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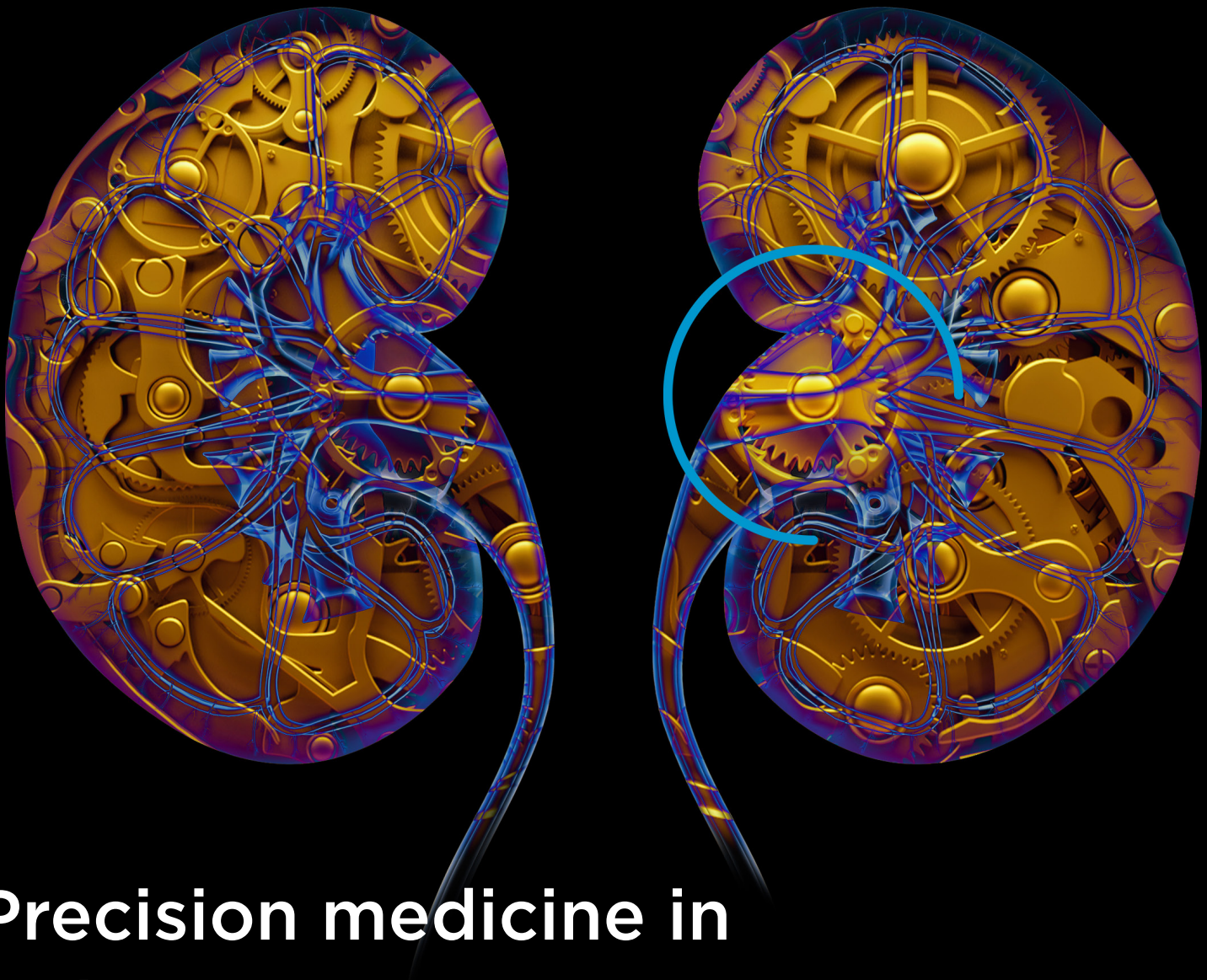

Precision medicine in Kidney transplantation

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Precision medicine in kidney transplantation

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TRANSPLANT TRIAL WATCH
published: 27 September 2023
doi: 10.3389/ti.2023.11920

Transplant Trial Watch

John Matthew O'Callaghan[1,2]* *and Simon Knight*[2,3]

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Keywords: randomised controlled trial, liver transplantation, heart transplantation, donation after brain death, donation after circulatory death (DCD)

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

Transplantation Outcomes with Donor Hearts after Circulatory Death.

by Schroder, J. N., et al. New England Journal of Medicine 2023; 388(23): 2121–2131.

Aims

The aim of this study was to investigate posttransplant outcomes of hearts obtained from donation after circulatory death (DCD) versus donation after brain death (DBD) donors.

Interventions

Participants were randomised to receive a heart from either a DCD or DBD donor.

Participants

297 adult candidates for heart transplantation were randomised, out of which 180 underwent transplantation.

Outcomes

The primary efficacy outcome was patient survival adjusted for prespecified donor and recipient risk factors. The secondary efficacy outcome was the donor-heart utilization rate.

Follow-Up

1 year posttransplantation.

CET Conclusion

This multicentre study randomised patients on the heart transplant waiting list to waiting for a standard, DBD organ; or to a DCD organ (assessed via *ex-vivo* perfusion) or DBD organ, whichever came first. 297 wait-listed patients were randomised, of whom 180 were transplanted in the study – 90 with DBD organs, and 90 with DCD organs. At 6 months post-transplant, there was no difference in risk-adjusted survival or other clinical outcomes between the two groups. This is a

Transplant Trial Watch

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very well-designed study. Studies that alter with organ allocation are challenging as they must not disadvantage patients by reducing the chances of an organ offer. By allowing patients in the DCD arm to receive a DBD organ if allocated, the investigators overcome this. At least in the short term, outcomes from DCD hearts assessed *ex-vivo* appear equivalent, and have the potential to increase the rate of transplantation – within the study, 67% patients randomised to the DCD cohort were transplanted compared to 39% in the DBD cohort.

Jadad Score
2.

Data Analysis
Per protocol analysis.

Allocation Concealment
No.

Trial Registration
ClinicalTrials.gov—NCT03831048.

Funding Source
Industry funded.

RANDOMISED CONTROLLED TRIAL 2

A randomized-controlled trial of ischemia-free liver transplantation for end-stage liver disease.

by Guo, Z., et al. Journal of Hepatology 2023 [record in progress].

Aims
To compare outcomes in the novel technique of ischaemia-free liver transplantation (IFLT) to conventional liver transplantation (CLT).

Interventions
The technique being tested is IFLT compared with CLT. IFLT is a complex technique in which during DBD donation the perfusion cannulas of a Liver Assist can be placed in the donor liver prior to cessation of donor circulation. The arterial canula placed via the splenic artery, portal vein via and vein graft and the outflow canula into the infra-hepatic cava. The perfusion can then seamlessly be transferred from donor circulation to NMP, the liver is then procured and continued NMP until implantation. The supra-hepatic caval (piggyback), portal vein and hepatic arterial anastomoses are then performed in the recipient while NMP continues, and once completed the NMP cannulas are removed, and hepatic perfusion transferred from NMP to recipient without interruption of perfusion.

Participants
65 adult whole liver-only transplant recipients.

Outcomes
The primary endpoint was early allograft dysfunction (EAD) within 7 days as defined by the Olthoff criteria. The secondary endpoints included primary non-function, post-reperfusion syndrome, biliary complications, post-reperfusion lactate, post-transplant LFTs, patient and graft survival at 1, 6, and 12 months, ITU stay and overall hospital stay.

Follow-Up
12 months.

CET Conclusion
This small unblinded randomised trial was conducted in a single high volume transplant centre in China by the group who have been pioneering the ischaemia-free liver transplant technique since its first publication in 2018. Images and videos of their technique have been included in their 3 publications on their reports and protocols. The IFLT cohort was $n = 32$ and the CLT $n = 33$, of these 2 (6%) in the IFLT experience EAD and 8 (24%) in the CLT ($p = 0.044$) which was the primary endpoint. In some of the secondary endpoints they found significant improvement with IFLT: peak ALT and ASK at 7 days, total bilirubin, post-op lactate positive perfusate microbial culture and non-anastomotic strictures at 12 months. When scrutinising these strictures, there were 2 in IFLT (one mild and one moderate) and 9 in CLT (five mild and four moderate) none of which required intervention. The marked reduction in post-reperfusion syndrome is important 3 (9%) in IFLT and 21 (64%) in CLT given the risk of post-reperfusion cardiac arrest. They found no significant differences in primary non-function, over-all hospital stay, anastomotic stenosis (though the rate was higher in IFLT) and, graft and patient survival. They present an impressive success given the complexity of the procedure, however this is its key limitation. Despite the improvement in EAD, strictures and post-reperfusion syndrome there was no measurable benefit in patient or graft survival within the first year and none of the strictures require intervention. It was done in a set of low risk DBD donors, a cohort in which similar benefits have been seen with NMP alone. There are technical limitations, it was performed with a liver assist device which is not transportable, thus donor and recipient must be in the same location. The technique is of interest and a great technical achievement, but a study of larger numbers with a wider range of DBD donors and longer-term follow-up is required.

Jadad Score
3.

Data Analysis
Modified intention-to-treat analysis.

Allocation Concealment
Yes.

Trial Registration
ChiCTR1900021158.

Funding Source
Non-industry funded.

CLINICAL IMPACT SUMMARY

This is a very interesting randomised controlled trial in liver transplantation that has the potential to significantly change practice and improve transplant outcomes. 68 liver transplant recipients from donation after brain death were randomised to standard treatment or for an "Ischemia-Free Liver Transplant" (IFLT). The trial was conducted at a single hospital in China. The study was adequately randomised, but the clinical team could not be blinded to the intervention, understandably. For the intervention group, the Liver Assist device (Organ assist, Netherlands) was used to establish *in situ* normothermic perfusion. The liver was then procured and moved to the reservoir of the Liver Assist for *ex situ* normothermic machine perfusion and moved to the recipient locality for transplant. For the liver implantation to the recipient, the anastomoses of the inferior vena cava, portal vein, and hepatic artery were performed under continuous *in situ* normothermic machine perfusion. Machine perfusion was discontinued after the donor liver had been revascularized. Then the biliary tract was reconstructed.

There was therefore zero cold ischemic time for the IFLT group. Mean cold ischaemic time in the standard care group was approximately 7 h, and mean normothermic perfusion time in the IFLT group was approximately 7 h.

The primary outcome was Early Allograft Dysfunction (EAD) and this was significantly reduced by IFLT (6% versus 24%), as were peak ALT, AST and bilirubin levels. Post-reperfusion syndrome was dramatically reduced, from 64% to 9%. Non-anastomotic biliary strictures were also significantly reduced (8% versus 36%), although this was recorded as seen on protocol MRCP.

This clinical trial has shown a dramatic reduction in the ischemia reperfusion injury of transplant livers through the novel use of technology to remove the cold ischemic phase of the organ preservation period. The donor liver is kept warm and perfused all through the process of procurement from the donor body, preservation outside the body, and during the implant into the recipient up until the moment of reperfusion with the recipient's blood. The technique clearly improved early transplant function. The reduction in non-anastomotic strictures was largely asymptomatic, so it remains to be seen if this technique can significantly reduce the risk of symptomatic strictures in higher risk livers.

AUTHOR CONTRIBUTIONS

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

CONFLICT OF INTEREST

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

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FORUM
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Is ABO Incompatible Living Donor Kidney Transplantation in Children a Better Option than the Use of Optimal Grafts From Deceased Donors? A Plea for Better Prioritization of Deceased Kidney Grafts for Children

Olivia Boyer[1*†] *and Lars Pape*[2†]

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Keywords: kidney, transplantation, graft, ABO incompatible, paediatric kidney transplantation, children, childhood

A Forum discussing:

Considering ABO Incompatible Living Donor Kidney Transplantation before Deceased Donor Kidney Transplantation in Children: A Letter to the Editor
by Stojanovic J and Paessler A (2023). Transpl Int 36:11613. doi: 10.3389/ti.2023.11613

Forum

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While we acknowledge that ABOi LDKTx can be successfully performed in children and has the advantage of reducing the waiting time and risks associated with prolonged dialysis whilst conferring the benefits of living donor transplantation [1], we would like to balance the conclusions made by the authors and outline valuable alternatives.

ABOi kidney transplantation carries a higher risk of rejection compared to ABO compatible transplantation, most particularly antibody-mediated rejection [2]. To overcome this, both extensive pre-transplant conditioning and additional pre-transplant immunosuppressive therapy are required and include desensitization techniques such as antigen-specific immunoadsorption, B cell-depleting monoclonal antibodies (mainly rituximab), and intensified immunosuppression protocols. Such complex treatments expose children to a higher risk of bacterial and viral infections [2], post-transplant lymphoproliferative disease, and other neoplasias. Apheresis techniques require central venous lines in the absence of an arteriovenous fistula, especially in children on peritoneal dialysis or with pre-emptive transplantation, and these procedures can be complicated by infection, thrombosis, or bleeding, and so jeopardize future access to dialysis. In addition, these techniques may be impractical or risky in young children due to the extracorporeal volume required during immunoadsorption sessions. ABO incompatible kidney transplantation is therefore rarely performed in children who weigh <20 kg.

From an economic standpoint, ABOi transplantation is more expensive and resource intensive than ABO compatible transplantation. Additional procedures, prolonged hospital stays, and specialized therapies required for desensitization significantly increase the overall cost of the transplant procedure. For this reason, it may not be available in every health framework. On the other hand, shorter dialysis times obviously spare costs. Moreover, living organ donation can have a financial impact on the donor and his or her family, depending on specific national policies and social security requirements.

Additionally, some parents may want to reserve the option of donating their kidney for a second transplant in adulthood, at an age when organ shortages can be even greater.

Furthermore, while transplants from living donors generally have a better prognosis than transplants from deceased donors, it should be noted that parents who are candidates for donation are increasingly older and have more co-morbidities [3], whereas children often receive transplants from young deceased donors whose parenchyma is generally well preserved at the time of donation. This may partly reduce the advantages of living donation in pediatric kidney transplantation.

We would therefore like to discuss alternatives to ABOi LDKTx in children.

Firstly, we call for better prioritization of the allocation of deceased donor kidney transplants in children who will eventually require several transplants over the course of a lifetime. Priority rules should include age-matching criteria that could guarantee prioritization of pediatric recipients for optimal transplants with shorter waiting times. The allocation policies for transplants vary between jurisdictions and healthcare systems. In France, for instance, absolute national priority is given to recipients under the age of 18 years for the two kidneys of any donor under the age of 18 [4]. Pediatric recipients are also given priority for one of the kidney transplants from donors aged between 18 and 29, in the absence of a recipient benefiting from a priority due to immunization or a multi-organ transplant. Pediatric priority is extended until the transplant if the candidate was under 18 at the start of dialysis. Similarly, in the United States, recipients younger than 18 have priority over donors under 35 years of age [5]. Spain, Italy, and Switzerland also have strong pediatric prioritization with short waiting times. However, this priority is more limited elsewhere, particularly in the Euro Transplant zone (comprising Holland, Belgium, Luxembourg, Germany, Austria, Croatia, and Slovenia) where it should be improved, as it is currently restricted to kidneys from donors aged under 18, who are allocated as a priority to recipients who are also younger than 18. The impact on waiting times for adults based on better pediatric prioritization would be very small because of the large difference in numbers on waiting lists. Moreover, the prioritization criteria would be regularly evaluated and refined to ensure equity, fairness, and transparency.

We agree with the authors that paired kidney exchange programs, also known as kidney swaps or paired donation, such as that in the United States, can be a good strategy for children. We are pleased to note that such a program has been initiated in the United Kingdom, and we hope that this will also be the case for other pediatric kidney transplant programs. Altruistic donation to children could also be allowed. We find it extremely difficult to understand why there is still so much political reluctance, particularly in countries like Germany and France.

Finally, the use of infant kidneys transplanted *en-bloc* in specialized centers may be an interesting alternative for reducing the waiting time for children on the list. Various series have shown good results with this strategy in specialized teams [6–9]. One retrospective study, for example, compared 72 children who had received an *en-bloc* kidney with 75 who had received a kidney from a living donor. The estimated glomerular filtration rate was significantly higher in children who had received an *en-bloc* kidney from the 5th to the 17th year after transplantation and the 25 years graft survival was similar in both groups [10]. Another option is the split of infant *en-bloc* kidneys and the allocation to two small pediatric recipients. This approach has been successful in specialized centers [11], and further increases the number of recipients.

Also of note, pediatric organ donation, which decreased significantly during the COVID-19 pandemic, as was also the case with adults, should be an absolute priority, and the rate of organ donation refusals must be reduced [12].

To conclude, it is essential to consider the advantages and disadvantages outlined above in the context of each child's specific medical condition and individual circumstances. The decision to pursue ABOi LDKTx should be made in consultation with the child's medical team, weighing the potential benefits against the associated risks. Regardless, pediatric organ donation must be promoted, and priority given to optimal kidneys for pediatric recipients, who will often undergo several kidney transplants in the course of their lives. This is described in detail in the position statement of the International Pediatric Transplant Association [13], which emphasizes the special obligations society has towards children, the fair innings argument, and cumulative and time-sensitive accrual of developmental morbidity.

AUTHOR CONTRIBUTIONS

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

CONFLICT OF INTEREST

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

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ESOT | Transplant International

SPECIAL ARTICLE
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Qualifying a Novel Clinical Trial Endpoint (iBOX) Predictive of Long-Term Kidney Transplant Outcomes

Amanda Klein[1†], Alexandre Loupy[2†], Mark Stegall[3†], Ilkka Helanterä[4†], Luke Kosinski[1†], Eric Frey[1], Olivier Aubert[2†], Gillian Divard[2†], Kenneth Newell[5†], Herwig-Ulf Meier-Kriesche[6], Roslyn Mannon[7†], Thomas Dumortier[8†], Varun Aggarwal[1†], Jagdeep T. Podichetty[1], Inish O'Doherty[1†], Ahmed Osama Gaber[9] and William E. Fitzsimmons[1†] on behalf of the Transplant Therapeutics Consortium*

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New immunosuppressive therapies that improve long-term graft survival are needed in kidney transplant. Critical Path Institute's Transplant Therapeutics Consortium received a qualification opinion for the iBOX Scoring System as a novel secondary efficacy endpoint for kidney transplant clinical trials through European Medicines Agency's qualification of novel methodologies for drug development. This is the first qualified endpoint for any transplant indication and is now available for use in kidney transplant clinical trials. Although the current efficacy failure endpoint has typically shown the noninferiority of therapeutic regimens, the iBOX Scoring System can be used to demonstrate the superiority of a new immunosuppressive therapy compared to the standard of care from 6 months to 24 months posttransplant in pivotal or exploratory drug therapeutic studies.

Keywords: kidney transplant, iBox, transplant outcomes, organ transplant, transplant clinical trial

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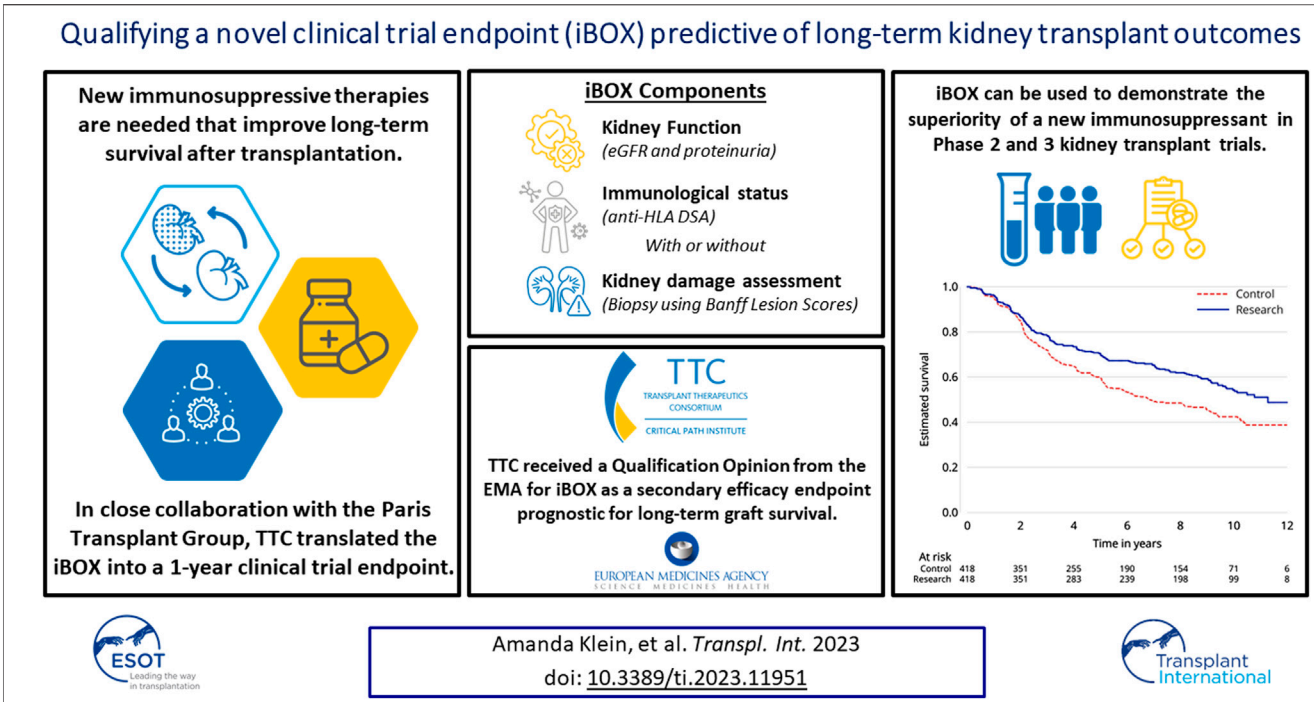
INTRODUCTION

Graft failure following kidney transplantation has significant negative implications, including return to dialysis, lower life expectancy, decreased quality of life, and need for retransplantation. Additionally, graft survival is the most important outcome for people living with a kidney transplant [1]. Currently, immunosuppressive therapies (ISTs) have improved short-term outcomes in kidney transplantation, with 1 year graft survival rates of over 90% [2–5]. Despite the relatively low rate of efficacy failure at 1 year posttransplant, long-term graft survival remains suboptimal. The 5 and 10 years graft survival rates are 77% and 49% for deceased donor and 86% and

Abbreviations: BELA, belatacept; CMA, conditional marketing authorization; CNI, calcineurin inhibitor; COU, context-of-use; DSA, donor-specific antibody; eGFR, estimated glomerular filtration rate; EMA, European Medicines Agency; FDA, Food and Drug Administration; IST, immunosuppressive therapy; RCT, randomized controlled trial; RLSE, reasonably likely surrogate endpoint; SOC, standard of care; TTC, Transplant Therapeutics Consortium.



