracic transplantation	Cardiothoracic
Histopathological analysis of pre-implantation donor kidney biopsy: Redefining the role in the process of graft assessment	Kidney
The value of monitoring (subclinical) DSA for kidney transplant outcomes	Kidney
Liver transplantation in patients with primary sclerosing cholangitis (PSC) and inflammatory bowel disease (IBD)	Liver
Clinical endpoints in liver transplantation according to value based care	Liver
Downstaging, bridging and immunotherapy in liver transplantation for hepatocellular carcinoma (HCC)	Liver
Role of pancreas machine perfusion to increase the donor pool for beta cell replacement	Pancreas
Prehabilitation for solid organ transplant candidates	Transversal
Molecular biology testing for non-invasive diagnosis of allograft rejection	Transversal

FIGURE 1 | Topics selected by ESOT Guideline Taskforce (GT) for consensus conference, TLJ 3.0, Prague November 2022.

TABLE 1 | Composition of the nine steering committees (SC).

Topic: Machine perfusion in cardiothoracic transplantation
Chairs: Arne Neyrinck, Cristiano Amarelli
Steering committee: Clemens Aigner, Irene Bello, Massimo Boffini, Stephan Clark, Marita Dalvindt, Julien de Wolf, Stephan Ensminger, David Gomez de Antonio, Martin Schweiger, Sandro Sponga, Bettina Wiegmann
Topic: Histopathological analysis of pre-implantation donor kidney biopsy: Redefining the role in the process of graft assessment (Part 1)
Chairs: Lucrezia Furian, Gianluigi Zaza
Steering committee: Jan Becker, David Cucchiari, Aiko de Vries, Albino Eccher, Sandrine Florquins, Jesper Kers, Lorna Marson, Marion Rabant, Michele Rossini
Topic: The value of monitoring (subclinical) donor specific antibodies (DSAs) for kidney transplant outcomes
Chair: Aiko de Vries
Steering committee: Dominique Bertrand, Klemens Budde, Emanuele Cozzi, Anthony Dorling, Marie Paule Emonds, Covadonga López del Moral, Soufian Meziyerh, Dennis van den Broek
Topic: Liver transplantation in patients with primary sclerosing cholangitis (PSC) and inflammatory bowel disease (IBD)
Chairs: Luca Belli, Silvio Nadalin
Steering committee: Annika Bergquist, Marco Carbone, Eleonora De Martin, Andrea Della Penna, Pal Dag Line, Chiara Mazzarelli, James Neuberger, Palak Trivedi
Topic: Clinical endpoints in liver transplantation according to value based care
Chairs: Umberto Cillo, Mario Strazzabosco
Steering committee: Marco Carbone, Agostino Colli, Costantino Fondevila, Anna Forsberg, Lorenzo Mantovani, Sandor Mihaly, Alessandra Nardi, James Neuberger, Wojtek Polak, Karen Rockell, Ian Rowe, Liz Schick
Topic: Downstaging, bridging and immunotherapy in liver transplantation for HCC
Chair: Christian Toso
Steering committee: René Adam, Sherrie Bhoori, Umberto Cillo, Marco Claasen, Constantino Fondevilla, Bastiaan Rakke, Maria Reig, Gonzalo Sapisochin, Dimitri Sneiders, Parissa Tabrizian
Topic: Role of pancreas machine perfusion to increase the donor pool for beta cell replacement
Chair: Joana Ferrer
Steering committee: Julien Branchereau, Jason Doppenberg, Cinthia Drachenberg, Marten A Engelse, Paul Johnson, Henri G. D. Leuvenink, Benoît Mesnard, Franka Messner, Ann Etohan Ogbemudia, Vassilios Papalois, Trevor Reichman, Fabio Vistoli, Steve White
Topic: Prehabilitation for solid organ transplant candidates
Chairs: Diethard Monbaliu, Sharlene Greenwood
Steering committee: Coby Annema, Ellen Castle, Stefan De Smet, Pisana Ferrari, Tania Januadis- Ferreira, Joost Klaasen, Evangelia Kouidi, Sunita Mathur, Yasna Overloop, Maria José Perez Saez
Topic: Molecular biology testing for non-invasive diagnosis of allograft rejection
Group: heart, Chair: Luciano Potena
Steering committee: Ingvild Birschmann, Maria Crespo Leiro, Kiran Khush, Annamaria Minervini, Andrianna Nikolova, Javier Segovia
Group: kidney, Chair: John Friedewald
Steering committee: Dany Anglicheau, Oriol Bestard, Sook Park, Joana Sellares, Claire Tinel
Group: liver, Chair: Marina Berenguer
Steering committee: Eleonora de Martin, Amelia Heissheimer, Josh Levitsky, Alina Lutu, Valeria Mas, Nabeel Wahid, Haseeb Zubair

SC members, consideration was given to: 1) representation of different disciplines and expertise; 2) gender balance; 3) broad geographic representation; 4) involvement of all health care professionals, if indicated and possible; 5) involvement of patient and public representatives if indicated; 6) involvement of members of ESOT YPT (young professionals in transplantation); 7) involvement of methodologists when indicated.

Some of the consensus topics are developed jointly with other international organizations. In those cases, representatives suggested by the partner organization were included as members of the SC and involved throughout the entire process.

The composition of the nine SC, including roles, is illustrated in **Table 1**.

Steering committee members participate on a voluntary basis and are not paid for their contribution. Travel and

accommodation costs for meetings are reimbursed according to the relevant ESOT travel and meetings policy.

Consensus Questions, Evidence Review and Formulation of Recommendations

A number of virtual meetings were held by the SC to define the scope and aims of their topics and to work on their particular consensus process. Further meetings are scheduled in the upcoming months. Key issues were identified and implemented in the process to be worked on. The agreed clinical questions were formulated according to the PICO methodology (PICO = Population, Intervention, Comparator and Outcome) (13). All PICO questions are listed in **Supplementary Appendix S1**. In some cases (i.e., VBHC endpoints in liver transplantation), the strict PICO format was methodologically not applicable (see below). PICO eliminations will be decided upon full agreement during the open discussion that will precede the conference or in the context of the meeting itself. All these changes will be accurately recorded and reported to assure full transparency of the process.

Following the definition of the PICOs, for each topic, literature searches were developed by expert staff from the CET who have expertise in conducting systematic reviews. The searches were conducted in the Transplant Library, Medline, and Embase with or without a date limit (dates differed for each of the groups) and the exact search date of each search was recorded (and will be reported in each consensus-dedicated publication). Bibliographic searches consisted of a combination of Medical Subject Headings and keywords. Search terms and strategies will be provided in the specific topic related publications. Searches, excluding grey literature (some SC included congress abstracts upon request) and following removal of duplicate references, resulted in unique references which were selected for title/abstract screening. If titles/abstracts appeared relevant to the PICO question, corresponding full texts were acquired and reviewed for possible inclusion and interactive reading, and to support the development of consensus statements. Due to the breadth of topics included, a full systematic review process for article review was not performed at this time. Rather, titles and abstracts were reviewed by CET members.

PRISMA flowcharts describing the number of studies identified by the literature search and number of studies selected for inclusion in the consensus statement will appear in the following topic-specific publications.

A short summary of the evidence addressing each key question by the included studies was prepared in an evidence table. The workgroup proposed a recommendation for each key question, based on the quality of evidence rated using the GRADE approach, with high quality rated as A, medium quality as B, and low quality as C; very low quality of evidence was not considered. In particular, in the evaluation of the quality of evidence according to GRADE the following features were considered: study design, risk of bias, inconsistency, indirectness, imprecision, number of patients, effect, importance (14). Strength of recommendation was rated as 1 (strong) or 2 (weak).

Jury Selection

The ESOT GT decided to maximize community involvement and inclusion of different perspectives while maintaining a high level of quality by assigning a panel to assess the documents prior to finalization. To establish these panels, an open call to attract jury members was launched in July 2022 *via* the ESOT webpage (15). Jury applicants register for the conference and specify their wish to be part of the recommendation voting process and the specific topic of interest. Jury member applicants' CVs are subsequently evaluated by the GT before acceptance to ensure they have the necessary experience allowing them to fairly assess the recommendations. Furthermore, due to the focus on patient-centered medicine, patients and patients' representatives are eligible to apply as jury members. Trainees will have the opportunity to follow the work of all included TLJ 3.0 panels as observers according to their particular interests (15). When jury members are appointed by the GT, conflicts of interests must be disclosed.

Jury members will receive the selected evidence as well as a preliminary version of the recommendations before the conference. They will be asked to provide the SC with comments and suggestions for potential changes and refinements before the start of the in-person meeting in Prague. In this way, a constructive discussion can take place during the face-to-face meeting.

Consensus Format

Working groups will include SC members and jury members. Working group processes will consist of the following: 1) SC leaders will introduce and present their topic to an extended panel composed of all working group members in addition to conference participants registered to participate in the in-person consensus discussion; 2) a single SC member, will provide an overview of the evidence for each key question and present the proposed recommendations; 3) feedback will be provided by working group members and conference participants with particular attention to the generation of clear and concise consensus statements taking into account the suggestions emerged by the discussion 4) the following day the consensus recommendations will undergo the jury vote. Consensus will be considered achieved will be considered as reached if an agreement rate of >80% is achieved; topic lectures and proposed consensus statements will be presented to the entire TLJ 3.0 audience in a dedicated session on the last day of the in-person meeting in Prague.

Consensus conference participants are selected and distributed amongst the working groups by the GT members. Complete information including the list of consensus conference working group domains, processes regarding consensus conference participant selection, development and refinement of consensus statements, and modified Delphi methodology including consensus polling will be also reported in Transplant International after the face-to-face meeting in Prague.

Validation Committee and AGREE

A validation committee, including experts in validation procedures, will be formed after the jury members have been

finalized. Consensus and recommendations will be reviewed by experts in validation according to the AGREE II guidelines: Appraisal of guidelines for research and evaluation II (16, 17). The complete validation and appraisal process will be published in due course after the in-person meeting in Prague.

SUMMARY AND NEXT STEPS

The 2022 ESOT Consensus Conference, as part of TLJ 3.0, will be the first consensus and guideline conference initiated by ESOT covering the entire field of organ transplantation including organ-specific as well as cross-cutting, inter- and multidisciplinary topics. This in-person event represents the impetus for the foundation of an ongoing consensus, recommendation, and guideline production process which launches also a permanent area, like a standing committee, within ESOT. All guidelines and recommendations produced and published by ESOT and its involved representatives will undergo a continuous review process to stay up to date. Pre-meeting responsibilities and activities included constitution of a taskforce, steering committees and their working group members, opening of the jury applications and their selection process. The guideline development process started with the identification of the topics of interest, formulation of PICO questions and the identification of the relevant evidence.

The consensus conference during the TLJ 3.0 consists out of discussion session on statements and generating recommendations including Delphi rounds in some cases, as well as a voting and a discussion session, on the last day during the in-person meeting (10). The TLJ 3.0 program, however, also includes educational sessions training on guideline and consensus statement production.

All recommendations and consensus statements produced for the nine selected topics will be published including the entire evidence-based consensus-finding process.

AUTHOR CONTRIBUTIONS

Involved in the conception or design of the work: all contributing authors. Literature screen and review: LP and CET (Centre for Evidence in Transplantation). Drafted the article: UC and AW. Critically revised the article: all contributing authors. Finally approved the version to be published: all contributing authors.

FUNDING

All costs related to taskforce and workgroup meetings were covered by ESOT, without external funding.

CONFLICT OF INTEREST

IJ received speaker fees from XVIVO perfusion paid to her institution. IJ is an ESOT Councilor for which she receives no reimbursement. JFe is recipient of a grant supported by Instituto de Salud Carlos III (ISCIII) through the project "PI18/00161 (Optimization of pancreas transplant graft: A multicentric study of histo-morphological and functional characteristics of unaccepted organs.)" and co-funded by the European Union. AdV received in the past speaker and consultation fees from Astellas, Chiesi, Hansa, Novartis, Sandoz, CSL Behring all of which paid to his institution. AdV is chair of the Dutch Kidney Advisory Committee (Landelijk Overleg NierTransplantatie LONT) for which he receives no reimbursement.

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

This manuscript and the ESOT Consensus Working Group is a collaborative work product of ESOT and its sections and committees. We would like to thank Devi Mey, Justyna Klimek, Irene Garcia, Giovanna Rossi and the entire ESOT staff for their tireless efforts to support this endeavor. We would also like to thank the YPTs for coordinating and performing the systematic literature searches which were additionally instrumental in this endeavor.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10915/full#supplementary-material

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ORIGINAL RESEARCH
published: 20 October 2022
doi: 10.3389/ti.2022.10712

Interleukin-18 and High-Mobility-Group-Protein B1 are Early and Sensitive Indicators for Cell Damage During Normothermic Machine Perfusion after Prolonged Cold Ischemic Storage of Porcine Liver Grafts

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In the era of organ machine perfusion, experimental models to optimize reconditioning of (marginal) liver grafts are needed. Although the relevance of cytokine signatures in liver transplantation has been analyzed previously, the significance of molecular monitoring during normothermic machine perfusion (NMP) remains elusive. Therefore, we developed a porcine model of cold ischemic liver graft injury after prolonged static cold storage (SCS) and subsequent NMP: Livers obtained from ten minipigs underwent NMP for 6 h directly after procurement (control group) or after 20 h of SCS. Grafts after prolonged SCS showed significantly elevated AST, ALT, GLDH and GGT perfusate concentrations, and reduced lactate clearance. Bile analyses revealed reduced bile production, reduced bicarbonate and elevated glucose concentrations after prolonged SCS. Cytokine analyses of graft perfusate simultaneously demonstrated an increase of pro-inflammatory cytokines such as Interleukin-1α, Interleukin-2, and particularly Interleukin-18. The latter was the only significantly elevated cytokine compared to controls, peaking as early as 2 h after reperfusion (11,012 ng/ml vs. 1,493 ng/ml; $p = 0.029$). Also, concentrations of High-Mobility-Group-Protein B1 were significantly elevated after 2 h of reperfusion (706.00 ng/ml vs. 148.20 ng/ml; $p < 0.001$) and showed positive correlations with AST ($r^2 = 0.846$) and GLDH ($r^2 = 0.918$) levels. Molecular analyses during reconditioning of liver grafts provide insights into the degree of inflammation and cell damage and could thereby facilitate future interventions during NMP reducing acute and chronic graft injury.

Keywords: ischemia-reperfusion injury, normothermic machine perfusion, cytokine, extended criteria donor organs, marginal organs, IL-18, HMGB1

OPEN ACCESS

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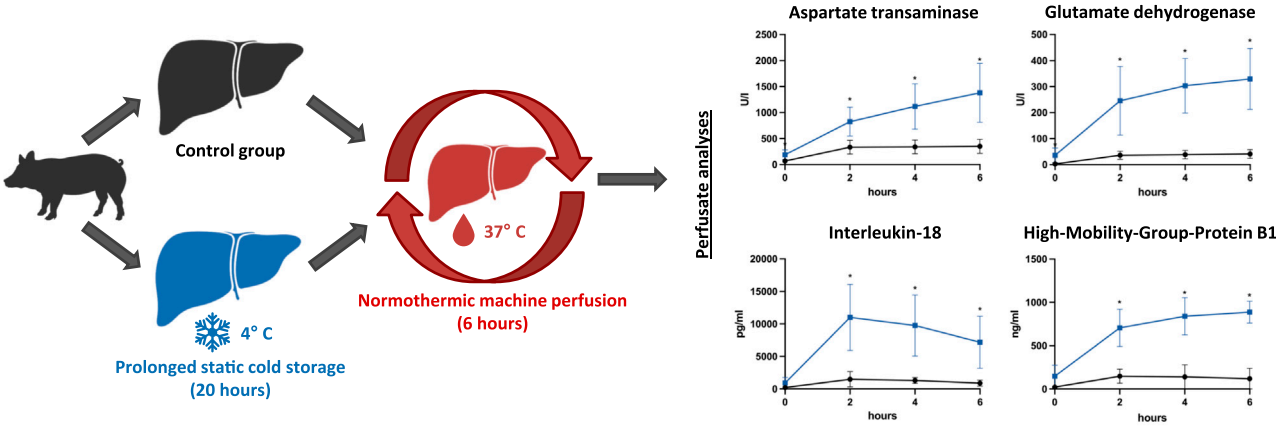
Received: *17 June 2022*
Accepted: *04 October 2022*
Published: *20 October 2022*

Citation:
Beetz O, Cammann S, Weigle CA, Sieg L, Eismann H, Johanning K, Falk CS, Krech T, Oldhafer F and Vondran FWR (2022) Interleukin-18 and High-Mobility-Group-Protein B1 are Early and Sensitive Indicators for Cell Damage During Normothermic Machine Perfusion after Prolonged Cold Ischemic Storage of Porcine Liver Grafts. Transpl Int 35:10712. doi: 10.3389/ti.2022.10712

Interleukin-18 and High-Mobility-Group-Protein B1 are early and sensitive indicators for cell damage during normothermic machine perfusion after prolonged cold ischemic storage of porcine liver grafts

In the era of organ machine perfusion, experimental models to optimize reconditioning of (marginal) liver grafts are needed.



Molecular analyses during reconditioning of liver grafts provide insights into the degree of inflammation and cell damage and could thereby facilitate future interventions during normothermic machine perfusion reducing acute and chronic graft injury.

Beetz, et al. *Transpl. Int.* 2022
doi: 10.3389/ti.2022.10712

GRAPHICAL ABSTRACT |

INTRODUCTION

In an era of worldwide organ shortage solutions to expand the donor pool and to reduce waitlist mortality of patients with end-stage liver disease are desperately needed. Accordingly, the increased utilization of marginal or extended criteria donor (ECD) organs has become clinical reality in many countries. Although there is no universally accepted definition of such organs, different donor-, graft- or storage-associated factors indicating a suboptimal graft quality, such as advanced donor age, hepatic steatosis or prolonged static cold storage (SCS) prior transplantation are most commonly being taken into account [1]. The short- and long-term success of this approach is critically limited by a higher vulnerability of ECD organs to an inevitable ischemia-reperfusion injury (IRI) induced and aggravated by the current state-of-the-art SCS and related early allograft dysfunction, bile duct complications and chronic allograft dysfunction [2, 3]. Hence, new challenges regarding optimal organ preservation and reconditioning have emerged.

The rediscovery and technical evolution of machine perfusion has the potential not only to adequately meet these demands but also to revolutionize the fields of organ repair, modification and, ultimately, transplantation.

Various forms of machine perfusion have been introduced and evaluated. Most prominently, the hypothermic (oxygenated) and the normothermic machine perfusion (NMP) are on the verge of entering clinical routine after being successfully assessed in large prospective multicenter trials [4, 5]. Although hypothermic machine perfusion, applying 4°C cold preservative (oxygenated) solution, has proven to be feasible and safe in several trials and reduces biliary complications as well as early allograft dysfunction, temperature-dependent downregulation of cellular metabolism impedes testing of hepatic function during perfusion, so far. NMP, on the other hand, is a more demanding technical procedure as it usually requires blood-based oxygenated perfusates at 37°C but thereby allows physiological aerobic metabolism and, hence, viability assessment of grafts prior to transplantation, which is essential to determine whether an ECD organ can be utilized or has to be discarded. Conventional parameters such as concentrations of aspartate or alanine transaminase and lactate, pH, glucose metabolism, bile production, adequate flow rates and a homogenous perfusion are currently applied experimentally and clinically, for example within the prospective VITTAL study [6, 7]. Although the relevance of cytokine signatures and damage-associated molecular patterns (DAMPs) in liver transplantation has been analyzed in the past [8, 9], the role of molecular monitoring during NMP and immunomodulatory effects of NMP especially on ECD organs remains surprisingly elusive. Ferdinand et al. recently reported increased inflammatory gene expression during NMP of human kidney grafts and demonstrated improved graft function upon adsorption of pro-inflammatory mediators [10]. In order to study these mechanisms and to evaluate therapeutic options for (marginal/ECD) liver grafts in the future, we developed a porcine model of cold ischemic liver graft injury after prolonged SCS of 20 h and subsequent NMP and investigated the inflammatory milieu in the perfusates.

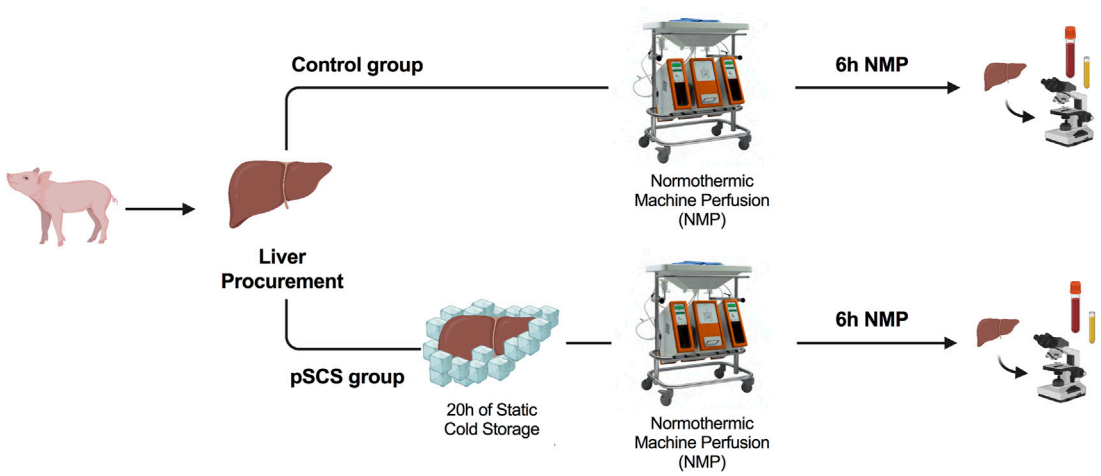


FIGURE 1 | Study design and time points of interventions. Normothermic machine perfusion (NMP) was performed directly (control group) or 20 h after liver procurement and static cold storage (prolonged SCS (pSCS) group), respectively. After 6 hours, animals were euthanized, serum samples were taken and liver and bile duct tissue was recovered for histological work-up.

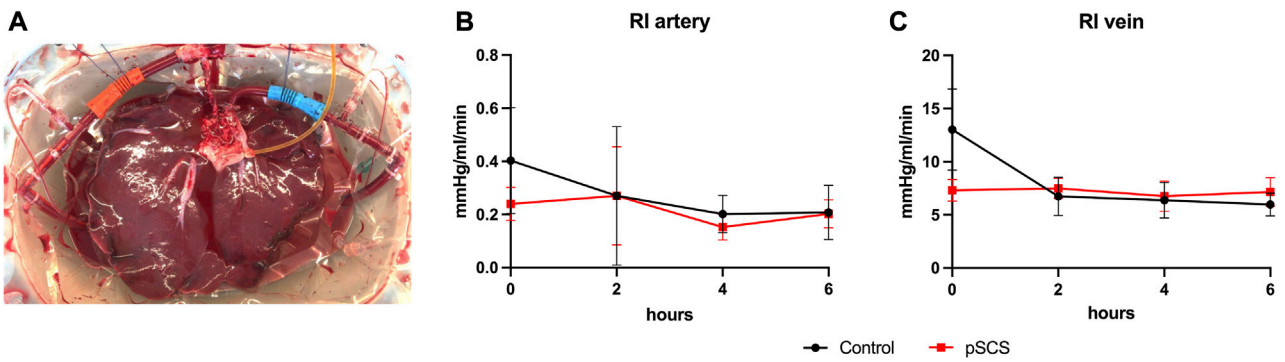


FIGURE 2 | Experimental set-up of the NMP. A representative example of a homogenous perfusion of a liver graft over the duration of 6 hours is depicted **(A)**. Courses of the computed arterial **(B)** and venous **(C)** resistance index (RI) during 6 hours of NMP are shown. Comparison of control group (black line) with prolonged SCS (pSCS) group (red line).

MATERIALS AND METHODS

Legal Approval

This study was performed at the Laboratory for Animal Science of Hannover Medical School after approval by the Lower Saxony regional authority for consumer protection and food safety (Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit (LAVES); 19/3146). The animals were kept under housing conditions of the EU-Guideline 2010/63 and valid German animal regulation act (Tierschutz-Versuchstierverordnung des deutschen Tierschutzgesetzes).

Experimental Design

The primary objective of this study was to introduce an experimental porcine model of cold ischemic liver graft injury after prolonged SCS in order to characterize the release of pro- and anti-inflammatory molecules during (re)perfusion under NMP.

Healthy pigs were divided into two groups: In the control group NMP was performed directly after liver procurement. In the group of prolonged SCS NMP was initiated after 20 h of SCS (4°C). All livers were perfused for a total of 6 hours. The experimental design is displayed in **Figure 1**.

Liver Procurement and Back-Table Preparation

Healthy female LEWE minipigs (n = 10) with a median age of 118 (117–126) days and median body weight of 52 (49–57) kg were used as liver donors. After premedication with zolazepam (5 mg/kg bodyweight) and atropine (0.02–0.04 mg/kg bodyweight) *via* intramuscular injection, anesthesia was induced by intravenously applied propofol (1.5–2.5 mg/kg

TABLE 1 | Applied scoring system for bile duct injury.

Bile duct wall component	Grade 0	Grade 1	Grade 2	Grade 3
Biliary epithelium	No loss	≤50% loss	>50% loss	n.a.
Mural stroma	No injury	≤25% necrotic	25–50% necrotic	>50% necrotic
Peribiliary vascular plexus	No injury	≤50% of vessels with changes	>50% of vessels with changes	Grade 2 + arteriolonecrosis
Thrombosis	Absent	Present	n.a.	n.a.
Intramural bleeding	None	≤50% of duct wall	>50% of duct wall	n.a.
Periluminal PBG	No injury	≤50% loss of cells	>50% loss of cells	n.a.
Deep PBG	No injury	≤50% loss of cells	>50% loss of cells	n.a.
Inflammation	None	At least 10 leukocytes/HPF	At least 50 leukocytes/HPF	n.a.

PBG, peribiliary glands; HPF, high-power field.

bodyweight). Upon endotracheal intubation anesthesia and analgesia were maintained with isoflurane (0.8–1.5 vol%) and fentanyl (0.003–0.007 mg/kg bodyweight) as previously reported [11, 12]. The procurement of the liver was performed as described in humans [13]. In brief, first the abdominal aorta and the inferior vena cava were exposed and the perfusion cannula was inserted cranial of the aortic bifurcation. Thereafter, the left lateral liver lobe was mobilized and upon transverse incision of the diaphragm the thoracic aorta was encircled. Before cross clamping of the supra-coeliac aorta, 25,000 I.E. heparin were administered intravenously. Exsanguination of the donor was then achieved by dissection of the suprahepatic vena cava inferior and collection of approximately 1,500 ml of blood in a container containing citrate-based anticoagulant (citrate-phosphat-dextrose solution with adenine). Afterwards, cold antegrade perfusion was performed with 3,500 to 4,000 ml of Custodiol (HTK)-solution (Dr. Franz Köhler Chemie GmbH, Bensheim, Germany) over a course of approximately 10–15 min followed by retrieval of the liver. Animals additionally received an intravenous bolus injection of a lethal dose (5000 mg) of pentobarbital sodium for intraoperative euthanasia.

After organ retrieval, the aortic segment was closed to one side by a doubled running non-absorbable monofilament suture (4-0 Prolene) and the aortic cannula was inserted on the opposite side and secured with a single purse-string suture (4-0 Prolene). Side branches of the aortic segment, the coeliac trunk and the hepatic artery were occluded with titanium clips. The portal vein cannula was inserted and secured in a similar fashion (**Figure 2A**). After ligation of the cystic duct, the common bile duct was cannulated and flushed with at least 20 ml of cold Custodiol (HTK)-solution.

Normothermic Machine Perfusion

NMP was performed with a Liver Assist device (Organ Assist, Groningen, the Netherlands). Approximately 1,500 ml of autologous whole blood, collected as described above, was used for perfusion. Colloid solution (e.g., Gelafundin) was added to achieve a total volume of approximately 2,000 ml. The perfusate was set to a temperature of 37°C and oxygenated with 100% oxygen at 0.5–1.0 L/min gas flow. The portal vein pressure was set at 8 mmHg. The hepatic artery was perfused with a pulsatile flow at a pressure of 60 mmHg. Potassium, insulin, calcium gluconate and sodium bicarbonate were added during the perfusion in order to achieve physiological conditions. Bile production was measured every 60 min and samples were taken every 2 hours after reperfusion (0, 2, 4, and 6 h).

Conventional Laboratory Parameters

Blood gas analyses (including lactate concentrations) were performed before and every 30 min after reperfusion. Aspartate transaminase (AST), Alanine transaminase (ALT), Glutamate dehydrogenase (GLDH), Alkaline phosphatase (ALP), Gamma-glutamyltransferase (GGT), bilirubin, urea and creatinine serum concentrations of perfusate samples were analyzed before and every 2 hours after the begin of perfusion.

Histological Analyses

Tissue samples for histology were obtained from the liver and the bile duct prior to reperfusion and after 6 hours of reperfusion. The tissue was fixed in buffered 4% formaldehyde and subsequently embedded in paraffin according to standard histopathological protocols. For histologic evaluation 4 µm thick sections were cut and stained with hematoxylin and eosin. Liver sections were semiquantitatively analyzed for the degree of inflammation (absent, mild, moderate, severe) as described by Ali et al [14]. Bile duct injury was assessed using a scoring system described by Hansen et al. and modified by op den Dries et al. ([15, 16]; see also **Table 1**). The histological samples were evaluated by a liver pathologist (TK) using a Zeiss Axio Imager A2 microscope, field number 25 (Zeiss, Germany). TK was blinded to the operative procedures. Microphotographs were generated using a Hamamatsu Nano zoomer S360 digital slide scanner (Hamamatsu, Japan).

Cytokine Multiplex Analyses

Luminex-based multiplex technology (Milliplex Porcine Cytokine/Chemokine Premixed 13-Plex Magnetic Bead Kit, Merck, United States) was used to generate cytokine profiles of perfusates, as previously reported [17]. Bio-Plex Manager 6.0 software was used to calculate standard curves and

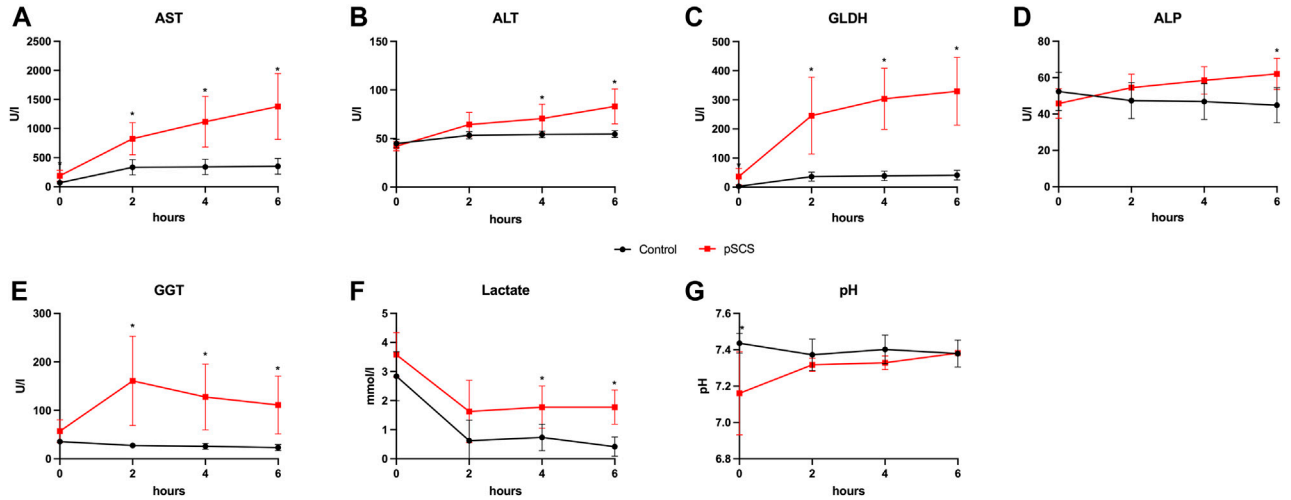


FIGURE 3 | Courses of perfusate parameters during NMP. **(A–G)** Courses of AST, ALT, GLDH, ALP, GGT, lactate and pH during 6 hours of perfusion are shown. Comparison of control group (black line) with prolonged SCS (pSCS) group (red line). *: $p < 0.050$.