GRAPHICAL ABSTRACT

64% for living donor transplants [4]. Therefore, there remains a significant unmet need for ISTs that improve long-term outcomes. One of the challenges for biopharmaceutical sponsors is executing registration trials of a feasible size and duration (1–2 years) to support superiority claims using the historically accepted primary efficacy failure composite endpoint consisting of death, graft failure, biopsy-proven acute rejection, and lost to follow-up. These current endpoints, while acceptable to regulators, are not optimized for short-term superiority of ISTs that are predictive of longer-term graft survival. Such studies would require extended duration (e.g., 5 years or more), which may be impractical and unfeasible.

TRANSPLANT THERAPEUTICS CONSORTIUM (TTC)—A REGULATORY-FOCUSED NEUTRAL CONVENER FOR TRANSPLANT

In 2014, the 2 major US transplantation societies, the American Society of Transplantation and the American Society of Transplant Surgeons, recognized the need for a pathway to develop new ISTs for transplant recipients [6]. In 2017, these societies partnered with Critical Path Institute and other transplant community members to create TTC (https://c-path.org/programs/ttc/). By facilitating a public–private partnership among scientists from the biopharmaceutical industry, diagnostics companies, academic institutions, professional societies, and government and regulatory agencies, TTC fosters consensus and data-driven research to increase speed in developing new therapies. TTC's primary focus is obtaining regulatory endorsement of an early novel endpoint capable of predicting long-term graft survival in pivotal clinical trials designed to support regulatory approval of new ISTs for kidney transplantation.

To develop a novel trial endpoint, it is important to understand the multifactorial causes of late kidney graft failure; predicting failure accurately with a single marker may not be optimal [3]. Several composite scores have been proposed as surrogates, but iBOX is based on the largest dataset and the only specifically designed multivariate model that predicts long-term death-censored graft failure [7, 8]. iBOX is a risk prediction tool that utilizes multiple clinically relevant features demonstrated to be mechanistically associated with an increased risk of late graft functional decline and failure. These features are estimated glomerular filtration rate (eGFR), proteinuria, anti-human leukocyte antigen donor-specific antibody, and kidney graft biopsy histopathology measured cross-sectionally at any time point posttransplantation. iBOX then integrates these parameters to generate individualized predictions of outcomes at 3, 5, and 7 years posttransplant. iBOX was originally designed to be used at the patient level to inform clinical care and management of kidney transplant patients. In close collaboration with the Paris Transplant Group, TTC translated this work into a clinical trial endpoint acceptable to European Medicines Agency (EMA), intending to streamline drug development by predicting long-term outcomes

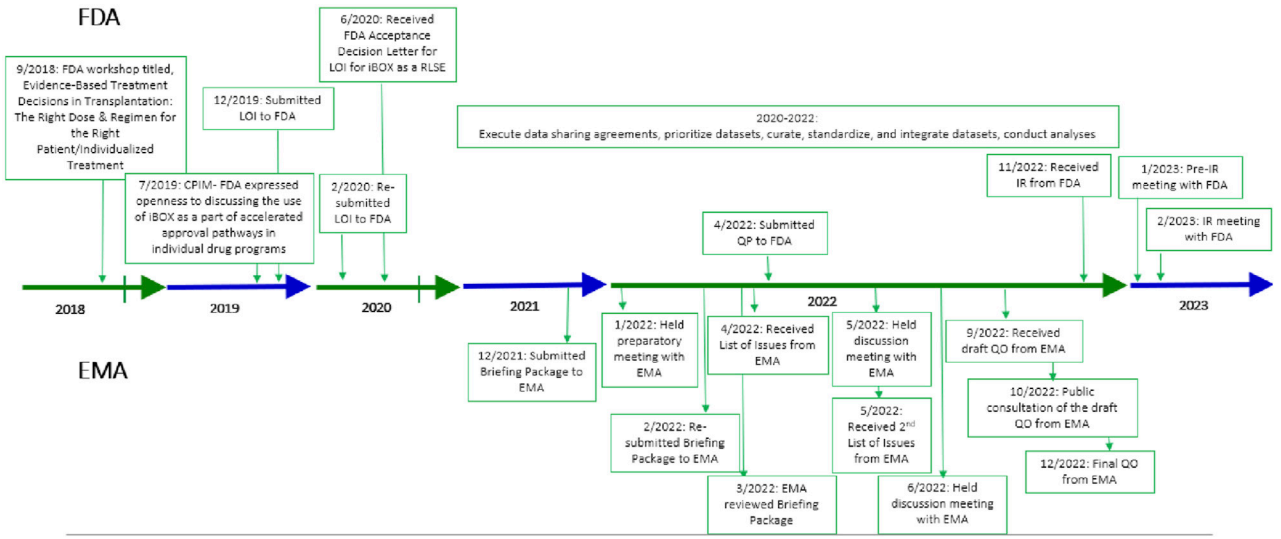


FIGURE 1 | Regulatory timeline with FDA and EMA. CPIM, Critical Path Innovation Meeting; EMA, European Medicines Agency; FDA, Food and Drug Administration; IR, information request; LOI, letter of intent; QO, qualification opinion; QP, qualification plan; RLSE, reasonably likely surrogate endpoint.

using short-term data, summarized in **Supplementary Table S1**. Additionally, the qualification of iBOX as a reasonably likely surrogate endpoint (RLSE) is proceeding with the US Food and Drug Administration (FDA). The regulatory process and timeline associated with FDA and EMA interactions are shown in **Figure 1**.

IBOX SCORING SYSTEM–FIRST QUALIFIED ENDPOINT IN TRANSPLANTATION

In December of 2022, EMA issued a qualification opinion for iBOX as a secondary endpoint prognostic for death-censored graft loss in kidney transplant recipients intended to be used in clinical trials to support the evaluation of novel IST applications [9, 10]. EMA qualified both a full iBOX (including biopsy), and an abbreviated iBOX (excluding biopsy), allowing flexibility in using this endpoint in studies with and without protocol/surveillance biopsies. Importantly, the component measures in iBOX are modifiable by IST interventions and are further described in **Table 1**. The iBOX is the first qualified endpoint in transplantation and the fifth qualified endpoint with EMA [10].

An important outcome of this qualification is that iBOX can be used as a key secondary endpoint to demonstrate superiority of a new IST compared with the standard of care (SOC) from 6 months to 2 years posttransplant in exploratory or pivotal drug therapeutic studies for regulatory purposes. The datasets supporting this regulatory endorsement represent adult kidney-only transplant recipients with varying underlying diagnoses, multiple donor types, various induction therapies, and either calcineurin inhibitor (CNI)-based or CNI-free therapeutic regimens. As a result, iBOX can be used in registration-driven trials representative of a broad population of kidney transplant recipients. The context-of-use (COU) for iBOX is summarized in **Table 2**.

Additionally, in Europe, sponsors and investigators will be able to assess and promote the potential superiority of novel ISTs when measured using iBOX. Further, iBOX will be included in the summary of product characteristics, claims, and other product labeling. Although conditional marketing authorization (CMA) is a separate consideration outside the purview of the Qualification of Novel Methodologies for Drug Development process, superiority to current SOC, thereby addressing an unmet need in kidney transplant, is one of the key criteria for CMA in the European Union [8, 10].

A COMMUNITY-BASED APPROACH TO ENDPOINT DEVELOPMENT

Datasets

A fundamental component of developing an evidentiary package that meets the requirements of regulatory endorsement for iBOX was the success of TTC's extensive global patient-level data-sharing initiative [9–11]. Datasets from relevant clinical trials, including those used by [7] in their 2019 publication and real-world data from international clinical transplant centers, were prioritized for acquisition. A flow diagram of the dataset selection process is shown in **Figure 2** with additional rationale provided in the **Supplementary Material**. The original iBOX [7] development included time posttransplant to account for varying iBOX assessments of individual patients and to assist in patient care and prognosis estimation. **Figure 3** from [7] shows the density of iBOX risk evaluation time points after transplantation. The derivation dataset included in the EMA qualification submission represents all 4,000 subjects described in [7]. For

TABLE 1 | Component measures of the full and abbreviated iBOX.

iBOX component measures	Detailed information on the iBOX measures
Time of posttransplant risk assessment (fixed time points)	Phase 2/proof-of-concept iBOX assessment: 6 months Phase 3 iBOX assessment: 1 year, 2 years
Kidney function (eGFR and UPCR proteinuria)	eGFR, where eGFR is measured in mL/min/1.73 m^2 Log transformed (UPCR value[a]), where UPCR is measured in gram per gram (g/g)
Immunological status (anti-HLA DSA MFI)	Anti-HLA DSA using a qualitative binary MFI cutoff • MFI <1,400 (References group) • MFI ≥1,400
Kidney damage assessment[b] (kidney allograft biopsy histopathology using Banff lesion scores)	Banff lesion score, interstitial fibrosis/tubular atrophy (IFTA score): Categorical variable with 3 levels • IFTA score = 0–1 (References group) • IFTA score = 2 • IFTA score = 3 Microcirculation inflammation (Banff lesion score, glomerulitis [g score] and Banff lesion score, peritubular capillaritis [ptc score]): Categorical variable with 3 levels • g and ptc score = 0–2 (References group) • g and ptc score = 3–4 • g and ptc score = 5–6 Banff lesion score, interstitial inflammation (i score) and Banff lesion score, tubulitis (t score): Categorical variable with 2 levels • i score and t score = 0–2 (References group) • i score and t score ≥3 Banff lesion score, presence/extent of glomerular base membrane double contours; transplant glomerulopathy (cg score): Categorical variable with 2 levels • cg score = 0 (References group) • cg score = ≥1

DSA, donor-specific antibody; eGFR, estimated glomerular filtration rate; HLA, human leukocyte antigen; MFI, mean fluorescence intensity; UPCR, urine protein-to-creatinine ratio.
[a]For proteinuria values below 0.05 g/g are replaced by 0.05 g/g before log-transformation.
[b]Omitted from abbreviated iBOX.

TABLE 2 | Context-of-use for the qualification opinion of the iBOX Scoring System.

General measurement	The iBOX scoring system is a secondary endpoint prognostic for death-censored graft loss (allograft failure) in kidney transplant patients to be used in clinical trials investigating novel immunosuppressive medicines
Timing of iBOX assessments	The iBOX Scoring System is an acceptable secondary measured between 6 and 24 months postkidney transplantation in pivotal or exploratory drug therapeutic studies for regulatory purposes. The iBOX Scoring System can be used to demonstrate the superiority of a new immunosuppressive therapy compared with the SOC at 6, 12, or 24 months postkidney transplant
Target population	Adult kidney-only transplant recipients from a living or deceased donor

SOC, standard of care.

application as a 1-year endpoint in a typical phase 3 clinical trial, we examined the number of subjects in the derivation dataset with iBOX assessments fixed at 1 year posttransplant and had outcome data of at least 5 years.

Five datasets supporting the regulatory endorsement of iBOX included data from clinical transplant centers (i.e., Loupy et al., 2019 derivation [7]. Mayo Clinic Rochester, and Helsinki University Hospital) and clinical trials (i.e., BENEFIT randomized controlled trial [RCT] [12] and BENEFIT-EXT RCT [13]) representing over 2,500 *de novo* kidney transplant recipients with 1-year iBOX assessments (**Table 3**). Participant consent was obtained from the transplant centers and clinical trials for primary uses. These datasets contained all elements necessary to assess the performance of iBOX as a pivotal trial endpoint including IST information, iBOX variables at 1 year posttransplant, and 5 years follow-up for death and graft loss of at least 5 years. Additionally, these datasets were accompanied by assay information for each component iBOX measure and laboratory certification documentation ensuring that the analytical methods were robust, reliable, and fit-for-purpose.

Clinical transplant center data are inherently heterogeneous and reflect the diversity of the kidney transplant recipient population in the United States and European Union. Datasets were curated, standardized, and aligned to conduct internal and external validation analyses to support the iBOX COU with EMA. Two clinical trial datasets have the most extensive CNI-free (belatacept [BELA]) patient-level data with the 4 core iBOX

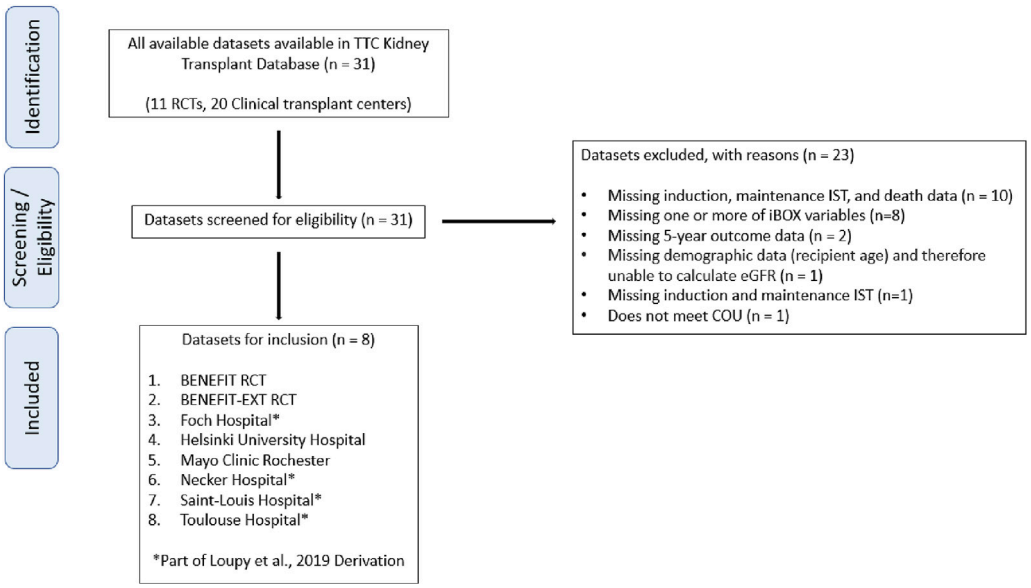


FIGURE 2 | Flow diagram of the data selection process. COU, context-of-use; RCT, randomized controlled trial; TTC, Transplant Therapeutics Consortium.

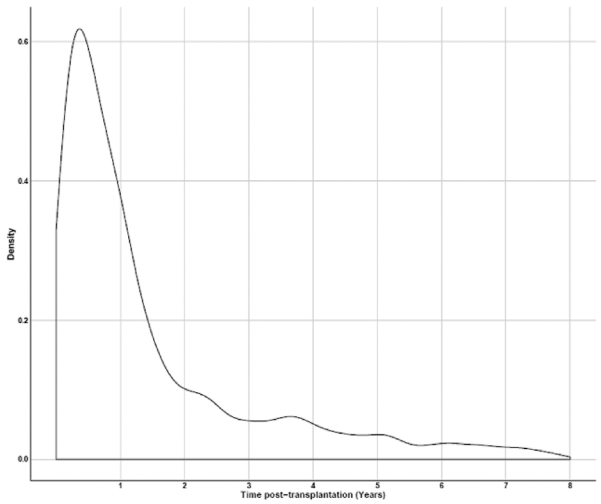


FIGURE 3 | Density of time points where iBOX assessments were made (*y*-axis), compared with the time posttransplant (*x*-axis) out to 8 years, as shown in [7].

variables and sufficient follow-up period available. This represents, as stated by EMA, "extensive global effort to collect clinical trials and real-world data" [10].

Critical Path Institute explored the number of transplant recipients with full and abbreviated iBOX assessments at varying times posttransplant in the already curated and aligned validation datasets. **Supplementary Figure S1** shows the distribution of assessment time points for donor-specific antibody (DSA) measurements up to 2 years postkidney transplant. DSA was selected for illustration because it is collected less frequently than eGFR and/or proteinuria and therefore acts as the key limiting factor for the availability of abbreviated iBOX measurements. The data distribution for iBOX assessments ranges from 6 months up to 2 years posttransplant. Helsinki University Hospital only assessed proteinuria and DSA data at 1 year posttransplant and therefore was excluded from the additional time points exploration. The number of transplant recipients with iBOX assessments at 6 months and 2 years posttransplant in the external validation datasets is shown in **Supplementary Table S2**. There were significantly more abbreviated iBOX assessments at the varying time points

TABLE 3 | Five-y posttransplant c-statistics values (SE) for the iBOX at 1 year posttransplant in the derivation and validation datasets.

Dataset		*n*	c-statistic (SE) for full iBOX at 1 year	c-statistic (SE) for abbreviated iBOX at 1 year
Derivation	[7] derivation	1174	0.85 (0.02)	NA
Validation	Mayo Clinic Rochester	483	0.93 (0.03)	0.84 (0.05)
	Helsinki University Hospital	344	0.78 (0.06)	0.77 (0.06)
	BENEFIT RCT	416	0.70 (0.09)	0.70 (0.08)
	BENEFIT-EXT RCT	260	0.81 (0.07)	0.78 (0.06)

NA, not applicable; RCT, randomized controlled trial; SE, standard error.

TABLE 4 | Poisson calibration for the full and abbreviated iBOX at 1 year posttransplant in the validation datasets.

	1 year Posttransplant			
Dataset	**Full iBOX**			
	n	**Observed graft loss events**	**Predicted graft loss events**	***p***
Mayo Clinic Rochester	483	18	24.34	.20
Helsinki University Hospital	344	21	14.40	.08
BENEFIT RCT	416	12	14.52	.51
BENEFIT-EXT RCT	260	12	14.97	.44
Dataset	**Abbreviated iBOX**			
	n	**Observed graft loss events**	**Predicted graft loss events**	***p***
Mayo Clinic Rochester	497	20	24.41	.37
Helsinki University Hospital	344	21	16.19	.23
BENEFIT RCT	515	15	18.77	.39
BENEFIT-EXT RCT	357	23	22.97	1.00

RCT, randomized controlled trial.

A p-value of <.05 would indicate a significant difference between the expected number of graft loss events as predicted by the iBOX versus the actual number of graft loss events.

because biopsies were more typically "for-cause" and not taken "per protocol" at 6 months or 2 years posttransplant. Although the full iBOX measurements at 2 years were limited due to lack of biopsy information, because the abbreviated iBOX performed well at this time point, the addition of biopsy information should only further improve the performance, and therefore, the full iBOX is expected to also perform well at 2 years.

Analyses

Validation analyses were performed to support the COU for iBOX with EMA for predicting death-censored graft loss. Both internal validations, evaluating iBOX on the data it was trained on (i.e., the derivation dataset), and external validation, evaluating iBOX on data it was not trained on, were performed. The abbreviated iBOX was treated as a modification of the full iBOX and not validated internally, save for checking the overall c-statistic. Both the full and abbreviated iBOX models were validated on 4 external datasets (i.e., validation datasets) (previously described above).

To avoid survivor bias, patients who did not reach their scheduled evaluation (i.e., those who lost their graft, died, or were lost to follow-up beforehand) were given an imputed worst-case iBOX score (**Supplementary Tables S3, S4**).

iBOX was validated by assessing its discrimination, the ability to rank individuals from a lower to a higher risk of graft loss, and its calibration, the ability to accurately predict absolute risk level [14]. Discrimination was assessed using Harrell's c-statistic [15], which gives the probability that, for any 2 randomly selected individuals, the individual with the higher iBOX score, i.e., the higher model-predicted hazard of graft loss, has a shorter death-censored graft survival time. A c-statistic value of 0.7 or greater indicates good discriminatory ability [16]. Secondly, calibration was evaluated by checking whether observed events (graft losses) matched predicted using a Poisson calibration method (see **Supplementary Material** for a summary of the method) [14].

The full iBOX discrimination in the derivation dataset, when restricted to transplant recipients with an iBOX score at 1-year posttransplant and follow-up to 5 years, had a c-statistic of 0.85, demonstrating iBOX discriminates appropriately among subjects for use in a phase 3 study (**Table 3**). In the validation datasets, c-statistics ranged from 0.70 to 0.93 (**Table 3**), and the predicted versus observed graft losses were not significantly different for iBOX assessments at 1 year posttransplant (**Table 4**).

Given that the iBOX models are trained primarily on subjects receiving CNI-based maintenance ISTs, it was unclear if iBOX would perform similarly in kidney transplant recipients not on CNI-based therapies. Internally, the iBOX was found to discriminate appropriately between higher- and lower-risk patients receiving mTOR inhibitor-based therapies (c-statistic >0.8) (**Table 5**). Externally, 5 years iBOX c-statistic values for CNI-free subjects, consisting primarily of patients on BELA-based regimens, at 1 year posttransplant in the validation datasets were evaluated; full and abbreviated iBOX c-statistics were 0.75 and 0.73, respectively (**Table 6**). These analyses demonstrate that iBOX can discriminate between subjects at higher and lower risk of death-censored graft loss in diverse datasets, including CNI and CNI-free populations, in clinical transplant centers and RCTs. Likewise, the results also showed that iBOX has good prediction accuracy based on calibration analyses (**Table 6**).

The performance of the full and abbreviated iBOX were also tested in the validation datasets at 6 months and 2 years posttransplant. The 5 years posttransplant discrimination (**Supplementary Table S5**) and calibration analyses (**Supplementary Table S6**) support the inclusion of time posttransplant in the iBOX model at 6 months and 2 years posttransplant.

Based on the iBOX formulas shown in **Table 7**, iBOX is not just the sum of the parts (i.e., the addition of components) but includes continuous and dichotomous variables weighted differently based on the beta coefficients. The c-statistic for eGFR alone and eGFR with proteinuria in comparison with the full and abbreviated iBOX is shown in **Table 8**, with calibration results in **Supplementary Tables S7, S8**, indicating that the iBOX score is influenced most by eGFR, and the other 3 components, proteinuria, anti-human leukocyte antigen DSA, and biopsy, all increase the predictive power.

In addition to validation, an analysis of the BENEFIT and BENEFIT-EXT RCTs included imputation of the worst-case iBOX scores at 1 year posttransplant for recipients who died or lost their

TABLE 5 | Five-y posttransplant c-statistics values for the full iBOX for subset of subjects in the derivation dataset.

Subset of subjects in the [7] derivation	n	Observed graft loss events	c-statistic (SE)
mTORi subjects (includes subjects on both mTORi and CNI therapies)	239	33	0.87 (0.03)
mTORi-only subjects	171	23	0.86 (0.04)

CNI, calcineurin inhibitor; mTORi, mammalian target of rapamycin signal inhibitor; SE, standard error.

TABLE 6 | Five-y posttransplant c-statistic values for the full and abbreviated iBOX for CNI and CNI-free subjects at 1 year posttransplant in the validation datasets.

Maintenance IST-based regimen	c-statistic (SE)	Observed graft loss events	Predicted graft loss events	*p*
Full iBOX				
CNI (TAC, CSA) *n* = 1045	0.82 (0.04) [TAC 0.86 (0.05), CSA 0.77 (0.05)]	50	51.6	.82
CNI-free (mTORi, BELA) *n* = 456	0.75 (0.08)[a]	13	16.6	.38
Abbreviated iBOX				
CNI (TAC, CSA) *n* = 1124	0.79 (0.04) [TAC 0.81 (0.05), CSA 0.77 (0.05)]	61	58.9	.78
CNI-free (mTORI, BELA) *n* = 587	0.73 (0.07)[a]	17	23.4	.26

BELA, belatacept; CNI, calcineurin inhibitor; CSA, cyclosporine; mTORi, mammalian target of rapamycin signal inhibitor; SE, standard error; TAC, tacrolimus.
[a]*The mTORi group only had 38 subjects with no graft loss events, so no breakdown of c-statistic by treatment was performed for the CNI-free group.*
A p-value of <0.05 would indicate a significant difference between the expected number of graft loss events as predicted by the iBOX versus the actual number of graft loss events.

TABLE 7 | Formulas to calculate full and abbreviated iBOX scores.

	$iBox_i = \sum_{j=1} \hat{b}_j x_{i,j}$ for subject i where	Full iBOX	Abbreviated iBOX
	Factor	HR (exp $[\hat{\beta}_j]$) (95% CI)[a]	
$X_{i,1}$	Time from transplant to evaluation (y)	1.08 (1.03–1.14)	1.12 (1.07–1.18)
$X_{i,2}$	eGFR (mL/min/1.73 m^2)	0.96 (0.95–0.96)	0.95 (0.95–0.96)
$X_{i,3}$	Log transformed UPCR proteinuria (g/g)	1.5 (1.39–1.62)	1.59 (1.48–1.71)
$X_{i,4}$	Anti-HLA DSA MFI		
	<1,400	1	1
	≥1,400	1.84 (1.44–2.34)	1.84 (1.44–2.34)
$X_{i,5}$	Interstitial fibrosis/tubular atrophy (IFTA score)		N/A
	0–1	1	
	2	1.14 (0.92–1.43)	
	3	1.41 (1.1–1.8)	
$X_{i,6}$	Microcirculation inflammation (g score and ptc score)		
	0–2	1	
	3–4	1.43 (1.11–1.85)	
	5–6	1.84 (1.25–2.7)	
$X_{i,7}$	Interstitial inflammation and tubulitis (i score and t score)		
	0–2	1	
	≥3	1.33 (1.06–1.68)	
$X_{i,8}$	Transplant glomerulopathy (cg score)		
	0	1	
	≥1	1.47 (1.14–1.9)	

CI, confidence interval; DSA, donor-specific antibody; HLA, human leukocyte antigen; HR, hazard ratio; MFI, mean fluorescence intensity; N/A, not applicable.
[a]$\hat{\beta}_j$ *= the log of the HR values.*
For categorical variables with more than 2 levels, e.g., IFTA score, the contribution of the variables was calculated as follows: $\beta_1 x_1 + \beta_2 x_2$. *If the IFTA score = 0 or 1, then* $x_1 = 0$ *and* $x_2 = 0$. *If the IFTA score = 2, then* $x_1 = 1$ *and* $x_2 = 0$. *If the IFTA score = 3, then* $x_1 = 0$ *and* $x_2 = 1$. β_1 *and* β_2 *refer to the beta coefficients for the IFTA scores = 2 and 3, respectively.*

graft in the first year (**Table 9**). This sensitivity analysis was performed to replicate the clinical trial setting where avoidance of survivor bias at 1 year would be necessary, and all randomized subjects would have an iBOX score at 1 year even if there were death or graft loss before that time. In both studies, the full and abbreviated iBOX score at 1 year was significantly lower in the BELA group than in cyclosporine, indicating a lower predicted risk of long-term graft failure. This corresponded to a statistically significant improvement in 5 years graft survival in the BENEFIT study. The BENEFIT-EXT study showed directionally higher 5 years death-censored graft survival. However, the difference was not statistically significant. The larger treatment

TABLE 8 | C-statistics for each validation dataset as parameters are removed in the iBOX with all parameters ("full"), without biopsy ("abbreviated"), without biopsy and DSA ("only eGFR and proteinuria"), and without biopsy, DSA, and proteinuria ("only eGFR").

Dataset	c-statistic (SE) at 1 year posttransplant			
	Full iBOX	Abbreviated iBOX	iBOX with only eGFR and proteinuria	iBOX with only eGFR
Mayo Clinic Rochester	0.93 (0.03)	0.84 (0.03)	0.80 (0.04)	0.75 (0.04)
Helsinki University Hospital	0.78 (0.06)	0.77 (0.06)	0.76 (0.06)	0.74 (0.06)
BENEFIT RCT	0.70 (0.09)	0.70 (0.08)	**0.69 (0.08)**	**0.69 (0.08)**
BENEFIT-EXT RCT	0.81 (0.07)	0.78 (0.06)	0.78 (0.06)	0.78 (0.06)

eGFR, estimated glomerular filtration rate; DSA, donor-specific antibody; RCT, randomized controlled trial; SE, standard error.
Bold text highlights c-statistics <0.7.

TABLE 9 | Treatment effect for 5 year graft survival with imputation (i.e., all-cause and death-censored) is the log HR, while the 1 year full and abbreviated iBOX scores are the difference in medians.

		BELA	CSA	Treatment effect	*p*
Full iBOX					
BENEFIT RCT (*n* = 466)	iBox score at 12 months: Median (SD)	−3.502 (0.07)	−2.915 (0.10)	−0.587	<.0001
	5 years KM survival probability % (SD)	96.0 (1.14)	89.7 (2.67)	−0.999	.02
BENEFIT-EXT RCT (*n* = 330)	iBox score at 12 months: Median (SD)	−2.6804 (0.065)	−2.1848 (0.12)	−0.4957	.0005
	5 years KM survival probability % (SD)	94.50 (1.55)	88.08 (3.43)	−0.8163	.071
Abbreviated iBOX					
BENEFIT RCT (*n* = 599)	iBOX score at 12 months: Median (SD)	−3.679 (0.05)	−3.042 (0.08)	−0.637	<.0001
	5 years KM survival probability % (SD)	96.3 (0.96)	89.7 (2.44)	−1.058	.006
BENEFIT-EXT RCT (*n* = 455)	iBOX score at 12 months: Median (SD)	−2.9057 (0.07)	−2.4255 (0.12)	−0.4803	.0007
	5 years KM survival probability % (SD)	85.05 (2.15)	78.54 (3.75)	−0.3292	0.2

BELA, belatacept; CSA, cyclosporine; KM, Kaplan-Meier; RCT, randomized controlled trial; SD, standard deviation.

difference in iBOX score at 1 year in the BENEFIT study compared with BENEFIT-EXT also corresponded to a larger treatment difference in graft survival. The lack of statistical significance on some of the 5 years graft survival analyses is related to limitations in the power to detect differences based on sample size.

Additional analyses were performed testing the performance of the full iBOX at 1 years posttransplant on all-cause 5 years graft loss (**Supplementary Tables S9, S10**). The discriminatory ability of iBOX for all-cause graft loss underperforms, with the full iBOX having reduced c-statistics, many of which are below 0.7, and poor all-cause calibration. This is expected given that iBOX was originally developed using variables more likely to impact risk of graft loss. Based on this evidence, iBOX was qualified with EMA with death-censored graft loss as the outcome measure.

SAMPLE SIZE CALCULATOR USING IBOX SCORES USING A PUBLIC-FACING GRAPHICAL USER INTERFACE

Separate from this EMA qualification submission, TTC developed a sample size calculator to assist sponsors in designing prospective clinical trials using iBOX as an endpoint. Sponsors can apply various inclusion/exclusion criteria and other specifications, consistent with the qualified COU, to calculate a sample size and project death-censored graft survival. This sample size calculator is publicly available at https://cpath.shinyapps.io/ibox_v3 to benefit the community and improve future clinical trial efficiency.

CONCLUSION AND FUTURE DIRECTIONS

The successful qualification opinion of iBOX by EMA is the first step in the process of providing an endpoint to allow the demonstration of superiority of new therapies and to stimulate the development of innovative therapies in kidney transplant. Validation analyses show that iBOX is suitable for predictions of graft loss events, with good performance based on c-statistics and the ability to predict numbers of graft loss events with reasonable margins of error, supporting the qualified COU with EMA. Although the original iBOX by [7] focused on the prognostic value for individual patient decision making, the tool was able to be adapted for regulatory purposes as a qualified clinical trial endpoint (**Supplementary Table S1**). iBOX as a secondary endpoint was put forward by EMA to further stimulate robust assessment of iBOX and may lend future opportunities to advance iBOX for other COUs, such as treatment of T cell-mediated or antibody-mediated rejection trials. Although this is an important step forward, it will not automatically lead to new innovative therapeutic development but must be applied strategically as an important tool in global development programs to demonstrate advantages over current SOC, which has good short-term results and is available as lower-cost generics.

Importantly, EMA has a higher evidentiary standard for qualifying a surrogate endpoint compared with the FDA. Unlike the FDA, EMA does not have a category of "reasonably likely" surrogate endpoints (RLSE), nor is CMA linked to surrogacy [17, 18] whereas the FDA has both an

RLSE and an accelerated approval pathway that is based on surrogate endpoints. To facilitate the harmonization of multinational trials, TTC submitted the iBOX as an RLSE to the FDA Biomarker Qualification Program, and it is currently under review by the FDA [19]. Recent TTC interactions with the FDA have focused on the needs of transplant recipients for new innovative therapeutics that have demonstrated superiority to the current SOC and the inadequacy of relying solely or primarily on the historical efficacy failure endpoint, which is driven by acute rejection. Ideally, we envision designing one phase 3 *de novo* trial with iBOX as a primary endpoint in the United States for Accelerated Approval (i.e., RLSE) and a secondary endpoint in the European Union after establishing noninferiority for efficacy failure, alongside pursuing CMA. The ability to conduct trials with sites in the United States and the European Union is critical to advancing the field and bringing new and improved therapies to kidney transplant recipients. As stated by the EMA in the qualification opinion, "The Committee for Medicinal Products for Human Use encourages the use of the iBOX scoring system as a secondary endpoint in future trials of kidney transplantation and further development of the scoring system targeting a potential future qualification as a surrogate endpoint" [10].

DATA AVAILABILITY STATEMENT

The aggregated dataset that was the basis for the work discussed in this publication is not publicly available as per requirements in the data contribution agreements. Requests to access these datasets should be directed to corresponding author.

AUTHOR CONTRIBUTIONS

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

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AUTHOR DISCLAIMER

The contents are those of the authors and do not necessarily represent the official views of, nor an endorsement by, FDA/HHS or the US Government.

CONFLICT OF INTEREST

AK, LK, EF, and VA are employed by C-Path. AL holds shares in Cibiltech. IH holds research funds from MSD and Hansa Biopharma, receives consultancy honoraria from Hansa Biopharma, MSD, Novartis, and Takeda, is an associate editor for American Journal of Transplantation, and is a Coordinating Committee member of TTC. H-UM-K is employed by Veloxis Pharmaceuticals. KN serves as a scientific advisory board member for Angion, Care Dx, CSL Behring, Hansa Biopharma, Immucor, Novartis, Sangamo Therapeutics, Sanofi, Takeda, Talaris, and Viela Bio. RM is employed by University of Nebraska Medical Center; serves as a consultant for Chinook Therapeutics and Olaris Inc; holds research funding from Transplant Genomics, Inc and Verici DX; has received honoraria from CSL Behring; holds patents or royalties with Eurofins; has an advisory or leadership role with Vitaerris VKTX01 IMAGINE Trial and Verici Dx; and is Chair, ASN Policy and Advocacy Committee; Immediate Past-Chair, Women in Transplantation; Member, ASN Grants Committee; Chair, SRTR Review Committee; Member, DSMB, NIDDK/NIH; and Deputy Editor, American Journal of Transplantation. AG receives research funding from Hansa Biopharma, Veloxis Pharmaceuticals, Novartis, and Medeor Therapeutics. WF serves on the Board of Directors of CTI Clinical Trial Services; is Adjunct Professor at University of Illinois at Chicago; is founder of Tutela Pharmaceuticals; and serves as a consultant to Azoth Immune Medicines, Tract Therapeutics, and Veloxis Pharmaceuticals.

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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Professional Societies
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Biopharmaceutical Industry and Diagnostic Companies
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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11951/full#supplementary-material

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ORIGINAL RESEARCH
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A Multi-Step Precision Pathway for Predicting Allograft Survival in Heterogeneous Cohorts of Kidney Transplant Recipients

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Accurate prediction of allograft survival after kidney transplantation allows early identification of at-risk recipients for adverse outcomes and initiation of preventive interventions to optimize post-transplant care. Many prediction algorithms do not model cohort heterogeneity and may lead to inaccurate assessment of longer-term graft outcomes among minority groups. Using data from a national Australian kidney transplant cohort (2008–2017) as the derivation set, we developed P-Cube, a multi-step precision prediction pathway model for predicting overall graft survival in three ethnic subgroups: European Australians, Asian Australians and Aboriginal and Torres Strait Islander Peoples. The concordance index for the European Australians, Asian Australians, and Aboriginal and Torres Strait Islander Peoples subpopulations were 0.99 (0.98–0.99), 0.93 (0.92–0.94) and 0.92 (0.91–0.93), respectively. Similar findings were observed when validating P-cube using an external dataset [Scientific Registry of Transplant Recipient Registry (2006–2020)]. Six sub-categories of recipients with distinct risk factor profiles were identified. Some factors such as blood group compatibility were considered important across the entire transplant population. Other factors such as human leukocyte antigen (HLA)-DR mismatches were unique to older recipients. The P-cube model identifies allograft survival specific risk factors within a heterogenous population and offers personalized survival predictions in a diverse cohort.

Keywords: kidney transplantation, kidney transplantation graft survival, allograft survival prediction, prediction model, ANZDATA

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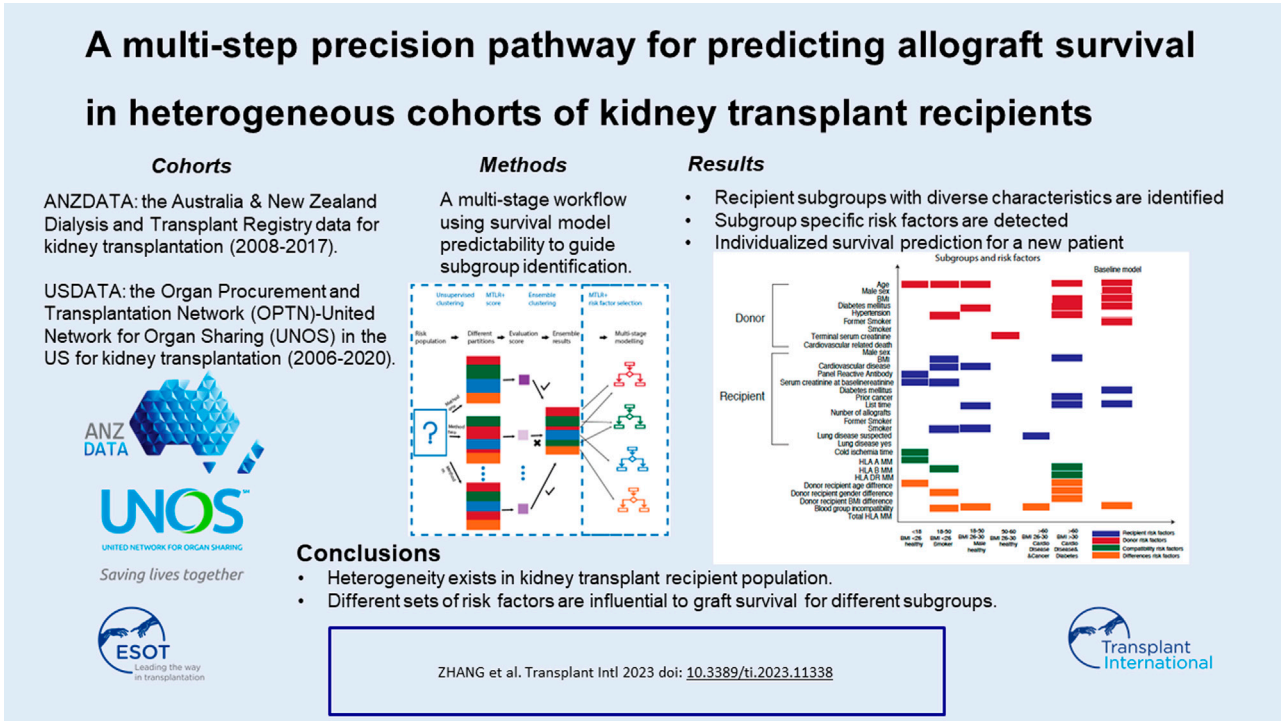
Abbreviations: ANZDATA, Australia and New Zealand Dialysis and Transplant Registry; C-index, concordance index; HLA, human leukocyte antigen; MTLR, multi-task logistic regression; P-Cube, multi-step precision prediction pathway; SRTR, Scientific Registry of Transplant Recipient.



GRAPHICAL ABSTRACT |

INTRODUCTION

Kidney transplantation confers significant survival and quality of life advantages compared with dialysis for patients with kidney failure [1]. Despite improvements in both short and longer-term allograft survivals in the last two decades, recipients' survival and quality of life remain inferior compared to the general population, attributed mainly to the complications of immunosuppression including infections, metabolic diseases, and cancer [2]. Maintaining optimal patient and graft survival are therefore the key priorities for transplant recipients, caregivers, and health professionals. Personalized predictions for those at risk of adverse events such as acute rejection, infections, cancer, and allograft loss allow early identification and interventions to optimize clinical care [3]. The derived probabilities of these predictive factors offer unique opportunities for health professionals to target appropriate management options such as immunosuppression strategies at the time of and after transplantation.

Over the past decade, several predictive factors for longer-term graft and patient outcomes have been identified as variables of importance using machine learning-based and traditional regression models [4]. However, prior studies have not accounted for the heterogeneity between subgroups within a transplant cohort [5, 6]. Allograft outcomes are consequences of many pre- and post-transplant events, precipitated by numerous known and unknown factors over the lifespan of a transplant recipient, and may differ between patient characteristics such as age, ethnicity, sex and gender, and other social determinants of health. Knowledge of these factors will guide individualized treatment plans and clinical decision making. Using an established evaluation framework, combined with novel supervised and unsupervised data driven approaches, we first identified the important characteristics that differentiate between distinct recipient subgroups for graft survival predictions. We then developed predictive models for longer-term allograft outcomes within the individual clusters. Finally, we externally validated these models to determine the reliability of their performance characteristics.

MATERIALS AND METHODS

Study Populations

Two separate cohorts, data from all deceased donor kidney transplants within the Australia and New Zealand Dialysis and Transplant Registry (ANZDATA) and the Scientific Registry of Transplant Recipient (SRTR) Registry, were used for the modeling step (**Figure 1**). The ANZDATA registry includes all kidney transplant recipients between 2008–2017 in Australia and New Zealand. The SRTR registry includes patients transplanted between 2006–2020 in the United States (US). The SRTR database includes data on all donors, waitlisted candidates, and transplant recipients in the US, submitted by the members of the Organ Procurement and Transplantation Network. In this analysis, we selected data from the Australian

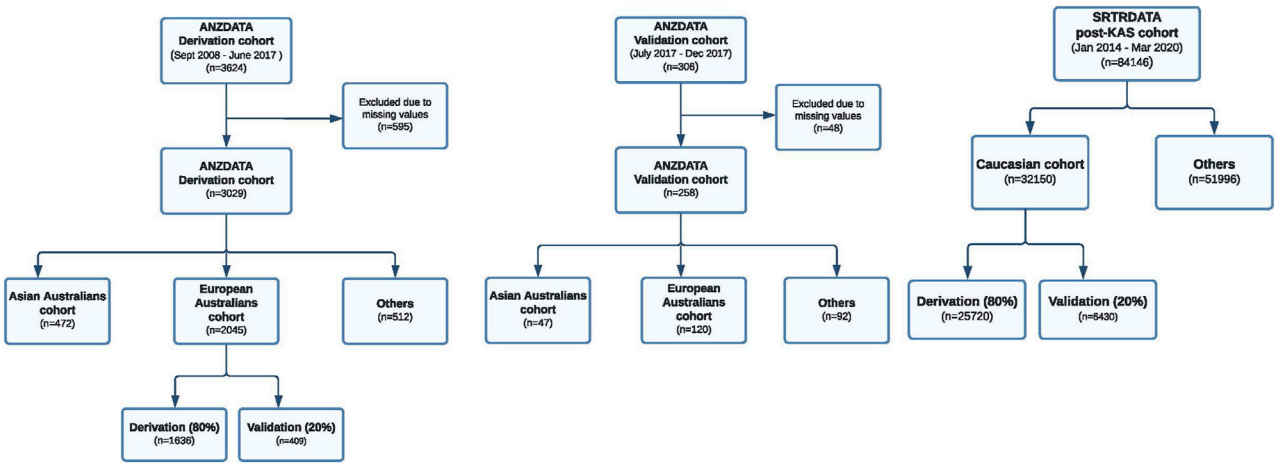


FIGURE 1 | Study flow of the Australian and US derivation and validation cohorts. ANZDATA derivation cohort, ANZDATA validation cohort and the US transplant cohort are shown.

populations and excluded all donor and recipient data from New Zealand. Data from New Zealand was excluded from the analyses because the deceased donor allocation algorithm (and systems) in New Zealand is different to Australia. There would be significant heterogeneity if both cohorts were combined as a training cohort. We followed 3,624 patients from September 2008 to June 2017 over the median graft survival period of 3.23 years (IQR: 1.79, 5.40 years).