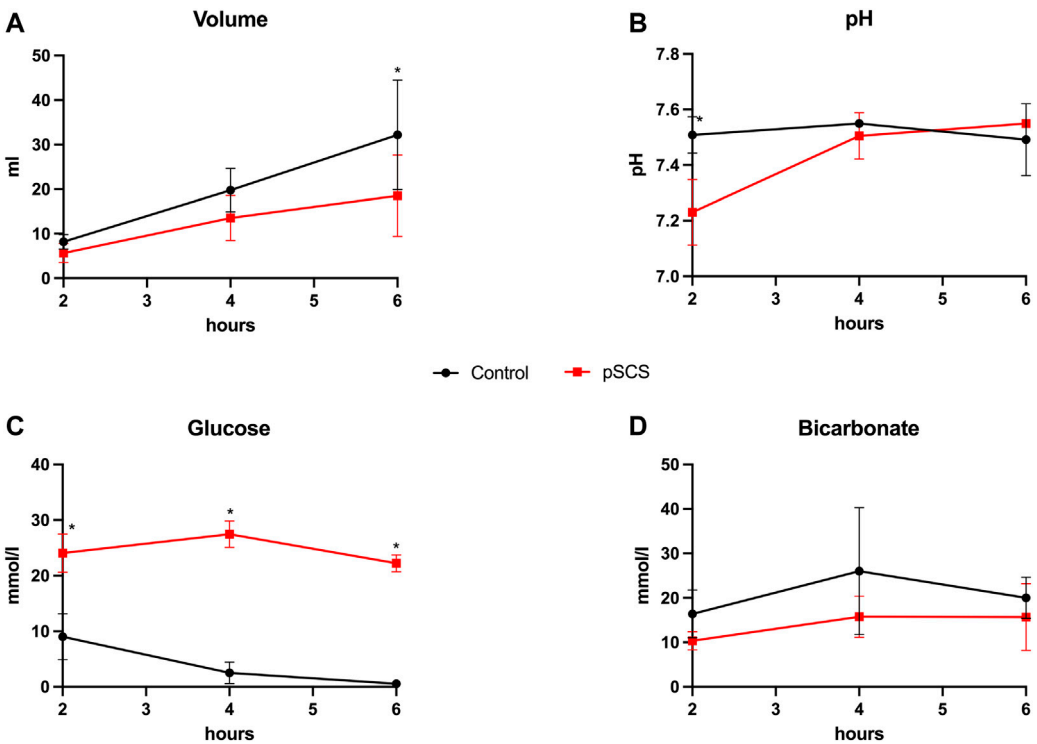


FIGURE 4 | Bile production and composition during NMP. **(A–D)** Courses of bile volume, pH, glucose and bicarbonate during 6 hours of perfusion are shown. Comparison of control group (black line) with prolonged SCS (pSCS) group (red line). *: $p < 0.050$.

cytokine concentrations. The detection limit of all proteins was 1–10 pg/ml.

Enzyme-Linked Immunofluorescent Assays

High-Mobility-Group-Protein B1 (HMGB1) serum concentrations were measured before and every 2 hours after reperfusion using a commercially available ELISA kit (Reference Number ST51011, Tecan—IBL International, Hamburg, Germany).

Statistical Analyses

Statistical analysis was performed using GraphPad PRISM 8.4.00 (GraphPad Software, Inc., La Jolla, CA). Comparison of mean

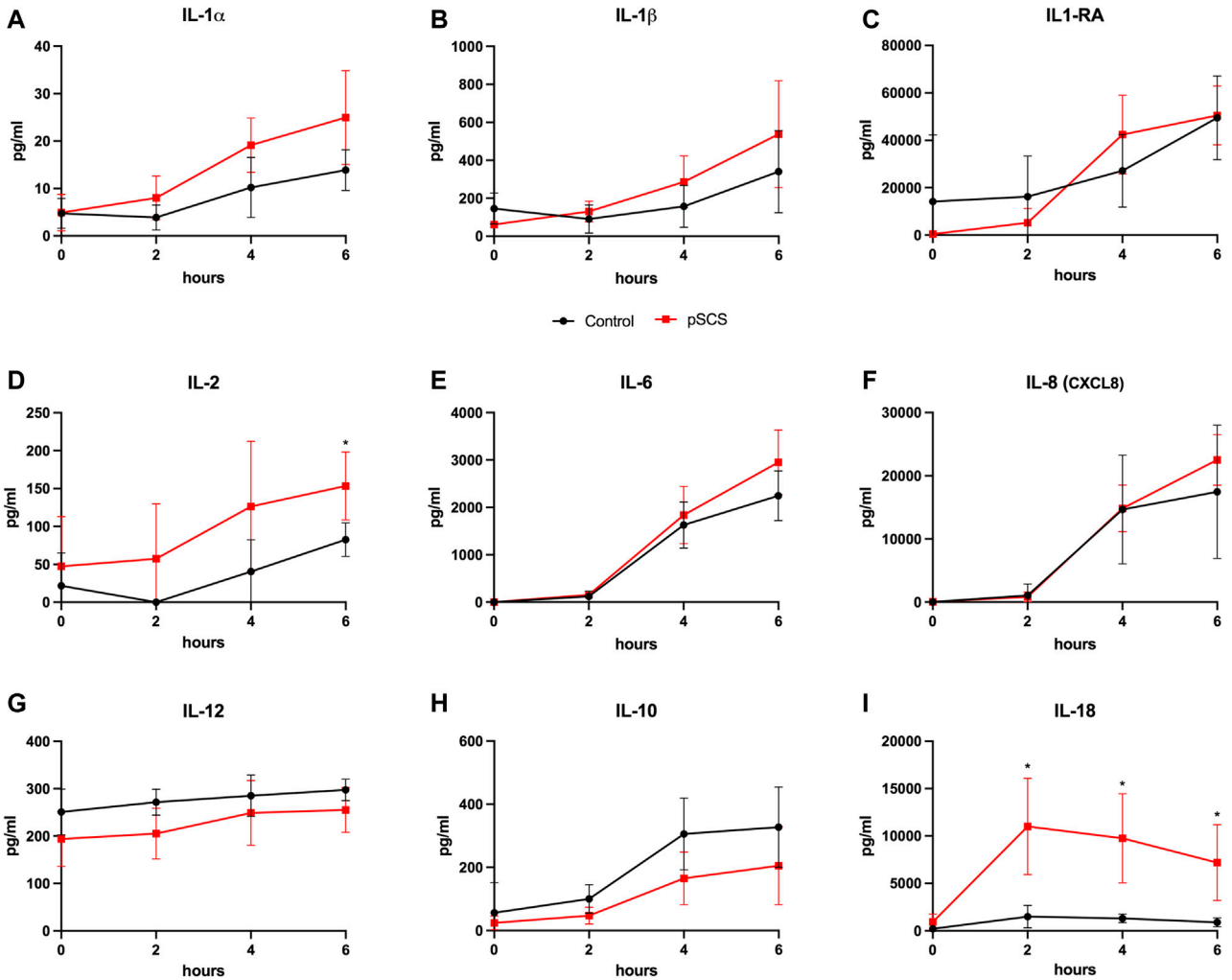


FIGURE 5 | Courses of cytokines during NMP. **(A–I)** Courses of IL-1α, IL-1β, IL-2, IL-6, IL-8, IL-12, IL-10 and IL-18 during 6 hours of perfusion are shown, respectively. Comparison of the control group (black line) with the prolonged SCS (pSCS) group (red line). *: $p < 0.050$.

values between both groups were performed with the Student's *t*-test in case of normal distribution or the Mann-Whitney *U* test, respectively. Differences were regarded statistically significant at p-values of < 0.050. Correlation between variables were expressed by the Pearson correlation coefficient. Results are expressed as mean ± standard deviation (SD) unless indicated otherwise.

RESULTS

Perfusion Parameters Were Not Influenced by Prolonged Static Cold Storage

None of the perfusions had to be terminated prematurely due to technical difficulties. Liver grafts of both groups showed homogenous perfusion over the duration of 6 h (**Figure 2A**).

Accordingly, computed resistance index (RI) did not vary over time and did not differ significantly between the prolonged SCS and the control group (**Figures 2B,C**). The mean arterial RI was 0.208 mmHg/L/min in the control group and 0.203 mmHg/L/min in the prolonged SCS group ($p = 0.926$). After 6 h of perfusion, the mean venous RI was 5.972 mmHg/L/min in the control group and 7.155 mmHg/L/min in the prolonged SCS group ($p = 0.184$).

Conventional Liver Function Parameters Were Elevated by Prolonged Static Cold Storage

Perfusate samples of grafts undergoing perfusion after SCS for 20 h revealed higher concentrations of AST, ALT and GLDH with an increasing difference over time, when compared to control grafts (control group): after 6 h of perfusion, the mean perfusate concentrations of AST, ALT and GLDH in the control group were 350.30 U/l, 54.50 U/l and 41.17 U/l, respectively, and stable throughout the previous 4 h of perfusion, whereas mean perfusate concentrations in the

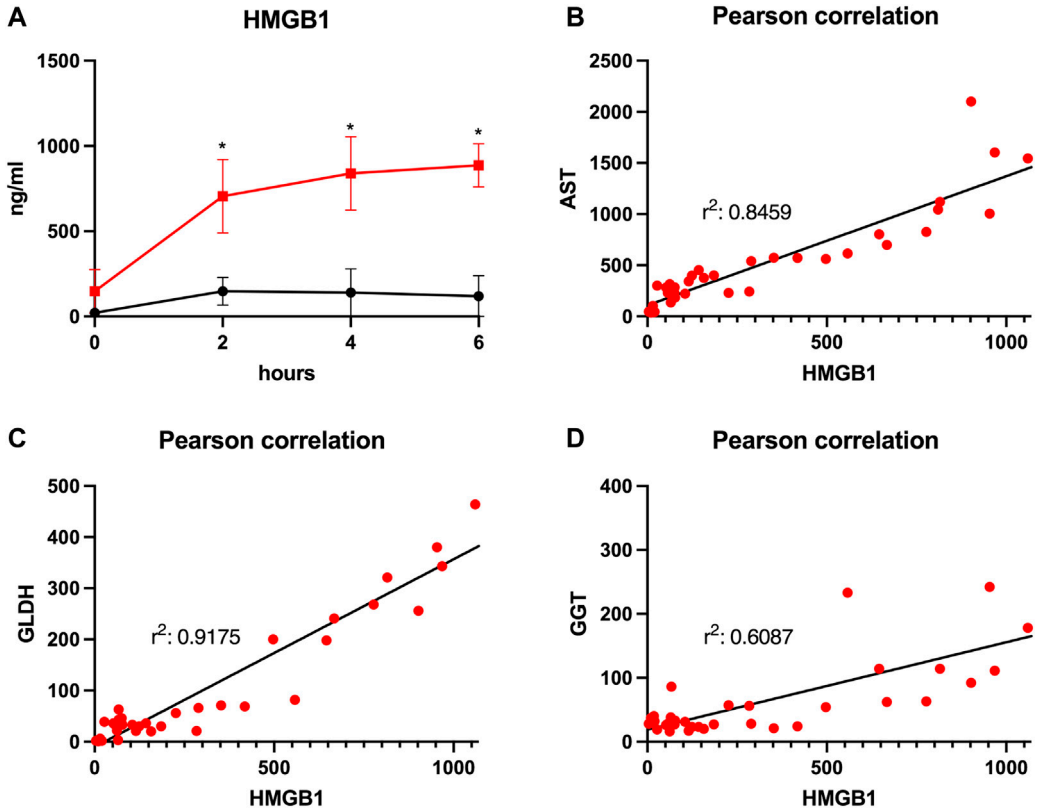


FIGURE 6 | Courses of HMGB1 during 6 h of perfusion are shown **(A)**. Comparison of the control group (black line) with the prolonged SCS (pSCS) group (red line). *: $p < 0.050$. Pearson correlations for HMGB1 with AST **(B)**, GLDH **(C)** and GGT **(D)**, respectively, are shown.

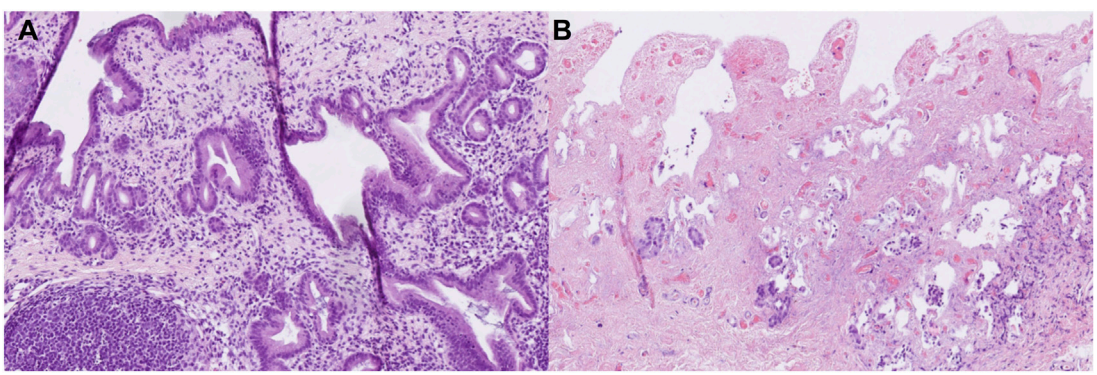


FIGURE 7 | Representative histological sections (hematoxylin-eosin staining, original magnification ×200) of bile ducts obtained from grafts undergoing 6 hours of NMP immediately after procurement **(A)** or after 20 h of SCS **(B)**. **(A)** shows a vital bile duct with dense inflammatory infiltrates (grade 2). **(B)** shows a largely necrotic bile duct without surface epithelium (grade 3), extensive necrosis of the mural stroma (grad 3), extensive damage to the periluminal peribiliary glands (grade 3) and mild inflammatory infiltrates (grade 1).

prolonged SCS group were 1,380.00 U/l, 83.00 U/l and 329.30 U/l ($p = 0.010$; $p = 0.024$; $p = 0.024$), respectively, and were rising throughout the length of perfusion (**Figures 3A–C**).

Accordingly, ALP and GGT perfusate concentrations indicating biliary damage were also elevated after prolonged SCS (**Figures 3D,E**). In this context, it is particularly noteworthy that the concentrations of GGT reached a

significantly higher level as early as 2 h after reperfusion when comparing both groups (160.80 U/l vs 27.67 U/l; $p = 0.010$).

Although lactate concentrations declined after 2 h and remained stable under 2 mmol/L with ongoing perfusion in both groups, grafts with prolonged SCS showed significantly reduced lactate clearance after 6 h of perfusion (1.775 mmol/L vs 0.417 mmol/L, $p = 0.010$; **Figure 3F**). The perfusate pH was kept stable throughout the perfusion in both groups (**Figure 3G**).

Bile Composition and Production Were Impaired by Prolonged Static Cold Storage

In line with the biliary damage indicated by the above mentioned perfusate analyses, grafts after prolonged SCS exhibited reduced bile production of only 18.50 ml compared to 32.20 ml ($p = 0.067$) over the course of perfusion, whereas analyses of the bile composition revealed lower bicarbonate concentrations by trend (19 mmol/L vs. 22 mmol/L; $p = 0.625$) and significantly higher glucose concentrations (22.20 mmol/L vs. 1.30 mmol/L; $p = 0.024$; **Figure 4**).

Pro-Inflammatory Cytokines and HMGB-1 Were Increased After Prolonged Static Cold Storage

A comparison of cytokine perfusate concentrations between both groups showed higher values but no significant differences regarding the pro-inflammatory cytokines IL-1α, IL-1β, IL-2, IL-6 and the chemokine CXCL8 (IL-8) at the end of perfusion in grafts with prolonged SCS (**Figures 5A–F**). IL-12 was the only pro-inflammatory cytokine with numerically higher values in the control group compared to the prolonged SCS group without significant differences after 6 h (297.5 pg/ml vs 255.3 pg/ml, $p = 0.200$) (**Figure 5G**). On the contrary, concentrations of the anti-inflammatory cytokine IL-10 were continuously lower in the prolonged SCS group, without reaching statistical significance (**Figure 5H**). More pronounced and significant differences, respectively, were observed for IL-18 and HMGB1 perfusate concentrations: The caspase-cleavage-dependent pro-inflammatory cytokine IL-18 increased after reperfusion, with an early peak after 2 h with 11,012.0 pg/ml and a slow decrease thereafter (7,195.0 pg/ml after 6 h) in the prolonged SCS group compared to stable concentrations of 1,493.0 pg/ml after 2 h ($p = 0.029$) and 896.5 pg/ml after 6 h ($p = 0.029$) in the control group (**Figure 5I**).

HMGB1, indicating inflammation and tissue damage, also rapidly increased during NMP after prolonged SCS and reached a plateau towards the end of perfusion with 887.3 pg/ml after 6 h (**Figure 6A**). HMGB1 perfusate concentrations in the control group peaked and then declined after approximately 2 h reaching a mean concentration of 119.3 pg/ml at the end of perfusion ($p = 0.010$). Of note, the increase in HMGB1 perfusate concentrations correlated well with liver function parameters such as AST and GLDH indicating hepatocyte and endothelial damage ($r^2 = 0.846$ and $r^2 = 0.918$, respectively; **Figures 6B,C**) as well as with GGT concentrations, indicating biliary injury ($r^2 = 0.609$; **Figure 6D**).

The following cytokines were detected with very low values in the majority of the obtained perfusate samples and were therefore not further analyzed: TNF-α, IL-4 and IFN-γ.

Histological Analyses

Histological analyses of liver and bile duct specimens obtained before and after 6 h of NMP did not reveal statistically significant differences between both groups, most likely as a result of the semiquantitative histological scoring system applied (see **Table 1**), the comparatively small sample number and statistical outliers for each histological item. However, there was an increase in the degree of liver inflammation by trend, which was largely absent prior to perfusion and increased to a mild or moderate degree in both groups, respectively. Furthermore, grafts after prolonged SCS showed increased biliary damage after 6 h of NMP when compared to control grafts. In more detail, there was a trend towards a higher degree of injury concerning the biliary epithelium (median (range): 2 (2) vs. 1 (1–2); $p = 0.200$), mural stroma (median (range): 2 (1–3) vs. 1 (0–2); $p = 0.500$) and periluminal peribiliary glands (median (range): 2 (1–2) vs. 1 (0–2); $p = 0.700$). **Figures 7A,B** depicts representative histological sections obtained from bile ducts of both groups after 6 h of NMP illustrating the spectrum of biliary damage.

DISCUSSION

Cold and warm ischemia during organ retrieval, storage and reperfusion inevitably results in IRI of liver grafts, i.e., hepatocytes, cholangiocytes, liver sinusoidal endothelial cells and non-parenchymal resident immune cells. The subsequent release of pro-inflammatory cytokines, such as IL-1β, IL-6, and CXCL8/IL-8 as well as DAMPs and reactive oxygen species initiates the development of an inflammatory environment: neutrophil recruitment and activation of Kupffer cells, leading to a further secretion of IL-1β and TNF-α and upregulation of adhesion molecules such as Mac-1 and ICAM-1, further promotes the infiltration of immune cells into the liver parenchyma and induces additional parenchymal injury [18, 19].

The current gold standard of organ preservation represented by SCS and an ongoing organ donor shortage with increased use of ECD organs induce or aggravate these mechanisms. In contrast, a variety of experimental and clinical studies have demonstrated that machine perfusion ameliorates the detrimental effects of IRI on liver grafts with regard to early allograft dysfunction or ischemic biliary complications [20, 21]. Most recently, Markmann et al. published data of a randomized clinical multicenter trial, including 293 patients, displaying a significant reduction of lobular inflammation after graft reperfusion with previous NMP [22]. Accordingly, Jassem et al. observed reduced numbers of pro-inflammatory cytokine producing T-cells among donor lymphocytes and higher numbers of $CD4^{pos}CD25^{high}CD127^{neg}FOXP3^{pos}$ regulatory

T-cells in the perfusate of 12 liver grafts after NMP, when compared to 27 grafts after SCS [23].

In the mentioned North American trial by Markmann et al. NMP was performed directly after organ procurement with a portable device showing a significant reduction of ischemic bile duct complications whereas a comparable European trial by Nasralla et al. performed NMP after a relevant cold ischemic time at the recipient center without observing positive effects on bile duct complications in the further course [22, 24]. Accordingly, Mergental et al. showed that although the application of NMP in a "back-to-base" approach is able to rescue ECD organs, development of ischemic bile duct complications is not prevented, which is in line with the results of our study which showed significant damage of the bile ducts despite NMP after a prolonged cold ischemia time [25].

As NMP allows assessment of grafts under physiological conditions prior transplant, simultaneous evaluation of the degree of IRI and molecular mechanisms influencing short- and long-term cell damage should be a central issue of future experimental and clinical studies.

To our knowledge, this is the first investigation into the kinetics of pro- and anti-inflammatory mediators during NMP of porcine livers. Our results show an increase of IL-1α, IL-1β, IL-2, IL-6 and IL-18, with the latter being the only significantly elevated cytokine, during NMP after prolonged SCS. The statistically significant and early increase of IL-18 is particularly interesting as its role in IRI is perceived as indicator of caspase-1 activation, which is required for the release of both IL-18 and IL-1β from stressed cells. Takeuchi et al. showed a significant reduction of IRI and a concomitant upregulation of anti-inflammatory cytokines such as IL-4 and IL-10 in a mice model under blockade of IL-18 [26]. In line with these results, Bal et al. demonstrated a protective effect of IL-18-binding protein on IRI induced liver injury in an experimental rat model [27]. Of note, our data showed similar kinetics of IL-18 and GGT perfusate concentrations (early increase, peak after 2 hours and decrease during ongoing perfusion) after prolonged SCS suggesting an association between IL-18 secretion and bile duct inflammation or injury.

More importantly, HMGB1, one of the most widely analyzed DAMPs in the transplant setting, is released early during NMP and correlates with the conventionally measured cell damage of hepatocytes (i.e., AST and GLDH concentrations) and cholangiocytes (GGT concentration). Of note, the corresponding histological analyses did not corroborate our findings in terms of proof for significantly increased hepatic or biliary inflammation and injury, respectively, probably due to the short *ex-vivo* observational follow-up of only 6 hours in this model.

Ilmakunnas et al. introduced HMGB1 as an early marker of hepatic injury after transplantation, peaking as soon as 10 minutes after portalvenous reperfusion with the highest concentrations being observed in the caval effluent. HMGB1 kinetics did not correlate with either IL-6 or TNF-α, but with the degree of graft steatosis and postoperative ALT levels [28]. In addition, anti-HMGB1 antibodies were shown to be protective against IRI and subsequent hepatocellular damage and cytokine upregulation [29, 30].

However, the diagnostic value and mechanistic role of HMGB1 during NMP is still elusive. Scheuermann et al. showed that elevated levels of inflammatory molecules, such as HMGB1, are associated with increased activation of toll-like receptors and apoptosis after liver reperfusion in a rat model [31].

Interestingly, Scheuermann et al. and Goldaracena et al. described that the amount of recirculating inflammatory molecules increases with higher perfusate temperature during machine perfusion most likely as a result of increased cell metabolism [31, 32].

Of note, absence of filtration and/or adsorber systems in current NMP devices allows continuous perfusate recirculation and hence potential accumulation of metabolic products and inflammatory molecules.

Different strategies have been established to reduce the accumulation of pro-inflammatory molecules during machine perfusion. A simple but effective idea was published by Obara et al.: replacement of the initial perfusate after 5 minutes of subnormothermic machine perfusion as an attempt to mimic a filter or dialyzer led to significantly lower concentrations of transaminases and lactate levels after reperfusion in a porcine model [33]. Haemoadsorption with an incorporated cytokine filter has been used to reduce the inflammatory response during kidney machine perfusion resulting in improved renal blood flow, albeit without significantly influencing renal function [34]. With regard to lung machine perfusion, filter-based cytokine removal has been shown to decrease the development of pulmonary edema with uncertain effects on clinical pulmonary function post engraftment [35].

A critical issue of perfusate exchange and cytokine filters may be the simultaneous removal of not only pro- but also important anti-inflammatory mediators. Thus, specific antibodies might be more effective in order to improve grafts during perfusion. Garcia-Aroz et al. showed that livers treated with monoclonal antibodies against CD47 before perfusion following 30 or 60 min of warm ischemia time showed significantly lower ALT levels and higher bile production compared with their respective control groups [36]. Further potentially effective strategies might include the use of regulatory cytokines and cell therapies during NMP in order to create an anti-inflammatory environment for organ (re)conditioning.

Our study has some important limitations: Although our model reflects suboptimal storage conditions (SCS >12 h; commonly defined as marginal or ECD organs [1]), liver grafts from young and healthy pigs do not resemble conditions of (marginal/extended criteria) human donors.

Furthermore, to reduce costs and logistical complexity we applied whole blood in our perfusion protocol at the expense of limiting the comparability with the clinical setting. However, Liu et al. demonstrated a trend toward superior functional and hepatocellular injury outcomes, with even lower AST release for porcine liver NMP with whole blood when compared to red blood cells and steen solution [6].

In the era of machine perfusion, the monitoring of cytokine profiles and DAMPs during *ex-vivo* preservation and (re) conditioning of liver grafts might serve as useful biomarkers for detection of inflammation and relevant IRI. This would enable sophisticated analyses of specific therapeutic interventions in order to promote an anti-inflammatory environment and

thereby reduce acute and chronic graft damage. Furthermore, the potential prognostic value of mentioned biomarkers for short- and long-term complications (such as biliary lesions with regard to the detrimental bile duct histology after prolonged SCS) could significantly improve organ assessment prior transplantation, despite the additional logistical and economic burden of corresponding analyses. Translational *ex-vivo* models, as presently described, but also long-term *in-vivo* models will play a crucial role in clarifying these aspects and optimizing machine perfusion based reconditioning protocols of marginal donor organs in the near future.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

This study was performed at the Laboratory for Animal Science of Hannover Medical School after approval by the Lower Saxony regional authority for consumer protection and food safety (Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit (LAVES); 19/3146).

AUTHOR CONTRIBUTIONS

Study conception and design: OB, FO, and FV. Acquisition of data: OB, SC, CW, LS, HE, CF, TK, and FO. Analysis and interpretation of data: OB, CF, TK, FO, and FV. Drafting of manuscript: OB, FO, and FV. Critical revision of manuscript: KJ, CF, and TK.

FUNDING

This study was funded by the MHH Transplant Centre (FV and CF).

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 want to thank Britta Trautewig, Corinna Löbbert, Ingrid Meder, Astrid Dinkel, Jana Keil and Kerstin Beushausen for excellent technical assistance.

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ORIGINAL RESEARCH
published: 12 October 2022
doi: 10.3389/ti.2022.10731

Donor Size Doesn't Impact *En Bloc* Kidney Transplant Outcomes: A Single-Center Experience and Review of Literature

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Few transplant programs use kidneys from donors with body weight (BW)<10 kg due to higher incidence of vascular and urological complications, and DGF. The purpose of this study was to investigate the non-inferiority of pediatric *en bloc* kidneys from donors with BW<10 kg. We performed a single-center retrospective analysis of *en bloc* kidney transplants from pediatric donor cohort (n = 46) from 2003 to 2021 and stratified the outcomes by donor BW (small group, donor BW<10 kg, n = 30; standard group, donor BW<10 kg, n = 16). Graft function, rate of early post-transplant complications, graft and patient survival were analyzed. Complication rates were similar between both groups with 1 case of arterial thrombosis in the smaller group. Overall graft and patient survival rates were similar between the small and the standard group (graft survival—90% vs. 100%, p = 0.09; patient survival—96.7 vs. 100%, p = 0.48). Serum creatinine at 1, 3, 5 years was no different between groups. Reoperation rate was higher in the small group (23.3% vs. 6.25%, p = 0.03). The allograft from small donors could be related to higher reoperation rate in the early post-transplant period, but not associated with lower long-term graft and patient survival.

Keywords: kidney transplant, *en bloc* kidney, body weight, pediatric donor, review of literature

OPEN ACCESS

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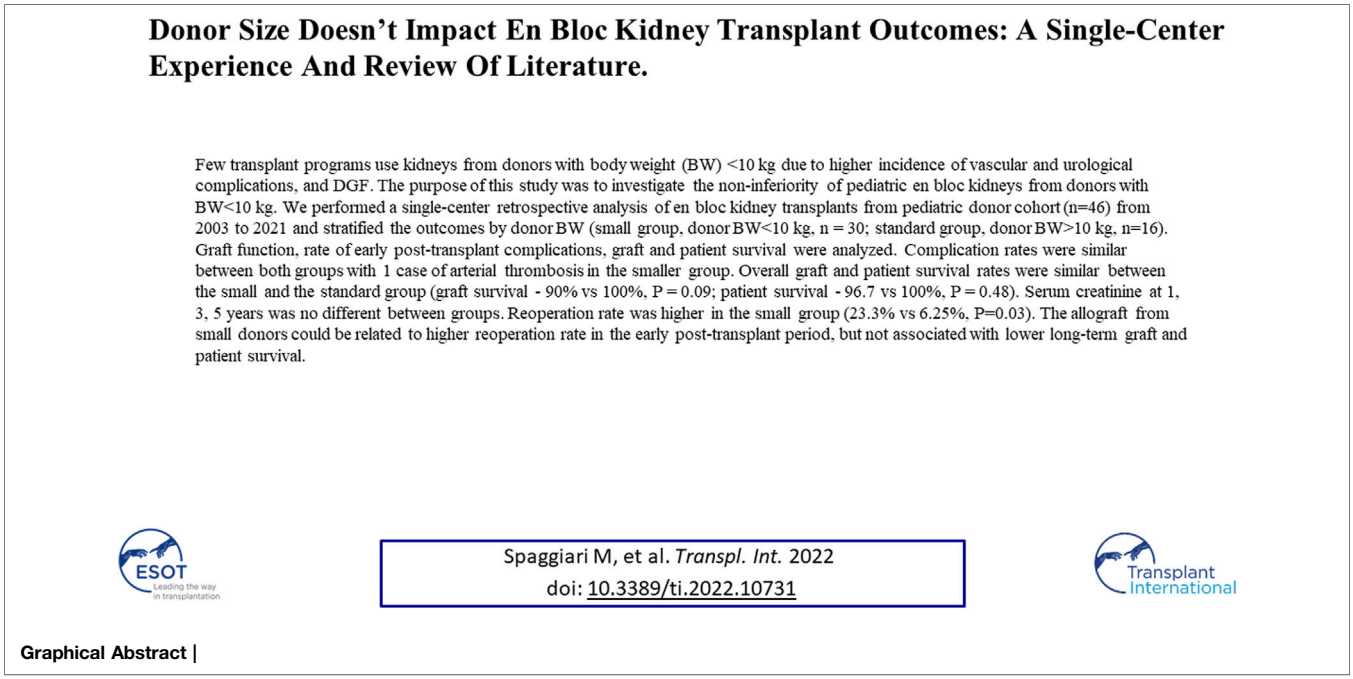
Received: *27 June 2022*
Accepted: *29 September 2022*
Published: *12 October 2022*

Citation:
Spaggiari M, Petrochenkov E, Patel H, Di Cocco P, Almario-Alvarez J, Fratti A, Tzvetanov I and Benedetti E (2022) Donor Size Doesn't Impact En Bloc Kidney Transplant Outcomes: A Single-Center Experience and Review of Literature. Transpl Int 35:10731. doi: 10.3389/ti.2022.10731

Abbreviations: DBW, donor body weight; BW, body weight; CIT, cold ischemia time; EBL, estimated blood loss; CMV, cytomegalovirus; eGFR, estimated glomerular filtration rate; ACR, acute cellular rejection; AMR, antibody mediated rejection; BMI, body mass index; DBD, donation after brain death; DCD, donation after cardiac death; EBK, *en bloc* kidney; DGF, delayed graft function; IQR, interquartile range; POD, postoperative day; PLEX, plasma exchange; IVIG, intravenous immunoglobulin; IVC, inferior vena cava; n, number of cases.



Graphical Abstract |

INTRODUCTION

The United States kidney transplant waitlist has been constantly growing (1). In 2020, 37,408 new patients were added to the waitlist and 23,642 kidney transplants were performed (2). A 36.8% gap between patients who need the transplant and those who receive it forces transplant centers to look for new sources of donor organs.