Study Design

Next, we present a general description of the two different models, a novel multi-step precision pathway and the classical regression model, for the prediction of overall graft survival after kidney transplantation. Epidemiological data have shown that post-transplant outcomes are not uniform for all transplant recipients. Allograft survival differs among gender, ethnic groups, socioeconomic status, and comorbidity status within a heterogenous kidney transplant cohort [7–11]. Therefore, if a group-blind classifier is trained on the entire cohort of transplant recipients for the prediction of allograft outcomes, this classifier will not fit well for all candidates. Rather, the optimal fit will likely apply to the majority, attributed largely to the large sample size, and ignore the minority groups. To address the issue of "fairness" in machine learning [12], we developed a precision prediction pathway (P-cube model) that considers the heterogenous characteristics within different subgroups.

The P-cube model was first developed using data from the European Australian sub cohort. We then assessed the predictive performances of this P-cube model for overall graft survival across all three different ethnic subgroups: European Australians, Asian Australians and Aboriginal and Torres Strait Islander Peoples. To explore the external validity of these models, we tested the modelled algorithm using data from the SRTR registry (n = 32,150). Here, we split the data (80:20) into a derivation cohort (n = 25,720) and independent validation cohort (n = 6,430). The classical regression model was developed using data from the entire Australian derivation cohort and did not account for cohort heterogeneity. We then compared the predictive performances of the P-cube and the classical regression models across all subgroups. The conduct and reporting of this study adhere to the transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): The TRIPOD statement [13].

Statistical Analysis

Model Building

Model I: The Precision Prediction Pathway

The precision prediction pathway (P-cube) model (**Supplementary Figure S1**) is a hybrid algorithm that incorporates techniques of supervised and unsupervised learnings. The P-cube model consists of two elements. First, we used a "modified consensus unsupervised clustering method" to segregate a heterogeneous population into homogeneous subgroups. Second, for each subgroup, a multi-task logistic regression was applied to determine the risk factors for overall graft survival. Within each subgroup, we estimated the probabilities of graft survival. Specific modeling strategies are detailed in the following.

Modified Consensus Unsupervised Clustering. First, we applied a collection of unsupervised clustering approaches (see **Supplementary Table S1**) such as the K-means and hierarchical clustering methods to define the recipient subgroups. A data-driven ensemble clustering method [14] was used to derive a compilation of stable and robust homogeneous subgroups.

Multi-Task Logistic Regression. Using the multi-task logistic regression (MTLR) [15], we determined the risk factors for overall graft survival for transplant recipients within individual subgroups. Implementation of this workflow was performed using R version 4.1.1 and the codes are available at https://

github.com/SydneyBioX/P3_model. Variables included in the P-cube model are shown in **Supplementary Table S2**.

Selection of Important Risk Factors for Allograft Survival Within Subgroups. We used the "elbow of the curve" method [16] to determine the important risk factors for overall graft survival. The knee of a curve was defined as a vertex of the graph. This corresponded with the graphical intuition where the curvature has a maximum. Specifically, for each subgroup, the "weights" of the selected risk factors from the MTLR model were ranked from the most to the least important. We then calculated the difference in these weights between two consecutive factors. After visual inspection by a single examiner, a stop line was determined if the differences (i.e., the amount of decrease in the exact weights of the risk factor) were less than a threshold value of 0.007. We have chosen a threshold value of 0.007 because this is the elbow point across all subgroups.

Model II: Regression-Based Model

A classical risk modeling strategy was used to build a regression model to determine the risk factors for overall graft survival within the entire derivation cohort, without accounting for recipient and donor heterogeneity.

Model Evaluation

We compared the P-cube model predictive performance with the classical regression-based model using Harrell's C-index [7]. Here, we fitted the classical regression-based and P-Cube models to the independent derivation cohorts and tested the performances of each model using data from the independent internal and external validation cohorts (**Figure 1**). We examined the stability and the performance of the P-cube model (Model I) using a perturbation strategy, whereby a subset of the derivation cohort was randomly selected (80% of the original cohort) and resampled to create a perturbated P-cube model. The predicted survival probabilities of the original and perturbated P-cube models were compared numerically using Pearson correlation and visually using a scatter plot. We also performed a sensitivity analysis on death censored graft survival using both C-index and the Brier Score. For overall patients' survival as the outcome of interest, we built the corresponding P-cube model and then assessed its performances.

Model Application

To apply the P-cube model in clinical settings, a "model decision tree" was built based on subgroup characteristics. The decision tree allowed us to define the most appropriate prediction pathway and the overall graft survival probability was then estimated for each hypothetical donor-recipient pair.

RESULTS

Baseline Characteristics of the Australian and US Cohorts

Within the Australian cohort**,** the average (SD) donor age of the derivation cohort was 48 (16.8) years, with the majority being male (54%), and 26% were from donors of circulatory (DCD). With regards to the recipient characteristics, the mean (SD) age of the derivation cohort was 52 (14.3) years, with the majority being men (65%), and 18% had diabetes at the time of transplantation. Similar characteristics were observed in the independent Australian validation cohort. Within the US cohort, the average (SD) donor age of the derivation cohort was 38 (15.4) years, with most of these deceased donors being male (62%). The mean recipient (SD) age of the derivation cohort was 54 (15.5) years, with the majority being men (61%), and 31% of recipients had diabetes mellitus at the time of transplantation. Similar characteristics were observed in the independent US validation cohort (**Supplementary Tables S3, S4**)**.**

Prediction Performances of the Classical Regression and P-Cube Models

For the classical regression model, the concordance index (C-index) (95% CI) was highest if the model was applied to the European Australian cohort: 0.95 (0.93–0.96), followed by the Asian Australian cohort: 0.87 (0.86–0.88) and the Aboriginal and Torres Strait Islander Peoples cohort: 0.78 (0.76–0.80). For the P-cube model, the C-index for the European Australians, Asian Australians, and Aboriginal and Torres Strait Islander Peoples cohorts were 0.99 (0.98–0.99), 0.93 (0.92–0.94) and 0.92 (0.91–0.93), respectively. The P-cube model was robust to small perturbations (**Supplementary Figure S2**). The Pearson correlation between the predicted survival probabilities using the original P-cube and the perturbed P-cube model was 0.92. The Brier Score (a lower score indicates better performance) for 5 years post-transplant using the classical regression model for the three cohorts compared with the P-cube model are as following: 0.217 vs. 0.216 (European Australians), 0.116 vs. 0.115 (Asian Australians), 0.218 vs. 0.216 (First Nation Peoples). Similarly, for 10 years post-transplant, the Brier Scores are 0.336 vs. 0.330 (European Australians), 0.124 vs. 0.123 (Asian Australians), 0.354 vs. 0.348 (First Nation Peoples). In our sensitivity analysis, we also found P-cube outperformed the classical regression model evaluated by both the C-index and Brier Score (**Supplementary Tables S6, S7**) for death censored graft and overall patient survivals. Similar recipient subgroups and risk factors were identified for patients' overall survival, indicating that patients' overall health level is critical for both allograft survival and post-transplant recovery (details can be found in **Supplementary Tables S6, S7**).

Defining the Individual Subgroups Using the P-Cube Model

Using an unsupervised data driven approach, six subgroups with unique recipient characteristics were identified (**Figure 2**). Each subgroup had unique features, including recipient age, comorbidities, and demographics. For example, group 1 included predominantly young transplant recipients (less than 18 years) and group 6 comprised of older recipients with

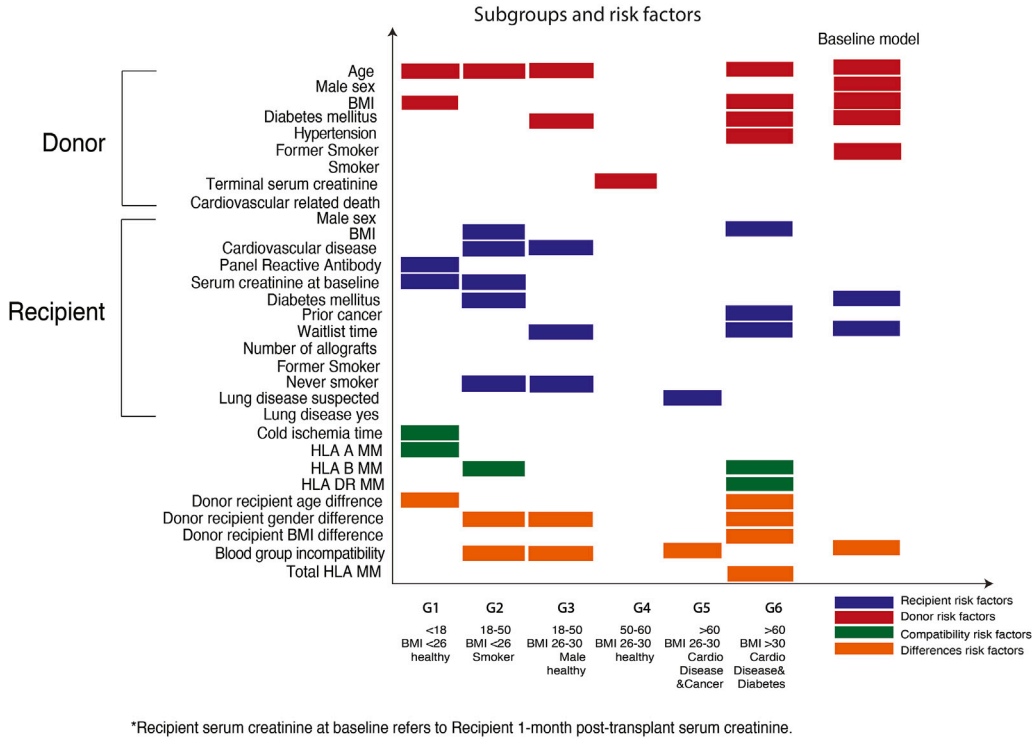


FIGURE 2 | Defining subgroups and the predictive factors for graft survivals within the Australian heterogenous populations. *X*-axis shows the six subgroups defined within the Australian transplant populations. *Y*-axis shows the predictive factors corresponding to each subgroup.

comorbidities such as cardiovascular disease and diabetes mellitus.

Risk Factors for Allograft Survival Within Subgroups

Using the elbow of the knee method (**Figure 3**), we identified the common risk factors for overall graft survival across all subgroups, and these included donor age and donor-recipient blood group compatibility. Moreover, unique predictive factors were also observed within the heterogenous subgroups. Within the pediatric sub cohort, donor age, recipient sensitization status (defined as panel reactive antibody), and donor-recipient age differences were the most important factors for allograft survival. Among the older recipients and those with comorbidities, human leukocyte antigen (HLA) DR mismatches were most predictive for overall graft survival.

External Validation Using Data From the SRTR Registry

When applied the modelling to the US cohort, the C-indices for the P-cube and classical regression models were 0.84, and 0.83, respectively. Within specific ethnic subgroups in the US, the predictive performances were comparable across the White and Asian sub-populations. Similarly, we also identified 8 subgroups within the US cohort (**Supplementary Figure S3**). Of all known risk factors, recipient-donor age difference was the most important predictive factor for overall graft survival within a sub-cohort (middle-aged recipient with comorbidities).

Applying the P-Cube Model in Clinical Settings

To test the application of the P-cube model in "real-life" settings, we applied the algorithm in three hypothetical transplant candidates with different characteristics (**Supplementary Figures S4, S5**). Three distinct predictive pathways were identified. The red curve represents a female paediatric recipient (aged 16 years) from subgroup 1 (pathway M1 in **Supplementary Figure S4**). The green curve represents a 45 years old male recipient without any major comorbidities such as cardiovascular or lung disease from subgroup 3, pathway M3 in **Supplementary Figure S4**. Lastly, the blue curve represents a 62 years old female recipient with diabetes and cardiovascular disease at the time of transplantation from subgroup 6 (pathway M6 in **Supplementary Figure S4**). The key factors that determined allograft survival in a young candidate were donor-recipient age differences, donor age and sensitization status of the recipient. In contrast, immunological mismatches and other recipients' characteristics such as co-existing cardiovascular disease and diabetes mellitus were predictive of overall graft survival in older candidates with comorbidities.

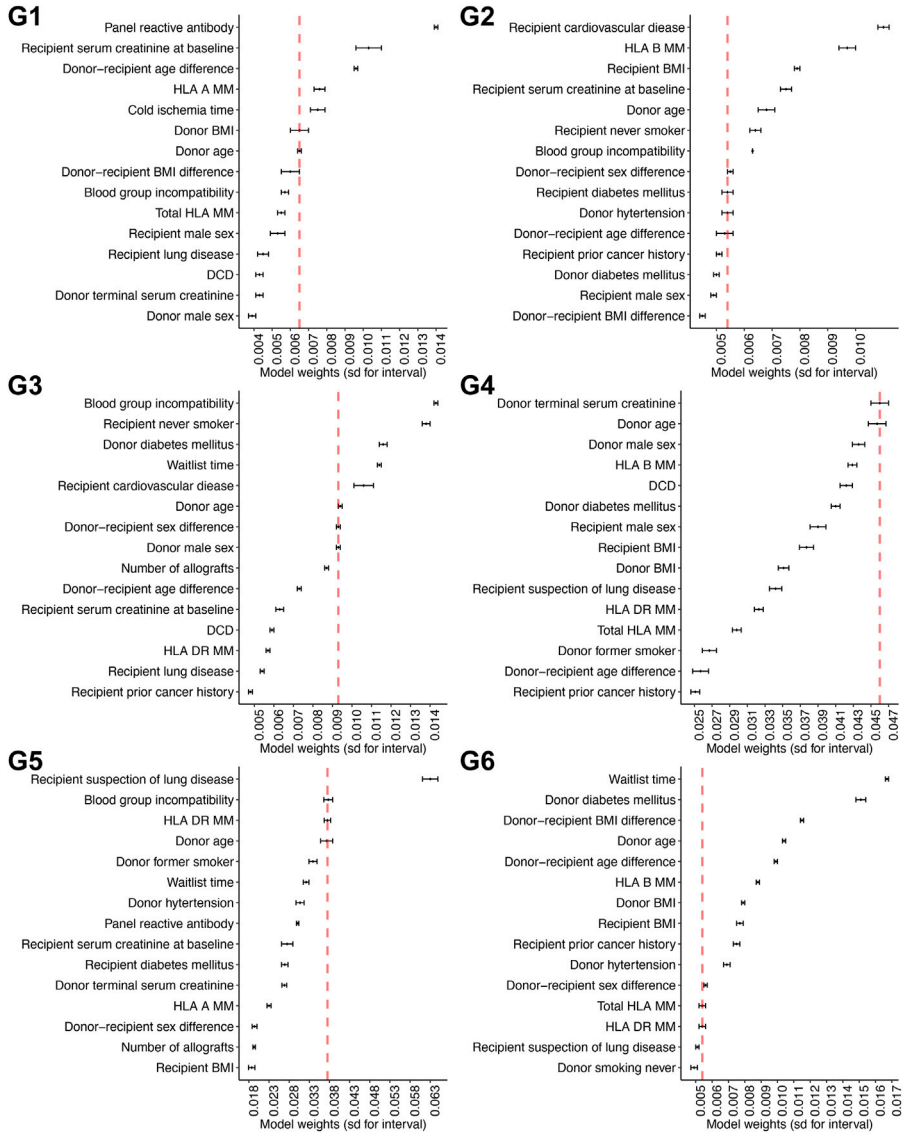


FIGURE 3 | Defining the weights for the individual predictive factors across the different subgroups Weights for each particular predictive factor within each subgroup are shown in this figure. Six panels are corresponding to six models for six subgroups.

DISCUSSION

Most of the published approaches to predict allograft and patient survival after transplantation use a one-size fits-all-model to apply to the entire transplant population and do not capture the heterogeneity within the population of interests. Many of these models construct a single risk score and apply it to the whole population without considering the nuances and the risk profiles of the individuals. Using data from the Australian kidney transplant population, we developed a novel prediction pathway using combined supervised and unsupervised data driven approaches to allow personalized prediction for allograft survivals in a heterogeneous cohort of kidney transplant recipients. The P-cube model has good discriminative power across all subgroups in the Australians cohort with improved predictive ability, particularly for minority groups such as our Aboriginal and Torres Strait Islander Peoples, when compared with the classical regression model. We have also demonstrated robustness and external validity of our modelling with good predictive ability for allograft survivals within the US transplant populations. Another novel aspect of the P-cube model is its ability to segregate and characterize the predictive factors within a homogenous subgroup. Our model recognized some of the features such as donor age that are consistently and equally important across all subgroups, while some factors such as HLA-DR mismatches and sensitization status are unique to certain membership within individual subgroups. Thus, allowing accurate survival predictions for patients and families in real time.

The P-cube model provides an opportunity for personalised prediction of longer-term allograft survival in kidney transplant recipients. Prior models depend largely on static and one-dimensional data at fixed time points and fixed covariates. The P-cube model is a flexible platform that allows identification of individuals who may be at a higher risk of experiencing allograft loss. This in turn allows clinicians to provide a more accurate prognosis for patients as well as potential for early intervention (such as modification of immunosuppression or to instigate other monitoring strategies). Understanding patients' graft and patient prognoses will facilitate access to certain services and benefits. In addition, knowledge of the transplant recipients' predicted long-term outcomes provides an opportunity to refine our allocation algorithm, with consideration of both donor and recipient characteristics to facilitate appropriate allocation pathways to maximise efficiency and efficacy of transplantation. Finally, it is important to emphasise that this model is not only limited to kidney transplant recipients, but can also be applied to other solid organ transplant recipients with input of appropriate variables.

Our P-cube model can be applied to the assessment of other subcategories across different transplant settings. Using an array of unsupervised learning approaches (partitioned and hierarchical-base methods), the P-cube model allows integration of other non-traditional clinical risk factors such as molecular immunological data (such as eplet mismatches or T cell epitope predictions) to allow for personalized risk predictions. The P-cube model can also handle regression, classification, and survival analysis in a streamlined algorithm. Our model can also be easily re-trained as new information becomes available and when clinical practices change with time.

Our modeling approaches, however, have several potential limitations. The computational time for this combined supervised and unsupervised learning strategy is lengthy and may take up to 24 h for processing time with currently available standard desktop computing. In future work, the selection of the threshold values for the determination of important risk factors for graft survival could be examined further as well as other methods such as bootstrapping and permutation tests. The ANZDATA registry does not routinely collect anti-HLA donor specific antibodies. Having access to these additional immunological data may enhance the model performance. We have validated the model in a single external validation dataset and assessed its performance within ethnics subgroups. Future research should test this algorithm in other subpopulations including different genders and socioeconomic groups.

In conclusion, we have developed a multistep prediction tool for allograft survival to guide clinical-decision making within a heterogenous cohort of kidney transplant recipient. This model can be extended to include other time to event endpoints such as patient and cause-specific survivals and acute rejection in future iterations. Findings derived from the P-cube model will provide health professionals and patients the relevant prognostic information to guide treatment decisions and contribute to personalized care.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://www.anzdata.org.au/anzdata/.

AUTHOR CONTRIBUTIONS

The model was designed by YZ with guidance from JY, SM, and GW. The implementation of the model and data analysis was done by YZ. The model validation was done by YZ with guidance from JY, SM, and GW. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

Author JY was employed by the company Laboratory of Data Discovery for Health Limited (D24H).

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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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11338/full#supplementary-material

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When There is No Guidance From the Guidelines: Renal Transplantation in Recipients With Class III Obesity

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Whilst renal transplantation is the optimal treatment for many patients with end-stage kidney disease, the latest international guidelines are unable to make recommendations for the management of patients with end-stage kidney stage kidney disease and Class III Obesity (BMI ≥40 kg/m^2). Data on all adult patients receiving a kidney-only-transplant in the UK between 2015–2021 were analysed from a prospectively collected database and interrogated across a range of parameters. We then analysed in detail the outcomes of patients transplanted at the highest-volume unit. There were 22,845 renal transplants in the study time-period; just 44 (0.2%) were performed in recipients with a BMI ≥40 kg/m^2. Most transplant centres did not transplant any patients in this category. In the centre with the highest volume, there were 21 transplants (9 living donor) performed in 20 individuals (13 male, median age 46 years). One-year patient and death-censored graft survival was 95% and 85%. Successful transplantation is possible in patients with BMI ≥40 kg/m^2 but carries additional risk. Obesity should not be the sole factor considered when deciding on transplant suitability. Restricting transplantation to a small number of high-volume centres in each country should be considered to optimize outcomes.

Keywords: end-stage renal disease, graft function, guidelines, kidney transplantation, transplant assessment

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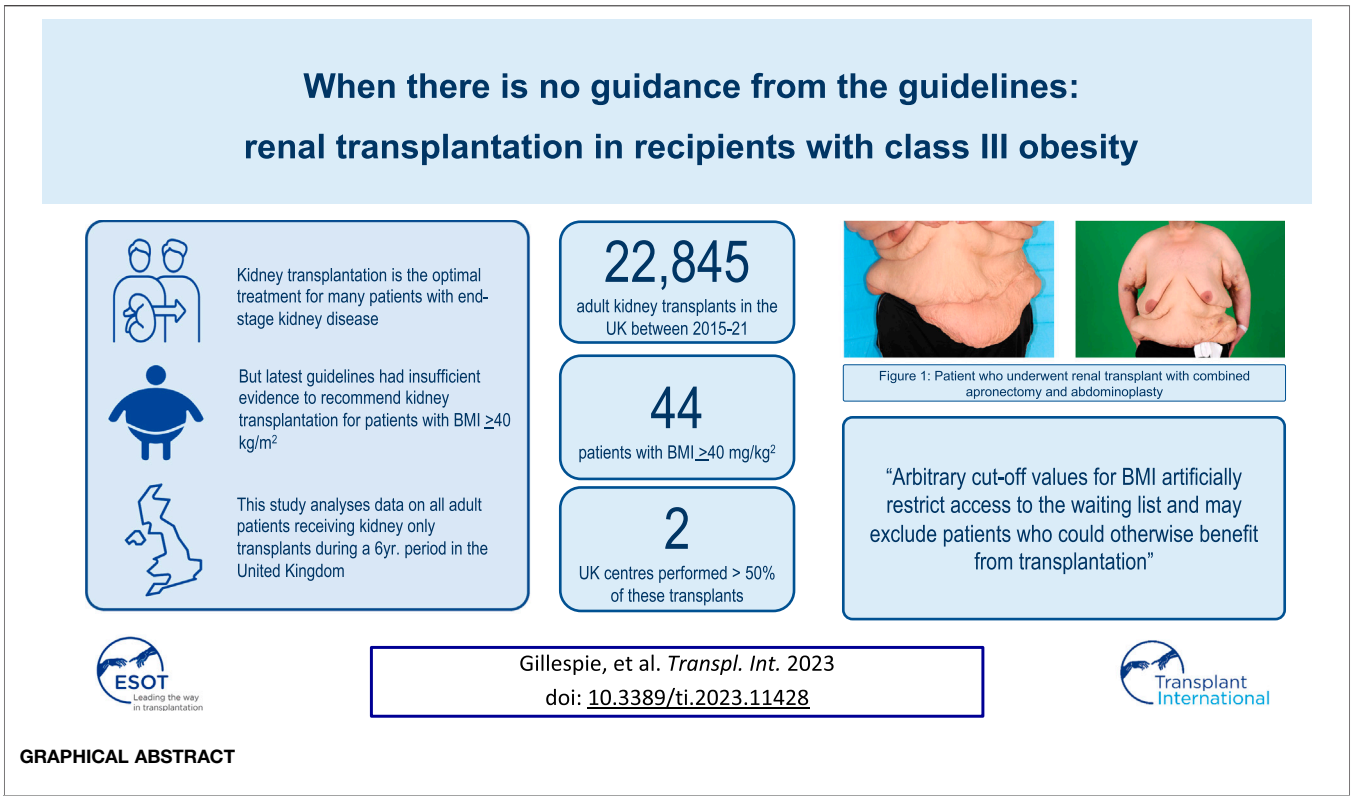
INTRODUCTION

Globally, the prevalence of obesity has tripled since 1975, with a current estimate that over 650 million adults across the world are obese [1]. The rising prevalence of obesity in the general population is mirrored in patients with end-stage kidney disease (ESKD). This trend poses challenges to nephrologists and transplant surgeons alike [2]. Obesity can be a causative or contributing agent to the development of ESKD, may accelerate the progression to renal failure [3], and limit management options or efficacy.

Traditionally the metric used to categorise obesity is body mass index (BMI). It is easy to measure using routinely collected health data, and so has become a useful tool to correlate weight with adverse health outcomes at a population level [4]. Although imperfect, it remains a commonly used, easily measured and a practically useful measurement [5]. Obesity is defined as a BMI ≥30 kg/m^2 and can be subdivided into classes I (BMI 30–34.9 kg/m^2), II (BMI 35–39.9 kg/m^2), and III (≥40 kg/m^2).

Kidney transplantation is the "gold-standard" form of renal-replacement therapy, offering patients both improved quantity and quality of life compared to maintenance dialysis therapy [6]. Additionally, it is more cost effective [7, 8]. Obesity, however, confers additional risks to patients undergoing transplant. First, the hazards associated with general anaesthesia are magnified [9–12]. Second, there are greater technical



GRAPHICAL ABSTRACT

challenges specific to transplant surgery including increased difficulty of vascular anastomoses, increased blood loss, potential for delayed graft function, and wound complications [13, 14]. And third, there is a higher likelihood of adverse outcomes related to long-term immunosuppression following transplantation, such as hypertension and diabetes mellitus [14–16].

Despite the rising prevalence of obesity within the ESKD population, only a small percentage are listed for transplantation [17]. Many guidelines exist to assist clinicians in assessing patients' suitability for renal transplantation. The European Renal Association (ERA) latest guidelines, published in 2021, suggest kidney transplantation is the optimal treatment for people with ESKD and a BMI up to 39.9 kg/m^2, but conclude there is insufficient data to make a recommendation for patients with a BMI ≥40 kg/m^2 [18]. The Kidney Disease: Improving Global Outcomes (KDIGO) guidelines suggest that transplantation in patients with BMI ≥40 kg/m^2 should be approached with caution, and patients should be counselled on the increased post-operative risks [19]. British Transplantation Society guidelines state that although obesity is not an absolute contra-indication to transplantation, individuals with a BMI ≥40 kg/m^2 are less likely to benefit [20].

Given this uncertainty within the clinical community, it is likely that many patients are denied transplantation on the basis of their BMI alone [21]. A recent survey of 23 transplant units in the UK showed that the overwhelming majority of units (20/23) had a BMI "cut-off"—by which patients who exceeded the BMI target were not considered for transplantation [22]. Others may be considered for transplantation upon reaching a target weight. The practice of delayed listing, however, may itself be harmful by increasing time spent on dialysis, thereby patients already at a higher risk for complications accrue further comorbidity [23, 24]. The dietary and lifestyle restrictions associated with ESKD, mean that achieving significant weight loss is particularly challenging for this cohort compared to the general population [25]. Latest guidelines support bariatric surgery for patients with BMI ≥40 kg/m^2, or BMI ≥35 kg/m^2 with additional co-morbidity, before transplantation [18]. However, access to timely bariatric surgery may be problematic in many regions.

It is accepted that transplantation confers a survival advantage for those patients with a BMI up to 39.9 kg/m^2 [18]. However comparable literature on outcomes for patients with Class III obesity (BMI ≥40 kg/m^2) is limited. We aimed to consider the graft and patient survival of recipients in the UK who had Class III obesity at the time of renal transplantation. Because national datasets are often unable to reliably capture relevant outcome measurements such as wound infections, biopsy-proven acute rejection, in-patient stay and other important metrics, we also analysed more granular short-, medium-, and long-term outcomes of the class III obese recipient cohort in the UK unit with the single greatest experience in this area.

MATERIALS AND METHODS

Setting

The United Kingdom (UK) has a population of 67 million people, which is served by 23 adult renal transplant units. National Health Service Blood and Transplant (NHSBT) provides transplant services to the NHS across the UK. NHSBT is

permitted to use patient identifiable information for service evaluation and safety monitoring without the consent of patients. Datasets are constructed based on information returned from individual transplant centres across the UK. The data available for analysis is anonymised at an individual level.

Northern Ireland (NI) is a distinct region within the UK with a population of 1.9 million people. All kidney transplants are performed in a single centre at the Regional Nephrology and Transplant Unit, Belfast City Hospital. Rates of obesity reflect those in the wider UK population [26], however, there is currently no bariatric surgery service available for patients in NI. Robot-assisted kidney transplantation is not utilised in NI. All transplant recipients are prospectively entered on the Northern Ireland Renal Transplant Database, which records patient characteristics and transplant outcomes. The Office of Research Ethics Committees Northern Ireland have given ethical approval for this database to be analysed to understand and improve renal services (Project IRAS ID 323151, REC Reference 23/NI/0034).

Data Collection

UK National Data

Data on all adult patients receiving a kidney-only-transplant in the UK between April 2015–March 2022 inclusive were interrogated until the date of extraction (July 2022):

1. Recipient characteristics: BMI, age, sex, cause of renal failure, number of previous renal transplants, duration of prior renal replacement therapy (RRT).
2. Donor characteristics: age, sex, and type (living donor, deceased donor after brain or circulatory death).
3. Unit: number of recipients with class III obesity at time of transplant per adult renal transplant unit.
4. Outcomes: graft function (reported by treating clinicians as immediate, delayed (at least one dialysis session required in first post-operative week), and primary non-function) and survival time in days, and all-cause patient survival.

NI Regional Data

The outcomes of all patients who received a renal transplant with BMI ≥40 kg/m^2 in NI between April 2015 and March 2022 were analysed until the date of extraction (February 2023). The median follow up time was 1740 days (range 483–2,930 days). Data were extracted from the prospectively collected NI Renal Transplant Database included, (in addition to the UK data):

5. Immunological details: HLA mismatch and the recipient's calculated reaction frequency.
6. Transplant outcomes:
   a. Short term: organ ischaemic time, time to function (post-operative day of creatinine fall by at least 10%), dialysis requirement, critical care admissions, return to theatre, biopsy-proven acute rejection within 10 days, and length of stay in hospital for the index admission.
   b. Medium term: wound complications (infection requiring treatment with oral or intravenous antibiotics, requirement for tissue viability nursing (TVN) support, hernia), development of new-onset diabetes after transplantation (NODAT) and change in BMI at 1 year post-transplant.
   c. Long term: major adverse cardiac events (myocardial infarction, stroke, cardiac death, heart failure requiring hospitalisation, revascularisation).

Statistics

In this study, parametric data were presented as mean ± standard deviation and non-parametric data as median and range. Analyses were performed on R v3.4.0 (R Foundation for Statistical Computing, Vienna, Austria). For national data, entries were checked for discrepancies between BMI at the time of listing and transplantation. For patients with a clear discrepancy between BMI at these two timepoints, we used height and weight to determine the accurate BMI. Erroneous entries were removed from the dataset before further analysis.

RESULTS

UK National Data

During April 2015–March 2022, there were 22,845 adult kidney-only transplant operations in UK, of which just 44 (0.2%) were performed in individuals with a BMI ≥40 kg/m^2 recorded at the time of transplantation. The median BMI was 46 kg/m^2, range 40–49.4 kg/m^2.

Donor Characteristics

Thirteen donors (29%) were living donors, 21 (48%) from donation after brain death and 10 (23%) from donation after circulatory death donors. Median donor age was 53 years (range 22–75 years). Donor characteristics are summarised in **Table 1**.

Recipient Characteristics

Twenty (45%) patients were male. The median age was 46 years (range 19–63 years). Only five (11%) patients were reported to have renal failure due to diabetic nephropathy, the prevalence of co-existent diabetes at the time of transplant is unknown. The primary renal disease was polycystic kidney disease in three patients (7%).

For most patients (n = 36, 82%) this was their first transplant. Five patients had one previous transplant, two had two previous transplants, one had three previous transplants. Six patients (14%) were transplanted pre-emptively, 35 (80%) were on dialysis at the time of transplant and RRT status at time of transplant was not recorded for three patients. Time on dialysis pre transplant ranged from 334–3,242 days (data available for 26/35 patients, median 1,232 days, mean 1,319 days). Recipient characteristics are summarised in **Table 2**.

Transplant Unit Details

Of the 23 adult renal transplant centres, the majority 12 (52%) did not transplant any patient with BMI ≥40 kg/m^2 in this period, and four centres undertook this for a single patient only

TABLE 1 | Summary of donor characteristics.

UK National Data			
Age	Median: 53 years Range: 22–75 years		
Type	Living Donor N = 13 29%	DBD N = 21 48%	DCD N = 10 23%
NI Regional Data			
Age	Median: 52 years Range: 22–58 years		
Sex	Male N = 11 52%	Female N = 10 48%	
Type	Living Donor N = 9 43%	DBD N = 5 24%	DCD N = 7 33%

TABLE 2 | Summary of recipient characteristics.

UK National Data			
Age	Median: 46 years Range: 19–63 years		
Sex	Male N = 20 45%	Female N = 24 55%	
RRT	Pre-emptive N = 6 14%	Dialysis N = 35 80%	Not recorded N = 3 6%
Previous transplant	None N = 36 82%	1 N = 5 11%	≥2 N = 3 7%
NI Regional Data			
Age	Median: 46 years Range: 22–58 years		
Sex	Male N = 13 62%	Female N = 8 38%	
RRT	Pre-emptive N = 3 14%	HD N = 14 67%	PD N = 4 19%
Previous transplant	None N = 19 90%	1 N = 0 0%	≥2 N = 2 10%

(**Figure 1**). Of the seven remaining centres, two were together responsible for transplanting 26 (59%) of the recipients with Class III obesity.

Survival Outcomes

Graft survival was recorded for 39 patients (median follow up 706 days, range 0–1,793 days). There was primary non-function in one, and death-censored transplant failure in two others (day 31 and day 226). The recorded graft survival was 36/39 (92%).

Patient survival is recorded for 34 patients (median follow up 710 days, range 24–1,793 days). Three deaths were recorded, at 24, 37, and 84 days post-transplant (90 days patient survival 31/34, 91%). Overall recorded patient survival is also 31/34 (91%).

NI Regional Data

There were 841 adult renal transplants carried out in this region of which 21 (2.5%) were performed in 20 individuals with a BMI ≥40 kg/m^2 at the time of transplantation. The mean BMI was 42 kg/m^2, range 40–46 kg/m^2. The median follow-up time is 57 months (range 15–96 months).

Donor Characteristics

Nine donors (43%) were living donors, 5 (24%) from donation after brain death and 7 (33%) from donation after circulatory death donors. Median donor age was 52 years (ranged 26–57 years).

Recipient Characteristics

Thirteen (62%) patients were male. The median age was 46 years (range 22–58 years). The most common cause (5, 25%) of renal failure was polycystic kidney disease (PKD). Only one patient had diabetic nephropathy, though 5 (25%) in total had diabetes at the time of transplant. One patient had a previous non-ST-elevation myocardial infarction (NSTEMI) with subsequent coronary stenting, and another was documented to have heart failure with preserved ejection fraction (estimated 55%).

Most transplants (19/21) were first transplants. One patient had three previous transplants, and one patient was transplanted twice during the study period. Three (14%) transplants were preemptive, 14 (67%) transplants were for patients on hemodialysis, and 4 transplants (19%) were for patients on peritoneal dialysis. The mean duration of renal-replacement therapy pre-transplant was 45 months (range 0–317 months). Recipient characteristics are summarised in **Table 2**.

Immunological Details

Most patients (15/20, 75%) were not previously sensitised. The calculated reaction frequency (cRF) ranged from 0%–97%. A-B-DR mismatches ranged from 1–6 (mean 3.4). Induction immunosuppression was 500 mg of intravenous methylprednisolone intra-operatively, and Basiliximab 20 mg pre-operatively and on day 4 post-transplant for younger patients (≤40 years) or if there was a poorer HLA match (2DR, or 2B & 1 DR). Standard oral immunosuppression was Prednisolone 20 mg OD, Mycophenolate Mofetil 1g BD, and Tacrolimus with a trough level typically 12 ± 2 μg/L.

Short-Term Outcomes

A single patient required admission to critical care. The first admission was unplanned (major post-operative haemorrhage with subsequent graft loss), and the second admission (following a subsequent transplant with combined apronectomy and abdominoplasty, **Figure 2**) was planned. No other patient in the cohort required admission to critical care during this period.

Nine (43%) patients required dialysis following transplantation, in 8 (including 3/9 from living donors) this was due to delayed graft function. One patient required dialysis due to primary graft failure. The median time to a 10% fall in creatinine was 5 days (range 1–56 days). Five patients (24%) developed biopsy proven acute cellular rejection (ACR) within 10 days of transplantation. All were

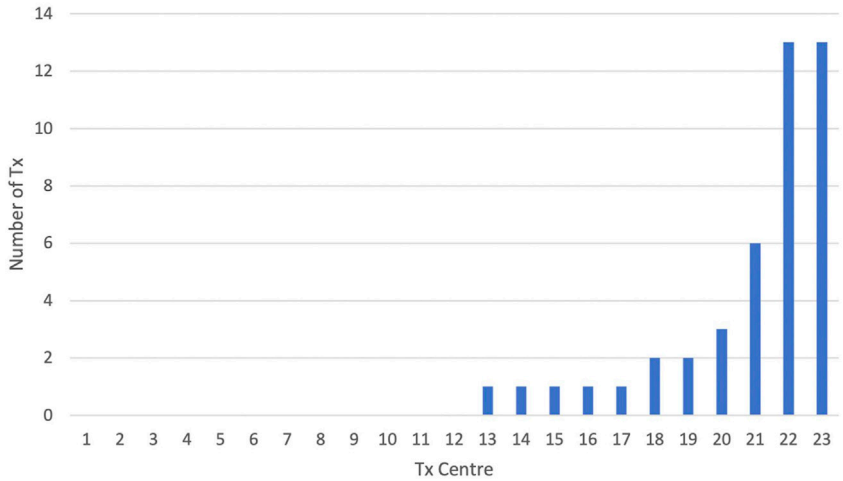


FIGURE 1 | Number of patients with BMI ≥40 kg/m^2 transplanted per UK transplant centre (n = 23) from 2015–2021.

managed successfully with intravenous methylprednisolone and up-titration of oral immunosuppression. There were no episodes of antibody-mediated rejection.

The median length of stay for the index admission was 9.0 days (range: 4–21 days). There was no significant difference between length of stay for deceased or living donor recipients.

Medium-Term Outcomes

Fourteen patients had wound related problems post-operatively. Five patients (24%) developed incisional hernia and five patients (24%) developed wound infection. There was impaired wound healing requiring specialist input in 6 patients (28%).

Five patients had diabetes at the time of transplant. Of the remaining patients 5 (31%) developed NODAT during the follow-up period. For those who did not have diabetes at the time of transplant, the median HbA1c pre-transplant was 31.5 mmol/mol (range 27–47 mmol/mol), and it was 36 mmol/mol at 1 year post-transplant (range 21–67 mmol/mol).

BMI at 1 year post-transplant was available for 16 of the 17 patients alive with a functioning graft. The median percentage-change in BMI was −2.7%, representing an overall trend of weight loss amongst patients in the cohort, though this ranged from −26% to +22%. The percentage-change in BMI at 3 years was available for all 9 patients alive with a functioning graft. Median percentage change in BMI at 3 years was −0.5%, with a range from −21% to +26%.

Long-Term Outcomes

One-year follow-up was available for all patients. Patient and death-censored graft survival was 95% and 85% at 1 year post-transplant. Three-year outcomes were available for 14 patients, patient and death censored graft survival was 79% and 82% respectively. Of 9 patients transplanted at least 5 years ago, 8 are still alive, and 7 have a functioning transplant (77% graft survival). Survival curves are presented in **Figure 3**.

The reasons for graft loss were varied: early failure secondary to graft thrombosis, non-recovery following recurrent acute kidney injury within a few weeks, late acute rejection secondary to non-compliance during COVID-19 pandemic, and acute cortical necrosis due to life-threatening ischaemic bowel.

In total, four patients died following transplantation. One patient died due to respiratory failure following COVID-19 infection, one patient died due to metastatic pancreatic cancer, one patient died due to bowel ischaemia, and one patient died of a presumed cardiac event. Amongst the remaining patients, there were no episodes of stroke, myocardial infarction, hospitalisation with heart failure, or requirement for coronary revascularisation following transplantation.

DISCUSSION

This study provides much needed evidence on the outcomes of kidney transplantation in patients with a BMI ≥40 kg/m^2. A particular strength of this study is our presentation of registry data alongside individual patient data from the highest-volume UK centre for transplants of this type, enabling a more detailed analysis across a range of parameters. The current evidence base for transplantation in individuals with Class III obesity does not allow for strong recommendations to be made [18]. This is partly due to the limited number of centres who perform transplantation at extremes of weight. Lack of support by national and international guidelines may perpetuate this reluctance, and subsequent paucity of data.

UK Transplant Practice

Two large registry studies have shown an overall mortality benefit of transplantation irrespective of BMI [17, 27]. with an appreciation that transplanting such individuals is generally

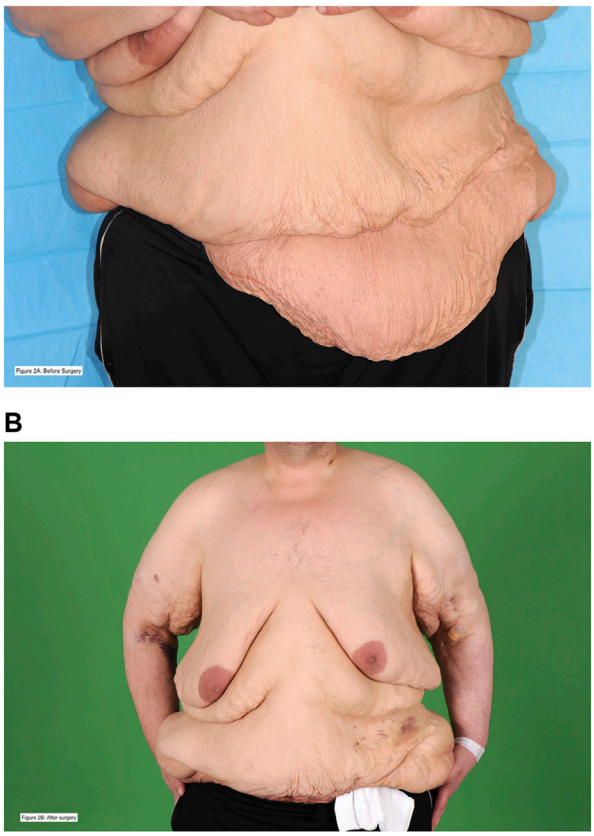


FIGURE 2 | This is a 38 year-old patient who was on haemodialysis at the time of transplant. He had a previous kidney transplant in the right iliac fossa that failed due to graft thrombosis. He developed an incisional hernia, underwent hernia repair, and after further weight loss was left with abdominal wall asymmetry and excess skin. He underwent combined renal transplantation in the left iliac fossa with apronectomy and abdominoplasty. Picture **(A)**, with a blue background, is before surgery. Picture **(B)**, with a green background, is post-surgery. The patient is alive with a functioning graft 4 years following transplantation. The patient has provided written and verbal consent for these photographs to be taken and shared for these purposes.

associated with good outcomes, although with increased morbidity and mortality compared to the non-obese recipient.