Pediatric deceased donor *en bloc* kidneys (EBK) grafts are an underutilized source of suitable kidneys from transplant. Because of the perceived higher risk of technical complications, transplantation from *en bloc* kidneys is routinely performed only at a few transplant centers. A few reports showed higher incidence of vascular and urological complications (3,4), rejection, and delayed graft function with *en bloc* kidneys grafts (5). The risk of technical complications and poor graft survival is perceived to being associated with donor size.

We report on a single centre retrospective analysis on *en bloc* kidney transplants emphasizing outcomes and technical complications between the group of "small donors" (donor body weight (DBW≤10 kg) and the group of "standard" *en bloc* kidney donors (DBW>10 kg). A review of the literature has been performed for reference and comparison.

METHODS

Study Population

This is a retrospective cohort analysis of *en bloc* kidney transplants in adult recipients, performed at an urban, academic institution between 2003 and 2021. Pediatric donors were stratified into 2 groups according to donor body weight (DBW): "standard group," with DBW greater than 10 kg and "small group," with DBW less than or equal to 10 kg. Donor demographics, including sex, race, age, weight, cause of death, cytomegalovirus (CMV) status, donation type (DBD/DCD) were obtained from United Network for Organ Sharing. This study was approved by IRB #2019-1320.

Transplant

During backbench preparation of the graft, the proximal stump of the inferior vena cava and the aorta are oversewn with 6.0 Prolene. The distal ends of the IVC and the aorta are used for the anastomoses. If the bifurcation into iliac vein and iliac artery are present, they are used to create a wide patch. All aorta and IVC lumbars as well as adrenal and gonadal vessels are secured with 4/0 silk ties (**Figure 1**). Then, the graft is flipped 180° in order to align the aorta and IVC with recipient external iliac artery and vein respectively. End-to-side arterial anastomosis between the distal aorta of the graft and the external iliac artery are performed with 6.0 Prolene suture. The venous anastomosis is an end-to-side anastomosis between the distal IVC of the graft and the external iliac vein of the recipients sutured with 6.0 Prolene. Two separated ureteroneocystostomy anastomoses over double-J stents are routinely performed and sutured with 5.0 PDS (**Figure 2**).

Induction therapy consists of rabbit antithymocyte globulin and methylprednisolone followed by a rapid, 5-day steroid taper. Maintenance was achieved using mycophenolate and tacrolimus (8–12 ng/ml for the first 2 months, then 5–10 ng/ml thereafter). Institutional immunosuppression regimen did not change during the study period. All patients received antimycotic prophylaxis with fluconazole 200 mg during the first postoperative week. The antimicrobial prophylaxis included ampicillin/sulbactam and

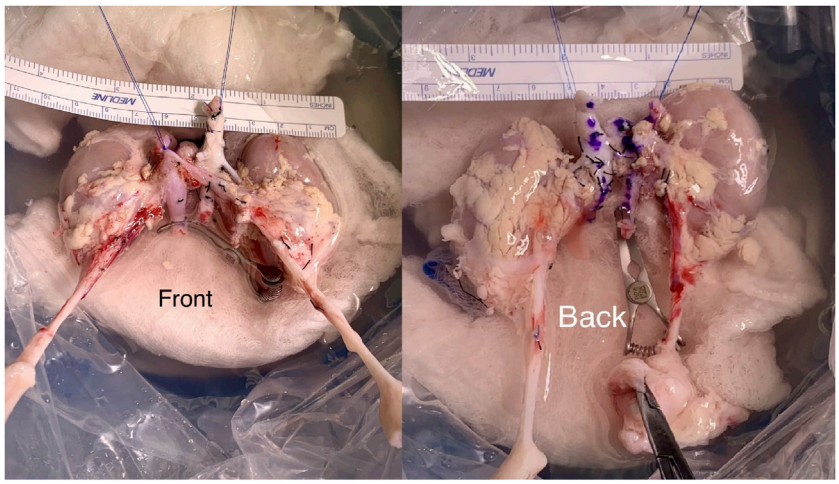


FIGURE 1 | *En bloc* kidney graft during the backbench preparation stage.

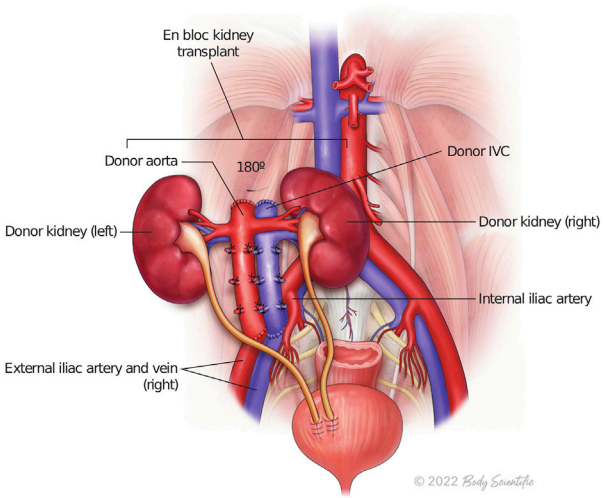


FIGURE 2 | The illustration represents *en bloc* kidney transplant.

vancomycin. Cytomegalovirus prophylaxis was provided by valganciclovir 450 mg daily for 6 months except those with negative CMV serology in both donor and recipient. In that case, 1 month of acyclovir was used for herpes simplex virus prophylaxis. Anticoagulation prophylaxis consisted of aspirin-dipyridamole 25 mg/200 mg every 12 h for 2 months, followed by lifelong 81-mg aspirin daily.

Outcomes

Cold Ischemia Time (CIT), Estimated Blood Loss (EBL) were analysed. Serum creatinine and eGFR values were collected at 6-months, 1-,3-,5-year follow up period. Delayed graft function (DGF) has been defined as the need of dialysis within the first week post-transplant. Rejection events, humoral (AMR), cellular (ACR), either empirically treated in case of sudden decrease of urine output associated with increase creatine or biopsy proven, have been reported. Post-transplant complications were collected: graft thrombosis, urinary leak, post-operative bleeding, and reoperation within the first 30 days.

Survival

Patient and graft survival rates were estimated using Kaplan-Meier curves and compared between the groups using a log-rank test. Patients lost at the follow-up with functioning graft were included in this analysis.

Statistical Analysis

Normally distributed continuous variables are expressed as mean ± standard deviation and non-normally distributed continuous variables as median (IQR). All continuous variables were normally distributed and reported as mean ± standard deviation and to compare between groups using analysis of variance test. Categorical variables were summarized as percentages and compared between groups using Fisher exact test. p values were calculated using 2-tailed tests and considered significant if less than 0.05. The statistical analysis was performed using IBM SPSS Statistics for Windows version 27 (IBM Corporation, Armonk, NY).

Literature Review

PubMed database was searched using the terms "pediatric *en bloc* kidney," "*en bloc* kidney," and "transplantation." We identified the studies published in the last 10 years, which included analysis of the EKT outcomes based on DBW or used DBW as the main criteria of the cohort stratification. The exclusion criteria from the literature research included the following: a cohort less than 10 patients; transplantation only to pediatric recipients, and transplantation of a single kidney. This yielded 6 articles which specifically detailed the outcomes of adult patients who received kidney grafts from pediatric donor (**Table 1**).

RESULTS

Study Population

Forty-six patients were identified for the analysis, 16 (34.78%) patients received the organ from donors with BW>10 kg (Range: 11.79–19.96) and 30 (65.22%) recipients had a donor with BW≤10 kg (Range: 3.18–9.98). Recipient baseline characteristics stratified by donor groups are presented in **Table 2**. The BMI of the recipients was significantly different between the groups (standard vs. small, 28.55 ± 6.88 kg vs. 24.39 ± 3.72 kg; $p = 0.04$). Fifteen (93.75%) out of 16 recipients in the standard group and 26 (86.7%) out 30 in the small group received dialysis pre-transplant. Duration of dialysis was not different between two groups (standard vs. small, 66.38 ± 36.83 vs. 50.63 ± 33.29; $p = 0.16$).

Donors in the small group were younger (standard vs. small, 24.0 ± 13.91 vs. 4.5 ± 8.03; $p = 0.00001$). Despite the difference in BW between the groups, Δ Weight (Recipient-Donor) kg was not significantly different ($p = 0.08$). Male sex and African American ethnicity were dominant in both groups, with anoxia as the leading cause of death. Five DCD donors were in the cohort, 3 in the standard and 2 in the small group. Mean final serum creatinine was higher in smaller donors but without significant difference (0.38 ± 0.15 vs. 0.33 ± 0.2, $p = 0.35$). Pediatric kidney grafts were procured by the regional Organ Procurement Agency (Region 7) in 40 (86.9%) cases. Six kidneys were imported outside of the region (Ohio-3, Mississippi-1, Kentucky-1, Indianapolis-1), with 4 donors with BW≤10 kg. Donor characteristic summary is presented in **Table 3**.

TABLE 1 | Literature review: pediatric kidney transplant to adult recipients.

	Period	Number of patients	Results
Current study 2022	2003–2021	DBW>10 kg, *n* = 16	DGF—0% Rejection rate—12.5% 5-y Graft survival—96.7% 5-y Patient survival—100%
		DBW≤10 kg, n = 30	DGF—3.3% Rejection rate—10% 5-y Graft survival—90% 5-y Patient survival—100%
Peng et al. (6) 2021	2015–2019*	DBW≤5 kg, *n* = 32	DGF—34.4% Rejection rate - 12.5% 5-y Graft survival—71.4% 5-y Patient survival—96.9%
		5 kg<DBW≤20 kg, *n* = 143	DGF—23.1% Rejection rate—10.5% 5-y Graft survival—89.5% 5-y Patient survival—94.4%
		DBW>20 kg, *n* = 110	DGF—16.4% Rejection rate—10.9% 5-y Graft survival—97.3% 5-y Patient survival—99.1%
Lopez-Gonsalez et al. (7) 2022	1999–2021	*n* = 42, (mean DBW 11.3 ± 3.6 kg)	DGF—NR Rejection rate—NR Graft survival—83.3% (mean follow-up 73 months) 5-y Patient survival - NR
Hafner-Giessauf et al(8) 2013	1990–2002	*n* = 13, (mean DBW 8 ± 3 kg)	DGF—NR Rejection - 7.7% 5-y Graft survival—84.6% Patient survival—NR
Mitrou et al. (9) 2018	2000–2017**	DBW<10 kg, *n* = 11	DGF—45.5% Rejection rate—9% 5-y Graft survival—81.8% 5-y Patient survival—100%
		DBW>10 kg, *n* = 17	DGF—23.5% Rejection rate—5.8% 5-y Graft survival—94.1% 5-y Patient survival—82.4%
Troppmann et al. (10) 2018	2007–2015	DBW≤10 kg, *n* = 130	DGF—19.2% Rejection rate—NR 5-y Graft survival—83.1% 5-y Patient survival—93.5%
Choi et al. (11) 2017	1996–2016	*n* = 15, (mean DBW 13.14 kg)	DGF—20% Rejection rate—13% 5-y Graft survival—92.9% 5-y Patient survival—NR

*n-number of patients; y-year; NR, not reported; DBW, donor body weight; DGF, delayed graft function; *—285 patients overall; **—28 patients overall.*

TABLE 2 | Recipient characteristics stratified by DBW.

	Standard group (*n* = 16)	Small group (*n* = 30)	Total (*n* = 46)	*p*-value
Age, (years)	45.59 ± 14.42	48.41 ± 14.89	47.43 ± 14.63	0.54
Weight, (kg)	74.81 ± 18.49	67.82 ± 9.97	70.25 ± 13.76	0.18
BMI, (kg/m^2)	28.55 ± 6.88	24.39 ± 3.72	25.84 ± 5.36	0.04
Sex, n (%)				0.99
• Male (%)	6 (37.5%)	17 (56.7%)	23 (50%)	
• Female (%)	10 (62.5%)	13 (43.3%)	23 (50%)	
Ethnicity, n (%)				0.1
• African-America	7 (43.75%)	13 (43.4%)	20 (43.47%)	
• Hispanic	8 (50%)	8 (26.6%)	16 (34.78%)	
• Caucasian	1 (6.25%)	3 (10%)	4 (8.6%)	
• Other	—	6 (20%)	6 (13.04%)	
CMV status, n (%)				0.4
• Positive	15	27	42	
• Negative	1	3	4	
Dialysis pretransplant, n (%)	15 (93.75%)	26 (86.7%)	41 (89.1%)	0.18
Duration of dialysis pretransplant, (month)	66.38 ± 36.83	50.63 ± 33.29	56.11 ± 34.99	0.16

n, number of cases; BMI, body mass index; CMV, cytomegalovirus.

TABLE 3 | Donor characteristics stratified by DBW.

	Standard group (*n* = 16)	Small group (*n* = 30)	Total (*n* = 46)	*p*-value
Age, (months)	24.0 ± 13.91	4.5 ± 8.03	15.35 ± 14.4	0.00001
Weight, (kg)	15.14 ± 2.7	7.09 ± 2.15	9.89 ± 4.52	0.00000
Δ Weight (Recipient-Donor), (kg)	59.67 ± 18.27	60.73 ± 9.37	60.36 ± 12.9	0.83
Sex, n (%)				0.86
• Male (%)	11 (68.75%)	17 (56.7%)	28 (60.7%)	
• Female (%)	5 (31.25%)	13 (43.3%)	18 (39.3%)	
Ethnicity, n (%)				0.1
• African American	7 (43.75%)	16 (53.3%)	23 (50%)	
• Hispanic	2 (13%)	4 (13.3%)	6 (13.04%)	
• White	5 (31.25%)	10 (33.3%)	15 (32.6%)	
• Other	2 (13%)	—	2 (4.3%)	
Cause of death				NA
• Stroke	—	1	1	
• Anoxia	9	14	23	
• Head trauma	7	13	20	
• Other	—	2	2	
DCD/DBD	3/13	2/28	5/41	NA
CMV status, n (%)				0.6
• Positive	5 (31.25%)	7 (23.33%)	13 (28.26%)	
• Negative	11 (68.75%)	23 (76.67%)	34 (71.74%)	
Final serum creatinine, (mg/dl)	0.33 ± 0.2	0.38 ± 0.15	0.37 ± 0.17	0.35
Area of procurement, n (%)				0.16
• Region 7	12 (75.5%)	28 (93.3%)	40 (86.9%)	
• Outside of the Region	4 (25.5%)	2 (6.7%)	6 (13.1%)	

Region 7, Illinois, Wisconsin, South Dakota, North Dakota, Minnesota; n, number of cases; BMI, body mass index; CMV, cytomegalovirus; DCD, donation after cardiac death; DBD, donation after brain death.

Outcomes

The mean follow-up in the standard group was significantly longer than in the small group (89.5 ± 62.55 vs. 51.92 ± 35.41 months; $p = 0.04$). No difference in intraoperative EBL was observed ($p = 0.8$). CIT was also similar between the standard and the small group, 13.8 ± 5.43 and 12.2 ± 5.7 h respectively ($p = 0.36$). The rate of reoperation within the first 30 days post-transplant was significantly higher in the group with DBW≤10 kg (6.25% vs. 23%; $p = 0.03$). Six (85%) out of 7 patients in the small group had a perinephric hematoma which required evacuation and additional hemostasis. No vascular thrombosis was observed in the standard group, while 1 out of 30 patients (3.3%) had arterial thrombosis in the small group. The thrombosis happened on POD 1 and led to graft loss. The rate of urological complications was not significantly different between the groups (standard vs. small, 6.25% vs. 67%; $p = 0.98$). Two patients in the small group had humoral rejection. Overall, 3 patients in the cohort experienced humoral

TABLE 4 | Outcomes and complications stratified by DBW.

	Standard group (*n* = 16)	Small group (*n* = 30)	Total (*n* = 46)	*p*-value
Cold ischemia time, (hours)	13.8 ± 5.43	12.2 ± 5.7	12.76 ± 5.65	0.36
Estimated blood loss, (ml)	136.56 ± 99.11	129.73 ± 93.10	131.63 ± 94.2	0.8
Follow-up period, (months)	89.5 ± 62.55	51.92 ± 35.41	64.99 ± 49.4	0.04
Reoperation, n (%)	1 (6.25%)	7 (23.3%)	8 (17.4%)	0.03
Urinary complications, n (%)	1 (6.25%)	2 (6.67%)	3 (6.5%)	0.98
Thrombosis rate, n (%)	—	1 (3.3%)	1 (2.1%)	NA
Rejection rate, n (%)	2 (12.5%)	3 (10%)	5 (10.9%)	0.58
Delayed graft function, n (%)	—	1 (3.3%)	1 (2.1%)	NA
Graft loss	—	3	3	0.58
Death with functioning graft	1	2	3	0.9
Patient death	1	3	4	0.41
Creatinine, (mg/dl)				
• 6 months	1.0 ± 0.23	1.45 ± 1.31	1.29 ± 1.08	0.09
• 1 year	0.94 ± 0.26	1.02 ± 0.35	0.99 ± 0.32	0.38
• 3 years	1.29 ± 1.66	1.60 ± 2.57	1.49 ± 2.26	0.69
• 5 years	1.26 ± 1.19	0.9 ± 0.36	1.05 ± 0.81	0.43
eGFR, (ml/min/1.73m^2)				
• 6 months	81.07 ± 17.81	71.17 ± 30.45	74.54 ± 27.01	0.18
• 1 year	88.64 ± 22.23	88.16 ± 27.2	88.32 ± 25.35	0.95
• 3 years	93.68 ± 38.15	77.37 ± 35.97	83.19 ± 36.92	0.28
• 5 years	79.91 ± 30.63	93.86 ± 41.26	87.66 ± 36.25	0.42

n, number of cases; eGFR, estimated glomerular filtration rate.

rejection, and all cases were confirmed by biopsy and successfully treated with PLEX and IVIG. Additionally, two patients from the standard group had AMR, one of them experienced graft loss and was retransplanted. Only one (3.3%) episode of DGF was observed in the cohort, and the patient received the organ from a donor with BW≤10 kg. He recovered normal graft function after additional hemodialysis. All the outcomes and complications can be seen in **Table 4**.

We did not observe any statistically significant differences in the graft function between the groups at 6-month, 1-, 3-, 5-year of follow-up (**Figures 3**, **4**). Mean serum creatinine and eGFR levels in the standard group after 5 years post-transplant were 1.26 ± 1.19 mg/dl and 79.91 ± 30.63 ml/min/1.73 m^2 respectively, and 0.9 ± 0.36 mg/dl and 93.86 ± 41.46 ml/min/1.73 m^2 in the small group. Detailed graft function is presented in **Table 4**.

Survival

Patient survival after 5 years was comparable among the groups (standard vs. small, 100% vs. 96.7%; $p = 0.48$), with median follow-up of 64.9 months (Range: 1–221) (**Figure 5**). Similar findings were observed in 5-year graft survival (standard vs. small, 100% vs. 90%; $p = 0.09$) (**Figure 6**). One graft was lost due to arterial thrombosis on POD1, one due to humoral rejection 32 months post-transplant in the setting of non-compliance, and the third one 11 years post-transplant. Three patient deaths were registered in the small group during 5-year follow-up; 2 of them occurred with functioning graft due to severe COVID-19 infection, and one patient had a myocardial infarction. The only deceased patient in the standard group passed due to COVID-19 infection. Three patients, all from the standard group, were lost in follow-up after 5, 4, and 4 years, respectively.

DISCUSSION

This study evaluates the outcomes of 46 pediatric *en bloc* kidney transplants using grafts from donors who weighed either greater or less than 10 kg. The primary outcome of this study is that renal function, graft and patient survival from donors with BW less than 10 kg are similar to such who received a pediatric transplant from donors with BW greater than 10 kg. We report excellent overall patient and graft survival rates for the cohort that included almost two-thirds of patients who received a kidney graft from extra small donors.

Recent publications have reported comparable graft survival between *en bloc* kidney transplant and both living and deceased donor adult kidney transplant (14, 13, 12). Suneja et al showed that the use of pediatric deceased donor kidneys has increased over the last few years but is still relative rare, especially from donors weighting <20 kg (13). Although it is a good source to expand the donor pool, almost 10% of kidneys from donors with BW≤20 kg are discarded (, 9, 14). A potential reason for that might be an extra degree of technical difficulties comparing to the grafts from adult donors, such as benching preparation of the organ or cystoureterostomy, so not every transplant center is comfortable with such procedures. As is reflected in our cohort, centers that do perform this procedure typically accumulate grafts from small donors from the different areas around them; almost 15% of the organs from this study were procured outside of the region and 25% outside of the state.

The largest number of EBK cases was reported by a group from China (6). Peng et al described 285 EBKs from 2015 to 2019 and showed how DBW affects the outcomes *via* a DBW<5 kg threshold. The authors demonstrated benefits

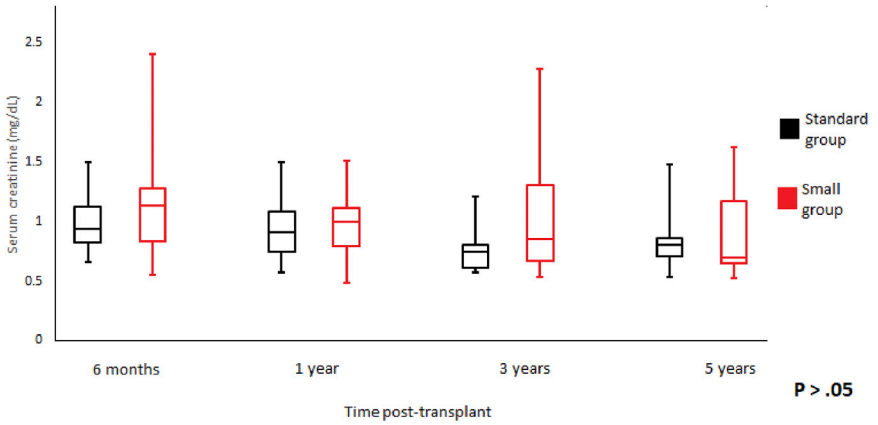


FIGURE 3 | Serum creatinine trend according to donor weight during the study period. Mean serum creatinine and standard error of mean over scheduled time points. $p > 0.05$ at all time periods.

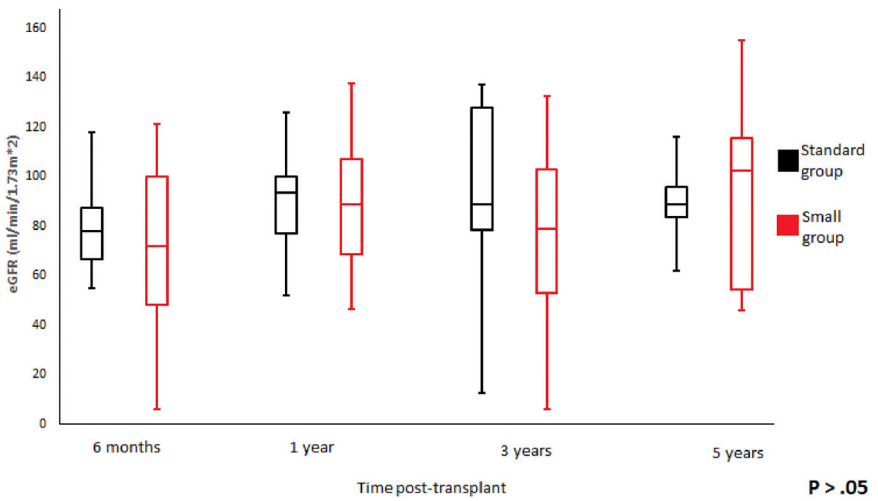


FIGURE 4 | Glomerular filtration rate trend according to donor weight during the study period. Mean glomerular filtration rate and standard error of mean over scheduled time points. $p > 0.05$ at all time periods.

for graft survival with increasing DBW by comparing groups with DBW<5 kg vs. 5 kg<DBW<20 kg vs. DBW>20 kg (71.4% vs. 89.5% vs. 97.3%; $p < 0.05$). No difference in patient survival, rates of thrombosis, urological complications, and acute rejection. That is the only study to our knowledge that analysed this extra-small group of DBW<5 kg.

Study published by Mitrou et al. was similar to ours by design. It described 28 *en bloc* kidney transplants, including 11 cases with DWB<10 kg and with an overall graft and patient survival rate of 81.8% and 100% respectively, among this group (9).

In our institution we do not apply any exclusion criteria for recipients of *en bloc* kidney transplant. However, we try to allocate *en bloc* grafts to patients smaller than 80 kg regardless of BMI.

We are reporting only 1 (2.1%) graft thrombosis in this study. This rate is comparable with the rate mentioned by Bakir et al in an adult single kidney transplant series (16). The patient received the graft from a donor with a body weight of 4.99 kg. On POD1 he was reoperated due to decreased urine output and absence of any flow in the graft on Doppler US. Complete arterial and venous thrombosis of the graft vessels was founded, and graftectomy was performed. The patient was then successfully retransplanted.

In terms of surgical complications, we believe that it is important to highlight that we did not observe any significant difference in urinary tract complications between the two groups. Only two patients out of 46 were reoperated on POD5 and POD6 due to urinary leakage from one of the two reimplanted ureters. Additionally, one patient had postoperative stricture of the reimplanted ureter, which complicated with hydronephrosis and multiple UTIs. The overall rate of urinary complications in the cohort was 7.8%. This is on the low side of the range from recently published literature, which varies from 2.5 to 21% (15).

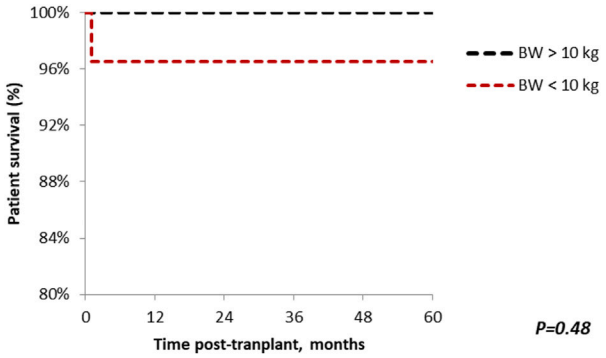


FIGURE 5 | The Kaplan-Meier patient survival plot for *en bloc* kidney transplant patients. Patient survival in the standard and small groups at 1, 3, 5 years are 100% and 96.7% respectively. $p > 0.05$ was estimated using log-rank test.

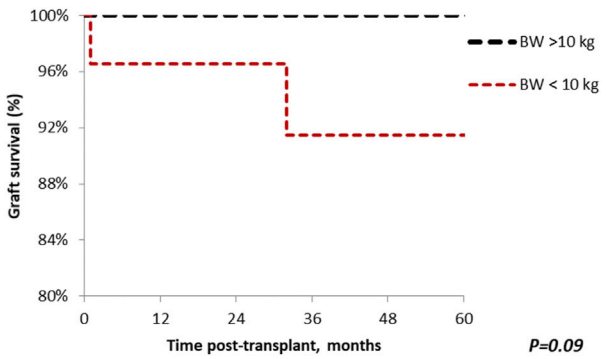


FIGURE 6 | The Kaplan-Meier graft survival plot for *en bloc* kidney transplant patients. Graft survival in the standard group at 1, 3 and 5 years is 100%; the small group 96.7%, 90% and 90%. $p > 0.05$ was estimated using log-rank test.

Fananapazir et al, in a cohort of 225 EBKs, showed that DBW<10 kg is a significant risk factor for such complications (total $n = 22$ (9.8%); 12% vs. 2% for EBK donors <10 vs. ≥ 10 kg; $p = 0.031$). Stricture of the ureter was the most common complication (55%), followed by urinary leak (41%). But in 50% of cases these can be managed nonoperatively, and they do not affect graft and patient survival (17). In our series we did not perceive any difference, possibly due to a small number of cases. We prefer to perform two separate ureter anastomoses. Alternative techniques, with the utilization of the bladder cuff, have been described (18). However, since the vascular supply of the bladder patch cannot be properly assessed (with a higher risk of ischemia in male donors), we deem it safer to perform two separate anastomoses with partially shortened ureters. The final position of the graft, flipped 180°, allows for easier access to the pelvis in the case of urological complications.

Overall reoperation rate in the first 30 days post-transplant was significantly higher for patients in the small group. Besides when the graft was removed due to arterial thrombosis, six patients needed additional hemostasis and evacuation of a perinephric haematoma (without renewal of the vascular anastomosis). All of them received the graft from donors with BW <10 kg. One patient had multiple reoperations in the early post-transplant period (POD1—relaparotomy, evacuation of perinephric hematoma, POD6—reformation of the cystoureterostomy, POD15—enterolysis, and small bowel resection due to SBO). Despite the complicated early post-transplant period, after more than 5 years of follow-up the patient has maintained stable graft function. We explain the higher rate of perinephric hematomas in the small group by additional technical difficulty of performing the "ideal" benching of the organ: the submillimeter size of the lumbar branches, either venous or arterial, sometimes makes the recognition and ligation particularly challenging and increases the risk of post operative hematoma. All hematoma washouts happened within the first 2 days post-transplant.

In our cohort we had 5 DCD donors, three in the standard group (18.75%) and two from donors with BW<10 kg (6%). In these 5 cases, we are reporting 100% 5-year death-censored graft survival. Due to a limited number of this type of patients, we believe, that it is impossible to make any significant conclusions regarding the safe use of kidney from DCD donor with extra small body weight from our series. However, in previous literature, Troppmann at el demonstrated that DCD status impacts early post-transplant graft function but does not appear to impact added risk graft loss and long-term kidney function (10). Analysing 120 EBKs (65 DBD vs. 65 DCD) from donors with BW<10 kg they showed a higher, but not statistically significant, rate of DGF (25% vs. 14%), urological complications (15% vs. 12%), and graft loss (23% vs. 11%) in DCD group. DCD vs. DBD 5-year graft and patient survival were 87% vs. 91% and 90% vs. 97% respectively.

The results of our study should be interpreted after an acknowledgement of its limitations. The main limitation is the relatively small cohort size, yet this is one of the largest series of EBKs from donors with BW < 10 kg. In our knowledge, there are only two similar publications with bigger cohorts, both were mentioned previously (, 13, 6). However, there are also multiple studies in the literature with a smaller number of patients (21, 20, 19). With constantly improving surgical technique and post-transplant management, the lowest limit of DBW for kidney transplantation is not yet clear. Therefore, to maximize utilization and avoid discarding organs, we think that further investigation in a multicenter study on a larger cohort scale is necessary.

CONCLUSION

To summarize, graft and patient survival rates after *en bloc* kidney transplantation from donors with BW<10 kg are not different from heavier donors. Renal function is unaffected by differences in DBW. The DBW<10 kg group is at an increased risk for surgical complications in early post-transplant period.

This study provides evidence that kidney transplant from donors with BW less than 10 kg, with experience, is a potentially important method for expanding the pool of kidney donors.

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 University of Illinois At Chicago Internal Review Board. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MS and EP participated in study design, statistical analysis, and writing. HP participated in statistical analysis. PC, JA-A, AF, IT, and EB participated in critical review and study design.

CONFLICT OF INTEREST

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

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ORIGINAL RESEARCH
published: 24 October 2022
doi: 10.3389/ti.2022.10839

Tacrolimus Monotherapy is Safe in Immunologically Low-Risk Kidney Transplant Recipients: A Randomized-Controlled Pilot Study

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In this randomized-controlled pilot study, the feasibility and safety of tacrolimus monotherapy in immunologically low-risk kidney transplant recipients was evaluated [NTR4824, www.trialregister.nl]. Low immunological risk was defined as maximal 3 HLA mismatches and the absence of panel reactive antibodies. Six months after transplantation, recipients were randomized if eGFR >30 ml/min, proteinuria <50 mg protein/mmol creatinine, no biopsy-proven rejection after 3 months, and no lymphocyte depleting therapy given. Recipients were randomized to tacrolimus/mycophenolate mofetil (TAC/MMF) or to taper and discontinue MMF at month 9 (TACmono). 79 of the 121 recipients were randomized to either TACmono (n = 38) or TAC/MMF (n = 41). Mean recipient age was 59 years and 59% received a living donor transplant. The median follow-up was 62 months. After randomization, 3 TACmono and 4 TAC/MMF recipients experienced a biopsy-proven rejection. At 5 years follow-up, patient survival was 84% in TACmono versus 76% in TAC/MMF with death-censored graft survival of 97% for both groups and no differences in eGFR and proteinuria. Eleven TACmono recipients had an infectious episode versus 22 TAC/MMF recipients (p < 0.03). Donor-specific anti-HLA antibodies were not detected during follow-up in both groups. Tacrolimus monotherapy in selected immunologically low-risk kidney transplant recipients appears safe and reduces the number of infections.