However, despite this, our review of national activity in transplantation for patients reported to have a BMI ≥40 kg/m^2, demonstrates no appreciable increase in transplantation rates for this patient cohort in the UK. From 2004–2010, 38 patients with a BMI ≥40 kg/m^2 were transplanted in the UK. In 2015–2021, 44 patients in this BMI category were transplanted. This static position in transplant numbers exists despite the substantial rise in the prevalence of obesity, including in those with ESRD, and the increase in renal transplant numbers overall in this period.

The detail of this study reveals that a reluctance to perform transplantation in this group of patients pervades the majority of UK transplant units. Only a quarter (6/23) of centres transplanted more than one patient with BMI ≥40 kg/m^2, and half (12/23) did not perform a single transplant for patients within this BMI category in this 7 years period. Just two units undertook the majority of recorded transplants (26/44). This corresponds to previous work, which showed that the majority centres in the UK operationalised "BMI cut-offs" [22].

Transplant Complexities

In our centre, the proportion of patients with Polycystic Kidney Disease (PKD) represented in the study is higher than expected (25% of patients with BMI ≥40 kg/m^2, compared to 15% of the total cohort of transplant patients in our centre in the study period). Large polycystic kidneys may contribute to some of the excess body weight for this cohort [28], and policies which operate BMI cut-offs may disproportionately disadvantage patients with PKD. Diabetic nephropathy, which may be anticipated to be more common in a cohort of patients with marked obesity, was the cause of ESKD in a single patient. Undoubtedly this reflects the careful selection in our Unit of the patients with class III obesity that proceed to transplant, with a nuanced and individualised consideration of the constellation of comorbidities for each. This is reflected in the age at transplantation, with the oldest recorded in our region being 58 years and nationally 63 years.

Critical in the selection of candidates with class III obesity for transplantation is consideration of the likelihood of complications and the physiological reserve to deal with a potential stormy post-transplant course. The granularity of the regional data allowed the nature and rate of complications to be detailed.

Delayed graft function is likely. The rate of delayed graft function is particularly unusual in those patients in receipt of a living donor transplant. In our centre in this period only 9% of living donor transplants did not function immediately, compared to 33% in this cohort (unpublished data). The rates of ACR are also higher: 7% of our patients overall compared to 24% in patients with BMI ≥40 kg/m^2 (unpublished data). It is important, however, to highlight that this can be successfully managed without deviation from normal protocol. The median length of stay was longer than typical for transplantation in our unit in this period (approximately 9 rather than 6 days), though not excessive. Within our practice the utilization of critical care is low and can be successfully anticipated for certain patients.

As expected, wound issues are common, though not inevitable (a third did not have any issue). This may require additional antibiotic therapy, input from a specialised Tissue Viability Team, and in certain cases, further operative treatment (e.g., hernia repair). NODAT developed in a substantial number, but not all patients, highlighting the need for regular monitoring and a multidisciplinary approach to post-transplant care.

Mortality is higher than our local and published national outcomes [31]. The 1 year patient survival (95%) is lower than the 98% for deceased donor and 99% for living donor kidney transplant recipients. There is an even greater difference in 1 year graft survival: 85% is considerably lower than our overall cohort, (92% in deceased donor and 99% in living donor transplantation). Interestingly only one patient had a (presumed) major adverse cardiac event, and the recorded

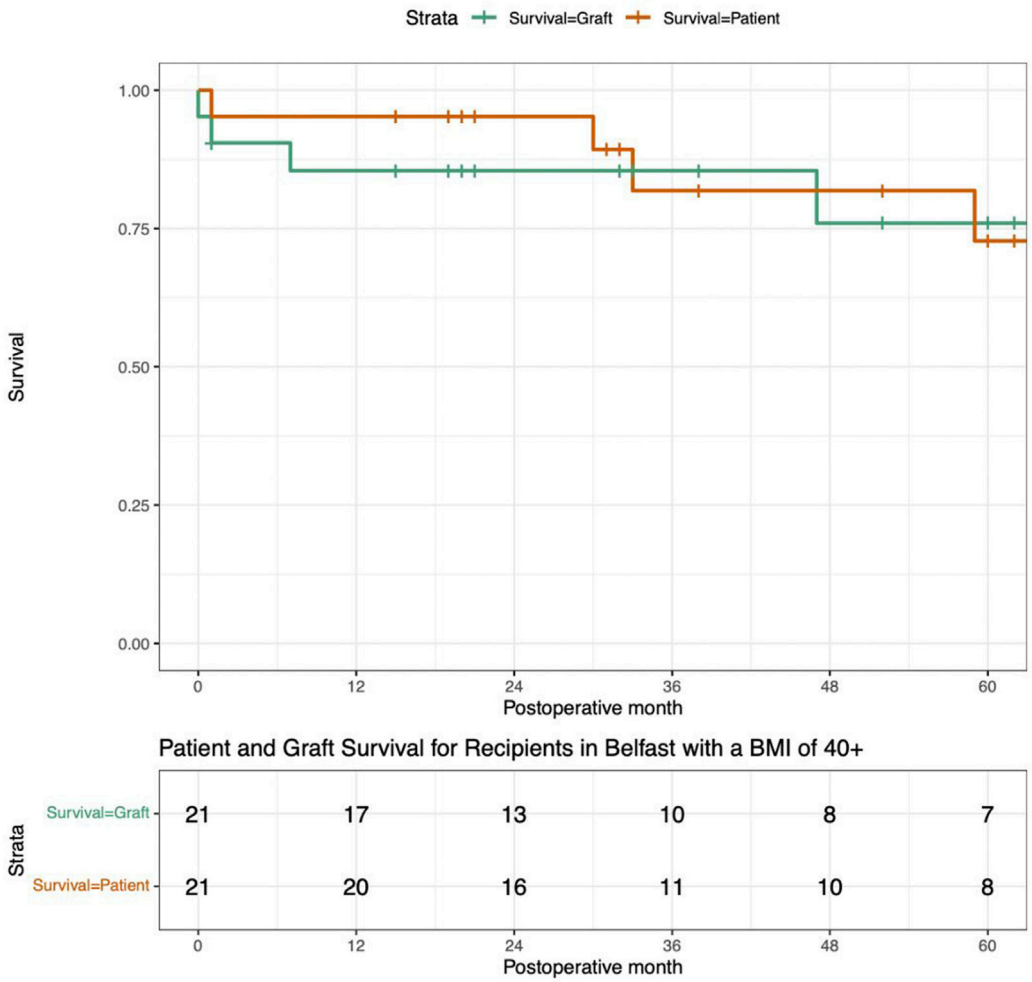


FIGURE 3 | These Survival Curves present the patient survival and death censored graft survival of this cohort of patients who underwent renal transplantation with a BMI ≥40 kg/m^2.

deaths were due to disparate causes, as may be anticipated in a group with class III obesity.

It is important to interpret the outcomes for this group with comparison to the expected mortality of living with obesity and CKD. The survival benefit of transplantation should be compared to the next best alternative, accounting for the potential difficulty in achieving adequate dialysis for patients with BMI ≥40 kg/m^2, particularly in the time constraints of in-centre haemodialysis.

Mitigation of Risk

Obviously, weight loss before transplantation is the one certain way to reduce the risks associated with renal transplantation and minimise subsequent complications. Achieving and sustaining weight loss is challenging even for those without renal failure and significant weight loss is unlikely for most patients with ESKD without surgical intervention. Recently published guidelines suggest that transplant candidates with BMI ≥40 kg/m^2 are considered for bariatric surgery before transplantation, with the intention of successful weight loss (to reduce BMI ≤39.9 kg/m^2) [18]. Bariatric surgery itself, however, is not without associated risk [31–33]. The majority of patients in our centre have ultimately had a successful transplant outcome, and if selection criteria can be further refined to identify such individuals, it could be argued that the risks of bariatric surgery, particularly combined with the increased wait-time to transplantation, may outweigh the risk of transplantation alone with BMI ≥40 kg/m^2. The evolution of new medications for the treatment of obesity, such as liraglutide, may change the risk vs. benefit profile of weight loss interventions pre-transplantation but their efficacy in patients with ESKD and Class III obesity remains uncertain [34].

If proceeding with transplantation in patients persistently with BMI ≥40 kg/m^2, then the risk of a poor outcome could be reduced by an elective operation with a living donor transplant. In our

centre this was the setting for almost half of the transplant procedures for those with class III obesity. This is reflective of our transplant practice overall but is in contrast to the national UK practice. Within the national cohort, there were just 13 living donor transplants in this cohort over the 7 years period. Yet the planned nature of this work affords the opportunity to optimise the patient's peri-operative status and arrange for experienced surgical and anaesthetic teams, in addition to preparing for critical care use for the small minority of patients who may require it. In these patients where the operative and peri-operative risk high, the benefits of living donation will exceed even the standard benefits of such transplants for patients with normal BMI. This ability to reduce some of the potentially avoidable risk may create a more favourable risk:benefit ratio in individualised decision making. Robot-assisted kidney transplantation has been reported to decrease wound morbidity in obese patients but practice is not yet widespread, and it is not available within our region [29, 30].

As with all clinical practice, experiential learning is of critical importance. For units with limited experience, embarking on the occasional transplant in a patient with a BMI $\geq$40 kg/m^2, is daunting and provides little opportunity to minimise the risks. A limited number of higher-volume centres are likely to provide a safer service model for this cohort of patients. It may be beneficial to have clear referral pathways to centres that will consider transplantation for patients where BMI alone is a precluding factor in their local unit, and thereby minimising inequity of access to transplantation.

Limitations

We are aware that BMI is an imperfect measure. Whilst this is a limitation, it reflects the data most likely to be available to clinicians at the time of assessment and transplant listing. Future work could look at the acceptability and feasibility of obtaining surrogate measures, such as waist circumference and waist to hip ratio, at clinic visits [18]. Furthermore, the fat distribution is likely to be relevant to outcomes: experientially central male adiposity is associated with greater complications than a female with relatively more adiposity in hips and thighs. The impact of this has not be described in terms of transplant outcomes.

A second limitation of this study is that we have only analysed the outcomes of patients who have been transplanted. Comparing outcomes to patients with lower or normal BMIs following transplantation is not useful, as the results for obese patients will inevitably be worse. It would be of interest to quantify the outcomes for patients with Class III obesity who are not transplanted. The most suitable comparator group may be those individuals who remain listed with BMI $\geq$40 kg/m^2. However, given the demonstrated reluctance, at least within the UK, to transplant such individuals, the comparator group is small, and would not include those otherwise suitable for transplantation who are not given the opportunity to be listed for transplantation.

A final limitation is that of registry data. Our analysis was limited by the amount of missing (or erroneous) data recorded in the National UK Transplant Registry. As has been reported in other studies, this restricts the potential usefulness of conclusions, particularly when analysing data for a very small number of patients [17]. It is notable that our centre, with reliable accuracy of data collection, had more patients transplanted with class III obesity than were recorded in the national statistics.

Future Studies

Further research may take the form of a prospective study, recording a variety of metrics of obesity, with long-term follow up from the point of initial assessment. It would also be of interest to understand how transplant nephrologists and surgeons make the complex decisions to list individuals with BMI $\geq$40 kg/m^2 for transplantation. Not all patients with a BMI $\geq$40 kg/m^2 were listed during the study period. We have presented the outcomes of a cohort of patients who had ESKD and BMI $\geq$40 kg/m^2 but whose other comorbidities and functional status, in combination with their BMI, meant they were deemed acceptable for transplantation. This sophisticated approach to listing, which assesses an individual's overall risk profile, rather than a single factor is likely to increase access to transplantation for all those who may benefit.

CONCLUSION

Renal transplantation is a lifesaving and life-changing intervention. Arbitrary cut-off values for BMI artificially restrict access to the waiting list and may exclude patients who could otherwise benefit from transplantation. This study shows that favourable outcomes for patients who undergo renal transplantation with BMI $\geq$40 kg/m^2 but that despite this, few centres in the UK offer this therapeutic option to their patients. Rather than a "BMI cut-off," patients will benefit most from an individualised approach to risk stratification; accounting for their BMI, other co-morbidities, the potential benefits of pre-emptive transplantation, and the adverse consequences of remaining on maintenance dialysis therapy. National consideration of concentrating expertise in this group of recipients in a smaller number of higher volume transplant centres may be useful.

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 humans were approved by The Office of Research Ethics Committees Northern Ireland (Project IRAS ID 323151, REC Reference 23/NI/0034). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

SO'N and AC contributed to conception and design of the study, oversaw statistical analysis, and actively contributed to writing of the manuscript. HG wrote the first draft of the manuscript. RC and CC contributed to the data retrieval and statistical analysis. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

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

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Impact of Calcineurin Inhibitor-Based Immunosuppression Maintenance During the Dialysis Period After Kidney Transplant Failure on the Next Kidney Graft Outcome: A Retrospective Multicenter Study With Propensity Score Analysis

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The impact of immunosuppressive therapy (IS) strategies after kidney transplant failure (KTF) on potential future new grafts is poorly established. We assessed the potential benefit of calcineurin inhibitor (CNI)-based IS maintenance throughout the dialysis period on the outcome of the second kidney transplant (KT). We identified 407 patients who underwent a second KT between January 2008 and December 2018 at four French KT centers. Inverse probability of treatment weighting was used to control for potential confounding. We included 205 patients with similar baseline characteristics at KTF: a total of 53 received at least CNIs on the retransplant day (G-CNI), and 152 did not receive any IS (G-STOP). On the retransplant date, G-STOP patients experienced a longer pretransplant dialysis time, were more often hyperimmunized, and underwent more expanded-criteria donor KTs than G-CNI patients. During the second KT follow-up period, rejection episodes were similar in both groups. The 10-year survival rates without death and dialysis were 98.7% and 59.5% in G-CNI and G-STOP patients, respectively. In the multivariable analysis, CNI-based IS maintenance was associated with better

Abbreviations: CI, confidence interval; CNI, calcineurin inhibitor; cPRA, calculated panel reactive antibody; DSA, donor-specific antibody; G-CNI, group with immunosuppressive therapy maintenance; G-STOP, group with discontinued immunosuppressive therapy; HLA, human leucocyte antigen; HR, hazard ratio; IPTW, inverse probability of treatment weighting; IS, immunosuppressive therapy; KT, kidney transplant; KTF, kidney transplant failure; PS, propensity score; p-y, patient-years.

survival (hazard ratio: 0.08; 95% confidence interval: 0.01–0.58, $p = 0.01$). CNI-based IS maintenance throughout the dialysis period after KTF may improve retransplantation outcomes.

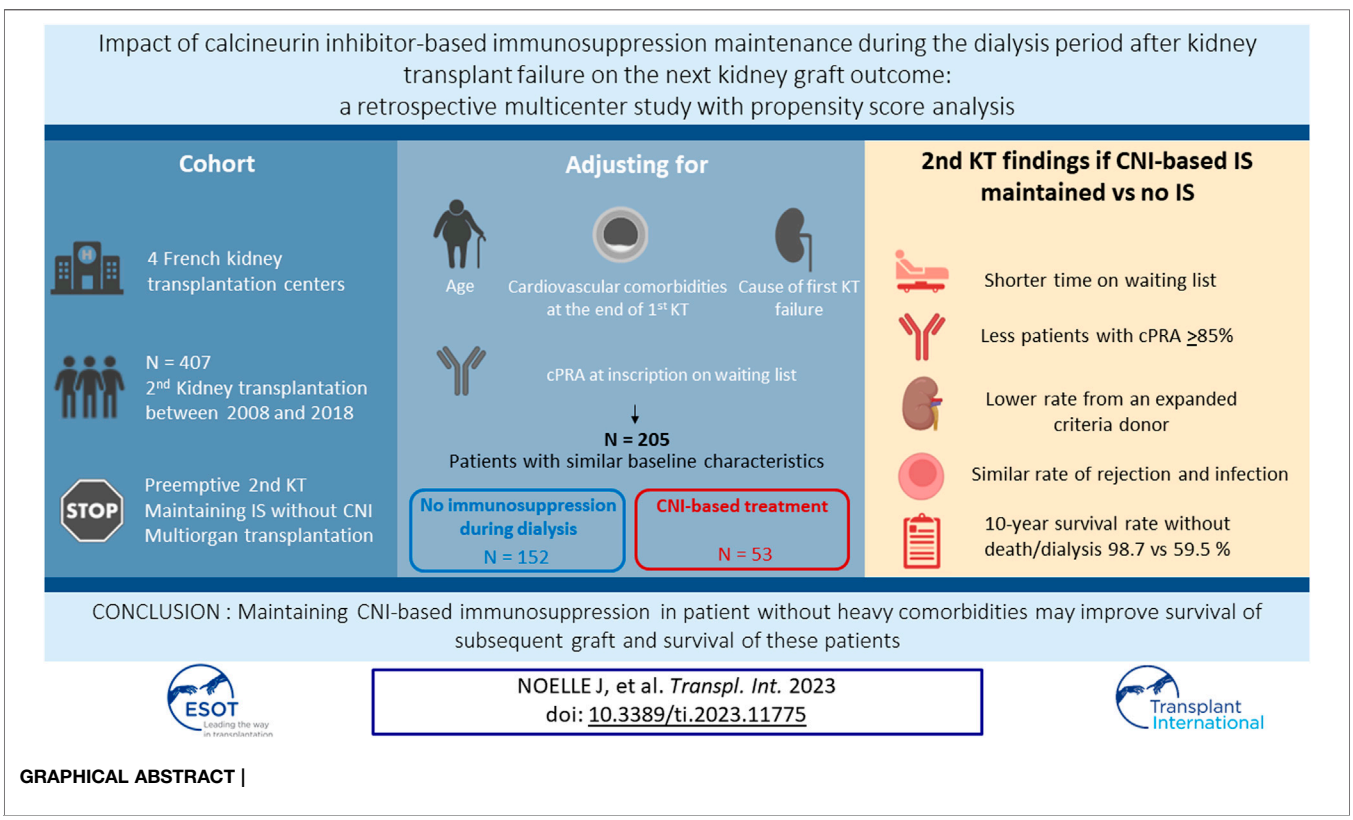
Keywords: kidney retransplant, kidney transplant failure, calcineurin inhibitor maintenance, waiting list, immunosuppression

INTRODUCTION

Since the 2000s, the number of patients waiting for a second transplant after kidney transplant failure (KTF) has increased year after year. Currently, they represent 13%–23% of patients on the waiting list ([1–4]) and approximately 14% of the transplantations performed in France [5]. The majority of these patients develop anti-human leucocyte antigen (HLA) antibodies after KTF, and immunosuppressive therapy (IS) is gradually withdrawn, thus limiting their access to a new transplant [6, 7]. They represent more than half of the hyperimmunized patients on the waiting list, defined by a calculated panel reactive antibody (cPRA) level ≥85% [1, 8, 9]. A prolonged wait time [1, 10] is associated with poorer survival of the second transplant [11–14] and increased mortality [11, 15, 16].

The optimal management of IS after KTF in potential candidates for a second kidney transplant (KT) remains uncertain [17]. Until recently, expert recommendations suggested a sequential decrease in IS with cessation of antimetabolites in the event of KTF, gradual withdrawal of calcineurin inhibitors (CNIs) with cessation between 1 month and 3 months, and a delayed cessation of steroids depending on residual diuresis and the occurrence of symptoms related to graft intolerance [18–20]. Recently, an American expert transplant group suggested stopping immunosuppressive drugs in the absence of transplantation 1 year after KTF [21]. IS withdrawal aims to minimize infectious, cardiovascular [22, 23], and neoplastic [24] risks in patients with KTF. On the other hand, the British Transplantation Society suggests maintenance of IS when a living donor transplant is planned in the year following KTF [25]. Indeed, recent studies have suggested a decrease in immunization that may allow better access to a subsequent KT if CNIs are maintained after KTF [26, 27], without an increased risk of cardiovascular or infectious events [28]. These divergences undoubtedly explain the very scarce literature on retransplant outcomes in patients with IS maintained throughout the dialysis period [29].

The objective of the present retrospective, multicenter, observational study was thus to evaluate the impact of CNI-based IS maintenance during the dialysis period until the new transplantation on the outcome of the second graft.



GRAPHICAL ABSTRACT |

MATERIAL AND METHODS

Study Population

This retrospective, multicenter study was performed at four French adult KT centers (Clermont-Ferrand, Bordeaux, Rouen and Poitiers). Patients were selected using the Cristal prospective database. The inclusion criteria were patients over 18 years old who had undergone a second KT between 1 January 2008 and 31 December 2020 at the Clermont-Ferrand, Rouen, or Poitiers transplant centers or between 1 January 2016 and 31 December 2020 at the Bordeaux transplant center (because of a change of the computerized patient record systems). The exclusion criteria consisted of second preemptive transplantations, continuation of IS treatment without CNIs, and multiorgan transplantations.

Data Collection

The following demographic, clinical, and biological data were collected: i) at the time of KTF—age, sex, body mass index, initial kidney disease, first transplant outcome and cause of allograft failure, PRA level, and the eventual presence of donor-specific anti-HLA antibodies (DSAs); ii) at the inscription on the waiting list for a second KT—PRA level and comorbidities (diabetes, stroke, ischemic heart disease, lower limb revascularization, neoplasia, and persistent post-KTF infection); iii) during the dialysis period—potential allograft nephrectomy, severe infection defined as an opportunistic infection [30] or requiring hospitalization [31], major cardiovascular events (hospitalization for acute coronary syndrome, cardiac arrhythmia, heart failure, lower limb revascularization, and stroke), and whether IS with CNIs was maintained; iv) at the retransplant initial hospitalization—induction therapy modalities, PRA level, eventual presence of DSAs (against the new KT), the type of donor (expanded criteria donor [32] or living donor), residual diuresis, and delayed graft function defined as the requirement of at least one dialysis session during the first week after transplantation [33]; and v) during the follow-up after the second KT—graft rejection episodes (Banff 2019 [34]), the appearance of DSAs, severe infection, major cardiovascular events, neoplasia, graft, and patient survival. Detection of anti-HLA antibodies was performed using the Luminex Single Antigen method (One Lambda, Canoga Park, CA) at the Clermont-Ferrand, Bordeaux, and Poitiers centers or Immucor Lifecodes (Immucor, Stamford, CT) at the Rouen center [35].

Oversight and study approval were provided by the Committee for Protection of Human Subjects (CPP SUD-EST VI) on 3 September 2019 (institutional review board 00008526) and by the National Consultative Committee on the Use of Health Research Information (14.510). No written consent was required for this study, but a non-opposition letter was sent to all patients in accordance with national legislation (MR-004 reference methodology) [36].

Definition of Groups

Two groups of patients were defined according to the modality of management of IS in the period between the two KTs: i) the CNI group (G-CNI), defined by the continuation of IS including CNIs either as monotherapy or in combination with other IS (i.e., steroids, mycophenolate mofetil, azathioprine, and mTOR pathway inhibitors) during the entire period between the two KTs, and ii) the stop group (G-STOP), defined by the cessation of all IS during the intertransplant period.

Statistics

Statistical analyses were performed with Stata software (version 15; StataCorp, College Station, Texas, USA). All tests were two-sided with an alpha level set at 5%. Categorical variables were expressed as number of patients and associated percentages, and continuous variables as mean ± standard deviation or median [25th; 75th percentiles], according to their statistical distribution.

Demographic and first transplant characteristics were compared between G-STOP and G-CNI using usual statistical tests: chi-squared test or Fisher's exact test for categorical variables and Student's t-test or Mann-Whitney test for continuous variables.

To assess the relationship between the group (G-STOP and G-CNI) and the primary and secondary endpoints, a propensity score (PS) analysis was implemented using the inverse probability of treatment weighting (IPTW) method [37, 38]. The PS was derived from the probability that treatment with a CNI would be continued for a given patient (G-CNI) conditional on confounders. The IPTW method consists of creating a "pseudo sample" of treated (G-CNI) and untreated (G-STOP) patients, weighting each patient by the inverse probability of receiving the treatment he or she actually received as follows: 1/PS in the G-CNI and 1/(1-PS) in the G-STOP. In practice, the probability of continuing CNI therapy was modeled using multiple logistic regression, and the estimated probability was used as the PS. Variables were selected for the PS based on clinical relevance: age at the end of the first transplant, cardiovascular comorbidities at the end of the first transplant, cause of first transplant failure, and cPRA level at the inscription on the waiting list for retransplant. Patients with missing cPRA levels at the inscription date were excluded from the analysis, as were patients with diabetes or infections because they were all G-STOP patients. Balance between groups was measured by standardized mean differences, calculated before and after weighting, and expressed as absolute values. A value greater than 0.2 was considered a sign of imbalance.

The primary outcome was a composite of dialysis and death after the second KT, presented as survival free of dialysis and death. This outcome was expressed as censored data and was estimated with the Kaplan-Meier method, and the groups were compared by the log-rank statistic. Multivariable analyses were performed with a Cox model (with the center as a random effect) considering covariables in terms of their significant results in univariate analysis ($p < 0.10$) as well as their clinical relevance[14,32]. The results are expressed as hazard ratios (HRs) and 95% confidence intervals (CIs).

Secondary outcomes were compared in both groups by mixed models, considering the center as a random effect: linear mixed models were used for continuous outcomes and generalized linear

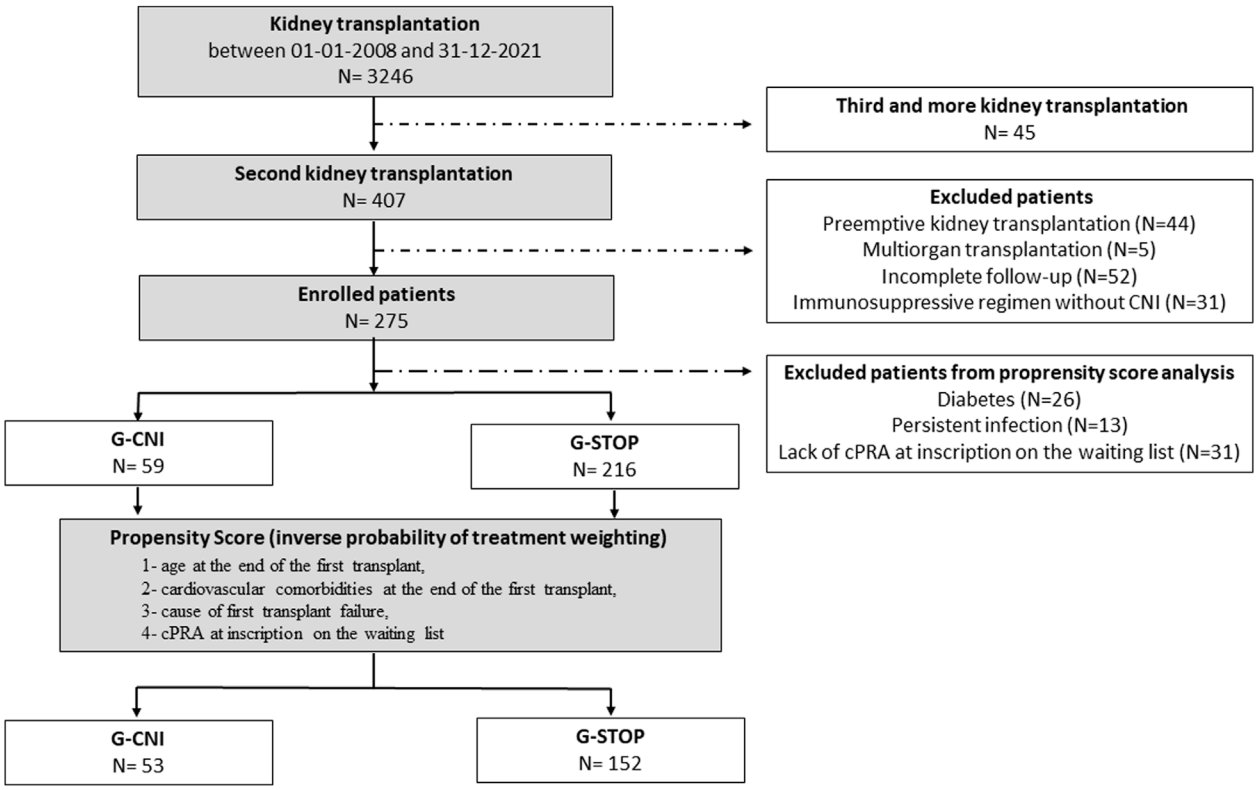


FIGURE 1 | Flow chart of the study. CNI, calcineurin inhibitor; cPRA, calculated panel reactive antibody; G-CNI, group with immunosuppressive therapy maintenance; G-STOP, group with discontinued immunosuppressive therapy.

mixed models with the logit link function were used for binary outcomes.

Finally, exposure-adjusted rates were calculated as the total number of event episodes (including recurrent events) over the total duration of follow-up and are expressed per 100 patient-years (p-y).

RESULTS

Patient Characteristics at the First-Graft Failure

Among the 3246 KTs performed at the four centers during the study period, 407 patients (12.5%) received a second KT (**Figure 1**). Five patients with multiorgan transplantation were excluded, as well as 44 patients with preemptive KT, 31 patients with IS without CNIs, and 52 due to lack of data. A total of 275 patients were included, 216 in the G-STOP and 59 in the G-CNI. The median follow-up time after the second KT was 3.6 years [2.0; 7.0].

The characteristics of the patients before IPTW are depicted in **Table 1**. The 275 patients included were mainly men (64.7%), aged 49.7 ± 13.6 years at KTF. The primary cause of the first graft loss was rejection (61.8%). G-STOP patients compared to G-CNI patients had more diabetes at the end of the first KT (11.6% vs. 1.7%, $p = 0.02$), a shorter transplantation survival (92 months [34; 163] vs. 133 [87; 220], $p = 0.003$), and a higher cPRA at inscription on the waiting list for retransplant (51% [0; 86] vs. 5% [0; 70], $p = 0.02$). The rate of hyperimmunized patients (cPRA ≥85%) in the G-STOP and G-CNI was 26.7% and 13.2%, respectively ($p = 0.04$). Among G-CNI patients, 36 (61.0%) were treated with tacrolimus and 23 (39.0%) with cyclosporine. IS maintenance until the second KT consisted of CNI monotherapy in 19 patients (32.2%) and CNI combined with an antimetabolite or corticosteroid therapy in 30 patients (50.8%). Ten patients (17.0%) received triple IS.

Patient Characteristics at the First-Graft Failure After IPTW

The characteristics at the time of KTF of the 205 patients included in the PS analysis are summarized in **Table 2**. After applying the IPTW method, the G-STOP and G-CNI were well balanced (standardized mean differences <20%) for the variables included in the IPTW model: age, cardiovascular comorbidities, the cause of first-transplant failure, and the cPRA level at inscription on the waiting list for retransplant.

TABLE 1 | Characteristics at the first kidney transplant failure date of patients with (G-CNI) or without (G-STOP) calcineurin inhibitor maintenance throughout the intergraft period.

	Total (*n* = 275)	G-STOP (*n* = 216)	G-CNI (*n* = 59)	*p*
Age at the end of G1 (years)	49.7 ± 13.6	49.1 ± 13.6	52.0 ± 13.3	0.15
Male sex	178 (64.7)	140 (64.8)	38 (64.4)	0.95
Body mass index (kg/m^2)	24.4 ± 4.6	24.6 ± 4.7	23.7 ± 3.9	0.13
Causal nephropathy				
Vascular nephropathy	14 (5.1)	13 (6.0)	1 (1.7)	0.14
Genetic nephropathy	57 (20.7)	40 (18.5)	17 (28.8)	
Glomerulonephritis	126 (45.8)	96 (44.4)	30 (50.8)	
Diabetic nephropathy	6 (2.2)	6 (2.8)	0 (0.0)	
Urological	44 (16.0)	39 (18.1)	5 (8.5)	
Other	28 (10.2)	22 (10.2)	6 (10.2)	
Comorbidities at the end of G1				
Diabetes	26 (9.5)	25 (11.6)	1 (1.7)	0.02
Cardiovascular disease[a]	37 (13.5)	28 (13.0)	9 (15.3)	0.65
Infections[b]	13 (4.7)	13 (6.0)	0 (0.0)	0.08
Solid cancer	25 (9.1)	18 (8.3)	7 (11.9)	0.40
Recurrent skin cancer	6 (2.2)	3 (1.4)	3 (5.1)	0.12
Hemopathy	5 (1.8)	4 (1.9)	1 (1.7)	1.00
G1 duration (months)	106 [43; 176]	92 [34; 163]	133 [87; 220]	0.003
Cause of G1 failure				
Rejection	170 (61.8)	132 (61.1)	38 (64.4)	0.11
Infection	10 (3.6)	10 (4.6)	0 (0.0)	
IFTA	28 (10.2)	20 (9.3)	8 (13.6)	
Vascular	37 (13.5)	33 (15.3)	4 (6.8)	
Causal nephropathy recurrence	30 (10.9)	21 (9.7)	9 (15.2)	
Presence of DSAs at the end of G1	62/228 (27.2)	46/173 (26.6)	16/55 (29.1)	0.72
cPRA at graft failure (%) (*n* = 244)	48 [0; 83]	51 [0; 86]	5 [0; 70]	0.02
cPRA at graft failure ≥85%	58/244 (23.8)	51/191 (26.7)	7/53 (13.2)	0.04

Data are expressed as the number of patients (associated percentage), mean ± standard deviation or median [25th; 75th percentiles]. cPRA, calculated panel reactive antibody; DSA, donor-specific antibody; G1, first graft; G-CNI, group with immunosuppressive therapy maintenance; G-STOP, group with discontinued immunosuppressive therapy; IFTA, interstitial fibrosis and tubular atrophy.

[a]*Cardiovascular comorbidities: cerebrovascular accident, ischemic heart disease and/or obliterating arteriopathy of the lower limbs (surgical treatment).*

[b]*Infections: numerous or persistent at the time of kidney transplant failure.*

Waiting Time and Characteristics of the Patients After the Second KT After IPTW

The median time on dialysis until the second KT was 21 months [11; 43]. This value was significantly lower in the G-CNI than in the G-STOP (16 [5; 26] vs. 37 [22; 64], respectively, $p < 0.001$). The waiting times from relisting to the second KT were 16 months [8; 23] in the G-CNI and 27 months [13; 48] in the G-STOP ($p = 0.06$) (**Table 3**).

G-CNI patients, compared to G-STOP patients, had a lower median cPRA level at the time of the second KT (67% [0; 84] vs. 87% [55; 96], $p = 0.001$). The rate of hyperimmunized patients was also lower in the G-CNI: 23.9% versus 55.2% in the G-STOP ($p < 0.001$). The numbers of patients transplanted with preformed DSAs and induction treatment were comparable between the groups (**Table 3**).

Patients in the G-STOP were more frequently transplanted with an expanded criteria donor graft (43.2% vs. 29.9% in the G-CNI, $p = 0.01$). Hyperimmunized patients, compared with patients with cPRA levels <85%, were more likely to receive a kidney transplant from an expanded criteria donor [44.1% and 32%, respectively ($p = 0.005$)]. On the day of the second KT, 44.1% of G-CNI patients had a residual diuresis ≥500 mL compared to 13.8% in G-STOP ($p = 0.06$). The delayed graft function rate was 9.9% in the G-CNI and 25.8% in the G-STOP ($p = 0.001$). The numbers of patients transplanted with preformed DSA and an induction treatment were comparable between the groups (**Table 3**). Data before IPTW are presented in **Supplementary Table SI**.

Outcome After the Second KT After IPTW

After the second KT, 10 years survival free of dialysis and death was significantly better in the G-CNI than in the G-STOP (HR: 0.06, 95% CI: 0.01–0.30, $p = 0.001$) (**Figure 2**), with 10 years survival rates of 98.7% and 59.5%, respectively. In multivariable analysis after adjustment for expanded criteria donor, rejection, delayed graft function, age at second KT, graft survival time from the primary transplant, and rejection as etiology of first graft failure, continuation of CNIs between the two KTs was associated with a better 10 years survival free of dialysis and death (HR: 0.08, 95% CI: 0.01–0.58, $p = 0.01$) (**Table 4**). The difference in survival also remained significant after sensitivity analysis excluding second living donor transplants, with a 10 years survival rate of 98.5% in the G-CNI versus 56.4% in the G-STOP (HR: 0.06, 95% CI: 0.01–0.30, $p = 0.001$). Data on survival before IPTW are presented in **Supplementary Figure S1**.

TABLE 2 | Characteristics at the first kidney transplant failure date of patients with (G-CNI) or without (G-STOP) calcineurin inhibitor maintenance throughout the intergraft period before and after applying inverse probability weighting.

	Before IPTW			After IPTW		
	G-STOP (*n* = 152)	G-CNI (*n* = 53)	SMD	G-STOP	G-CNI	SMD
Age at the end of G1 (years)	47.9 ± 13.8	51.9 ± 13.2	0.29	48.9 ± 14.0	48.4 ± 12.8	0.04
Male sex	97 (63.8)	35 (66.0)	0.05	(64.7)	(59.2)	0.11
Body mass index (kg/m^2)	24.4 ± 4.7	23.7 ± 3.7	0.18	24.4 ± 4.7	23.5 ± 3.6	0.22
Causal nephropathy						
Vascular nephropathy	11 (7.2)	1 (1.9)	0.26	(8.1)	(1.3)	0.32
Genetic nephropathy	25 (16.5)	15 (28.3)	0.29	(16.1)	(36.3)	0.47
Glomerulonephritis	73 (48.0)	26 (49.1)	0.02	(48.2)	(44.3)	0.08
Diabetic nephropathy	0 (0.0)	0 (0.0)	NA	(0.0)	(0.0)	NA
Urological	26 (17.1)	5 (9.4)	0.23	(16.4)	(8.0)	0.26
Other	17 (11.2)	6 (11.3)	0.00	(11.2)	(10.1)	0.04
Comorbidities at the end of G1						
Diabetes	0 (0.0)	0 (0.0)	NA	(0.0)	(0.0)	NA
Cardiovascular disease[a]	18 (11.8)	8 (15.1)	0.10	(12.7)	(11.6)	0.03
Infections[b]	0 (0.0)	0 (0.0)	NA	(0.0)	(0.0)	NA
Solid cancer	13 (8.6)	7 (13.2)	0.15	(9.8)	(10.4)	0.02
Recurrent skin cancer	1 (0.7)	3 (5.7)	0.29	(0.7)	(4.1)	0.22
Hemopathy	3 (2.0)	1 (1.9)	0.01	(1.8)	(1.4)	0.03
G1 duration (months)	92 [36; 167]	133 [90; 217]	0.44	104 [43; 172]	120 [44; 205]	0.16
Cause of G1 failure						
Rejection	96 (63.2)	34 (64.1)	0.02	(63.5)	(59.5)	0.08
Infection	0 (0.0)	0 (0.0)	NA	(0.0)	(0.0)	NA
IFTA	14 (9.2)	6 (11.3)	0.07	(9.6)	(7.7)	0.07
Vascular	23 (15.1)	4 (7.6)	0.24	(13.4)	(20.1)	0.18
Causal nephropathy recurrence	19 (12.5)	9 (17.0)	0.13	(13.5)	(12.7)	0.02
Presence of DSAs at the end of G1	30/120 (25.0)	13/49 (26.5)	0.04	(22.9)	(29.7)	0.15
cPRA at graft failure (%)	50 [0; 84]	5 [0; 70]	0.32	44 [0; 83]	56 [0; 83]	0.06
cPRA at graft failure ≥85%	37 (24.3)	7 (13.2)	0.29	(22.2)	(23.8)	0.04

Data are expressed as the number of patients (associated percentage), mean ± standard deviation, or median [25th; 75th percentiles]. cPRA, calculated panel reactive antibody; DSA, donor-specific antibody; G1, first graft; G-CNI, group with immunosuppressive therapy maintenance; G-STOP, group with discontinued immunosuppressive therapy; IFTA, interstitial fibrosis and tubular atrophy; IPTW, inverse probability of treatment weighting; NA, not applicable; SMD, standardized mean difference.

[a]*Cardiovascular comorbidities: cerebrovascular accident, ischemic heart disease, and/or obliterating arteriopathy of the lower limbs (surgical treatment).*

[b]*Infections: numerous or persistent at the time of kidney transplant failure.*

A return to dialysis was observed in 18.3% of G-STOP patients compared to 1.3% of G-CNI patients ($p = 0.004$). The main cause of graft loss was rejection (45.1%). The number of humoral rejections and the occurrence of DSA were comparable in the two groups, but there was less cellular rejection in the G-CNI than in the G-STOP (2.1% vs. 8.8%, respectively, $p < 0.001$). All deaths were observed in the G-STOP (**Table 3**; **Supplementary Table SI**).

Major Cardiovascular, Infectious, and Neoplastic Events

In the period between the two KTs, the serious infectious event rates and their exposure-adjusted rates (patient-years) in the G-CNI and G-STOP were similar (**Figure 3A**; **Supplementary Figure S2**). The rates of cardiovascular events and neoplasia and their exposure-adjusted rates were significantly lower in the G-CNI than in the G-STOP (**Figure 3A**; **Supplementary Figure S2**).

At the last follow-up after the second KT, the rates of patients with neoplastic events were similar in the G-CNI and G-STOP (**Figure 3B**). The rate of cardiovascular events was lower in the G-CNI than in the G-STOP (7.9% and 15.9%, respectively, $p = 0.04$) (**Figure 3B**). The serious infectious event rates were similar in the G-CNI and G-STOP (**Figure 3B**), but the exposure-adjusted rate was higher in the G-CNI than in the G-STOP (28.2/100 p-y and 22.8/100 p-y, respectively; $p = 0.02$) (**Supplementary Figure S2**).

Overall, after the first KTF, patients in the G-CNI and the G-STOP had a higher exposure-adjusted rate of serious infection (22.0/100 p-y and 15.3/100 p-y, respectively; $p < 0.001$) but a lower rate of major cardiovascular events (1.5/100 p-y and 4.8/100 p-y, respectively; $p < 0.001$). The exposure-adjusted rate of neoplasia was similar in both groups (**Supplementary Figure S2**).

DISCUSSION

To our knowledge, this retrospective multicenter study is the first report relative to the impact of maintaining IS with CNIs in patients with KTF throughout the dialysis period on the second KT. Our results show that the maintenance of CNI-based IS therapy during the dialysis period is associated with a lower HLA immunization rate, lower waiting time before retransplantation,

TABLE 3 | Intergraft period and second transplantation outcomes after inverse probability weighting.