Keywords: kidney transplantation, infection, rejection, tacrolimus, immunosuppression reduction, mycophenolate mofetil

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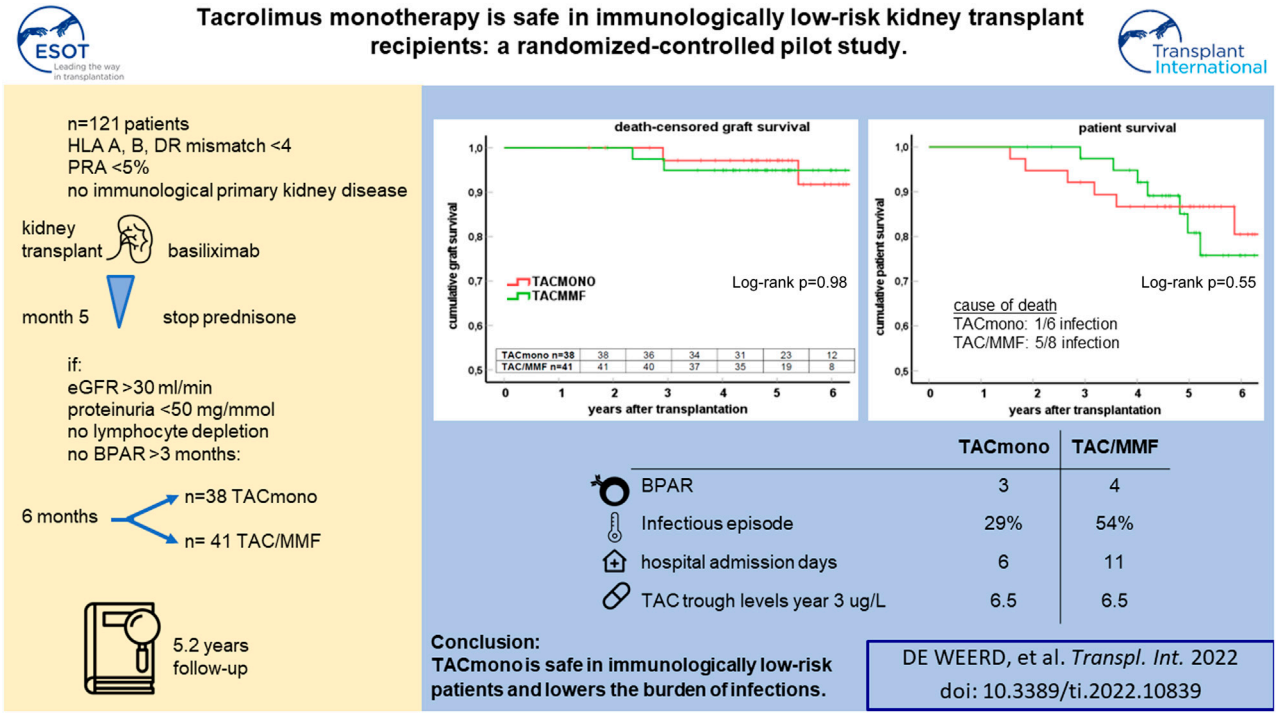
Received: *16 August 2022*
Accepted: *12 October 2022*
Published: *24 October 2022*

Citation:
de Weerd AE, Fatly ZA, Boer-Verschragen M, Kal-van Gestel JA, Roelen DL, Dieterich M and Betjes MGH (2022) Tacrolimus Monotherapy is Safe in Immunologically Low-Risk Kidney Transplant Recipients: A Randomized-Controlled Pilot Study. Transpl Int 35:10839. doi: 10.3389/ti.2022.10839

Abbreviations: BPAR, biopsy-proven acute rejection; CDC, complement-dependent cytotoxicity; CNI, calcineurin inhibitors; DSA, donor-specific antibodies; DSMB, data safety monitoring board; eGFR, estimated glomerular filtration rate; FACS, flowcytometry; HLA, human leucocyte antigen; IL-2RAb, interleukin-2-receptor antibody; IQR, interquartile range; MPGN, membranoproliferative glomerulonephritis; MMF, mycophenolate mofetil; PCR, polymerase-chain reaction; PRA, panel-reactive antibodies; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TAC, tacrolimus; TACmono, tacrolimus monotherapy.



GRAPHICAL ABSTRACT |

INTRODUCTION

Kidney transplant recipients use life-long immunosuppression to prevent rejection and subsequent allograft loss. The only exceptions to this maintenance are recipients of a monozygotic twin donor kidney and the very rare recipients who demonstrate operational tolerance after discontinuation of immunosuppression. The most widely used combination of immune suppressive drugs consists of tacrolimus combined with mycophenolate mofetil in over 90% of recipients (1). As the risk for rejection is the highest in the first months after transplantation, induction therapy is administered at transplantation with a T-cell depleting agent in over 60% of recipients (1). Triple immunosuppression with steroids is used in the vast majority of recipients and over 60% use steroids after 1 year (2). Well-known side effects of immunosuppressive drugs are infection, malignancy and cardiovascular disease. The current SARS-CoV-2 pandemic for example has demonstrated that immunosuppression intensity puts solid organ transplant recipients at high risk of unfavorable outcomes (3). Immunosuppression, and specifically the use of mycophenolate mofetil results in worse vaccination responses (4, 5). Risk factors for rejection such as HLA-immunization and HLA mismatch can guide the choice for the initial immunosuppressive regimen with or without T cell depleting induction therapy. The risk for acute rejection declines rapidly after the first months after transplantation which follows the decrease in frequency of donor-specific alloreactive T cells (6). In accordance, most post-transplantation immune suppression protocols allow for a gradual stepdown in dose or number of immune suppressive drugs but there is currently a lack of reliable markers to guide weaning of immunosuppression.

Calcineurin inhibitors are the cornerstone of immunosuppressive regimens, and in previous weaning trials, discontinuation of tacrolimus led to a higher percentage of biopsy-proven acute rejections (BPAR) (7). Tacrolimus monotherapy has resulted in good outcomes when combined with depleting induction therapy (8). However, tacrolimus monotherapy after interleukin-2 receptor antibody induction in immunologically low-risk kidney transplant recipients (based on the frequency of interferon-γ expressing donor-specific alloreactive T cells, as described by Bestard et al.) increased acute rejection rates as compared to standard of care triple immunosuppression (9).

Based on these studies, it appears that tacrolimus monotherapy without T cell depletion early after kidney transplantation and tacrolimus withdrawal late after transplantation leads to a higher rejection incidence, even in immunologically low-risk patients. However, in older steroid withdrawal studies there is experience in tacrolimus monotherapy after non-depleting induction, demonstrating excellent graft outcomes despite a higher early rejection rate in the Atlas study (10). Tacrolimus monotherapy initiated at a later point in time after transplantation and without a prior severe

rejection in the early post-transplantation period, may therefore still be an option and could reduce the incidence of adverse events in the long-term.

In this pilot study, lowering to tacrolimus monotherapy after non-depleting induction therapy was initiated 6 months after transplantation in immunologically low risk kidney transplant recipients, who were included at time of transplantation. The aim of this pilot study is to investigate the feasibility of a non-inferiority trial to determine the safety of tacrolimus monotherapy in immunologically low-risk kidney transplant recipients. Safety in terms of rejection, graft survival and donor-specific anti-HLA antibody (DSA) formation was assessed.

METHODS

Study Design and Patients

We performed a randomized controlled, investigator-driven, open-label, single center pilot study from August 2014 till April 2018. Follow-up for data analysis was until March 2022. All recipients scheduled to receive either a deceased donor or a living donor kidney were screened for eligibility. Inclusion criteria were age 18 years and older, peak panel reactive antibodies (PRA) of <5% and HLA mismatches with the donor of ≤3. Re-transplantation was allowed when meeting these before mentioned inclusion criteria. Exclusion criteria were HLA-identical living-related transplantation, the presence of an immunological-mediated disease requiring (additional) immunosuppression, ABO-blood group incompatibility, a complement dependent cytotoxicity (CDC) or flowcytometry (FACS) positive cross-match, a combined liver/kidney or pancreas/kidney transplantation, the participation in another clinical trial and females of childbearing potential unwilling to use effective means of contraception. All recipients provided written informed consent before entry of the study during admission for kidney transplantation. This study is approved by the Medical Ethical Committee of the Erasmus Medical Center, conducted according to the Declaration of Helsinki and Declaration of Istanbul and registered in the Netherlands Trial Register [NTR4824, www.trialregister.nl].

Randomization and Study Medication

All recipients were treated with the interleukin-2-receptor antibody (IL-2RAb) basiliximab, steroids, tacrolimus (TAC) and mycophenolate mofetil (MMF). Prednisolone 20 mg daily was tapered and discontinued at month 5 post-transplantation, target trough levels were for TAC 5-8 ug/L (once daily formulation Advagraf^{R}) and MMF 1.5–3 mg/L from 3 months onwards in accordance with the standard protocol in our clinic. Recipients were included during admission. After a run-in period of 6 months they were randomized in a 1:1 ratio to either continue TAC and MMF (standard arm) or to halve their MMF dose at month 6 and discontinue MMF at month 9 while targeting for the same trough TAC levels (intervention arm). Randomization was carried out by an independent researcher with random allocation cards using computer-generated random numbers. Randomization criteria were eGFR >30 ml/min/1.73 m^2 (CKD-EPI formula), proteinuria <0.5 mg protein/mmol creatinine in spot urine, freedom of biopsy-proven acute rejection (BPAR) from month three till six and the absence of lymphocyte depleting anti-rejection therapy. The full inclusion, exclusion and randomization criteria of this pilot study are described in **Supplementary Table S1**. **Supplemental Figure S1** depicts the immunosuppressive regimens and trough levels.

Study Objectives

The aim of this pilot study is to investigate the feasibility of a non-inferiority trial to determine the safety of tacrolimus monotherapy in immunologically low-risk kidney transplant recipients.

The feasibility objectives of this pilot study are:

1. Methodology: biopsy-proven acute rejection (BPAR)-rate
2. Process: willingness to participate in weaning study
3. Biological plausibility: (surrogate) parameters to assess treatment effect of less intensive immunosuppression:
   a. Biological plausibility of the benefit of discontinuing mycophenolate mofetil: hospital admission and infections.
   b. Secondary safety objectives: patient survival, death-censored graft survival, kidney allograft function and proteinuria.

No superiority or non-inferiority assessments are performed in this pilot study. We performed a post-hoc analysis on the number of symptomatic SARS-CoV-2 infections before the SARS-CoV-2 vaccination campaign till April 2021. This report analyzes these objectives, while detailed vaccination responses will be analyzed in a separate report [NL4824, www.trialregister.nl].

Outcomes

BPAR was scored using the Banff-classification biopsies on *for cause* renal biopsies (11). Kidney function was measured with the CKD-EPI formula. Hospital admissions were defined as total number of (overnight) admissions in the transplant center till month 15 and in the referring hospitals thereafter. Total number of admitted days was also recorded. Infectious burden was defined as the sum of antibiotic use and CMV replication. Antibiotic use for at least three consecutive days was systematically recorded between month 6 and 15 (thereafter, due to referral to different hospitals, documentation of antibiotic use was more error prone). Serum CMV replication was measured by indication and 1 year after transplantation with polymerase-chain reaction (PCR). HLA antibodies were measured both 15 months and 4 years after transplantation with Luminex screening assay (Thermo Fisher Scientific, Waltham, MA). When present, HLA antibodies were further characterized with the Luminex single-antigen bead assay (12). SARS-CoV-2 infections were documented before April 2021 when the SARS-CoV-2 vaccination campaign commenced. A SARS-CoV-2 infection was scored when recipients were admitted to the hospital with positive polymerase chain reaction SARS-CoV-2 swab.

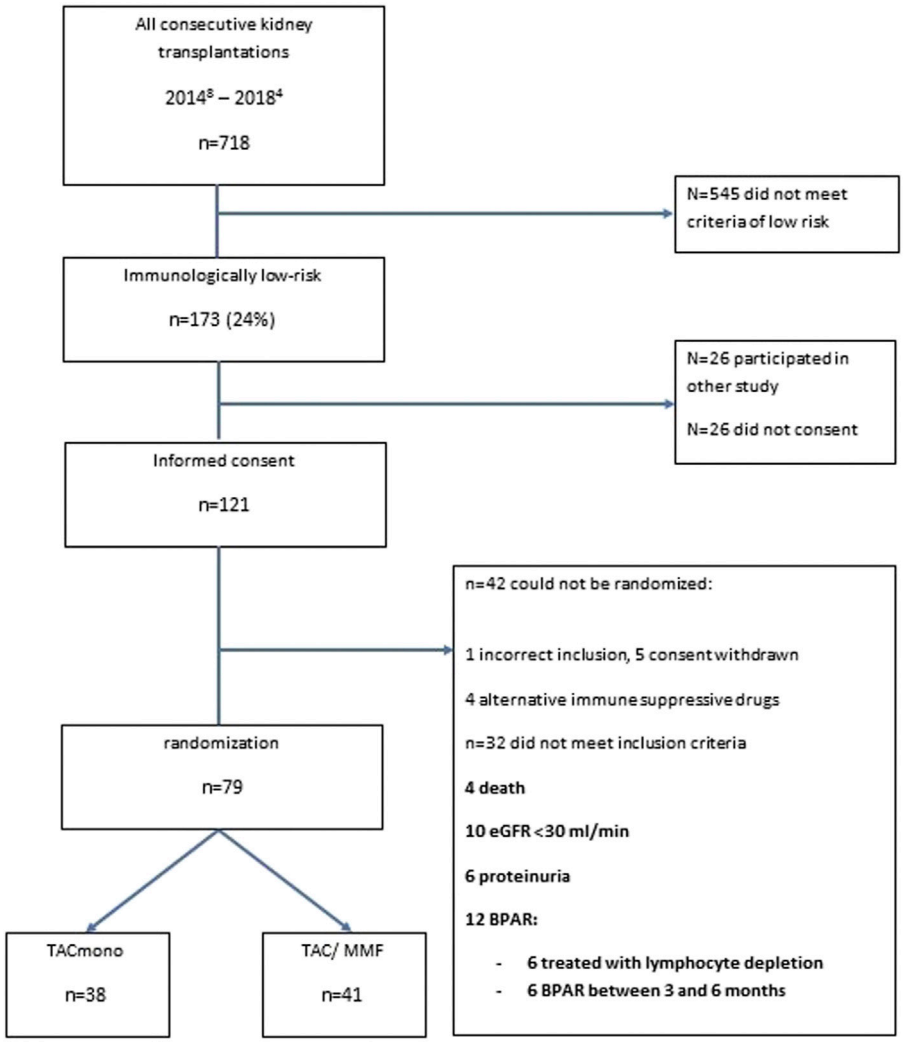


FIGURE 1 | Flowchart of the selection of recipients included in the study.

Statistical Analysis

The sample size for this pilot study was calculated for the expected recruitment rate, one of the feasibility objectives. It was estimated that randomization of 80 patients would allow for a reasonable estimate of safety. Based on our historical data, it was expected that one third of recipients would not meet randomization criteria at month six because of rejection, low eGFR or proteinuria. It was estimated that if 120 patients gave consent out of 171 eligible patients, we could be 95% sure that the true consent rate will be between 63 and 77% (95% confidence interval of one proportion). The rough estimate therefore was that consent of at least 120 patients could determine the feasibility of the recruitment process and to allow for randomization of 80 patients. A planned interim analysis was performed after 40 patients had completed follow-up. The Data Safety Monitoring Board (DSMB) could advise to terminate the study if less than 15 recipients were included per year and when a difference in BPAR rate was observed between the treatment arms ("a sound clinical judgement that continuation of the study will harm recipients"). All patients who were randomly allocated to treatment were included in the analysis (intention-to-treat principle). Baseline characteristics were described according to distribution and type of data. We presented frequencies and proportions for categorical variables, means for normally distributed continuous variables and medians for continuous variables with a skewed distribution. Patient and graft survival was analyzed with the Kaplan-Meier log-rank test using SPSS version 21. Kidney function and proteinuria were analyzed with the Mann-Whitney-U test for differences between groups.

RESULTS

Baseline Characteristics of Randomized Recipients

Between August 2014 and April 2018, 718 adult kidney transplantations were performed. 170 (24%) of these

TABLE 1 | Baseline characteristics of kidney transplant recipients 6 months after transplantation, randomized to either tacrolimus monotherapy or dual tacrolimus and mycophenolate mofetil.

	TACmono *n* = 38	TAC/MMF *n* = 41
Age recipient (range)	59.6 (37–71)	59.0 (29–80)
Male (%)	76	71
Kidney disease (n)		
Diabetic nephropathy	11	11
Hypertension	7	10
ADPKD	4	4
Other	16	16
Age donor (SEM)	48.5 (2.3)	48.8 (2.7)
Total HLA mismatches (SEM)	2.1 (0.15)	2.4 (0.15)
PeakPRA (SEM)	2.4 (0.35)	3.4 (0.85)
Retransplantion (n)	1	1
Pre-emptive (%)	37	34
eGFR (CKD-EPI, ml/min/1.73 m^2) (IQR)	53.8 (44–69)	50.4 (43–61)
Proteinuria (spot urine g/mol)	15.4 (1.5)	19.3 (6.0)
TAC trough ug/L (mean)	7.5 (0.43)	7.2 (0.38)
MMF trough mg/L (mean)	2.0 (0.18)	2.0 (0.18)
BPAR within 3 months after transplantation	4	1

ADPKD, autosomal-dominant polycystic kidney disease; HLA, human leucocyte antigen; IQR, interquartile range; MMF, mycophenolate mofetil; PRA, panel-reactive antibodies; BPAR, biopsy-proven acute rejection; SEM, standard error of the mean; TACmono, tacrolimus monotherapy.

procedures were in recipients who met our inclusion criteria of immunologically low risk recipients: 30% of deceased donor recipients *versus* 21% of living donor recipients. 147 recipients were counseled for this study of whom 121 gave written informed consent (consent rate 82%, **Figure 1**). After the run-in period of 6 months, 79 recipients could be randomized. Of the 42 non-randomized recipients, 12 had experienced BPAR of whom six had been treated with lymphocyte depleting anti-rejection treatment. Four of the non-randomized patients had been changed to an alternative immunosuppressive regimen: methotrexate for arthritis, continuation of prednisone with TAC trough level of 3 ug/L for arteriolar hyalinosis, and in two patients azathioprine for MMF-induced diarrhea.

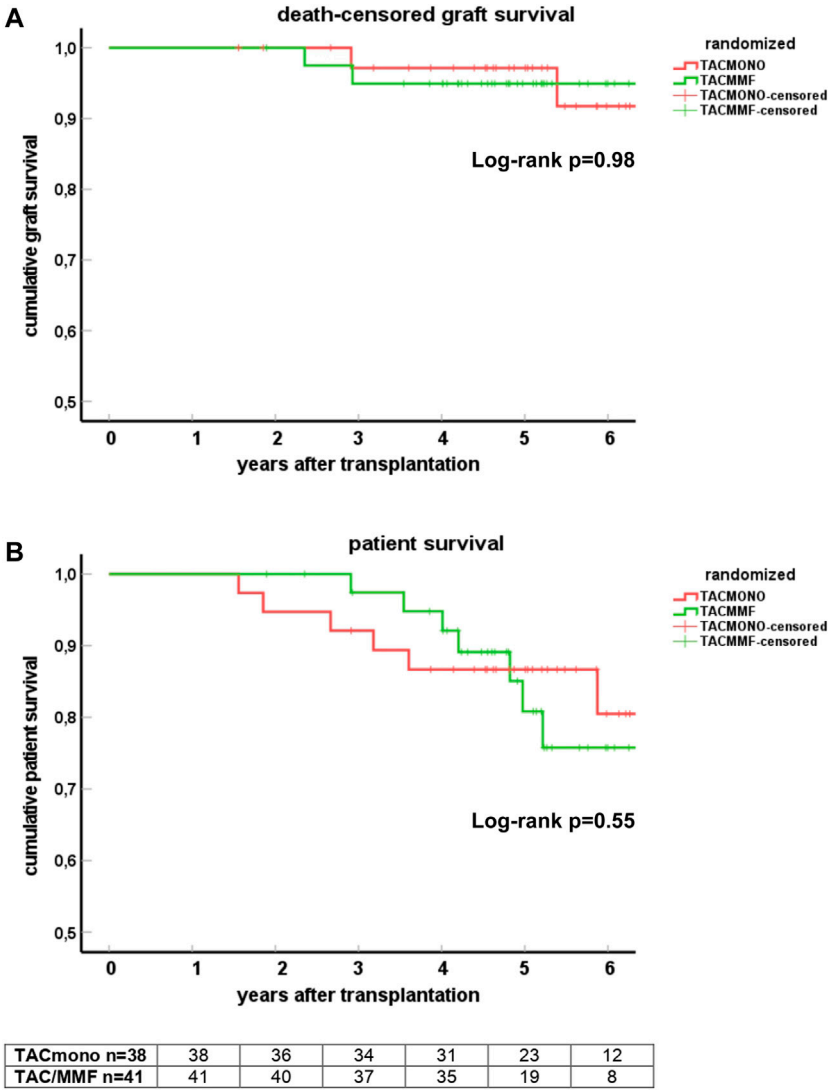
Of the 79 recipients in study, 41 were randomized to standard TAC/MMF and 38 were randomized to the intervention TACmono. Mean age was 59.3 and 37% of recipients were 65 years of age and older (**Table 1**). The majority were male (73%), 59% received a living donor transplant, 3% received a second kidney transplant and 35% were transplanted pre-emptively. Diabetic nephropathy was the cause of end-stage kidney disease in 28%, hypertension in 22% and autosomal dominant polycystic kidney disease (ADPKD) in 10%. As steroid-responsive rejection in the first 3 months was not an exclusion criterion, 5 randomized recipients had experienced prior rejection. Of note is that four of these recipients were randomized to TACmono. Mean kidney function at month 6 was 55 ml/min with 17 mg protein/mmol creatinine in the urine. Six months after transplantation, mean TAC and MMF trough levels were 7.3 ug/L and 2.0 mg/L respectively at randomization.

Patient and Graft Survival

At 5 years follow-up, patient survival was 86% in the TACmono group *versus* 76% in the TAC/MMF group with 97% death-censored graft survival for both groups (log-rank test patient survival $p = 0.55$ and death-censored graft survival $p = 0.98$, **Figure 2**). Six TACmono and eight TAC/MMF patients died (**Table 2**). Causes of death were infection in one (diabetic ulcers), malignancy in two (stomach respectively pulmonary carcinoma), cardiovascular in two (heart failure, sudden cardiac death) and "other" (dementia) in one TACmono recipient. In contrast, causes of death in TAC/MMF recipients were sepsis in five (pneumonia, SARS-CoV-2 infection, decompensated hepatitis B, septic shock, urosepsis), malignancy in one (pulmonary carcinoma), cardiovascular in one (sudden cardiac death) and "other" in one (liver cirrhosis). Graft failure occurred in two TACmono recipients: one recipient lost his graft 35 months after transplantation due to chronic prostatitis with biopsy signs of urinary tract infection and borderline rejection (after experiencing Banff IA rejection at month 11). The other TACmono recipient lost his allograft 65 months after transplantation due to membranoproliferative glomerulonephritis (MPGN), which was interpreted as a probable recurrence of a previously undiagnosed MPGN. Two TAC/MMF recipients restarted dialysis, one recipient with mixed Banff IIA/antibody-mediated rejection due to non-adherence at month 28, and one recipient with Banff IIA vascular rejection at month 35.

Rejection Episodes After Randomization

During follow-up, seven recipients had experienced BPAR (**Table 3**). Three TACmono recipients experienced acute cellular rejection Banff IA 8, 10 and 11 months after transplantation (all within 3 months after discontinuation of MMF). These rejections were reversible with pulse methylprednisolone and thereafter their initial MMF dosage was restarted. One of these rejecting TACmono recipients had recurrent prostatitis and lost his graft 35 months after transplantation with biopsy signs of urinary tract infection and borderline rejection. Four TAC/



TACmono n=38	38	36	34	31	23	12
TAC/MMF n=41	41	40	37	35	19	8

FIGURE 2 | Death-censored graft survival **(A)** and patient survival **(B)** in kidney transplant recipients treated with either tacrolimus monotherapy or with standard tacrolimus/mycophenolate mofetil. Survival is shown by Kaplan-Meier cumulative survival curves. The group of recipients randomized to tacrolimus monotherapy (TACmono) or standard tacrolimus with mycophenolate mofetil (TAC/MMF) are shown as separate curves.

TABLE 2 | Cause of death in kidney transplant recipients, randomized to either tacrolimus monotherapy or dual tacrolimus and mycophenolate mofetil.

	TACmono	**TAC/MMF**
Number of recipients	38	41
Follow-up in months after transplantation (median and range)	64 (19–90)	60 (23–87)
Deceased at follow-up	6	8
Time to death after transplantation (median and range)	35 (19–70)	54 (42–86)
Cause of death		
Infection	1	5
Malignancy	2	1
Cardiovascular	2	1
Other	1	1
Graft loss other than death	2	2

TACmono, tacrolimus monotherapy; MMF, mycophenolate mofetil.

TABLE 3 | Biopsy-proven acute rejections in kidney transplant recipients, randomized to either tacrolimus monotherapy or dual tacrolimus and mycophenolate mofetil.

	TACmono	TAC/MMF
n	38	41
follow-up in months (median, range)	64 (19–90)	60 (23–87)
BPAR	3	4
Type of rejection	Banff IA *n* = 0.3	Borderline rejection *n* = 1 Histology of c-aABMR without DSA *n* = 1 Banff IIA and mixed rejection without DSA *n* = 1 Banff IIA *n* = 1

TAC, tacrolimus; MMF, mycophenolate mofetil; MPS, methylprednisolone; AMR, antibody-mediated rejection; caABMR, chronic-active antibody-mediated rejection; IVIG, intravenous immunoglobulins.

MMF recipients experienced BPAR: one borderline cellular rejection at 9 months; one Banff IIA vascular rejection at 34 months; one histology of chronic-active antibody-mediated rejection, however without detectable DSA, after a CMV infection at 11 months; and one Banff IIA and mixed rejection (C4d positive, however without detectable DSA) due to non-adherence at 28 months after transplantation. Glomerulonephritis was diagnosed in two TACmono patients (with unknown primary kidney disease); membranoproliferative glomerulonephritis for which MMF was reinitiated and one IgA nephropathy with endocapillary proliferation which was treated with high dose steroids.

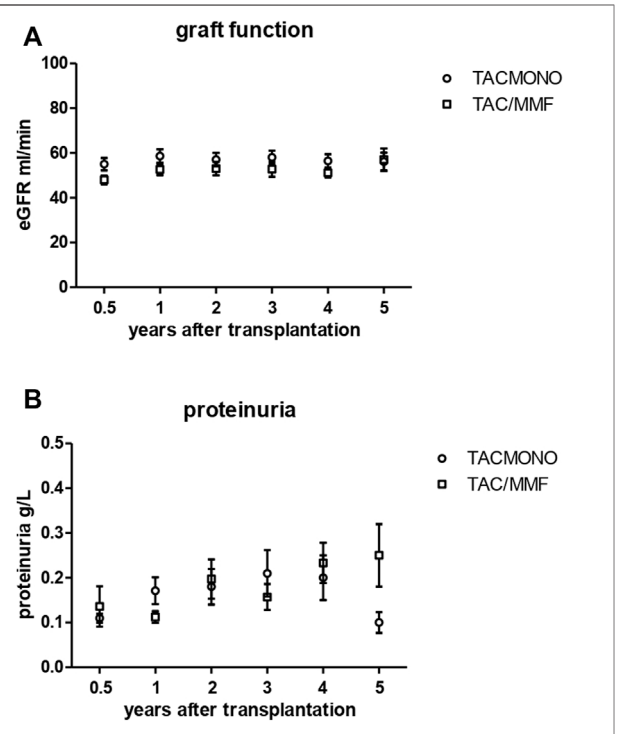


FIGURE 3 | Kidney function **(A)** and proteinuria **(B)** in kidney transplant recipients treated with either tacrolimus monotherapy or with standard tacrolimus/mycophenolate mofetil. The eGFR (CKD-EPI formula) and proteinuria (in spot urine) is shown in the years after transplantation. The group of recipients randomized to tacrolimus monotherapy (TACmono) or standard tacrolimus with mycophenolate mofetil (TAC/MMF) are shown as separate data points.

Kidney Function, Proteinuria, DSA and TAC Trough Levels

At 1, 3 and 5 years of follow-up, kidney function and proteinuria were comparable between TACmono and TAC/MMF (**Figure 3**): eGFR was 58 vs. 52 ml/min at month 6 (p = 0.16) and 59 vs. 58 ml/min at year 3 (p = 0.98) in TACmono vs. TAC/MMF. Proteinuria was 0.15 *versus* 0.19 g/L at month 6 (p = 0.55) and 0.10 *versus* 0.25 g/L at year 5 (p = 0.53) in the TACmono *versus* TAC/MMF group. DSA were not detectable at time of transplantation. 15 months after transplantation Luminex screening did not reveal HLA-antibodies in randomized recipients. Four years after transplantation in only one recipient HLA-antibodies were detectable, which were non-donor HLA directed (in one TAC/MMF recipient after experiencing rejection). Tacrolimus trough levels 1 year, 3 years and 5 years posttransplant were 6.3, 6.5, 6.4 ug/L in TACmono vs. 6.2, 6.5 and 6.2 ug/L in TAC/MMF (**Table 4**).

Infectious Burden and Hospital Admissions After Randomization

Eleven TACmono *versus* 22 TAC/MMF recipients experienced infectious burden defined as antibiotic use and viral replication (p = 0.03, **Table 4**). Between 6 and 15 months after transplantation, infections needing antibiotics were recorded 12 times in 9 TACmono *versus* 24 times in 14 TAC/MMF recipients. Two TACmono recipients had detectable but asymptomatic CMV viral replication in serum 1 year after kidney transplantation. Four TAC/MMF recipients had an episode of symptomatic CMV viremia 1 year after transplantation, of which two developed CMV disease. The median number of hospital admissions was 1 (IQR 0–3.25) in TACmono *versus* 2 (IQR 1–3) in TAC/MMF (p = 0.32), with a total number of admitted days of 6 (IQR 0–17) *versus* 11 (IQR 3.5–24.5) in the TACmono vs. TAC/MMF, respectively (p = 0.18). At the onset of the SARS-CoV-2 pandemic in 2020 and before the vaccination campaign, two TACmono recipients and four TAC/MMF recipients were admitted to the hospital because of SARS-CoV-2 infection. One TAC/MMF recipient died of SARS-CoV-2 infection.

TABLE 4 | Hospital admissions, antibiotic use and CMV replication in kidney transplant recipients, randomized to either tacrolimus monotherapy or dual tacrolimus and mycophenolate mofetil.

	TACmono	TAC/MMF	*p*-value
n	38	41	
follow-up in months (median, range)	64 (19–90)	60 (23–87)	0.25
Infectious burden	11 (29%)	22 (54%)	0.03
CMV viremia month 12	2 (5%)	4 (10%)	
Number of antibiotic treated recipients from month 6 till month 15	9 (24%)	14 (34%)	
Total of antibiotic courses	12	24	
Hospital admissions during follow-up			
Admissions (median, IQR)	1 (0–3.25)	2 (1,2,3)	0.32
Admitted days (median, IQR)	6 (9–17)	11 (3.5–24.5)	0.18
Tacrolimus trough levels (mean, SEM)			
month 6	7.5 (0.43)	7.2 (0.38)	0.63
year 1	6.3 (0.24)	6.2 (0.25)	0.72
year 2	6.7 (0.26)	6.4 (0.23)	0.76
year 3	6.5 (0.40)	6.5 (0.22)	0.10
year 4	6.4 (0.33)	6.8 (0.25)	0.56
year 5	6.4 (0.29)	6.2 (0.38)	0.41

IQR, interquartile range; SEM, standard error of the mean.

DISCUSSION

In this randomized controlled pilot study, tacrolimus monotherapy without mycophenolate mofetil appeared safe in immunological low-risk kidney transplant recipients and was associated with a lower rate of infections.

Rejection episodes were not increased in the tacrolimus monotherapy group. The few BPARS that occurred after randomization were easily reversible after steroid treatment. As one quarter of all consecutive kidney transplant recipients in our center met the immunological low-risk criteria as defined in this study, the identification of kidney transplant recipients who could benefit from less immunosuppression is relevant for a substantial portion of kidney transplant recipients. As our center has a large living donor program (65%) and a substantial number of immunized retransplant candidates, this portion of low risk recipients could likely be over one third of transplant recipients in other centers.

The introduction of calcineurin inhibitors (CNI) cyclosporin and tacrolimus in the standard immune suppressive regimen has dramatically reduced rejection incidence and subsequent increase graft survival in the short term (13). The potential nephrotoxicity of CNI has led to a number of studies aiming to lower or discontinue CNI in either recipients with a low immunological risk and/or long after transplantation when direct alloreactive T cell responses have declined. However, previous attempts to minimize calcineurin inhibitors early following kidney transplantations have shown discouraging high rejection rates in trials (7). Attempts to postpone weaning of CNI, after at least 4 years posttransplant, were also terminated prematurely: all five consecutive stable recipients who discontinued tacrolimus with or without steroids in Dugast et al. experienced either rejection or developed anti-HLA antibodies (14). Of note, the rationale for a CNI-free immune suppressive regimen in these studies was the potential nephrotoxicity of CNI. This side-effect was believed to be the most important cause for a relative lack in improvement of long-term graft patency observed after introduction of CNI. Most recent studies however, have pointed to chronic humoral rejection as the major cause for long-term graft failure and stress the importance of adequate trough levels and low intrapatient variability (15–19). Tacrolimus with trough levels above 5.0 ug/L have indeed been associated with improved graft survival (20, 21). A different strategy to minimize immunosuppression is therefore to maintain tacrolimus as the cornerstone of modern post kidney transplant immunosuppression, and to wean both steroids as well as mycophenolate mofetil. The recent CELLIMIN trial treated pre-transplant donor-specific IFN-γ T-cell ELISPOT (and DSA) negative recipients with tacrolimus monotherapy, but found high rejection rates comparable to ELISPOT-positive recipients treated with standard of care immunosuppression (9). Indeed, tacrolimus monotherapy without an increase in rejection incidence has only been achieved after depleting induction therapy (8, 10, 22). Despite higher early rejection rates in the Atlas study, basiliximab with tacrolimus monotherapy led to excellent graft outcomes (10).

The rationale behind the design of the current pilot study has taken these different observations into account and aimed for tacrolimus monotherapy in recipients with an *a priori* low risk for rejection and at a later point in time after transplantation. For extra safety, when immunological low risk recipients did have a severe rejection in the first 3 months or a late rejection after 3 months, poor graft function or proteinuria, they were not randomized. The results show that such an approach, pre-transplant immunological criteria combined with the clinical course in the first 6 months, identified recipients in whom weaning to tacrolimus monotherapy gave excellent outcomes. In addition, with an average tacrolimus trough level of 6 ng/L the graft function remained stable, no DSA developed and adherence to the once daily immunosuppressive regimen in the TACmono group was significantly better than in the TAC/MMF group (23). Another benefit TACmono recipients experienced, was

improvement in diarrhea complaints after discontinuing MMF, as assessed with standardized questionnaires on gastro-intestinal symptoms (24).

The benefits of TACmono in terms of less adverse events is of course difficult to quantify in a pilot study. In our relatively small cohort, significantly less infections were noted and there was a trend towards less antibiotic use, and a trend towards less and shorter hospital admissions in recipients without MMF. Also, 5 out of 8 deceased TAC/MMF recipients died of an infectious cause of death, *versus* only 1 out of 6 deceased TACmono recipients. This pilot study however is not designed to dissect random errors from causality in graft survival differences and weaning immunosuppression. The goal of this pilot was to assess the feasibility of a larger weaning trial: the assuring comparable rejection rates in both groups, the willingness of recipients to lower their immunosuppression and the lower infection rates indicate that such a trial is worthwhile conducting. A strong indication for the benefit of tailored weaning was demonstrated by the severely hampered vaccination responses in TAC/MMF recipients in our cohort: in a substudy on SARS-CoV-2 vaccination only 7% of TACmono recipients were non-responders *versus* 38% non-responders in TAC/MMF (25).

There are a number of limitations of this study, apart from the obvious relative small cohort size as described above. The recipient age was 59 years and over one third of recipients was older than 65 years of age. This was a consequence of the exclusion of immunized recipients or those with an underlying immunological kidney disease. Elderly recipients age is associated with less rejection (26–29) and increased vulnerability for infections and these recipients may benefit specifically from minimized immune suppression. Whether the results can be generalized to younger recipients cannot be inferred from the current study.

This pilot approach with traditional immunological criteria as HLA matching and antibody screening can be implemented relatively easily. It is not known whether more granular immunological information such as HLA eplet matching can be more precise for identifying patients in whom tacrolimus monotherapy is safe.

To conclude, tacrolimus monotherapy from 9 months after transplantation appears safe in selected recipients with a proven low risk of acute rejection and is associated with a reduced risk of infection.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Medical Ethical Committee of the Erasmus Medical Center. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AdW participated in research design, writing of the paper, performed the research and participated in data analysis. ZF participated in writing of the paper and participated in data analysis. MB-V performed the research and participated in data analysis. JK participated in data analysis. DR participated in data analysis. MD participated in data analysis. MB participated in research design, writing of the paper, performed the research and participated in data analysis.

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

Simone Brand-Schaaf kindly provided the Luminex screening and single antigen bead data.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10839/full#supplementary-material

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ORIGINAL RESEARCH
published: 29 September 2022
doi: 10.3389/ti.2022.10656

Combined Donor-Recipient Obesity and the Risk of Graft Loss After Kidney Transplantation

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Background: As the prevalence of obesity increases globally, appreciating the effect of donor and recipient (DR) obesity on graft outcomes is of increasing importance.

Methods: In a cohort of adult, kidney transplant recipients (2000–2017) identified using the SRTR, we used Cox proportional hazards models to examine the association between DR obesity pairing (body mass index (BMI) >30 kg/m^2), and death-censored graft loss (DCGL) or all-cause graft loss, and logistic regression to examine risk of delayed graft function (DGF) and ≤30 days graft loss. We also explored the association of DR weight mismatch (>30 kg, 10-30 kg (D>R; D<R) and <10 kg (D = R)) with each outcome, stratifying by DR obesity pairing.

Results: Relative to non-obese DR, obese DR were highest risk for all outcomes (DCGL: HR 1.26, 95% CI 1.22–1.32; all-cause graft loss: HR 1.09, 95% CI 1.06–1.12; DGF: OR 1.98, 95% CI 1.89–2.08; early graft loss: OR 1.34, 95% CI 1.19–1.51). Donor obesity modified the risk of recipient obesity and DCGL [p = 0.001] and all-cause graft loss [p < 0.001] but not DGF or early graft loss. The known association of DR weight mismatch with DCGL was attenuated when either the donor or recipient was obese.

Conclusion: DR obesity status impacts early and late post-transplant outcomes.

Keywords: graft loss, weight mismatch, obesity, kidney transplant outcomes, body mass index, obesity pairing

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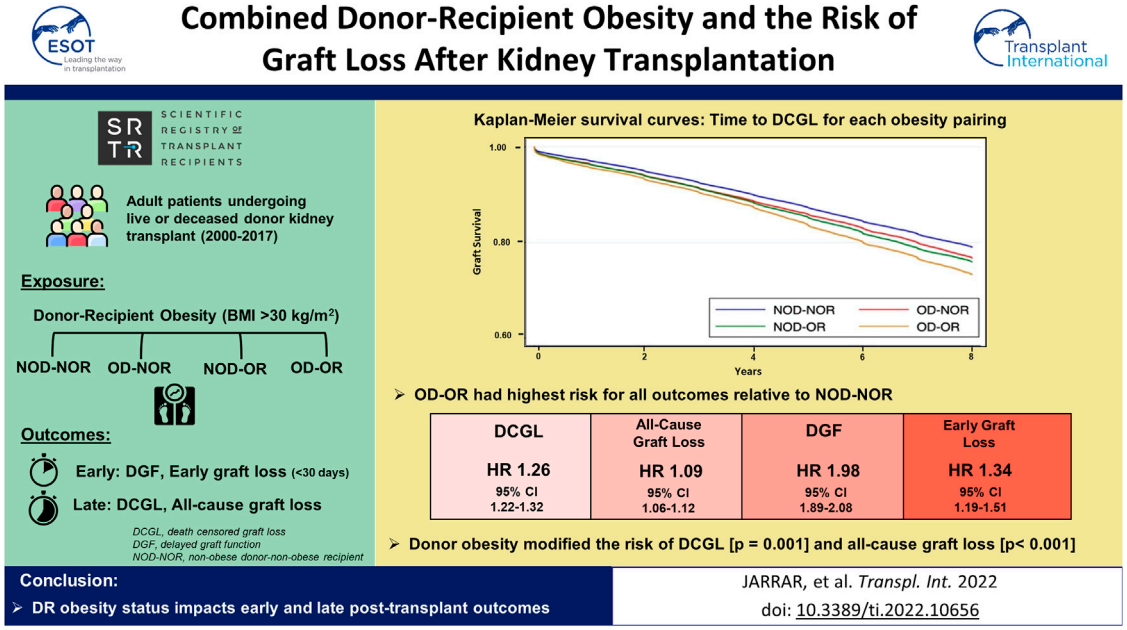
Received: *18 May 2022*
Accepted: *12 September 2022*
Published: *29 September 2022*

Citation:
Jarrar F, Tennankore KK and Vinson AJ (2022) Combined Donor-Recipient Obesity and the Risk of Graft Loss After Kidney Transplantation. Transpl Int 35:10656. doi: 10.3389/ti.2022.10656

Abbreviations: SRTR, Scientific Registry of Transplant Recipients; BMI, Body Mass Index; DCGL, Death-censored graft loss; DGF, Delayed graft function; DR, Donor and recipient; HR, Hazard ratio; OR, Odds ratio; NOD-NOD, Non-obese donor and recipient; OD-NOR, Obese donor-non obese recipient; NOD-OR, Non obese donor-obese recipient; OD-OR, Obese donor and recipient; ESKD, End-stage kidney disease; HLA MM, Human leukocyte antigen mismatch; PRA, Peak panel reactive antibody.



GRAPHICAL ABSTRACT |

INTRODUCTION

Obesity has become a major public health concern worldwide (1), with data classifying more than one third of adults as obese in the United States (2). The global rise in obesity is reflected in the kidney transplant population, with the proportion of recipients with a body mass index (BMI) in excess of 30 kg/m^2 doubling every 15 years (3). As obesity rates increase in the general population, the number of obese transplant candidates and kidney donors, both living and deceased, is also expected to increase (4).

The increased prevalence of obesity has important implications for both kidney transplant recipients and transplant programmes. Although not considered a contraindication for kidney transplantation according to most clinical practice guidelines (5), recipient obesity is associated with increased risk of death-censored graft loss (DCGL) (6, 7, 8, 9, 10), delayed graft function (DGF) (6, 11, 12, 13, 14), increased peri- and post-operative complications (6, 15, 16) and prolonged hospitalizations (7, 8). Meanwhile, donor obesity has been linked with increased incidence of recipient DGF and DCGL (11, 17, 18), though its exact influence on graft outcomes is less clear. No studies to date have assessed the potential interaction between donor and recipient obesity on graft outcomes. Importantly, weight mismatch between kidney donors and recipients (DR) has been shown to associate with graft outcomes; recipients receiving organs from relatively smaller donors experience significantly worse outcomes than those receiving kidneys from weight-matched or larger donors (19, 20, 21, 22, 23). However, whether donor and/or recipient obesity modifies the association between DR weight mismatch and transplant outcomes has not been previously examined.

In this study, we aimed to describe the changing prevalence of donor and recipient obesity at the time of transplantation and explore whether *combined* DR obesity status impacts early (DGF, ≤30 day graft loss) and/or late (DCGL, all-cause graft loss) post-transplant outcomes. We also explored whether DR obesity status modifies the known relationship between DR weight mismatch and graft outcomes after kidney transplantation.

METHODS

Subject Selection

We conducted a retrospective cohort study of adult patients who received a first living or deceased donor kidney transplant in the United States (US) between 1 January 2000, and 31 December 2016, identified using the Scientific Registry of Transplant Recipients (SRTR) database. Exclusion criteria included those <18 years of age, those receiving a second transplant, or those missing either donor or recipient data for weight or body mass index (BMI). Donors and recipients with BMI values <10 and >100 kg/m^2 were excluded, as these were assumed to represent coding errors.

Exposure

The primary exposure was donor and/or recipient obesity status. Obesity status was dichotomized at a BMI cut point of >30 kg/m^2 versus ≤30 kg/m^2 according to standard guidelines (24) to identify four DR obesity pairings: i. non-obese DR (NOD-NOR), ii. obese donor-non obese recipient (OD-NOR), iii. non obese donor-obese recipient (NOD-OR), and iv. obese DR (OD-OR).

A secondary exposure was combined donor and/or recipient obesity and DR weight mismatch. We categorized DR absolute weight difference as >30 kg, 10-30 kg (donor < recipient, D<R; or donor > recipient, D>R) and <10 kg (D = R) as per previous literature (19), stratified by the four aforementioned DR obesity pairings (NOD-NOR, OD-NOR, NOD-OR and OD-OR).

Outcome

The primary outcome was death-censored graft loss (DCGL). Graft loss was defined as need for return to chronic dialysis or repeat transplantation. Secondary outcomes included the composite of graft failure or death (i.e., all-cause graft loss), delayed graft function (DGF), defined as need for dialysis within the first 7 days following transplantation, and early (≤30 days) graft loss. Censoring occurred at losses to follow-up and at the date of last follow-up.

Data Collection

We adjusted for known literature predictors of graft loss including donor and recipient age, race, and sex, recipient end-stage kidney disease (ESKD) cause, dialysis vintage, pre-emptive status, cold-ischemia time (CIT), previous kidney transplant, human leukocyte antigen (HLA) mismatch (MM), peak panel reactive antibody (PRA), and recipient medical comorbidities including type 2 diabetes, hypertension, coronary artery disease and peripheral vascular disease. These co-variates were selected *a priori*. For the primary analysis, missing data was treated by case wise deletion.

The SRTR data system includes data on all donor, wait-listed candidates, and transplant recipients in the US, submitted by the members of the Organ Procurement and Transplantation Network (OPTN). The Health Resources and Services Administration (HRSA), US Department of Health and Human Services provides oversight to the activities of the OPTN and SRTR contractors.

Analysis

Descriptive statistics were reported for baseline characteristics. Means and standard deviations and medians and interquartile range were used for continuous normal and continuous non-normally distributed variables. Baseline donor and recipient characteristics were reported for all patients in each of the DR obesity pairing groups.

Primary Analysis

Temporal Changes in DR Obesity Pairing Over Time

We examined temporal trends in the incidence of each DR obesity pairing at the time of transplantation over the study period.

Association of DR Obesity Pairing With DCGL

For the outcome of DCGL, we used a multivariable Cox proportional hazards model to determine the adjusted hazard ratio (HR) for DCGL for each DR obesity pairing (OD-NOR; NOD-OR; OD-OR), relative to NOD-NOR. Time to DCGL was demonstrated visually using Kaplan Meier survival curves. Proportionality was confirmed with visual examination of log-log plots.

Secondary Analyses

Association of DR Obesity Pairing With Secondary Outcomes

In a secondary analysis, we used a multivariable Cox proportional hazards model to determine the adjusted HR for all-cause graft failure for each DR obesity pairing (OD-NOR; NOD-OR; OD-OR), relative to NOD-NOR. Multivariable logistic regression was used to determine the adjusted odds ratio (OR) for the outcomes of DGF and early (≤30 days) graft loss associated with each DR obesity pairing relative to NOD-NOR. Finally, we determined if donor obesity modified the association of recipient obesity with each of DCGL, all-cause graft loss, DGF and early graft loss, by including an interaction term between donor and recipient obesity status in each regression model.

Association of Combined DR Weight Mismatch & Obesity Status With DCGL

For the outcome of DCGL, we used multivariable Cox proportional hazards models to determine the adjusted relative hazard ratio (HR) for each DR weight mismatch category relative to weight-matched DR (<10 kg absolute weight difference), stratified by DR obesity status. Weight-matched NOD-NOR was the reference category for all comparisons, irrespective of DR obesity status. Proportionality was confirmed with visual examination of log-log plots.

Association of Combined DR Weight Mismatch & Obesity Status With Secondary Outcomes

We repeated the above analysis examining DR weight mismatch stratified by DR obesity status to examine the outcome of all-cause graft loss. We also examined the effect of combined DR obesity and weight mismatch on DGF and early graft loss, using multivariable logistic regression, adjusting for the same factors listed above.

Sensitivity Analyses and Subgroup Analysis

We repeated our primary analysis (DR obesity pairing) for the following:

(i) Adjusting for era effect for the outcome of DCGL.
(ii) Excluding donors and recipients with a BMI <18 for early and late graft outcomes.
(iii) Adjusting for donation after circulatory death (DCD) vs. donation after neurologic death (DND) status for the outcome of DCGL in deceased donor transplant recipients.
(iv) Adjusting for donor kidney side (right vs. left) for early and late outcomes.

We repeated our secondary analysis (combined DR weight mismatch & obesity status) for:

(i) Combined DR weight mismatch and obesity status using a reference category of weight-matched DR (D = R) within each DR obesity pairing (as opposed to D = R NOD-NOR).

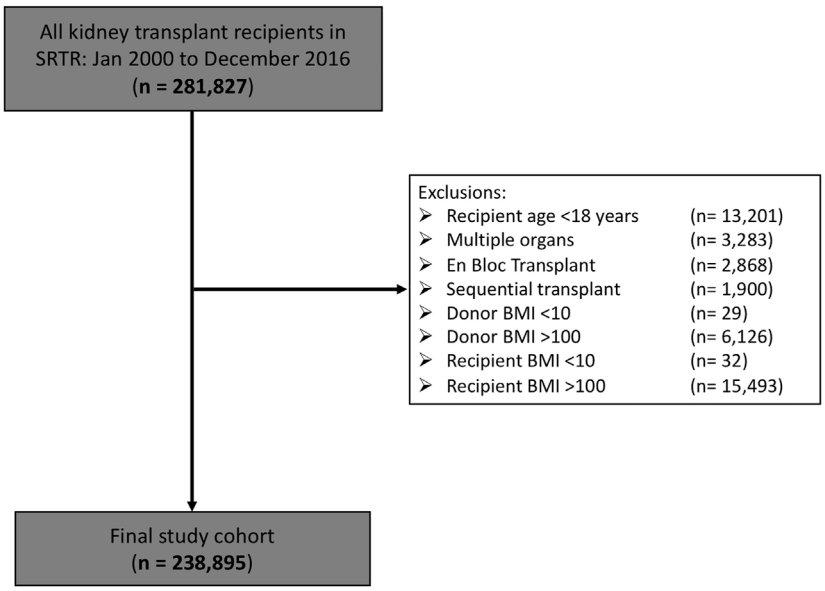


FIGURE 1 | Final study cohort following exclusions.

(ii) Combined DR weight mismatch and obesity status separately in living donors and deceased donors.
(iii) Using DR height mismatch instead of weight mismatch. For this analysis, we categorized DR absolute height difference as >15 cm, 5-15 cm (D<R; D>R) and <5 cm (D = R), as per previous literature (20). A <5 cm difference between donor and recipient height was used as the reference category for height mismatch. Similar to the primary analysis, we examined the association of DR height mismatch with DCGL within each DR obesity pairing.
(iv) Using higher BMI cut points (>35 kg/m^2 and >40 kg/m^2) to define DR obesity status; the reference category was patients with a BMI of 18–25 kg/m^2.

Ethics approval for this study was provided through the Nova Scotia Health Research Ethics Board. All statistical analyses were performed using Stata version 13.1 (Stata Corp., College Station, TX). For statistical comparisons, a $p < 0.05$ was deemed the threshold for statistical significance.

RESULTS

Baseline Characteristics

Our final study cohort consisted of 238,895 kidney transplant recipients (**Figure 1**). Baseline characteristics are shown in **Table 1**. A total of 154,125 (64.5%) were from deceased donors and 84,770 (35.5%) from living donors. Mean donor and recipient BMIs were 27.1 ± 6.0 kg/m^2 and 27.7 ± 5.6 kg/m^2, respectively, with 40.0% and 49.7% of donors and recipients noted to be obese, respectively. Median absolute DR weight difference was −2.10 kg (Q1-Q3 −19.26 to 14.80 kg); recipients being slightly larger than donors. Overall, DCGL occurred in 30,132 patients (12.6%), all-cause graft loss in 82,372 (34.9%), DGF in 83,374 (18.1%) and early graft loss in 4778 (2%). Median follow-up time was 4.15 years (Q1–Q3 1.97–7.71 years).

Temporal Changes in DR Obesity Pairing

There was a decrease in the incidence of NOD-NOR from 62% to 45% over time (**Figure 2**). Of the DR obesity pairings, NOD-OR had the greatest absolute increase over time (18% to 26%; 43.3% relative increase). OD-OR experienced the greatest relative increase over time, from 6% to 11% (91.4% relative increase).

DR Obesity Pairing

DR Obesity and DCGL

Examining the effect of DR obesity status on DCGL (relative to NOD-NOR), the adjusted relative hazard was highest in the OD-OR pairing (HR 1.24, 95% CI 1.19–1.30), **Table 2**. This was followed by NOD-OR (HR 1.16, 95% CI 1.12–1.20). OD-NOR pairing was not associated with risk of DCGL. The fully adjusted multivariable model is available in **Supplementary Table S1A**. Time to DCGL for each of the DR obesity pairings is shown in **Figure 3**.

DR Obesity and Secondary Outcomes

Combined donor and recipient obesity (OD-OR) was also associated with the highest risk for all-cause graft loss, DGF and early graft loss, **Table 2**. OD-NOR pairing was associated with DGF and early graft loss but not with all-cause graft loss. NOD-OR pairing was associated with both early and late outcomes. The fully adjusted multivariable models are available in **Supplementary Tables S1B–D**.

TABLE 1 | Baseline characteristics by donor-recipient obesity pairing.

Characteristics	Categories			
N = 238,895 (%)	NOD-NOR	OD-NOR	NOD-OR	OD-OR
	N = 123,449 (51.7)	N = 38,969 (16.3)	N = 53,964 (22.6)	N = 22,513 (9.4)
Donor age (Q1, Q3)	39 (26, 50)	43 (33, 52)	40 (27, 51)	43 (33, 52)
Recipient age (Q1, Q3)	51 (39, 60)	53 (41, 62)	53 (43, 61)	54 (44, 61)
Donor sex (F)	57,937 (46.9)	19,357 (49.7)	24,145 (44.7)	11,360 (50.5)
Recipient sex (F)	47,994 (38.9)	14,876 (38.2)	21,739 (40.3)	8,994 (40.0)
Donor race				
White	102,958 (83.4)	32,371 (83.1)	45,214 (83.8)	18,481 (82.1)
Black	14,613 (11.8)	5,585 (14.3)	7,016 (13.0)	3,497 (15.5)
Other	5,863 (4.8)	1,008 (2.6)	1,727 (3.2)	534 (2.4)
Recipient race				
White	85,145 (69.0)	25,557 (65.6)	36,067 (66.8)	14,695 (65.3)
Black	27,699 (22.44)	10,315 (26.5)	15,628 (29.0)	6,904 (30.7)
Other	10,605 (8.6)	3,097 (8.0)	2,266 (4.2)	914 (4.06)
Pre-emptive	24,115 (19.5)	6,373 (16.4)	9,423 (17.5)	3,795 (16.9)
HLA MM				
0	11,179 (9.1)	3,161 (8.1)	4,436 (8.2)	1,679 (7.5)
1	4,678 (3.8)	1,255 (3.2)	1,711 (3.2)	741 (3.3)
2	11,279 (9.1)	3,179 (8.2)	4,166 (7.7)	1,893 (8.4)
3	22,529 (18.3)	6,790 (17.4)	9,271 (17.2)	4,126 (18.3)
4	26,176 (21.2)	8,810 (22.6)	12,282 (22.8)	5,045 (22.4)
5	30,677 (24.9)	10,401 (26.7)	14,289 (26.5)	5,962 (26.5)
6	15,979 (12.9)	5,165 (13.3)	7,411 (13.7)	2,955 (13.1)
Previous transplant	17,333 (14.0)	5,389 (13.8)	4,670 (8.7)	1,768 (7.9)
Recipient diabetes	31,117 (25.2)	11,157 (28.6)	23,003 (42.6)	9,983 (44.3)
Recipient hypertension	93,868 (76.0)	29,970 (76.9)	41,896 (77.6)	17,436 (77.5)
Cause of ESRD				
Diabetes	24,229 (19.6)	8,788 (22.6)	17,906 (33.2)	7,822 (34.7)
Glomerulonephritis	32,830 (26.6)	9,391 (24.1)	11,637 (21.6)	4,628 (20.6)
PCKD	12,610 (10.2)	3,754 (9.6)	4,617 (8.6)	1,807 (8.0)
HTN	28,089 (22.8)	9,670 (24.8)	12,607 (23.4)	5,462 (24.3)
Hereditary	2,943 (2.4)	820 (2.1)	671 (1.2)	257 (1.1)
Drugs	2,897 (2.4)	841 (2.2)	824 (1.5)	350 (1.6)
Other	14,295 (11.6)	4,074 (10.5)	4,198 (7.8)	1,611 (7.2)
Median CIT (Q1, Q3)	11.5 (2.0,19.4)	13.2 (4.0, 20.7)	12.45 (2.75, 20.0)	12.48 (2.71, 20.0)
DR weight mismatch				
D>R, 10–30 kg (N = 48,908)	28,657 (23.3)	14,031 (36.0)	1,216 (2.3)	5,004 (22.2)
D>R, >30 kg (N = 25,552)	6,293 (5.1)	16,936 (43.5)	55 (0.1)	2,268 (10.1)
D = R, <10 kg (N = 74,555)	49,896 (40.4)	6,991 (17.9)	9,478 (17.6)	8,190 (36.4)
D<R, 10–30 kg (N = 56,617)	29,908 (24.2)	958 (2.5)	20,433 (37.9)	5,318 (23.6)
D<R, >30 kg (N = 33,263)	8,695 (7.0)	53 (0.1)	22,782 (42.2)	1,711 (7.7)

Proportion missing: human leukocyte antigen mismatch (0.8%); pre-emptive (0.48%); recipient diabetes (0.87%); recipient hypertension (12.7%); end-stage renal disease (3.9%); PRA (18.0%); donor race (0.01%); recipient race (0.003%); donor BMI (1.7%); recipient BMI (2.9%); CIT (11.0%).

BMI, body mass index; ESRD, end-stage renal disease; HLA, human leukocyte antigen; HTN, hypertension; PCKD, polycystic kidney disease; CIT, cold ischemia time; NOD-NOR, non-obese donor-non-obese recipient; OD-NOR, obese-donor-non-obese recipient; NOD-OR, non-obese donor-obese recipient; OD-OR, obese-donor-obese-recipient.

Donor obesity modified the risk of recipient obesity on both DCGL ($p = 0.001$) and all-cause graft loss ($p < 0.001$), while no interaction was observed between donor and recipient obesity for DGF ($p = 0.559$) or early graft loss ($p = 0.208$).

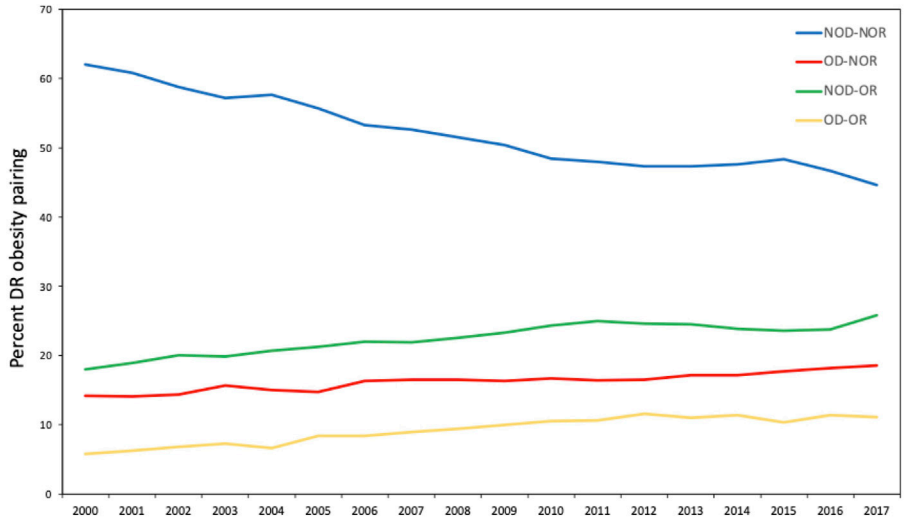
Combined DR Weight Mismatch & Obesity Pairing

Association With DCGL

Amongst NOD-NOR, both D>R by 10–30 kg (HR 0.94, 95% CI 0.90–0.99) and 30 kg (HR 0.84, 95% CI 0.77–0.92) were protective against DCGL and D<R by 10–30 kg (HR 1.12, 95% CI 1.07–1.17) and 30 kg (HR 1.42, 95% CI 1.33–1.52) were risk factors for DCGL versus no weight difference, **Table 3**. In all DR obesity pairings, there was a trend towards increased risk of DCGL as the recipient size increased relative to the donor and when either the donor or recipient were obese, D>R was no longer protective. In OD-OR, all DR weight mismatch categories were associated with an increased risk of DCGL relative to weight-matched NOD-NOR.

Association With Secondary Outcomes

Amongst NOD-NOR, D>R was not protective against all-cause graft loss, but a larger recipient than donor was significantly higher risk than no weight difference, **Supplementary Table S2**. Amongst OD-OR, all DR weight mismatch categories (except D>R by >30 kg) were higher risk for all-cause graft loss than a weight matched NOD-NOR; no significant association was seen for OD-NOR and NOD-OR.



	NOD-NOR	OD-NOR	NOD-OR	OD-OR
Starting, Jan 2000 (%)	62.1	14.2	18.0	5.8
Ending, Dec 2016 (%)	44.6	18.5	25.8	11.1
Absolute change (%)	- 27.4	4.3	7.8	5.3
Relative change (%)	- 44.1	30.3	43.3	91.4

FIGURE 2 | Temporal changes in donor-recipient obesity pairing over time. The accompanying table displays descriptive statistics for each of the donor-recipient obesity pairings.

TABLE 2 | Adjusted risk for post-transplant adverse outcomes for each DR obesity pairing.

	DCGL	All-cause graft loss	DGF	Early (≤30 days) graft loss
	Hazard ratio (95% CI)	Hazard ratio (95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)
NOD-NOR	Ref.	Ref.	Ref.	Ref.
OD-NOR	1.01 (0.98–1.05)	0.99 (0.97–1.02)	**1.36 (1.31–1.42)**	**1.20 (1.08–1.34)**
NOD-OR	**1.16 (1.12–1.20)**	**1.05 (1.02–1.07)**	**1.49 (1.43–1.54)**	**1.19 (1.08–1.31)**
OD-OR	**1.24 (1.19–1.30)**	**1.08 (1.04–1.11)**	**1.98 (1.88–2.08)**	**1.32 (1.16–1.51)**

Green (HR < 1.0), yellow (HR 1-1.2), orange (HR 1.2-1.4), red (HR > 1.4) (Colors only apply to significant results).
Models were adjusted for known literature predictors of graft loss, including donor and recipient age, race, sex, recipient end-stage kidney disease (ESKD) cause, cold ischemia time (CIT), dialysis vintage, pre-emptive status, previous kidney transplant, human leukocyte antigen (HLA) mismatch, peak panel reactive antibody (PRA), and recipient medical comorbidities (coronary artery disease, hypertension, peripheral vascular disease, type 2 diabetes).
NOD-NOR, non-obese donor-non-obese recipient; OD-NOR, obese-donor-non-obese recipient; NOD-OR, non-obese donor-obese recipient; OD-OR, obese-donor-obese-recipient; DCGL, death-censored graft loss; DGF, delayed graft function.

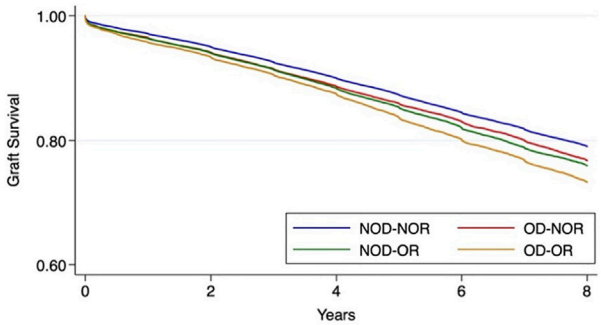
Amongst NOD-NOR, a 30 kg difference between donor and recipient (D<R) was the highest risk for DGF (OR 1.24, 95% CI 1.14–1.34) relative to no weight mismatch, **Supplementary Table S3**. Though not always significant, when stratified by DR obesity status, all DR weight mismatch categories were associated with DGF. Risk of DGF was most pronounced for OD-OR and highest at extremes of weight mismatch (>30 kg difference) for both D>R and D<R.

Results for early graft loss are shown in **Supplementary Table S4**. D<R by 30 kg was highest risk in each DR obesity pairing.

Sensitivity Analyses

Transplant Era Effect

When we repeated the primary analysis adjusting for transplant era, we found that the effects of DR obesity persisted and were similar to those seen in our primary analysis. The adjusted



DR Obesity pairing	0-2 years	2-4 years	4-6 years	6-8 years	8+ years
NOD-NOR	123449	93132	68329	47815	31232
OD-NOR	38969	28061	19856	13353	8336
NOD-OR	53964	39454	27755	18061	10918
OD-OR	25513	16040	10973	6833	3946

FIGURE 3 | Kaplan-Meier survival curves for time to death-censored graft loss for each donor-recipient obesity pairing. A number at risk table is included below the figure. *The log-rank *p*-value is <0.001.

relative hazard was highest in the OD-OR pairing (HR 1.28, 95% CI 1.23–1.34), followed by NOD-OR (HR 1.18, 95% CI 1.14–1.21). OD-NOR pairing was not associated with risk of DCGL.

Exclusion of Donors and Recipients With BMI <18

When we repeated the primary analysis excluding donors and recipients with BMI <18, the same trends were observed for both early and late outcomes (**Supplementary Table S5**).

DND vs. DCD Status (Deceased Donors)

When we repeated our primary analysis adjusting for DCD vs. DND status in deceased donor transplant recipients, we found no significant association between DCD status and risk of DCGL (HR 0.97, 95% CI 0.92–1.02).

Donor Kidney Side

When we repeated our primary analysis adjusting for transplant kidney side, we found no significant association between right-sided donor transplants and risk of DCGL (HR 0.99, 95% CI 0.97–1.02) or all-cause graft loss (HR 0.99, 95% CI 0.97–1.01). A significant association was found between right-sided donor transplants and both DGF (OR 1.08, 95% CI 1.05–1.11) and early graft loss (OR 1.12, 95% CI 1.03–1.22).

Association of Combined DR Weight Mismatch & Obesity With DCGL; Modified DR Reference Category

When we used a weight matched reference category within each DR obesity pairing (as opposed to D = R in NOD-NOR for all comparisons), overall D>R was protective against DCGL and D<R was a risk for DCGL, **Figure 4**, **Supplementary Table S6**. In NOD-NOR, point estimates were more pronounced for D>R by 30 kg (HR 0.83, 95% CI 0.76–0.91) and D<R by 30 kg (HR 1.42, 95% CI 1.32–1.52) compared to D = R. Amongst OD-OR, DR weight mismatch was not associated with DCGL.

Height Mismatch

Amongst the entire cohort, risk of DCGL increased as recipient height increased relative to donor, though not all results reached statistical significance (**Supplementary Table S7**). A donor >15 cm taller than their recipient was protective against DCGL in the overall cohort (HR 0.91, 95% CI 0.87–0.94) and NOD-NOR (HR 0.89, 95% CI 0.84–0.95); this protective effect was not significant in any of the other DR obesity pairings.

Living vs. Deceased Donors

There was a trend towards increased risk of DCGL as the recipient-to-donor weight increased in most DR obesity pairings, though results did not always reach statistical significance (**Supplementary Tables S8, S9**). This analysis was limited by small subgroup sample sizes, particularly for OD-NOR in living donor transplants.

TABLE 3 | Hazard ratios for death-censored graft loss for each DR weight mismatch category stratified by DR obesity status. Reference category used for all DR obesity pairings was weight-matched (D = R) NOD-NOR.

	Hazard ratio for DCGL (95% CI)			
DR Weight Mismatch (kg)	**NOD-NOR**	**OD-NOR**	**NOD-OR**	**OD-OR**
	N = 123,449	**N = 38,969**	**N = 53,964**	**N = 22,513**
>30 (D>R)	**0.84 (0.77–0.92)**	0.99 (0.94–1.05)	0.54 (0.20–1.44)	**1.29 (1.15–1.46)**
10-30 (D>R)	**0.94 (0.90–0.99)**	1.05 (0.99–1.11)	1.14 (0.96–1.35)	**1.19 (1.09–1.30)**
< 10 (D = R)	Ref.	**1.15 (1.07–1.24)**	1.06 (0.99–1.14)	**1.24 (1.16–1.33)**
10-30 (D<R)	**1.12 (1.07–1.17)**	1.19 (0.98–1.46)	**1.12 (1.07–1.18)**	**1.38 (1.27–1.50)**
>30 (D<R)	**1.42 (1.33–1.52)**	1.88 (0.98–3.61)	**1.32 (1.26–1.39)**	**1.46 (1.28–1.67)**

Green (HR < 1.0), yellow (HR 1-1.2), orange (HR 1.2-1.4), red (HR > 1.4) (Colors only apply to significant results).
NOD-NOR, non-obese donor-non-obese recipient; OD-NOR, obese-donor-non-obese recipient; NOD-OR, non-obese donor-obese recipient; OD-OR, obese-donor-obese-recipient; DCGL, death-censored graft loss.
Models were adjusted for known literature predictors of graft loss, including donor and recipient age, race, sex, recipient end-stage kidney disease (ESKD) cause, cold ischemia time (CIT), dialysis vintage, pre-emptive status, previous kidney transplant, human leukocyte antigen (HLA) mismatch, peak panel reactive antibody (PRA), and recipient medical comorbidities (coronary artery disease, hypertension, peripheral vascular disease, type 2 diabetes).

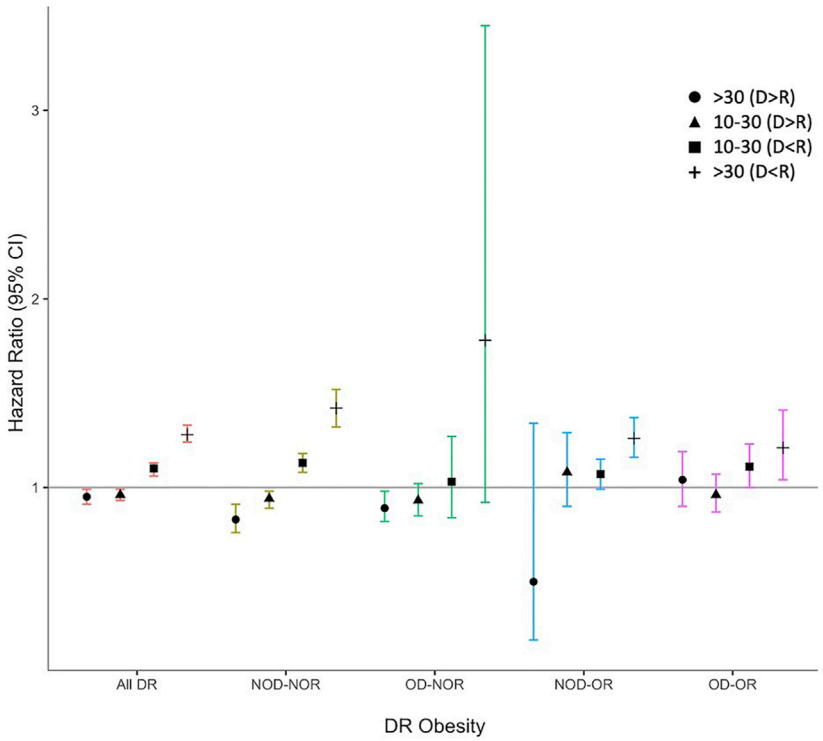


FIGURE 4 | Hazard ratio plot for death-censored graft loss for combined donor-recipient weight mismatch, stratified by donor-recipient obesity. Models were adjusted for known literature predictors of graft loss, including donor and recipient age, race, sex, recipient end-stage kidney disease (ESKD) cause, cold ischemia time (CIT), dialysis vintage, pre-emptive status, previous kidney transplant, human leukocyte antigen (HLA) mismatch, peak panel reactive antibody (PRA), and recipient medical comorbidities (coronary artery disease, hypertension, peripheral vascular disease, type 2 diabetes).

Extremes of BMI

Relative to NOD-NOR, risk of DCGL was highest for OD-OR using both >35 kg/m^2 (HR 1.45, 95% CI 1.31–1.60) and >40 kg/m^2 (HR 1.41, 95% CI 1.05–1.90) cut-offs, followed by NOD-OR (BMI ≥35 kg/m^2: HR 1.26, 95% CI 1.18–1.35; BMI 40 kg/m^2: HR 1.36, 95% CI 1.20–1.54). OD-NOR was not significantly associated with DCGL for either BMI cut-offs, (data not shown). Sample sizes were small in the OD-OR subgroup at BMI 40 kg/m^2 (n = 203).

DISCUSSION

In this study, we describe the changing demographics of obesity at the time of kidney transplantation and explore how DR obesity pairing impacts early and late graft outcomes. We also investigate whether obesity status modifies the known relationship between DR weight mismatch and graft outcomes after kidney transplantation.

Previous studies have found a significant increase in the prevalence of overweight and obese recipients at time of transplantation (3, 16). We demonstrate a substantial increase in the prevalence of obesity in both kidney donors and recipients over time, with relative increases in NOD-OR transplants by 43.3% and OD-OR by over 91.4% over our study period.

When examining the effect of DR obesity pairing on late graft outcomes, OD-OR and NOD-OR were both associated with risk of DCGL and all-cause graft loss; OD-OR was highest risk for both outcomes. Isolated recipient obesity has been linked to a multitude of adverse graft outcomes, including DCGL (6, 25, 26) and early events including wound-related morbidity and acute rejection (27, 28), which likely compound the risk of long-term failure. Obesity is associated with chronic medical conditions including type 2 diabetes, cardiovascular disease, and chronic respiratory disorders, which are associated with increased morbidity and mortality in the general population and kidney transplant recipients (29, 30, 31, 32). Obesity also causes various structural, hemodynamic, and metabolic alterations in the kidney (33). It has been hypothesized that a kidney that is small for the metabolic needs of an individual may experience a triad of glomerular hypertension, hypertrophy, and hyperfiltration that eventually leads to progressive glomerulosclerosis, proteinuria, and loss of function (17, 33, 34, 35); these renal complications are seen in obesity-related glomerulopathy (ORG) (36, 37). Damage to transplanted kidneys may be caused by similar pathophysiologic mechanisms to those which occur in the native kidneys of obese patients, contributing to downstream adverse effects in recipients (38, 39). We demonstrate for the first time that donor obesity modifies the known association between recipient obesity and DCGL

and all-cause graft loss. This interaction likely relates to additive harms when an obese donor kidney (with some element of pre-existing pre-terminal hyperfiltration and ORG) is transplanted into an obese recipient wherein pre-existing vascular disease, longer operative times and surgical complications may compound risk (17, 27).

Notably, risk of DCGL was more exaggerated than that of all-cause graft loss in both NOD-OR and OD-OR. This finding is in keeping with other studies which have shown a comparable mortality risk between obese recipients and those with a normal BMI (4, 6, 12). While this appears counter-intuitive given the greater burden of co-morbidities in obese individuals and the association of obesity with mortality in the general population (40), there are a number of possible explanations. First, the J-shaped relationship between BMI and survival in the prevalent dialysis population is important to consider, wherein both high and low BMIs are associated with increased mortality (41, 42). This likely reflects a combination of underlying comorbidity, protein-energy malnutrition, or the existence of a chronic inflammatory state as opposed to a directly protective effect of adiposity (41, 43). Second, renal transplant recipients have a substantial increased risk of cardiovascular morbidity by virtue of an accumulation of traditional and transplant-related risk factors (44, 45). It is thus possible that the additional mortality risk conferred by obesity is overshadowed by the significant cardiovascular risk in this unique population.

We found an increased risk of DGF when either the donor or recipient was obese, with the risk greatest in OD-OR. This is in agreement with previous retrospective studies which have separately correlated recipient and donor BMI with incidence of DGF (11, 12, 13, 14). DGF is a consequence of mostly, but not exclusively, nonimmunological factors (e.g., hypoxia during cold or warm ischemic periods) and ischemia-reperfusion–mediated immunological factors (46, 47). Previous studies have shown that obese recipients are more likely to experience protracted operative times, early post-operative complications (27, 28, 42), acute rejection (14) and prolonged warm ischemia times (48, 49). Donor obesity has also been linked with increased nephrectomy operation times as well as prolonged cold and warm ischemia times (18, 50). The association between BMI and ischemia-reperfusion injury has not been well studied, however, obesity is considered a proinflammatory environment marked by an increased activation of innate and adaptive immune responses (4). Adipocytes and immune cells within adipose tissue are known to produce proinflammatory cytokines including IL6, TNF-alpha and IL1-beta, while anti-inflammatory mediators are simultaneously suppressed (4, 51, 52). After transplant surgery, obesity-related proinflammatory cytokines may stimulate an exaggerated ischemia-reperfusion injury–mediated immunological response, contributing to both DGF and early graft loss. Further, venous thromboembolism, risk of which is higher in obese patients (53), may contribute to the early outcomes seen in obese recipients (54).

Our analysis demonstrates an attenuation of the protective effects of a larger donor than recipient (16, 17, 18, 19, 20, 24, 55) when either the donor or recipient is obese. This finding may similarly be explained by the nephron underdosing hypothesis (39, 56) whereby the relatively smaller renal mass in smaller donors results in increased single nephron glomerular filtration rate and increased risk of hyperfiltration injury over time (34, 38, 57, 58, 59). While recipients are typically protected by larger donors because of the greater nephron load afforded, there is likely paradoxical nephron underdosing when larger donors are obese. Nephron load is thought to be a correlate of lean body mass, not actual body mass in obese individuals, (33, 36) and as such, larger donors due to increased adiposity would not be expected to yield a greater nephron supply. Additionally, glomerular hyperfiltration, which occurs in the context of the increased metabolic needs of obesity, may lead to the development of glomerulomegaly and glomerulosclerosis in a manner analogous to that described in reduced renal mass states (36, 37, 60). This has been observed in patients with biopsy-proven ORG (37). Obesity therefore mitigates the protective association seen when donors are larger than their recipients given the combined effect of lower nephron density per unit mass and underlying glomerulosclerosis in the obese donor kidney at the time of donation.

Interpretation of the findings regarding obesity and graft outcomes requires caution. Although this study demonstrates the potential detriments of donor and recipient obesity on outcomes following transplantation, we do not suggest discard of obese donor kidneys or that obese recipients be declined access to transplantation. Evidence suggests that in most cases, kidney transplantation in obese patients affords better survival than remaining on dialysis (4). Glanton et al. reported doubled mortality rates for obese patients who stayed on the waiting list compared to those who received a kidney transplant, though this survival benefit was not achieved in patients with BMI $\geq$40 kg/m^2 (61). Our study highlights the importance of counseling potential recipients on achieving a healthy pre-transplant BMI to optimize post-transplant outcomes.

While likely of benefit, there are insufficient data to assess the impact of pre-transplant interventions, such as planned weight reduction strategies, among potential recipients. The role of bariatric surgery in the dialysis population and transplant candidates is becoming an increasingly salient issue, with many studies showing promising results (62, 63, 64). Pending more evidence, encouraging kidney transplant candidates living with obesity to lose weight and have their nutritional status supervised by a multidisciplinary weight-management team remains important (5). Obese transplant candidates should continue to be carefully optimized prior to surgery to minimize peri- and post-operative morbidity and post-operative graft injury. This may include strategic pairing of donors and recipients to minimize additive insults from suboptimal DR weight mismatch and obesity pairing.

There are several limitations to our study for consideration. First, while BMI is often used as a surrogate marker of obesity and suitability for kidney transplantation, some studies have shown waist-to-hip ratio and waist circumference to be

stronger predictors of cardiovascular death than BMI (65). Waist circumference is currently not collected in the SRTR, but its application and comparison to BMI in future analyses is important. Second, the internal consistency of BMI in donors and recipients may be questioned; it is plausible that an elevated BMI in donors and recipients is associated with significant differences in lean body masses. As demonstrated by previous literature, many patients with ESKD are in a catabolic state manifested by a combination of underlying comorbidity, protein-energy malnutrition, and a chronic inflammatory state (43). In such states, a higher BMI may reflect lower overall risk. As such, examination of potentially more reliable clinical markers, such as BSA, are warranted in future investigations. Third, our study dichotomized DR obesity at a BMI cut point of 30 kg/m^2 as defined by earlier literature (66). Ideally, further sub-categorization of BMI would be undertaken to better understand how varying degrees of donor and/or recipient obesity influence graft outcomes, however, this as demonstrated by our sensitivity analysis examining OD-OR defined using a BMI cut point of 40 kg/m^2 limited the available sample sizes and the validity of the results. Additionally, we could not access any histologic parameters of the allograft such as implantation biopsy, percentage of global glomerulosclerosis, or health of the tubulointerstitium, which could provide important insights on histopathologic changes related to obesity. A prospective study at an appropriate center could allow for exploration of implantation biopsies at the time of organ retrieval. Moreover, immunosuppressive data including details regarding changes over time, are not robustly captured by the SRTR and were therefore not included in our multivariable models. Finally, we could not access specific causes of graft loss; these may have provided pathophysiologic explanations as to how DR obesity status influences early and late graft loss. As such, we could not establish the relative impact of specific factors for a given recipient on graft loss. We also could not access donors' cause of death as this is not reliably reported in the SRTR.

In summary, we report an increased proportion of obese donors and recipients between 2000 and 2017, with the greatest relative increase in OD-OR followed by NOD-OR. We demonstrate the combined exposure of an obese donor and obese recipient to be associated with the greatest risk of short and long-term complications after transplant. Finally, we demonstrate that donor and/or recipient obesity attenuates the protective signal typically seen in the setting of a larger donor-to-recipient size. Our findings highlight the importance of informed consent procedures for obese donors and transplant candidates. Further, our data indicate that obesity status should be considered when considering the implications of DR weight matching.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: Scientific Registry of Transplant Recipients.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Nova Scotia Health Research Ethics Board. 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 participated in the research design. AV did the initial data analysis with input from KT and FJ. KT provided feedback and suggestions to make the analysis more robust. FJ wrote the initial manuscript, and AV and KT provided several rounds of feedback leading to production of the final article. All authors reviewed and approved the final manuscript.

AUTHOR DISCLAIMER

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

CONFLICT OF INTEREST

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

ACKNOWLEDGMENTS

The data reported here have been supplied by the Minneapolis Medical Research Foundation as the contractor for the Scientific Registry of Transplant Recipients (SRTR).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10656/full#supplementary-material

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BRIEF RESEARCH REPORT
published: 20 October 2022
doi: 10.3389/ti.2022.10753

Incidence of Gastrointestinal Bleeding After Transesophageal Echocardiography Use in Orthotopic Liver Transplantation

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The risk of upper gastrointestinal bleeding (UGIB) after transesophageal echocardiography (TEE) in patients with high grade esophageal varices (EV) that are undergoing Orthotopic Liver transplantation (OLT) is poorly understood. This was a retrospective single-centre cohort study in all patients that underwent OLT at Queen Elizabeth Hospital Birmingham between September 2016 and September 2018. The primary outcome was to determine the incidence of UGIB in patients that have undergone OLT with EV that received TEE. 401 patients were included in the study, of which 320 (80%) received TEE. The incidence of post-operative UGIB in patients that received TEE was 1.6% (5/320) in the entire cohort: 2.7% (4/149) in patients with no evidence of EV and 0.6% (1/171) in patients with EV. UGIB occurred in 1 patient with grade 2 EV and did not occur in patients with grade 1 or 3 EV. The incidence of UGIB in patients that received TEE was not statistically different to patients that did not: 1.6% (5/320) vs. 3.7% (3/81) p = 0.218. In conclusion, in patients that underwent OLT, intra-operative TEE use was associated with low rates of UGIB, even in cohorts with high grade EV. This suggests that TEE is a relatively safe method of haemodynamic monitoring in patients undergoing OLT.

Keywords: liver transplantation, cardiovascular, liver, transesophageal echocardiography, echocardiography

OPEN ACCESS

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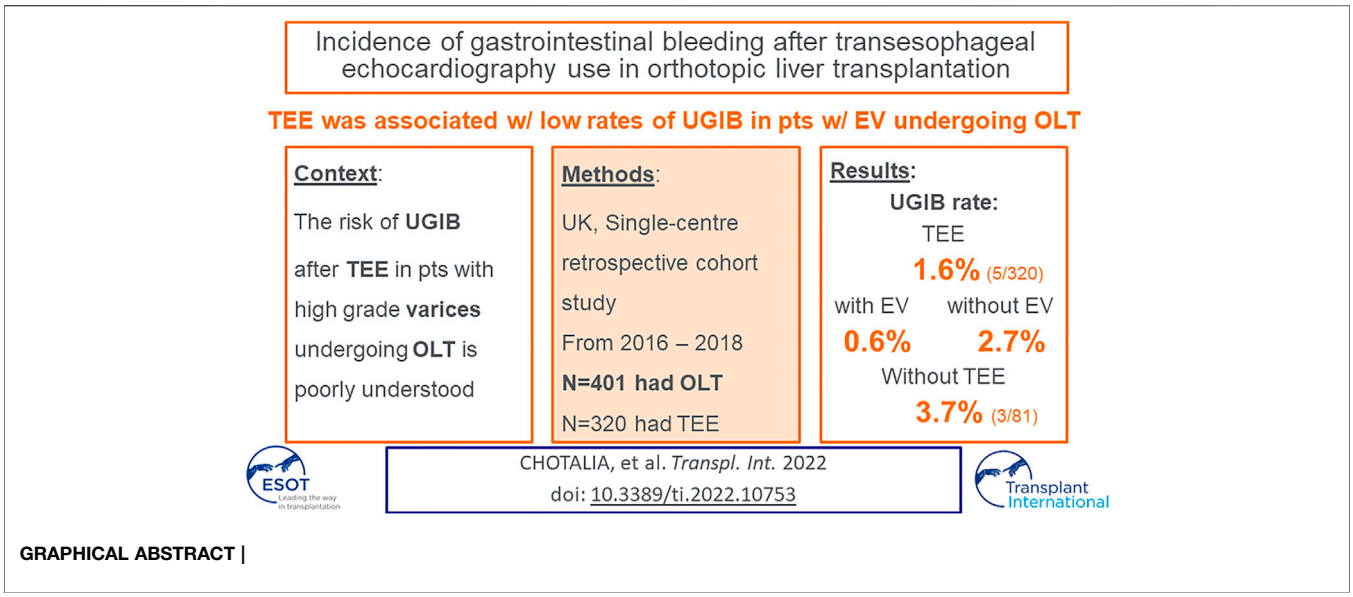
***Received:** 08 July 2022*
***Accepted:** 29 September 2022*
***Published:** 20 October 2022*

Citation:
Chotalia M, Topiwala U, Iqbal A, Parekh D, Isaac JL, Perera MTPR and Arshad MA (2022) Incidence of Gastrointestinal Bleeding After Transesophageal Echocardiography Use in Orthotopic Liver Transplantation. Transpl Int 35:10753. doi: 10.3389/ti.2022.10753

Abbreviations: UGIB, upper gastrointestinal bleeding; TEE, transesophageal echocardiography; EV, esophageal varices; OLT, orthotopic liver transplantation; TIPPS, transjugular intrahepatic portosystemic shunt; DBD, donation after brain death.



GRAPHICAL ABSTRACT |

INTRODUCTION

Cardiovascular instability is common during Orthotopic Liver Transplantation (OLT) and may be precipitated by cross-clamping the inferior vena cava and portal vein, surgical manipulation and reperfusion [1]. Haemodynamic monitoring is therefore vital in administering fluid/blood products and vasoactive agents during OLT and transesophageal echocardiography (TEE) is increasingly being utilised in this regard [1]. TEE has the ability to quickly detect rare but devastating intraoperative complications during OLT, such as intracardiac thrombosis and pulmonary embolism, as well as guide therapy for them. Numerous studies have demonstrated the effectiveness of TEE in the diagnosis and treatment of cardiovascular perturbations during OLT [2].

Large multicentre studies have established that TEE is a relatively safe procedure [3,4], but patients with esophageal varices (EV) were excluded from these analyses as TEE has previously been considered relatively contraindicated in this patient cohort due to concerns of precipitating upper gastrointestinal bleeding (UGIB) [5]. Recently small retrospective studies in patients with EV that received TEE demonstrated similarly low rates of UGIB, however the number of patients with high grade EV (grade 2–3) were small [6,7,8,9,10,11]. As the risk of bleeding is proportional to the size of the varix [12], this is an important omission.

As EV are present in almost 3/4 patients with end-stage liver disease (ESLD) awaiting OLT [13] and bleeding from EV is a serious complication with a 20% mortality rate [14], evaluating the safety of TEE in patients with high grade varices undergoing OLT is of paramount importance. Therefore, the main aim of this study was to determine the incidence of UGIB in patients with EV that received TEE during OLT. Secondary aims were to compare the rates of UGIB in patients with different grades of varices and in patients that underwent OLT with and without TEE.

METHODS

Ethical Approval

This study was a retrospective service evaluation of anonymised, routinely collected data as defined by the UK NHS Health Research Authority (http://www.hra.nhs.uk). The study was registered with the hospital's clinical audit registration system (CARMS-14529) and specific ethical permissions were not required.

Data Collection

This was single-centre retrospective cohort study of patients that underwent OLT at the Queen Elizabeth Hospital Birmingham (University Hospitals Birmingham NHS Foundation Trust) between September 2016 and September 2018. Data were retrieved retrospectively from the hospital's electronic patient records, surgical and anaesthetic records and included demographic data, MELD score, blood test results on the day of OLT (biochemistry, full blood count and coagulation profile), blood product transfusion during OLT and medical history of previous EV treatments including beta blocker, transjugular intra-hepatic portosystemic shunt (TIPSS) insertion, band ligation or sclerotherapy. Varices were graded in accordance with the modified Paquet classification [15]. UGIB was defined as the presence of blood in the oesophagus or stomach at the time of oesophago-gastric duodenoscopy. Clinically significant UGIB was defined by a transfusion requirement of packed red cells or if there was a drop in haemoglobin of >2 g/dl.

Statistical Analysis

All statistical analysis was performed using GraphPad Prism v.8.0. Categorical data are presented as n (%) and compared using a chi squared test. Continuous data were tested for normality using Shapiro-Wilk's test. If not normally distributed, continuous were presented as median (interquartile range) and were compared using a Mann-

TABLE 1 | Comparing clinical and demographic parameters in OLT patients that did and did not receive TEE.

Demographic	All (*n* = 401)	Received TEE (*n* = 320)	No TEE (*n* = 81)	*p* value
Age (years)	56 (46–64)	56 (46–64)	57 (46–64)	0.975
Sex (%male)	263 (65.6)	210 (65.6)	53 (65.4)	0.755
MELD score	13.9 (10.2–18.8)	14.1 (10.2–18.9)	13.8 (10.2–15.8)	0.283
Indication for OLT				0.757
Alcoholic	104 (25.9)	80 (25.0)	24 (29.6)	
PSC	68 (17.0)	53 (16.6)	15 (18.5)	
NASH	54 (13.5)	45 (14.1)	9 (11.1)	
PBC	41 (10.2)	35 (10.9)	6 (7.4)	
Hepatitis C	26 (6.5)	21 (6.6)	5 (6.2)	
Other	108 (26.9)	86 (26.9)	22 (27.2)	
Grade of varices				0.228
None	193 (48.1)	149 (46.6)	44 (54.3)	
1	133 (33.2)	107 (33.4)	26 (32.1)	
2	61 (15.2)	54 (16.9)	7 (8.6)	
3	14 (3.5)	10 (3.1)	4 (4.9)	
Bilirubin μmol/L	35 (17–64)	35 (16–65)	37 (17–57)	0.905
INR	1.4 (1.2–1.6)	1.4 (1.2–1.6)	1.3 (1.2–1.5)	0.330
Platelets x10^9/L	92 (65–144)	91 (65–143)	105 (66–154)	0.435
Donor type (%DBD)	267 (66.6)	212	55	0.778
Blood product transfusion (units)				
Packed red cells	2 (0–4)	2 (0–4)	2 (0–4)	0.674
FFP	4 (0–6)	4 (0–6)	4 (0–6)	0.771
Platelets	1 (0–10)	1 (0–5)	0 (0–5)	0.145
Cryoprecipitate	0 (0–0)	0 (0–0)	0 (0–0)	0.210
Cell saver (mls)	450 (0–780)	450 (0–770)	460 (0–990)	0.924
UGIB incidence	8 (2.0)	5 (1.6)	3 (3.7)	0.218
OGD performed	18 (4.5)	14 (4.4)	4 (4.9)	0.827
ICU mortality	15 (3.7)	10 (3.1)	5 (6.1)	0.197

Legend: OLT, orthoptic liver transplantation; TEE, transesophageal echocardiography; PSC, primary sclerosing cholangitis; PBC, primary biliary cirrhosis; NASH, non-alcoholic steatohepatitis; MELD, model for end-stage liver disease; INR, international normalized ratio; DBD, death brain stem donation; FFP, fresh frozen plasma; UGIB, upper gastro-intestinal bleeding; OGD, oesophago-gastric duodenoscopy; ICU, intensive care unit.

Whitney U test. This was a pragmatic study and post-hoc power calculations to determine study size were not performed. All tests performed were two-sided and a p value < 0.05 was considered statistically significant.

Transesophageal Echocardiography

The decision to perform a TEE was at the discretion of the treating consultant liver transplant anaesthetist. The echocardiogram was conducted and interpreted by this anaesthetist, who had relevant experience in perioperative TEE use. A standardised protocol of obtaining mid-esophageal and transgastric views was followed using a Phillips TEE probe and Phillips CX50 ultrasound machine (Phillips Healthcare, Andover, MA, United States). The TEE probes were routinely inserted after induction of anaesthesia and placement of an endotracheal tube and withdrawn at the closure of the abdomen at the end of the surgery.

RESULTS

401 patients were included in the study and had a median age of 56 (IQR 46–64), were 66% male and had a median MELD score of 14 (IQR 10–19). The most common indication for OLT was alcoholic cirrhosis (n = 119, 30%) and the most common graft type was donation after brain death (DBD) (n = 267; 67%). ICU mortality for the entire cohort was 4% (n = 15). Additional demographics are listed in **Table 1**.

Of the 401 patients, 320 (80%) received TEE. Of these patients, 149 (47%) had no evidence of EV, 107 (33%) had grade 1 EV, 54 (17%) had grade 2 EV and 10 (3%) had grade 3 EV. No episodes of intra-operative UGIB occurred. The incidence of post-operative UGIB in patients that received TEE was 1.6% (5/320) in the entire cohort: 2.7% (4/149) in patients with no evidence of EV and 0.6% (1/171) in patients with EV. A post-operative UGIB occurred in 1 patient with grade 2 EV, however this was not associated with a drop in haemoglobin or red blood cell transfusion. An UGIB did not occur in patients with grade 1 or 3 EV. The rates of UGIB were not statistically different between patients with and without EV and across different grades of EV. There were no incidences of clinically significant UGIB in patients that underwent TEE. Patients with high MELD scores (≥18) had no statistically significant difference in UGIB incidence compared to those with low MELD scores (<18; 2/93 (2.2%) vs 3/227 (1.3%); p = 0.630).

Comparison to Patients That did not Receive TEE

81 patients underwent OLT but did not receive a TEE. There were no differences in the demographics or incidence of EV between

patients that did and did not receive TEE (**Table 1**). The incidence of UGIB in patients that received TEE was not statistically different to patients that did not [1.6% (5/320) vs 3.7% (3/81); $p = 0.218$]. The number of blood products transfused intra-operatively were also similar between cohorts, as was the ICU-mortality rate.

DISCUSSION

In one of the largest studies in this field to date, we demonstrate a low rate of gastro-intestinal bleeding (<1%) following TEE in patients with EV undergoing liver transplantation. This relatively low risk of bleeding was also present in patients with high grade EV (Grade 2 or 3; 1.6%), a cohort that has previously been sparsely assessed in the literature. Furthermore, the rate of UGIB in patients that received TEE was no different to those that did not receive TEE during their OLT. Altogether, this suggests the relative safety of this semi-invasive monitoring technique in patients undergoing OLT, although larger, multi-centre studies are required to validate these findings. It is worth noting that this patient cohort (by definition) are all intubated, have excellent IV access and have available cross matched blood prior to TEE insertion. This provides a safety net should UGIB occur.

COMPARISON TO PREVIOUS LITERATURE

Numerous large, multi-centre studies have demonstrated the relative safety of TEE, with GI bleeding rates of 0.02–1% and a GI tract perforation risk of 0.01% [3, 4]. However, these studies largely excluded patients with EV, likely secondary to the historic recommendation that the presence of portal hypertension or EV were relative contraindications to TEE examination [5]. Since then, smaller retrospective studies have demonstrated a low bleeding risk following TEE in hospitalised patients with EV [6–9]. However, to the best of our knowledge, only one patient with grade 3 varices was included in these studies. Furthermore, portal venous pressures during the process of liver transplantation are likely to be markedly different to hospitalised patients with EV, hence the risk profile identified in these studies may not be directly applicable to TEE use in OLT.

In patients undergoing OLT with varices, a similarly low risk of GI bleeding following TEE was identified by Burger-Klepp et al [10] and Pai et al [11], however only 7 patients had Grade 3 varices. Here we identified 171 patients with EV, 10 of whom had grade 3 varices and also demonstrated a <1% risk of GI bleeding with TEE in these patients. If data from all of these studies are combined, the rate of UGIB following TEE is 0.2% (3/619) in patients with EV undergoing OLT. In the present study, there were no incidences of clinically significant UGIB (necessitating > 2 units packed red blood cell transfusion or drop in haemoglobin by 2 g/dl) following TEE. Importantly, this is also the first study to our knowledge to demonstrate equivalent UGIB rates in patients that underwent OLT with and without TEE, suggesting that the rates of bleeding identified may be independent of TEE use. This finding is corroborated by reports that variceal rupture is precipitated more commonly by intrinsic pressure in the portal system, after clamping the portal system at the start of the anhepatic phase, rather than direct external pressure [15]. Unfortunately, we were unable to analyse the duration of the anhepatic phase comprehensively in all patients to test this hypothesis. Furthermore, rates of UGIB were equivalent in patients with and without EV, suggesting that the presence of EV should not be a contraindication to intra-operative TEE examination during liver transplantation.

Strengths and Limitations

Despite assessing bleeding risk following TEE in the largest number of patients with grade 3 EV to date, the small patient numbers with high grade EV and low event rate of UGIB means that the study lacks sufficient power to detect clinically significant complications of TEE in this patient cohort and is at risk of type 2 statistical error. The retrospective nature of the study may have led to reporting bias, with only clinically significant bleeding being documented in the notes. Nevertheless, occult UGIB that does not precipitate OGD examination, RBC transfusion or drop in haemoglobin, is unlikely to contribute significantly to patient morbidity. The cohort had lower median MELD scores (13.9 (IQR 10.2–18.8) than other published OLT cohorts [8–11] and therefore the generalisability of these findings may not extend to patients with very severe hepatic insufficiency. However, there was no statistically significant difference in the incidence of UGIB in patients with high MELD scores (≥18) compared to low MELD scores (<18). We therefore have no evidence to suggest that TEE is unsafe/precipitates UGIB in patients with a greater severity of hepatic insufficiency. Lastly, the study may have been influenced by selection bias, as the choice to perform TEE was at the discretion of the treating anaesthetist, and patients that did not receive TEE may have had a clinically perceived increased risk of variceal bleeding. However, variceal grade, severity of liver disease and markers of coagulopathy did not differ between patients that did and did not receive TEE.

CONCLUSION

In patients that underwent OLT, intra-operative TEE use was associated with low rates of UGIB, even in cohorts with high grade EV. This suggests that TEE is a relatively safe method of haemodynamic monitoring in patients undergoing OLT.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation

and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

UT, MC, and MA collected the data. MC and MA performed the analysis. MA, AI, JI, and MP conceived and designed the analysis. MC and MA wrote the first draft of the manuscript and UT, AI, JI, DP, and MP revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

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

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BRIEF RESEARCH REPORT
published: 04 October 2022
doi: 10.3389/ti.2022.10721

Outcome of COVID-19 in Kidney Transplant Recipients Through the SARS-CoV-2 Variants Eras: Role of Anti-SARS-CoV-2 Monoclonal Antibodies

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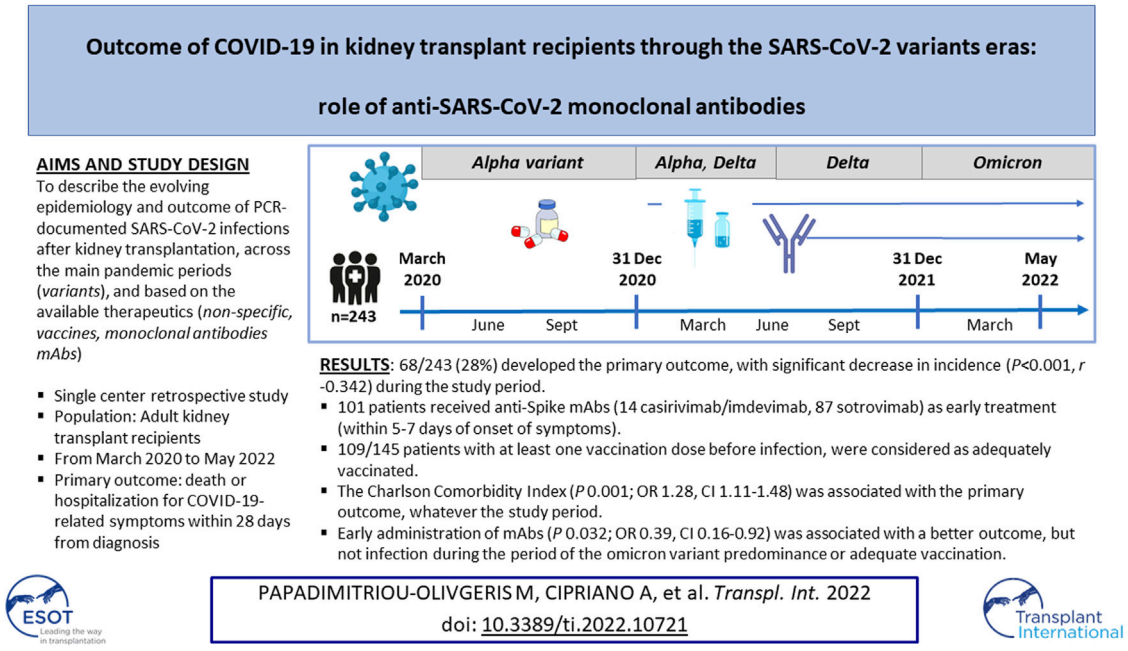
Received: *23 June 2022*
Accepted: *20 September 2022*
Published: *04 October 2022*

Citation:
Papadimitriou-Olivgeris M, Cipriano A, Guggisberg N, Kroemer M, Tschopp J, Manuel O and Golshayan D (2022) Outcome of COVID-19 in Kidney Transplant Recipients Through the SARS-CoV-2 Variants Eras: Role of Anti-SARS-CoV-2 Monoclonal Antibodies. Transpl Int 35:10721. doi: 10.3389/ti.2022.10721

Kidney transplant recipients (KTR) are at increased risk for COVID-19-associated complications. We aimed to describe the evolving epidemiology and outcome of PCR-documented SARS-CoV-2 infection in KTR followed at our institution from March 2020 to May 2022. The primary endpoint was hospitalization for COVID-19-related symptoms or death within 28 days from diagnosis. Overall, 243 cases were included of which 68 (28%) developed the primary outcome. A significant decrease in the incidence of the primary outcome was observed ($p < 0.001$, r –0.342) during the study period. Anti-Spike monoclonal antibodies (mAbs) were administered as early treatment (within 5–7 days of onset of symptoms) in 101 patients (14 with casirivimab/imdevimab and 87 with sotrovimab). Among 145 patients who had received at least one vaccination dose before infection, 109 patients were considered as adequately vaccinated. Multivariate analysis revealed that the Charlson Comorbidity Index (*P* 0.001; OR 1.28, CI 1.11–1.48) was associated with the primary outcome, while early administration of mAbs (*P* 0.032; OR 0.39, CI 0.16–0.92) was associated with a better outcome, but not infection during the period of the omicron variant predominance or adequate vaccination.

Keywords: COVID-19, kidney transplantation, vaccination, outcome, monoclonal antibodies, SARS-CoV-2



Graphical Abstract |

INTRODUCTION

Kidney transplant recipients (KTR) represent a high-risk group for adverse outcomes of Coronavirus Disease 2019 (COVID-19) due to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), because of the burden of immunosuppression and the presence of comorbidities (obesity, diabetes mellitus, hypertension and cardiovascular diseases) (1, 2). In the first wave of the pandemic before specific anti-SARS-CoV-2 treatments were available, the overall mortality varied between centers, ranging from 19% to 50% (1–3). Acute kidney injury (AKI) was seen in 30%–89% of hospitalized patients and reported graft loss ranged between 4% and 11% (1, 2). These early studies usually included patients with moderate or severe disease, due to lack of testing for mild cases. As the pandemic evolved, subsequent studies showed an overall decrease of mortality, mostly attributed to earlier diagnosis (due to greater accessibility of testing), improvements in supportive care, and potential impact of preventive and therapeutic measures such as the use of corticosteroids, tocilizumab, anti-SARS-CoV-2 monoclonal antibodies (mAbs) and vaccination (4, 5).

Despite the availability of vaccination, solid organ transplant (SOT) recipients are known to elicit reduced humoral responses to mRNA SARS-CoV-2 vaccines, compared to the immunocompetent population (6–10). Variables described to be associated with lower or nonresponse to vaccination were older age, high dose corticosteroids, maintenance under triple immunosuppressive treatment and in particular the use of mycophenolic acid (MPA) (8). Some studies have additionally shown a higher risk for breakthrough COVID-19 in vaccinated SOT recipients as compared to the general population, although vaccinated patients had lower rates of hospitalization as compared to unvaccinated KTR (11, 12). The administration of early treatment with mAbs (casirivimab/imdevimab and sotrovimab) targeting the spike protein of SARS-CoV-2 has been used for high-risk patients with mild to moderate COVID-19, with promising results by reducing morbidity and mortality (13, 14). However, data on the efficacy of mAbs in the KTR population remain scarce, especially regarding sotrovimab (14–17). Some case-control studies performed in KTR showed that the administration of mAbs halted the progression of COVID-19 symptoms and decreased the number of hospitalizations related to COVID-19, with a good safety profile (15–18). In Switzerland, two mAbs became available in 2021: casirivimab/imdevimab and sotrovimab.

In this study, we aim to describe the evolving epidemiology of SARS-CoV-2 infections in Swiss KTR since the beginning of the pandemic, to assess the overall morbidity and mortality as well as the potential beneficial impact of anti-SARS-CoV-2 vaccination and mAbs on patients and grafts outcomes.

PATIENTS AND METHODS

Study Design

This observational retrospective study was conducted at the Lausanne University Hospital (Lausanne, Switzerland), a 1500-bed tertiary care hospital and one of the six kidney transplantation centers in Switzerland. Our institution performs around 60 kidney transplantations per year and

regularly follows around 1000 KTR. The study was approved by the institutional ethics review board (Swissethics Project-ID 2022-00324) for the retrospective use of clinical data.

Patients

All adult (≥18 years old) KTR followed at our Transplantation Center who were diagnosed with a microbiologically-proven SARS-CoV-2 infection by real-time PCR between March 1st, 2020 and May 20th, 2022, were included in the analysis. Subsequent episodes of COVID-19 were included if they occurred at least 3 months after the previous one, based on reappearance of typical COVID-19 symptoms and *de novo* positive SARS-CoV-2 real-time PCR. Patients that had previously refused the institution's general consent and those with graft loss (re-initiation of dialysis at the time of the study) were excluded. Patients were identified by the preexistent database including all KTR followed at our center. All patients were instructed to contact the transplantation center in case of COVID-19-compatible symptoms and following a positive antigenic test or PCR for SARS-CoV-2 irrespective of symptoms. Nephrologists responsible for the care of patients in other associated centers were additionally instructed to communicate with our center in the event of a positive case. Data were prospectively collected for all cases of COVID-19 in KTR in a secured database.

Immunosuppressive Protocols

Depending on their immunological risk, KTR received basiliximab or anti-thymocyte globulins induction therapy (Thymoglobulin®). Maintenance immunosuppressive protocol generally consisted of the combination of a calcineurin inhibitor (CNI; mainly tacrolimus, TAC), mycophenolic acid (MPA), and prednisone following a tapering protocol during the first year. Beyond the first year, prednisone (5 mg/day) was only maintained in high immunological risk recipients. TAC doses were adjusted according to therapeutic drug monitoring and MPA according to digestive and haematological tolerability. All patients received co-trimoxazole prophylaxis during the first 6 months, and valgancyclovir or valacyclovir during the first 3 to 6 months according to donor/recipient serostatus.

Management of Patients With COVID-19

Prevention and treatment of COVID-19 in KTR varied over time according to the availability of the different drugs and vaccines. From March 2020 to June 2020, only investigational drugs were used *via* the inclusion in clinical trials (hydroxychloroquine, lopinavir, remdesivir). Since June 2020, dexamethasone was used in all patients needing supplemental oxygen therapy. Tocilizumab was administered in selected patients not responding to dexamethasone. Remdesivir was not used in hospitalized patients on a routine basis. The vaccination campaign started in January 2021 and KTR were considered as a priority group for vaccination. Two doses of an mRNA vaccine (mRNA-1273 or BNT162b2) were proposed initially, with a third dose proposed from September 2021. Casirivimab/imdevimab (2400 mg) was available since July 2021. Sotrovimab (500 mg) was available in Switzerland since September 2021, although it was used at our institution only from end of December 2021, based on data regarding the reduced activity on the omicron variant of casirivimab/imdevimab as compared to sotrovimab (19, 20). Anti-Spike mAbs were proposed to all KTR with documented mild or moderate COVID-19 within 5–7 days of onset of symptoms (considered in this study as "early treatment"). From March 15th, 2022, the dose of administered sotrovimab was doubled to increase its activity against the predominant omicron BA.2 variant (21). In addition, casirivimab/imdevimab was used in selected patients with severe COVID-19 and negative SARS-CoV-2 serology, according to the Recovery study (22). In this case, we used the term "late treatment" with mAbs. Following a positive SARS-CoV-2 test, MPA dosage was reduced by 50% or even stopped depending on the severity of the disease and/or concomitant administration of high dose corticosteroids. TAC trough levels were also decreased by around 30%.

Outcomes and Data Collection

The primary outcome was death or hospitalization for COVID-19-related symptoms within 28 days from the diagnosis of infection. The secondary outcome was defined as need for oxygen therapy within 28 days. Data regarding demographics (age, sex), comorbidities, transplantation characteristics (date of transplantation, immunosuppression, graft function), vaccination status (BNT162b2 or mRNA-1273), SARS-CoV-2 serology, specific anti-SARS-CoV-2 treatments including mAbs (casirivimab/imdevimab and sotrovimab), and complications were collected in the patients' electronic health records. SARS-CoV-2 serology (IgG) was performed using a previously described Luminex-based (Luminex Corp) assay quantifying antibody binding to the trimeric form of the SARS-CoV-2 S-protein and divided by the negative control; a ratio of ≥5.9 was considered positive (23).

All data were collected, stored and managed using REDCap electronic data capture tools hosted at Lausanne University Hospital. REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies (24, 25).

Definitions

The date of the first positive SARS-CoV-2 PCR was defined as infection onset. Acute kidney injury (AKI) was defined according to the 2012 Kidney Disease Improving Global Outcome (KDIGO) guidelines. Reduction of immunosuppressive treatment was defined as at least 50% MPA or 30% TAC dose decrease. Adequate vaccination was defined as having received three doses before infection or developing infection within 4 months after two doses. By using the data from the Swiss Federal Office of Public Health (26) that monitored the circulation and prevalence of SARS-CoV-2 variants, we divided the study in four periods: Period 1 (March to December 2020): pre-vaccination period, with the initial virus or alpha variant; Period 2 (January to June 2021): vaccination available but before mAbs, with the alpha and delta variants; Period 3 (July to December 2021): vaccination available and mAbs, with the delta variant; and Period 4 (January to May 2022): vaccination available and mAbs, with the omicron variant.

TABLE 1 | Patients' characteristics depending on the period of SARS-CoV-2 diagnosis.

Characteristics	Period 1 (*n* = 63)	Period 2 (*n* = 24)	Period 3 (*n* = 41)	Period 4 (*n* = 115)	All episodes (*n* = 243)
Demographics					
Male sex	40 (64%)	18 (75%)	27 (66%)	72 (63%)	157 (65%)
Age (years)	62 (50–70)	60 (48–68)	55 (43–67)	57 (43–66)	58 (45–68)
Co-morbidities					
Coronary heart disease	8 (13%)	3 (13%)	4 (10%)	19 (17%)	34 (14%)
Congestive heart failure	1 (2%)	1 (4%)	2 (5%)	5 (4%)	9 (4%)
Chronic obstructive pulmonary disease	2 (3%)	2 (13%)	2 (5%)	6 (5%)	13 (5%)
Diabetes mellitus	16 (25%)	7 (29%)	10 (24%)	25 (22%)	58 (24%)
Malignancy (solid organ or hematologic)	9 (14%)	1 (4%)	0 (0%)	5 (4%)	15 (6%)
Obesity	10 (16%)	7 (29%)	10 (24%)	26 (23%)	53 (22%)
Charlson Comorbidity Index	4 (3–6)	5 (3–6)	4 (2–5)	4 (2–6)	4 (2–6)
Transplantation data					
Years from transplantation	6 (3–12)	7 (3–12)	6 (3–11)	6 (3–11)	6 (3–12)
Combined kidney and other organ transplantation	3 (5%)	1 (4%)	3 (7%)	7 (6%)	14 (6%)
Immunosuppressive treatment					
Tacrolimus	50 (79%)	23 (96%)	39 (95%)	101 (88%)	213 (88%)
Cyclosporine	5 (8%)	0 (0%)	1 (2%)	6 (5%)	12 (5%)
Mycophenolic acid	42 (71%)	20 (83%)	32 (78%)	91 (79%)	188 (77%)
Azathioprine	2 (3%)	1 (4%)	7 (17%)	9 (8%)	19 (8%)
Prednisone	43 (68%)	16 (68%)	26 (63%)	84 (73%)	169 (70%)
Other	2 (3%)	1 (4%)	1 (2%)	8 (7%)	12 (5%)
Triple immunosuppressive treatment	31 (49%)	13 (54%)	25 (61%)	75 (65%)	144 (59%)
Rituximab or Thymoglobulin (within the last year)	2 (3%)	1 (4%)	1 (2%)	6 (5%)	10 (4%)
Vaccination status					
No vaccination	63 (100%)	18 (75%)	6 (15%)	11 (10%)	98 (40%)
One dose	0 (0%)	1 (4%)	2 (5%)	1 (1%)	6 (3%)
Two doses	0 (0%)	3 (13%)	23 (56%)	18 (16%)	44 (18%)
Three doses	0 (0%)	0 (0%)	10 (24%)	85 (74%)	95 (39%)
Adequate vaccination	0 (0%)	3 (13%)	15 (37%)	91 (79%)	109 (45%)
Serology before infection (among 103 episodes)[a]	—	—	6.5 (0.9–29.1)	28.9 (8.8–83.4)	27.0 (3.5–72.9)
Positive serology	—	—	11 (61%)	66 (80%)	83 (77%)
SARS-CoV-2 infection					
Community	59 (94%)	20 (83%)	40 (98%)	111 (97%)	230 (95%)
Nosocomial	4 (6%)	4 (17%)	1 (2%)	4 (4%)	13 (5%)
Reduction of immunosuppression	23 (37%)	6 (25%)	16 (39%)	8 (7%)	53 (22%)
Monoclonal antibodies (as early treatment)	0 (0%)	0 (0%)	19 (46%)	82 (71%)	101 (42%)
Casirivimab/imdevimab	0 (0%)	0 (0%)	14 (34%)	0 (0%)	14 (6%)
Sotrovimab	0 (0%)	0 (0%)	5 (12%)	82 (71%)	87 (36%)
Hospitalization (within 28 days)	34 (54%)	8 (33%)	15 (37%)	20 (17%)	77 (32%)
Hospitalization due to COVID-19	31 (52%)	6 (30%)	14 (34%)	15 (15%)	66 (30%)
Need for oxygen therapy (secondary outcome)	21 (33%)	5 (21%)	12 (29%)	6 (5%)	44 (18%)
Non-mechanical ventilation or Optiflow	7 (11%)	4 (17%)	4 (10%)	2 (2%)	17 (7%)
Intensive Care Unit hospitalization	8 (13%)	4 (17%)	6 (15%)	2 (2%)	20 (8%)
Mechanical ventilation	4 (6%)	2 (8%)	4 (10%)	1 (1%)	11 (5%)
Treatment					
Convalescent plasma	2 (3%)	4 (17%)	0 (0%)	2 (2%)	8 (3%)
Lopinavir/ritonavir	2 (3%)	0 (0%)	0 (0%)	0 (0%)	2 (1%)
Hydroxychloroquine	3 (5%)	0 (0%)	0 (0%)	0 (0%)	3 (1%)
Remdesivir	3 (5%)	0 (0%)	0 (0%)	0 (0%)	3 (1%)
Tocilizumab	0 (0%)	2 (8%)	3 (7%)	0 (0%)	5 (2%)
Casirivimab/imdevimab (as late treatment)	0 (0%)	0 (0%)	6 (15%)	0 (0%)	6 (3%)
Dexamethasone	16 (25%)	5 (21%)	11 (27%)	6 (5%)	38 (16%)
Death (within 28 days)	5 (8%)	1 (4%)	1 (2%)	1 (1%)	8 (3%)
Primary outcome (death or hospitalization for infection-related symptoms or complications)	32 (51%)	7 (29%)	14 (34%)	15 (13%)	68 (28%)
Acute complications					
Acute kidney injury	7 (11%)	4 (17%)	5 (12%)	3 (3%)	19 (8%)
Community-acquired pneumonia	4 (6%)	3 (13%)	4 (10%)	3 (3%)	14 (6%)
Renal function at 28 days					
Creatinine increase >15% from baseline (among 175 episodes)	6 (13%)	5 (28%)	5 (15%)	8 (10%)	24 (14%)
Creatinine increase ≥ AKIN stage I (among 175 episodes)	5 (11%)	4 (22%)	5 (15%)	3 (4%)	17 (10%)
De novo donor-specific anti-HLA antibodies (among 112 episodes)	3 (7%)	0 (0%)	1 (4%)	1 (2%)	5 (4%)

Data are depicted as number and percentage or median and Q1-3.

[a]*Six cases that belong in Periods 1 and 2 are not included.*

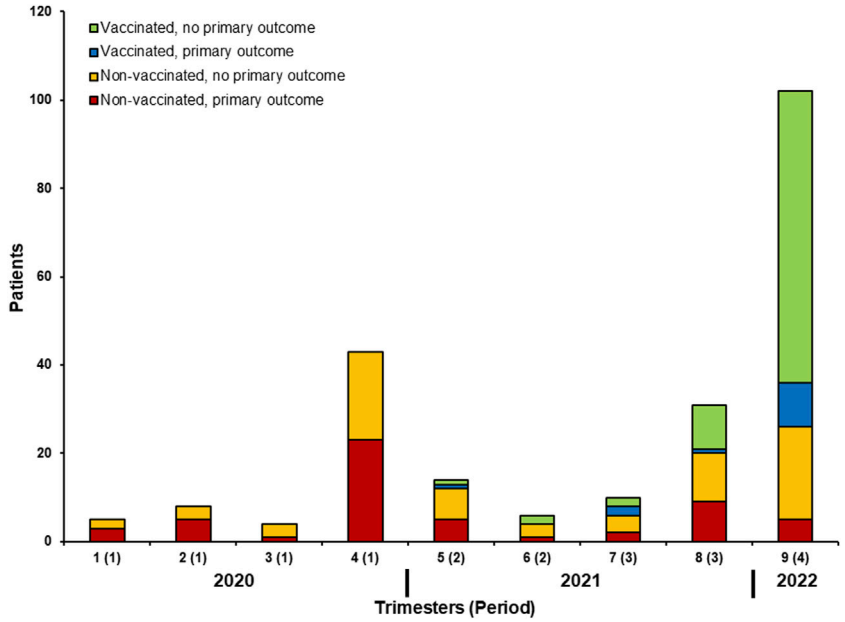


FIGURE 1 | Number of patients with the primary outcome depending on adequate vaccination and timing of SARS-CoV-2 infection.

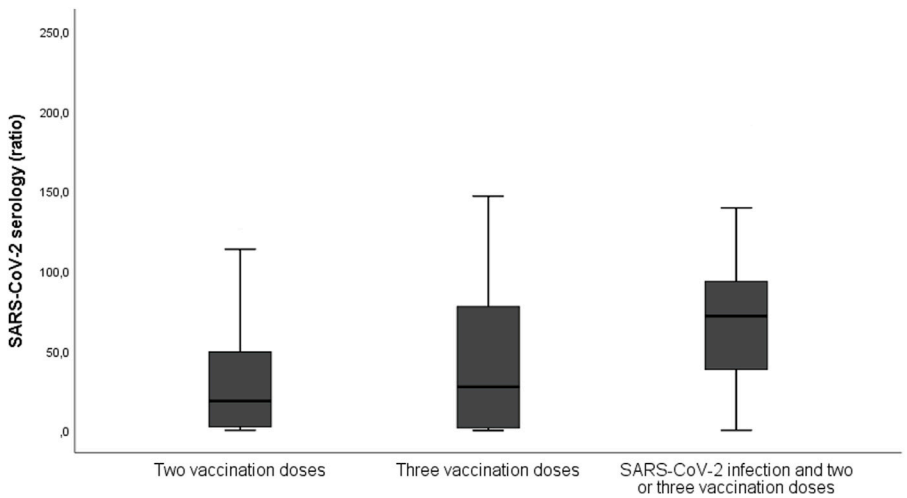


FIGURE 2 | Results of SARS-CoV-2 serology depending on its timing (after vaccination and/or SARS-CoV-2 infection). The serology was performed using Luminex-based assay quantifying antibody (IgG) binding to the trimeric form of the SARS-CoV-2 S-protein and divided by the negative control; a ratio of ≥5.9 was considered positive. The median ratio for patients with two vaccination doses without prior infection was 18.5, those with three vaccination doses without prior infection was 27.4, and for those with two or three doses and prior SARS-CoV-2 infection the ratio was 71.7.

Statistical Analyses

The SPSS version 26.0 (SPSS, Chicago, IL, United States) software was used for data analysis. Categorical variables were analyzed using the *chi*-square or Fisher exact test and continuous variables with Mann-Whitney *U* test. Two multivariate logistic regression analyses were performed with primary and secondary outcomes, respectively, as the dependent variables. Four variables from the univariate analysis with $p < 0.05$ (Charlson Comorbidity Index, adequate vaccination, mAbs as early treatment, Period 4) that did not contribute to multicollinearity were used in multivariate logistic regression model. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to evaluate the strength of any association. The primary and secondary outcomes trends during the pandemic periods were assessed using Spearman's correlation analysis. All statistic tests were 2-tailed and $p < 0.05$ was considered statistically significant.

TABLE 2 | Univariate and multivariate analyses among patients with and without the primary outcome.

Characteristics	Univariate analysis			Multivariate analysis	
	No primary outcome (*n* = 175)	Primary outcome (*n* = 68)	*P*	OR (95% CI)	*P*
Demographics					
Male sex	115 (66%)	42 (62%)	0.563		
Age (years)	55 (42–67)	63 (52–69)	0.001		
Co-morbidities					
Coronary heart disease	23 (13%)	11 (16%)	0.541		
Congestive heart failure	5 (3%)	4 (6%)	0.271		
Chronic obstructive pulmonary disease	6 (3%)	7 (10%)	0.033		
Diabetes mellitus	36 (21%)	22 (32%)	0.053		
Malignancy (solid organ or hematologic)	7 (4%)	8 (12%)	0.024		
Obesity	37 (21%)	16 (24%)	0.686		
Charlson Comorbidity Index	4 (2–5)	5 (4–7)	<0.001	1.28 (1.11–1.48)	0.001
Transplantation data					
Years from transplantation	7 (3–12)	5 (2–12)	0.492		
Combined kidney and other organ transplantation	9 (5%)	5 (7%)	0.507		
Immunosuppressive treatment					
Tacrolimus	154 (88%)	59 (87%)	0.793		
Cyclosporine	9 (5%)	3 (4%)	1.000		
Mycophenolic acid	137 (78%)	51 (75%)	0.610		
Azathioprine	16 (9%)	3 (4%)	0.291		
Prednisone	121 (69%)	48 (71%)	0.826		
Other	9 (5%)	3 (4%)	1.000		
Triple immunosuppressive treatment	110 (63%)	34 (50%)	0.067	0.83 (0.44–1.48)	0.574
Rituximab or Thymoglobulin (within the last year)	8 (5%)	2 (3%)	0.730		
Periods					
Period 1	31 (18%)	32 (47%)			
Period 2	17 (10%)	7 (10%)			
Period 3	27 (15%)	14 (21%)			
Period 4	100 (57%)	15 (22%)	<0.001[a]	0.60 (0.23–1.54)	0.288[a]
Vaccination status					
No vaccination	55 (31%)	43 (63%)			
One dose	4 (2%)	2 (3%)			
Two doses	31 (18%)	13 (19%)			
Three doses	85 (48%)	10 (15%)	<0.001[b]		
Adequate vaccination	95 (54%)	14 (21%)	<0.001	0.44 (0.18–1.09)	0.077
Serology before infection (among 109 episodes)	28.7 (6.4–81.1)	3.8 (0.6–36.2)	0.008		
Positive serology	77 (81%)	6 (46%)	0.005		
Monoclonal antibodies (as early treatment)	89 (51%)	12 (18%)	<0.001	0.39 (0.16–0.92)	0.032
Casirivimab/imdevimab	12 (7%)	2 (3%)	0.240		
Sotrovimab	77 (44%)	10 (15%)	<0.001		
Sotrovimab (double dose)	15 (9%)	2 (3%)	0.164		

Data are depicted as number and percentage or median and Q1-3.

[a]*Comparison of Period 4 to all other periods.*

[b]*Comparison between patients having received three doses and those that have not.*

RESULTS

Patients Characteristics

Overall, 246 KTR with at least one episode of COVID-19 were identified, for whom 243 episodes were included in the study corresponding to 237 patients (6 patients had two episodes of COVID-19 during the study period). Among the 9 patients that were excluded, 4 patients were excluded for refusal of general consent and 5 due to graft loss at the time of study initiation. Patients' characteristics according to the time-period of SARS-CoV-2 diagnosis are shown in **Table 1**. Overall, there was no significant difference in the demographic characteristics of the infected patients during the different pandemic periods. The majority of patients were middle-aged men with 22% suffering from obesity, 24% from diabetes, and/or 14% from coronary heart disease, representative of the general KTR population. The majority were on CNI-based (mainly TAC) triple immunosuppressive therapy, including prednisone (70%) and MPA (77%). No patient was on belatacept maintenance immunosuppressive therapy and only a minority of the study population (4%) had received T- or B-cell depleting agents in the previous year before suffering from COVID-19, and one patient received eculizumab every 3 weeks for the treatment of recurrent glomerulonephritis.

Outcomes

In total, 77 patients (32%) were hospitalized within 28 days from diagnosis; 66 patients were hospitalized due to COVID-19 symptoms and 44 patients needed oxygen therapy. Eight patients (3%) died within 28 days from the diagnosis of infection. Sixty-eight patients (28%) developed the primary outcome (hospitalization for COVID-19-related symptoms or death within 28 days from infection diagnosis) and 44 (18%) the secondary endpoint (need for oxygen therapy within 28 days). Hospitalization for COVID-19-related symptoms or death was seen in 45% (39/87) of patients during Period 1 and 2 and 19% (29/156) of patients during Period 3 and 4. A significant decrease in the incidence of primary ($p < 0.001$, r −0.342) and secondary outcomes ($p < 0.001$, r −0.311) was observed during the consecutive study periods. Overall, AKI (≥ AKIN stage I) was observed in 8% of KTR, and the same proportion (10–14%) of patients had persisting moderate to severe graft dysfunction at 28 days. Four patients lost their graft and returned to dialysis following severe COVID-19. Among 112 patients in whom anti-HLA Abs could be screened after the episode of SARS-CoV-2 infection, 5 (4%) developed *de novo* donor-specific anti-HLA Abs (DSA). There was however no episode of acute cellular or antibody-mediated rejection that could be associated with the infection.

Use of mAbs

In total, mAbs were administered as early treatment in 101 patients (14 with casirivimab/imdevimab and 87 with sotrovimab), and 6 (3%) additional patients received casirivimab/imdevimab as a late treatment (**Table 1**). Double dose of sotrovimab was administered in 17 patients, of whom two were hospitalized due to COVID-19 symptoms and one needed oxygen therapy.

Vaccination and SARS-CoV-2 Serostatus

Among 145 patients that had received at least one vaccination dose before infection, 109 (45% of all infection episodes) were considered as adequately vaccinated. **Figure 1** shows the number of patients with the primary outcome depending on adequate vaccination and timing of SARS-CoV-2 infection. Serology was performed in 109 patients at the time of SARS-CoV-2 infection diagnosis and it was positive in 83 (76%). Among 108 patients for whom serology was performed after two or three doses (without documented prior infection), 83 (77%) had positive serology. **Figure 2** shows the results of SARS-CoV-2 serology depending on the timing of sampling (after vaccination and/or SARS-CoV-2 infection). Serology results of patients with two or three vaccine doses and with prior SARS-CoV-2 infection (median ratio of 71.7) were significantly higher ($p < 0.001$) than for those who received two (median ratio of 18.5) or three doses (median ratio of 27.4) without prior infection.

Variables Associated With the Primary and Secondary Outcomes

Multivariate analysis revealed that the Charlson Comorbidity Index (*P* 0.001; OR 1.28, CI 1.11–1.48) was associated with the primary outcome, while administration of mAbs as early treatment (*P* 0.032; OR 0.39, CI 0.16–0.92) was associated with a better outcome (**Table 2**). Of note, adequate vaccination and infection during Period 4 were associated with improved primary outcome in the univariate analysis, but this was not confirmed in the multivariate analysis. In the multivariate analysis for the secondary outcome (hospitalization for need of oxygen), the Charlson Comorbidity Index (*P* 0.001; OR 1.30, CI 1.11–1.51) increased the risk of secondary outcome, while administration of mAbs as early treatment (*P* 0.009; OR 0.19, CI 0.06–0.66) was associated with a reduced risk for the secondary outcome. Similarly, adequate vaccination and infection during Period 4 were not associated with the secondary outcome.

DISCUSSION

The first aim of this study was to describe the epidemiology of SARS-CoV-2 infection in at-risk immunosuppressed KTR, based on the evolution of the pandemic and the availability of preventive and therapeutic measures. Interestingly, we observed that adverse outcomes related to COVID-19 (death, SARS-CoV-2-related hospitalizations) declined over time (51% in Period 1 to 13% in Period 4), similar to what has been described in the general population (27). As the patients' demographic characteristics did not significantly differ over time, these outcomes could be mainly explained by the pathogenicity of the prevalent variants during the different periods of the study, together with better preventive and therapeutic management of KTR with COVID-19. An important finding of this study is that administration of mAbs as ealry treatment was associated with lower rates of adverse outcomes (mortality or hospitalization). Only 12% of patients who received mAbs were hospitalized for SARS-CoV-2-related symptoms or died within 28 days of the diagnosis of infection. These results are similar to what was previously reported in two studies using bamlanivimab or casirivimab/imdevimab in SOT recipients (16, 28), although another study did not confirm this positive impact in immunosuppressed SOT recipients (29). To the best of our knowledge, this is the largest study in kidney transplantation that describes patients' management and outcomes over time during the 2 years of SARS-CoV-2 pandemic. In addition, we report a beneficial effect of sotrovimab administration in KTR, with a significant reduction of deaths or hospitalizations within 28 days of infection diagnosis. Our results corroborate a recent publication that describes the benefit of an early use of mAbs in KTR with a mild form of COVID-19 (30). This is also the first study, reporting the preventive use of a double dose of sotrovimab against omicron BA.2 variant, with only one patient (6%) subsequently admitted for oxygen therapy. While in Switzerland mAbs are used only as an early treatment, neutralizing anti–SARS-CoV-2 mAbs such as casirivimab/imdevimab were used as pre-exposure prophylaxis in SOT recipients with weak or no humoral response after vaccination (3 doses of an mRNA vaccine). This latter strategy was shown to be efficient in preventing COVID-19 incidence in SOT, compared to untreated controls (17).

An important observation in our study is that the humoral response to adequate vaccination was higher than previously reported (14%–38%) among KTR (8, 31, 32). A possible explanation could be the different testing methods used and the absence of a well-established protective antibody titer. For the chosen cut-off of positivity defined at a ratio >5.90, the assay used in the present study has shown a sensitivity and specificity of 97% and 98%, respectively, in hospitalized patients (23). As compared to a healthy control population, the predictors of failure for SOT recipients to mount a humoral response were described to be higher age, need for high-dose corticosteroids during the last year, maintenance under triple immunosuppressive therapy, and a regimen that included MPA (8, 31). In our study, no factor among the studied ones was found to be associated with the humoral response in KTR.

Patients with an increased Charlson Comorbidity Index, incorporating age and comorbidities, had a higher risk of death or hospitalization within 28 days from infection diagnosis, whatever the study period. While in previous reports SOT recipients' characteristics differed between the various waves of the SARS-CoV-2 pandemic, with higher rates of high-risk comorbidities (cardiovascular, pulmonary) in the earlier periods (4), no such difference was found in the present study. Thus, comorbidities did not play a role in the lower mortality observed in the later periods of our study.

The study has several limitations. First, it is a retrospective monocentric study including a relatively moderate number of patients. Second, there is a selection bias towards symptomatic patients, as paucisymptomatic or asymptomatic KTR that did not seek medical attention and did not have a PCR-documented infection were not included in the study. This bias should be minimal, since KTR were strongly advised to be tested and to contact their physician at the occurrence of the first symptoms. Third and more importantly, the study included patients during a 2-year period with a changing viral epidemiology, SARS-CoV-2 variants associated with diverse pathogenicity (33), and different therapeutic (mAbs) or preventive modalities (vaccination); all factors influencing the outcomes. We cannot exclude that some confounders were not adjusted in the multivariate analyses. Finally, viral sequencing was not routinely available, so that we used the period of infection as a proxy for the different variants, as done in other epidemiological studies (27). Thus, some misclassification cannot be excluded.

In conclusion, we observed a decrease in unfavorable outcomes of infected KTR in the last wave of the pandemic. Although these changes are probably due to a combination of factors, we identified the use of mAbs as the only measure significantly associated with a better outcome. Prospective studies are needed to better delineate the role of mAbs and vaccination in preventing COVID-19-associated complications in immunocompromised patients, particularly in the era of the new variants.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The study involving human participants was reviewed and approved by the institution's Ethics Committee (CER-VD, 2022-00324) for the retrospective use of clinical data. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors contributed in collecting the clinical data and in the care of the patients. MP-O, AC, NG, OM, and DG drafted the paper. MP-O, AC, OM, and DG reviewed and analyzed the data. MP-O, OM, and DG reviewed the final version of the paper.

CONFLICT OF INTEREST

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

ACKNOWLEDGMENTS

We thank all patients who participated in the study, the nurses, and the physicians and nephrologists responsible for the care of kidney transplant recipients in our Transplantation Center as well as in other centers associated with Lausanne University Hospital.

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LETTER TO THE EDITOR
published: 10 October 2022
doi: 10.3389/ti.2022.10740

Successful Heart Transplantation After 7 h of Cold Storage and Paracorporeal Donor Heart Resuscitation

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Keywords: heart transplantation, donor heart recuscitation, cold storage, *ex vivo* perfusion, cytokine adsorption

Dear Editors,

The need of organs for transplantation is increasing worldwide, though the availability of them does not meet the existing demand (1). In some countries, factors limiting even "ideal" heart utilization include the distance between the donor and recipient hospitals, weather conditions (2). "Suboptimal" organs are more often used for transplantation (3). The risk of primary graft failure and death rises dramatically for the heart as ischemic time increases (4). Real life practice demonstrates that accidental rescue might become an acceptable strategy in the future.

We report a case of paracorporeal donor heart resuscitation followed by successful heart transplantation after 7 h of cold storage.

Fifty-five years old male patient was diagnosed with dilated cardiomyopathy at 2012 years. He underwent a HeartMate3 left ventricular assist device (LVAD; Abbott Inc.) implantation as a bridge to heart transplantation (HTx) in January 2018. His clinical status was complicated by a deep driveline infection. After initial antibiotic therapy, the patient underwent two driveline site debridement surgeries in May and September 2019. Recurrent infections with resistant pathogens (*Burkholderia cepacia*, *Staphylococcus aureus*) resulted in a higher waitlist status priority for HTx.

A suitable donor heart became available from a 42-year-old male who had had a hemorrhagic stroke. Before explant for hemodynamic stabilization, he needed infusion of norepinephrine 0.1 mcg/kg/min and dobutamine, 15 mcg/kg/min. Echocardiographic evaluation of the donor heart showed (LVEF 62%) no abnormalities, 2 days in ICU and 1 day on ventilator. Donor and recipient had the blood group O (I) Rh-positive and AB (IV) Rh-positive status, respectively. Our institution is sole transplant center in the country, and donor hearts are often retrieved from distant regions to be transplanted (5). This time the distance between the two hospitals was 1,000 km. Furthermore, poor winter weather conditions delayed the flight of organ retrieval team. Therefore, travel time on a return journey was estimated to be at least 6 h. The donor heart was arrested with the standard heart preservation solution (4°C Custodiol) and preserved in standard way of cold ischemic storage.

When the donor heart arrived to the clinic, the time of cold ischemic storage was already 430 min. Severe, time consuming, and technically demanding reoperation for LVAD explantation anticipated additional time. Due to the high risk of graft failure and post implantation dysfunction, the decision

Abbreviations: ICU, intensive care unit; LVAD, left ventricular assisted device; CPB, cardiopulmonary bypass; LVEF, left ventricular ejection fraction; S1LV, myocardial velocity associated with isovolumic contraction of left ventricle; S1RV, myocardial velocity associated with isovolumic contraction of right ventricle.

OPEN ACCESS

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Received: *04 July 2022*
Accepted: *29 September 2022*
Published: *10 October 2022*

Citation:
Kaliyev R, Lesbekov T, Bekbossynova M, Nurmykhametova Z, Medressova A, Kuanyshbek A, Faizov L, Samalavicius R and Pya Y (2022) Successful Heart Transplantation After 7 h of Cold Storage and Paracorporeal Donor Heart Resuscitation. Transpl Int 35:10740. doi: 10.3389/ti.2022.10740

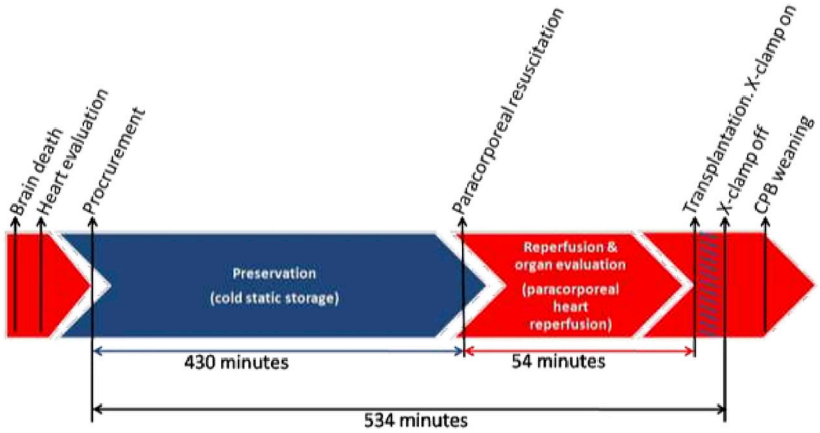

FIGURE 1 | Process of heart transplantation.

was made to perfuse the allograft through cardiopulmonary bypass (CPB) circuit of recipient and assess its function after reperfusion. The ascending aorta of donor heart was connected in end-to-end manner to splitted systemic flow line *via* 3/8 connector. No vents were used making open blood drainage from organ chambers into container and then to cardiotomy reservoir through suction pump. Standard gases and lactate samples of freely draining blood were collected from the container and measured during paracorporeal resuscitation stage.

Following 6 min of reperfusion of the heart, stable sinus rhythm was restored, using the 15 J shock from external biphasic defibrillator. The standard CPB roller pump was used (Stockert S5, LivaNova Deutschland GmbH), with the mean aortic pressure between 68 and 84 mmHg and coronary blood flow between 800–860 ml/min at a temperature of 35C. The graft was conditioned with 45 μg/kg Levosimendan infusion in the coronary perfusion and cytokine adsorption (CytoSorb, Cytosorbents Inc., NJ, United States) with a blood flow of 200–300 ml/min was applied from recipient's CPB circuit. Periodic arterial and venous blood samples were taken from donor heart during perfusion period of 54 min. After organ evaluation, the decision was made to use the heart for transplantation based on surgeon observation, good visual contractility, stable sinus rhythm, and lactate values. The donor heart was arrested with 1 L of regular normothermic blood cardioplegia solution just before transplanting. It took two cardioplegia doses before resuming systemic blood flow in the heart. The bi-atrial technique of performing orthotopic cardiac transplantation has been utilized. In 52 min, the heart rhythm was restored. Transplantation and preoperative care proceeded according to the standard procedures of our center. The next day after HTx the remnants of driveline and ICD with leads were extracted. Prolonged suppressive antibiotic therapy with Colistin, Meropenem and Vancomycin in combination with Cansidas was used up to 4 weeks.

Total cold ischemic time was 430 min and duration of coronary perfusion was 54 min. This resulted in a total cross-clamp to cross-clamp time of 534 min (**Figure 1**). Venous lactate taken from freely draining blood were collected from the donor heart container at the start of coronary perfusion was 2.2 mmol/L, and 3.0 mmol/L at the end. Total CPB time of the recipient was 183 min including 40 min of warm reperfusion after transplantation. Patient was weaned from cardiopulmonary bypass administrating norepinephrine 0.1 mcg/kg/min and dobutamine 7 mcg/kg/min infusion. The recipient was extubated 23 h following surgery, discharged from the hospital 10 days after. The heart function was measured by Swan-Ganz catheter showing 6.4 L/min of cardiac output and 4 L/min/$m^2$of cardiac index. Echocardiography parameters confirmed normal biventricular function: S^1LV lateral 21 cm/s, S^1LV medial 7.8 cm/s, S^1RV 7.6 cm/s, LVEF 68%. For over 9 mon post-transplant, there is no evidence of rejection or cardiac dysfunction.

With prolonged ischemic time, the donor graft can be transported for longer distances and increase the chances of gaining a matching donor graft. We report a successful heart transplantation following donor heart resuscitation after 7 h of cold storage using recipient's cardiopulmonary bypass circuit prior to organ implantation. This maneuver allowed to start resuscitation of the donor organ by means of controlled warm reperfusion, medical treatment and cytokines adsorption. It is remarkable that this scenario was covered by recipient's physiologic conditions, i.e., patient's multiorgan system reserve capacity.

To the best of our knowledge, this is the first reported case of paracorporeal resuscitation of donor heart after 7 h of cold static storage using recipient's cardiopulmonary bypass circuit to be successfully transplanted.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Local Bioethics Committee of the National

Research Cardiac Surgery Center (No. 01-74/2021 from 10/06/20). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

RK, concept/design, approval of article; TL, data analysis/interpretation, critical revision of article; MB, concept/design; ZN, data analysis/interpretation, critical revision of article, data collection; AM, data collection; AK, data collection; LF, data collection; RS, statistics, data interpretation; YP, approval of article. All authors read and approved the final manuscript.

FUNDING

This research has been funded by the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan (Grant No. BR10965200) https://www.ncste.kz/en/main. The funders had no role in study design, data collection and analysis, decision to publish, or preparation 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.

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LETTER TO THE EDITOR
published: 03 October 2022
doi: 10.3389/ti.2022.10846

First Report on Successful Conversion of Long-Term Treatment of Recurrent Atypical Hemolytic Uremic Syndrome With Eculizumab to Ravulizumab in a Renal Transplant Patient

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Keywords: kideny transplantation, ravulizumab, eculizumab, atypical hemolytic uremic syndrome, C5 inhibition

Dear Editors,

Ravulizumab is a long-acting C5-complement monoclonal antibody developed through targeted modifications of eculizumab to significantly extend the half-life of the drug with comparable affinity and specificity to eculizumab (approx. 52 days vs. approx. 11 days) [1]. The efficacy and safety of ravulizumab in patients with aHUS treated with or without complement inhibitors has been adequately studied in adults [2] and pediatric patients [3] and recently led to the approval of the drug by the European Medicines Agency and the US Food and Drug Administration (Ultomiris® SmPC). During 26 weeks of treatment, ravulizumab provided rapid and effective complement inhibition with no unexpected safety issues.

In renal transplant patients, there has been only a single report of ravulizumab use. Ravulizumab was successfully administered in the case of a living kidney donation in a patient with aHUS over the reported treatment period of 6 months [4].

Here we report the results of a young woman who was successfully switched from chronic aHUS treatment with eculizumab to ravulizumab after kidney transplantation.

Back in 2013, we published on the long-term eculizumab treatment of a kidney transplant patient who had a relapse of her aHUS shortly after a living kidney donation [5]. The cause of the aHUS relapse was an MCP mutation and, as was determined in a later analysis, also a factor H mutation. Recurrent aHUS attributable to both complement factor mutations requires lifelong anti-C5 treatment due to high risk [6]. Our patient had been treated with eculizumab administered every 14 days for more than 10 years. As shown in **Figure 1**, the complete available laboratory data of creatinine, hemoglobin, and platelets show a very stable course of the patient. Remarkably, only one episode of fever occurred during the entire observation period, the cause of which remained unclear. However, the patient achieved *restitutio ad integrum* with short-term inpatient treatment with piperacillin/tazobactam. Because eculizumab has been shown to be effective after renal transplantation for treatment of aHUS and because ravulizumab is a modified version of eculizumab, we expected comparable efficacy and safety of both products [7]. Immunosuppressive therapy consisted of tacrolimus (target through 4–6 ng/ml), low dose mycophenolate mofetil, and prednisolone. At the time of conversion, our 39-year-old patient (body weight 70 kg, BMI 19.6 kg/m^2) had a serum creatinine of 1.66 mg/dl (eGFR 39 ml/min/1.73 m^2 (CKD-EPI formula)), hemoglobin concentration of 11.6 g/dl, and platelet count of 359 × 10^3/mm^3). After 22 months of therapy with ravulizumab 3,300 mg every 8 weeks following an induction therapy with additional administration of 3,300 mg 2 weeks after the first infusion according to the prescribing

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Received: *19 August 2022*
Accepted: *22 September 2022*
Published: *03 October 2022*

Citation:
Jehn U, Altuner U, Pavenstädt H and Reuter S (2022) First Report on Successful Conversion of Long-Term Treatment of Recurrent Atypical Hemolytic Uremic Syndrome With Eculizumab to Ravulizumab in a Renal Transplant Patient. Transpl Int 35:10846. doi: 10.3389/ti.2022.10846

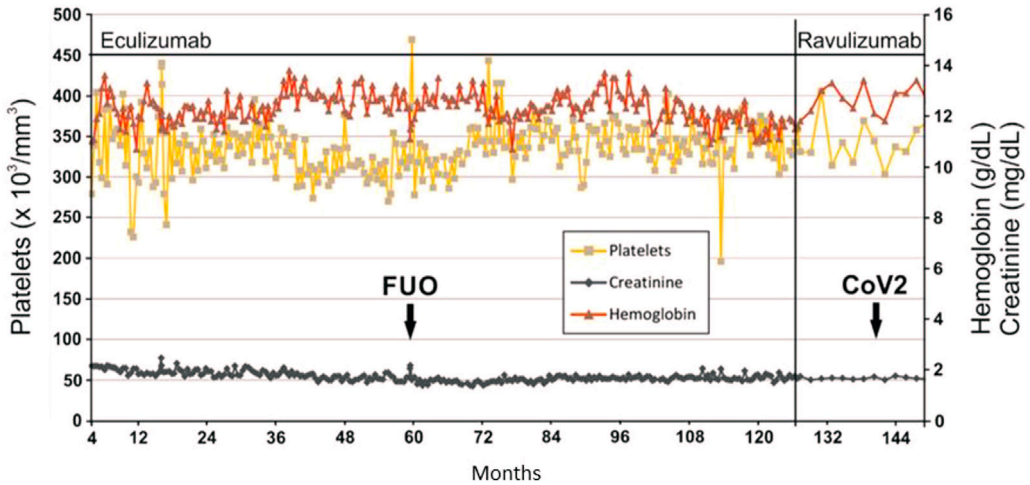

FIGURE 1 | Laboratory values under complement inhibition with eculizumab or ravulizumab. FUO, fever of unknown origin; CoV2, SARS CoV2 infection.

information, serum creatinine [1.63 mg/dl (eGFR 39 ml/min/1.73 m^2)], hemoglobine (12.6 g/dl), and platelet count ($367 \times 10^3/mm^3$) were stable over time (**Figure 1**). However, 14 months after conversion, SARS-CoV-2 infection was diagnosed out-of-hospital between two infusion appointments without our knowledge. The patient, who had been vaccinated three times had severe illness lasting 10 days, but without respiratory distress or graft failure. The patient's migraine was not changed by the switch to ravulizumab.

We present this case report because ravulizumab therapy offers improvement in health-related quality of life and greater cost-effectiveness compared with eculizumab therapy because of the longer interval between infusions [8]. The presented case demonstrates that switching C5 inhibition to ravulizumab is safe and effective in renal transplant patients with genetic aHUS, even after decades of therapy with eculizumab. It should be noted that meningococcal vaccination or prophylaxis must be continue with ravulizumab administration (Ultomiris* SmPC). Because ravulizumab-based therapy offers significant health-related quality of life and cost-effectiveness benefits, it may be the therapy of choice for these patients.

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 case sudy was approved by the local ethics committee (Ethik Kommission der Ärztekammer Westfalen-Lippe und der Medizinischen Fakultät der Westfälischen Wilhelms-Universität). It was performed in accordance with the current transplantation guidelines and the 1964 Declaration of Helsinki. Written informed consent was obtained from the patient for publication of this case report.

AUTHOR CONTRIBUTIONS

UJ and SR collected the data and wrote the letter, UA and HP revised it.

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

FUNDING

The APC was funded by the Open Access Publication Fund of University of Münster.

CONFLICT OF INTEREST

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

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LETTER TO THE EDITOR
published: 14 October 2022
doi: 10.3389/ti.2022.10693

Enteric Budesonide in Transplant and Native IgA Nephropathy: Real-World Clinical Practice

Marina Lopez-Martinez[1,2], Irina Torres[1,2], Sheila Bermejo[1,2], Francesc Moreso[1,2], Clara Garcia-Carro[3,4], Ander Vergara[1,2], Natalia Ramos[1,2], Manel Perello[1,2], Alejandra Gabaldon[2,5], M. Antonieta Azancot[1,2], Monica Bolufer[1,2], Nestor Toapanta[1,2], Oriol Bestard[1,2], Irene Agraz-Pamplona[1,2†] and Maria Jose Soler[1,2*†]*

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Keywords: IgA nephropathy, IgAN recurrence in transplant kidney, proteinuria reduction, TRF-budesonide, CKD progression

Dear Editors,

Targeted-release-formulation of budesonide (TRF-budesonide) has demonstrated promising results in terms of proteinuria and renal function in patients with immunoglobulin A nephropathy (IgAN) (1). Regarding its well tolerance, enteric budesonide may become the first step of immunosuppressive treatment of IgAN, although real world clinical practice publications are lacking (2-4). We evaluated the effect of budesonide in our cohort of patients affected by IgAN. We included all patients, either transplanted kidney or native, which were diagnosed of IgAN and were treated with enteric budesonide in our center from December 2017 to January 2022. At baseline clinical and analytical parameters were collected during the next 3, 6, 12, and 24 months. We also assessed the occurrence of budesonide-related adverse events.

A total of 14 patients were included in the study. Nine of the patients had IgAN in their native kidneys (7 males) and 5 were transplanted (5 males), age of 46 ± 17.21 years. The relative decrease of proteinuria was of 33.1% and 54.6% after 3 and 6 months of treatment with budesonide, respectively ($p < 0.05$) (**Table 1**). Evaluating native and transplant kidney separately, proteinuria in transplant kidney also significantly decreased (26.7%) after 3 months of treatment (**Table 1**). These results are in line with previous literature (2–6). There is increasing evidence about the role of gut-associated lymphoid tissue and complexes with Gd-IgA1 deposition in IgAN pathogenesis (7). The first study that evaluated TRF-budesonide published a significant albuminuria reduction of 40% in 16 patients with IgAN after 2 months of treatment (2). Afterwards, the phase 2b clinical trial NEFIGAN demonstrated significant proteinuria reduction (21%–27%) in 199 patients with IgAN after 9 months of treatment with TRF-budesonide (5). This latest trial justified to carry out the phase 3 trial NEFIGARD, where TRF-budesonide significantly reduced UPCR by 27% of 199 patients (6). There is only another retrospective study that evaluated the effect of TRF-budesonide in native kidneys IgAN with significant proteinuria reduction (3). This constant effect of local budesonide in proteinuria reduction is quite remarkable, as proteinuria is considered as the main sign of disease progression in IgAN (7) and a surrogate marker of kidney outcome in IgAN (8).

To our knowledge, there is only a case report published that described a successful post-transplant IgAN treated with TRF-budesonide (4). As 58% of IgAN recurs post-transplant (4,9) and 20%–40% progress to end-stage chronic kidney disease (9,10), TRF-budesonide could be a promising effective treatment in these patients. None of the patients experimented any adverse event. HbA1c, LDL and body mass index, whose increment could be considered as adverse events of steroid therapy,

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Received: *07 June 2022*
Accepted: *04 October 2022*
Published: *14 October 2022*

Citation:
Lopez-Martinez M, Torres I, Bermejo S, Moreso F, Garcia-Carro C, Vergara A, Ramos N, Perello M, Gabaldon A, Azancot MA, Bolufer M, Toapanta N, Bestard O, Agraz-Pamplona I and Soler MJ (2022) Enteric Budesonide in Transplant and Native IgA Nephropathy: Real-World Clinical Practice. Transpl Int 35:10693. doi: 10.3389/ti.2022.10693

TABLE 1 | Change from baseline of analytical parameters.

	Baseline	3 months	6 months	12 months	24 months
UPCR native-kidney (Relative decrease(Relative decrease%)	1.2 {0.70–2.80)	0.81{0.8–0.93) –32.5	0.53 {0.18–0.85) –55.8	1.10 {0.16–1.35) –8.3	0.74 {0.30–1.70) –38.3
UPCR transplant-kidney Relative decrease (%)	1.5 {0.78–4.3)	1.1{0.39–1.55)* –26.7	0.59 {0.17–1.40) –60.7	0.75 {0.16–1.33) –50.0	1.27 {0.55–1.70) –15.3
UPCR total (Relative decrease%)	1.3 {0.72–4.23)	0.87 {0.54–1.18)* –33.1	0.59 {0.17–0.85)* –54.6	1.10 {0.21–1.30) –15.4	0.92 {0.36–1.83) –29.2
Creatinine (mg/dl)	1.66± 0.83	1.89 ± 1.62	1.41± 0.53	1.37±0.60	1.78 ± 0.49
CKD-EPI {ml/min)	57.14 ± 24.49	57.07 ± 27.71	62.33 ± 23.00	65.63 ± 25.39	57.40 ± 25.47
Haematuria (yes %)	9 {69.2)	8 {61.5)	5 {50)	6 {75)	5 {100)
BMI (Kg/m')	27.19 ± 6.70	27.19 ± 6.70	27.19 ± 6.70	27.19 ± 6.70	27.19 ± 6.70
LDL (mg/dl)	57.14 ± 24.49	57.14 ± 24.49	57.14 ± 24.49	57.14 ± 24.49	57.14 ± 24.49
HbA1c{%)	5.89 ± 0.98	5.40 ± 0.30	6.07 ± 1.57	5.53 ± 0.85	5.22 ± 0.40
Clinical adverse events (yes%)	0	0	0	0	0

*Median values (interquartile range 25/75 within parentheses) and relative decrease percentage of urine protein to creatinine ratio {UPCR), mean values {SD) of creatinine and estimated glomerularfiltration rate by Chronic Kidney Disease Epidemiology Collaboration Equation {CKD-EPI), presence of haematuria, body mass index {BMI), low density lipoprotein {LDL), HbAlc and clinical adverse events before and after 3,6, 12, and 24 months treatment with budeson ide was started.*p < 0.05 versus baseline.*

remained stable (**Table 1**). NEFIGAN and NEFIGARD trials corroborate this well tolerance (5,6). Local steroid therapy, like enteric budesonide, provides the immunosuppressive result directly in the IgAN origin and avoids serious side effects usually present in systemic steroid treatment.

Our results support that TRF-budesonide causes significant proteinuria reduction and maintain eGFR stable without adverse events in IgAN. Remarkably, the effect of local steroid treatment in transplant kidneys should also be analyzed in proper designed randomized clinical trials. Targeting intestinal mucosal immune system seems to be a good therapeutic strategy of IgAN treatment which will probably replace systemic steroids.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by (AG) 252/2018. 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

MS, IA-P, and FM collaborated on the original idea and study design. IA-P, ML-M, MS, SB, NR, MA, FM, OB, IT, CG-C, AV, MB, and NT contributed to the inclusion of patients in the cohort. ML-M and MS collaborated on the statistical analysis and wrote the paper. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

ML-M received support for attending meetings and/or travel from Sanofi and AstraZeneca. SB received consulting fees and payment for lectures, presentations, speakers, bureaus, manuscript writing or educational events, participation on a data safety monitoring board or advisory board from AstraZeneca and Mundipharma. CG-C has received travel and congress fees support from AstraZeneca, Esteve, Novo Nordisk, Boehringer Ingelheim Lilly, Astellas, Otsuka, Novartis and Baxter, and has given scientific lectures and participated in advisory boards organized by AstraZeneca, Boehringer Ingelheim Lilly, Mundipharma and Novo Nordisk. AV received grants or contracts from Instituto Carlos III (ISCIII) and Fundación Alfonso Martín Escudero, support for attending meetings and/or travel from Mundipharma, Sanofi, and Novo Nordisk. NR Received grants from the participation on a data safety monitoring board or advisory board from Alexion. MS received grants or contracts from Boehringer, ISCIII, and Marató TV3; honoraria for lectures from NovoNordisk, Jansen, Boehringer, Mundipharma, AstraZeneca, Ingelheim Lilly, Vifor, ICU Medical, Fresenius, and Travere Therapeutics; support for attending meetings from Travere; participation on a data safety, monitoring board or advisory board from NovoNordisk, Jansen, Boehringer, Mundipharma, AstraZeneca, Ingelheim Lilly, Vifor, ICU Medical, Bayer, GE Healthcare, and Travere Therapeutics. MS has the following leadership or fiduaciary roles: SEC Board member, SEN board member, Ex ERA board member, Ex-ASN Board News, Ex-ERA-EDTA SAB, Ex-Council member ERA, Elected EIC CKJ.

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

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Transplant International

Official journal of the European Society for Organ Transplantation

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