	Total	G-STOP	G-CNI	*p*
Intergraft period				
Pretransplant dialysis time (months)	21 [11; 43]	37 [22; 64]	16 [5; 26]	<0.001
Time on the waiting list (months)	19 [9; 37]	27 [13; 48]	16 [8; 23]	0.06
Transplantectomy and causes	(24.5)	(34.1)	(15.4)	0.06
Thrombosis	(39.5)	(34.6)	(48.3)	0.94
Graft intolerance syndrome	(52.2)	(52.5)	(51.7)	
Infection	(1.4)	(2.2)	(0.0)	
Surgical reason	(1.2)	(1.8)	(0.0)	
Other	(5.7)	(8.9)	(0.0)	
Second transplantation				
cPRA at D0 (%)	76 [25; 93]	87 [55; 96]	67 [0; 84]	0.001
cPRA at D0 ≥ 85%	(39.4)	(55.2)	(23.9)	<0.001
Anti-HLA antibodies at D0	(82.0)	(91.5)	(72.9)	0.047
Presence of DSAs at D0	(16.5)	(16.7)	(16.4)	0.99
HLA-A/B/DR antigen mismatches (0–6)	4 [2; 4]	3 [2; 4]	4 [2; 4]	0.92
HLA- DR antigen mismatches, N = 2	(14.5)	(16.9)	(12.3)	0.39
Cold ischemia time (minutes)	940 [688; 1,110]	960 [742; 1,208]	935 [620; 1,051]	0.03
Living donor	(10.6)	(7.8)	(13.3)	0.26
Expanded criteria donor	(36.4)	(43.2)	(29.9)	0.01
Residual urine output ≥500 mL	(29.1)	(13.8)	(44.1)	0.06
Induction treatment				
No induction treatment	(0.5)	(1.0)	(0.0)	0.27
Thymoglobulin	(78.8)	(84.0)	(73.8)	
Basiliximab	(20.7)	(15.0)	(26.2)	
Delayed graft function	(17.7)	(25.8)	(9.9)	0.001
Evolution after second transplantation				
Rejection	(18.7)	(22.1)	(15.5)	0.47
Humoral	(14.0)	(14.6)	(13.4)	0.95
Cellular	(5.4)	(8.8)	(2.1)	<0.001
Development of DSA	(9.7)	(10.4)	(8.9)	0.52
Return to dialysis and causes	(9.6)	(18.3)	(1.3)	0.004
Rejection	(45.1)	(48.4)	(0.0)	NA
Infection	(2.9)	(3.1)	(0.0)	
IFTA	(27.9)	(29.9)	(0.0)	
Vascular	(24.1)	(18.6)	(100.0)	
Causal nephropathy recurrence	(0.0)	(0.0)	(0.0)	
Death and causes	(4.7)	(9.7)	(0.0)	<0.001
Infection	(19.4)	(19.4)	—	NA
Cancer	(7.8)	(7.8)	—	
Cardiovascular	(29.9)	(29.9)	—	
Other	(42.9)	(42.9)	—	

Data are expressed as the number of patients (percentage) or median [25th; 75th percentiles]. cPRA, calculated panel reactive antibody; D0, day of transplantation; DSA, donor-specific antibody; G-CNI, group with immunosuppressive therapy maintenance; G-STOP, group with discontinued immunosuppressive therapy; HLA, human leucocyte antigen; IFTA, interstitial fibrosis and tubular atrophy; NA, not applicable.

and less use of expanded criteria donors. Remarkably, G-CNI patients had a better survival free of dialysis and death at 10 years than G-STOP patients.

In the literature, the negative impact of the dialysis waiting time after KTF on the subsequent KT outcome and increased mortality is well documented [11, 14, 21]. In a recent study of 911 patients from the ANZDATA registry, each year spent on dialysis after KTF was associated with a 5% increase in the risk of death (mainly from cardiovascular or infectious events) as well as a greater risk of acute rejection and graft failure after the second KT [11]. The impact of the second KT on survival seems to be particularly beneficial when it takes place in the first 3 years after the return to dialysis [15]. One way to explain the two-fold shorter dialysis wait time in the G-CNI compared to the G-STOP in our study is the lower immunization after KTF in the G-CNI before and after IPTW. Indeed, despite a similar cPRA level at re-registration after PS analysis, G-CNI patients had a significantly lower median cPRA level at the time of the second KT. Moreover, the rate of hyperimmunized subjects was also lower in the G-CNI than in the G-STOP. These results are consistent with those previously reported in the literature. Thus, in 77 Spanish patients who experienced KTF, the cessation of CNIs in the first 6 months was significantly associated with the development of DSA with respect to the first graft (odds ratio: 23.2, 95% CI: 5.3–100.6, $p < 0.001$) [27]. In another study performed in the USA in 119 patients with KTF, 68% of patients with discontinued IS were hyperimmunized after 24 months, compared to 8% of patients with IS continuation that included a CNI ($p < 0.001$) [26]. The latter had better access to retransplantation (46% vs. 29%) and a shorter median waiting time between relisting and the

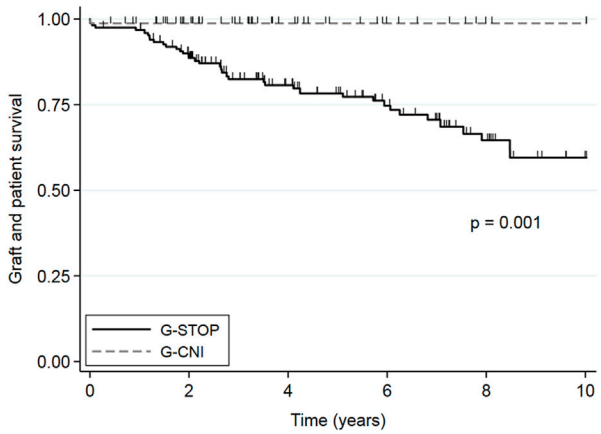


FIGURE 2 | Overall second kidney transplant survival (without death or kidney transplant failure) in patients with immunosuppressive therapy maintenance until the second graft (G-CNI) or discontinued therapy (G-STOP). Data are presented after the inverse probability of treatment weighting.

TABLE 4 | Multivariable analysis of the factors associated with 10 years survival free of dialysis and death in patients after second renal transplantation after inverse probability weighting.

	HR	95% CI	*p*
Second transplant			
CNI maintenance (vs. stop)	12.50	1.72; 100.0	0.01
Expanded criteria donor	0.40	0.09; 1.79	0.23
Recipient age (years)	0.97	0.94; 1.01	0.06
Rejection	0.32	0.20; 0.52	<0.001
Cold ischemia time (minutes)	1.00	1.00; 1.01	0.77
First transplant			
Graft survival (years)	1.01	1.01; 1.01	0.002
Rejection	0.42	0.27; 0.67	<0.001

CI, confidence interval; CNI, calcineurin inhibitor; HR, hazard ratio.

second KT (17 [7; 55] vs. 36 [3; 72] months) [23]. The significantly higher rate of hyperimmunized patients in the G-STOP group may explain in part why these patients received more expanded criteria deceased donor allografts [32]. Indeed, French biomedical agency gives priority access to KT for patients with PRA levels >85%. In comparison with other donor types, the use of expanded criteria deceased donor kidneys for transplantation has a significant negative impact on graft survival [39–41] and death [40], whether it is a first transplant or a retransplant [14].

While the rate of humoral rejection was similar in both groups, we observed a lower rate of cellular rejection in the G-CNI than in the G-STOP after the second KT. The rates of second transplants with preformed DSAs and *de novo* DSAs were similar in the two groups, which may explain the similar humoral rejection rates in the two groups [13]. Healthy et al. previously reported risk factors for acute rejection after retransplant as a shorter primary graft survival, rejection in the first KTF, and a long time spent on dialysis [14]. We can hypothesize the role of alloreactive memory T-cells [42, 43] acquired during the first allograft period but also during the dialysis period [29]. Indeed, a recent German retrospective study [29] reported a significantly lower rate of T-cell-mediated rejection of the second KT and better graft survival ($p = 0.02$) in patients with *in situ* previous transplants who also usually had CNI maintenance compared to patients with first allograft nephrectomy who also usually discontinued therapy. The authors observed less T-cell alloreactivity measured by ELISPOT assay against the pretransplant donor in the group with CNI maintenance for a prolonged period compared to patients with discontinued treatment due to transplantectomy [29].

The benefit-risk balance of IS maintenance until a new KT is widely debated in the literature. Some retrospective cohort studies have observed higher rates of major cardiovascular, infectious, or tumor events in patients with IS maintenance [22, 23]. However, the IS regimens continued after KTF are highly variable and could include only low-dose corticosteroids. In our previous work, we reported an increased risk of infection associated with the continuation of corticosteroids but not with CNI maintenance therapy [44]. In the present work, we did not observe an increase in these adverse events before the second KT in the G-CNI. Our results are similar to the most recent data available [26]. In a study of 102 patients with KTF, mortality was similar in patients in whom IS was discontinued early within 3 months after KTF ($n = 52$) and in patients ($n = 50$) in whom IS was continued with antimetabolites and/or CNIs [45]. A Canadian prospective registry did not observe any difference in the infectious rate between patients in whom IS was continued after KTF and those in whom IS was discontinued [28]. However, we observed higher exposure-adjusted rates (p-y) of serious infectious events in G-CNI after the second KT. Future studies will have to be vigilant regarding this point.

The current work includes several limitations. First, due to the retrospective nature of the study, major differences between the two groups were observed, such as the rates of diabetes at the end of the first KT, persistent infections at the time of KTF, and PRAs level at relisting in the G-CNI. We thus proposed a PS analysis using the IPTW method to reduce the effect of these confounding factors that may have influenced survival. However, we cannot exclude the existence of factors not accounted for [46]. Indeed, there seem to be patient profiles in which IS is more likely to be maintained, such as the persistence of significant diuresis [47] or a living donor transplant [48]. Recently, a prospective Canadian study showed a similar profile of patients on IS therapy after KTF. Other underlying confounding factors are probably unknown, such as social level [49] and ease of access to care [50, 51]. One of the main decision-making factors remains the prescribing habits of transplant nephrologists, as highlighted by recent surveys in the USA [48, 52] and France [44]. Only a prospective randomized study will be able to overcome the confounding factors. Second, as this study focused on patients who had access to a second KT, we cannot exclude the possibility that patients who had continued CNI-based IS after KTF experienced serious adverse events with abandonment of the retransplant plan or even death without being counted. Additionally, we were not able to access the date of cessation of IS treatment and thus establish its temporality in relation to the possible occurrence of an adverse event. However, we previously carried out a preliminary

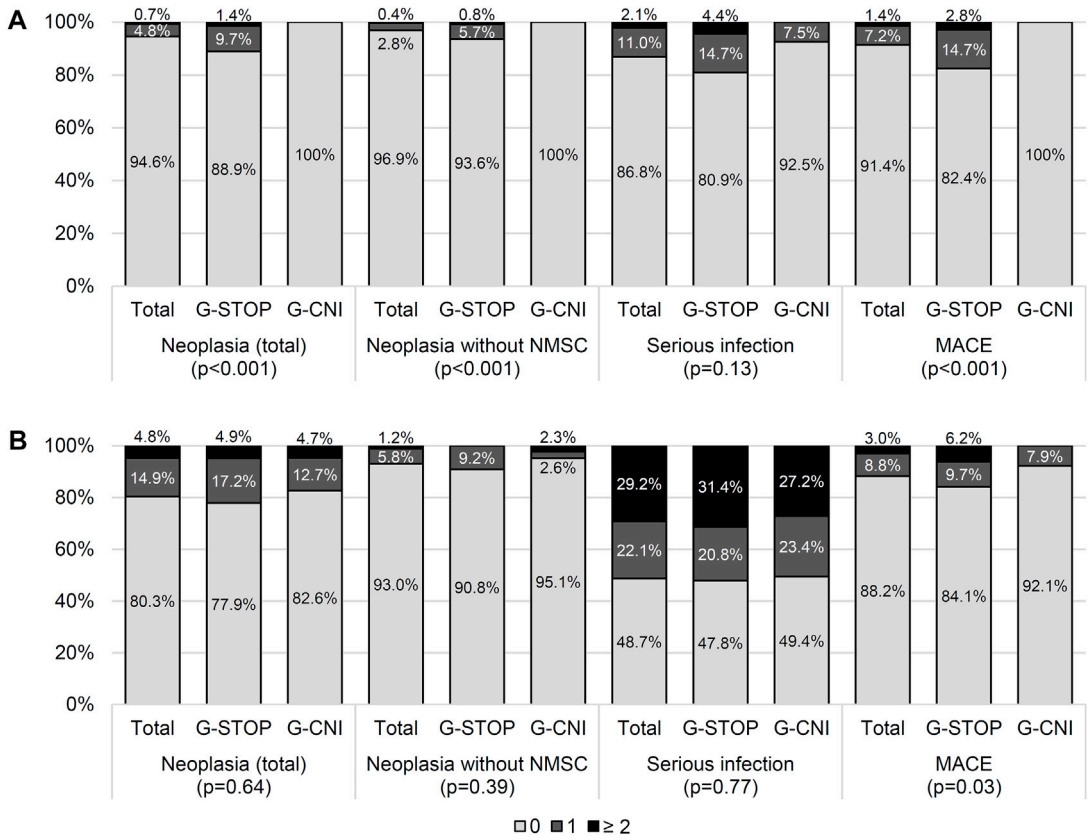


FIGURE 3 | Adverse events between the two kidney transplants **(A)** and after the second transplant **(B)** in patients with immunosuppressive therapy maintenance (G-CNI) or discontinued therapy (G-STOP). Data are presented after the inverse probability of treatment weighting. MACE, major cardiovascular event; NMSC, non-melanoma skin cancer.

retrospective study of 119 KT patients relisted after KTF at four French adult KT centers. We did not report an increased risk of infectious, neoplastic, or cardiovascular events or death in patients in whom a CNI was continued for more than 3 months after KTF [44]. Furthermore, in the present cohort, according to the records, only one patient who was not immunized had IS interruption due to infection 2 months before retransplant. He subsequently developed acute antibody-mediated rejection with preformed DSAs against the new transplant. Finally, we chose to consider the maintenance of IS treatments only if CNIs were maintained. Indeed, only CNIs were associated with lower immunization during the inter-transplant period [27, 44, 53]. For the cohorts reported in the literature [48], G-CNI patients received heterogeneous treatments, with only one-third of patients on CNIs alone and almost one-fifth of patients on triple IS. Furthermore, residual CNI levels are rarely measured in patients after KTF and therefore were not collected. Only a recent English study of 48 adult KTF transplant recipients reported a residual tacrolimus level ≥3 ng/ml as protective against the development of alloimmunization [54]. Further studies are necessary to determine the optimal CNI-based IS protocols after KTF.

Our study shows that after KTF, maintaining CNI-based IS in a cohort of patients without heavy comorbidities may reduce the risk of immunization, shorten the waiting time, and provide better access to standard criteria donor grafts. These strategies may improve the survival of the subsequent graft and these patients.

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 humans were approved by Oversight and study approval were provided by the Committee for Protection of Human Subjects (CPP SUD-EST VI) on September 03, 2019 (institutional review board 00008526) and by the National consultative committee on the use of health research information (14.510)—No written consent was required for this study but a notice of non opposition letter was send to all patients in accordance with the national legislation (MR-004 reference methodology) [36]. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for

participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

JN collected data, analysed data, and wrote the paper. VM designed the research/study, analysed data, and wrote the paper. CéL, analysed data and contributed important reagents. LC collected data. BC collected data. AT collected data. LE collected data. DB collected data. ChL collected data. RL collected data. ClG collected data. MF collected data. A-EH collected data. P-OR designed the research/study and analysed data and contributed important reagents. CyG designed the research/study, analysed data, and wrote 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.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11775/full#supplementary-material

Supplementary Figure S1 | Overall second kidney transplant survival (without death or kidney transplant failure) in patients with immunosuppressive therapy maintenance until the second graft (G-CNI) or discontinued therapy (G-STOP). Data are presented before the inverse probability of treatment weighting.

Supplementary Figure S2 | Exposure-adjusted rates of serious complications in patients with immunosuppressive therapy maintenance until the second graft (G-CNI) or discontinued therapy (G-STOP). Data are presented after the inverse probability of treatment weighting. KT, kidney transplant; MACE, major cardiovascular event; NMSC, non-melanoma skin cancer. *$p < 0.05$.

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ABO Incompatible Kidney Transplantation Without B-cell Depletion is Associated With Increased Early Acute Rejection: A Single-Center Australian Experience

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We performed a single-center retrospective cohort study of 66 consecutive ABO incompatible kidney transplants (ABOiKT) performed without B-cell depleting therapy. Outcomes were compared to an earlier era performed with rituximab (n = 18) and a contemporaneous cohort of ABO compatible live donor transplants (ABOcKT). Acute rejection within 3 months of transplant was significantly more common after rituximab-free ABOiKT compared to ABOiKT with rituximab (OR 8.8, p = 0.04) and ABOcKT (OR 2.9, p = 0.005) in adjusted analyses. Six recipients of rituximab-free ABOiKT experienced refractory antibody mediated rejection requiring splenectomy, and a further two incurred early graft loss with no such episodes amongst ABOiKT with rituximab or ABOcKT cohorts. Patient and graft survival were similar between groups over a median follow-up of 3.1 years. This observational evidence lends strong support to the continued inclusion of rituximab in desensitization protocols for ABOiKT.

Keywords: ABO incompatible, kidney transplantation, rituximab, immunosuppression, rejection

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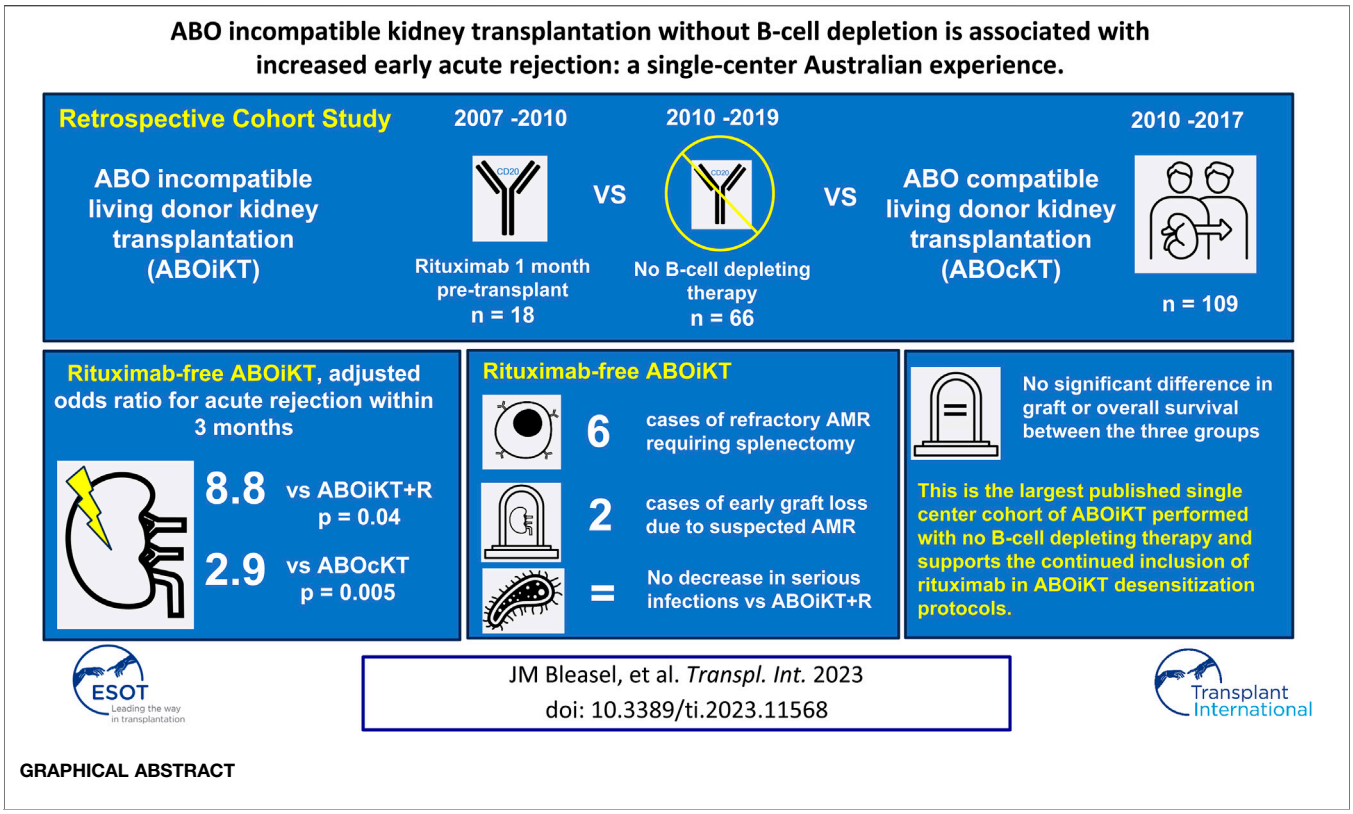
INTRODUCTION

Kidney transplant offers the best survival and quality of life for most patients with end stage kidney disease [1, 2]. Limited availability of living donors and long waiting times for deceased donor allocation leave many transplant candidates to accrue significant morbidity and healthcare expenditure on dialysis [3, 4]. Since the pioneering case series using extracorporeal antibody removal and splenectomy [5], kidney transplantation between ABO incompatible individuals (ABOiKT) has developed as a viable strategy to increase the living donor pool. The anti-CD20 monoclonal antibody rituximab has now replaced splenectomy as pre-transplant B-cell depleting

Abbreviations: ABOcKT, ABO compatible kidney transplant; ABOiKT, ABO incompatible kidney transplant; ABOiKT + R, ABO incompatible kidney transplant with rituximab; AMR, antibody mediated rejection; CDC, complement dependent cytotoxic; CI, confidence interval; DGF, delayed graft function; DSA, donor specific antibody; ESKD, end stage kidney disease; HLA, human leukocyte antigen; HR, hazard ratio; IQR, interquartile range; IV, intravenous; IVIg, intravenous immunoglobulin; OR, odds ratio; cPRA, calculated panel reactive antibody; SD, standard deviation.



GRAPHICAL ABSTRACT

therapy in almost all ABOiKT programs, with excellent outcomes in terms of rejection and graft survival reported by individual centers [6–8]. However, increased rates of infection and death from infection have been observed in ABOiKT recipients compared to their ABO compatible counterparts, raising concerns about the degree of immunosuppression required for the procedure [9–12]. Our center initiated an ABOiKT program in 2007 employing pretransplant rituximab and immunoadsorption. Three years later rituximab was excluded from the desensitization protocol due to concerns about infection risk and following reports of successful ABOiKT with no B-cell depleting therapy at other centers [13, 14]. Here we evaluated outcomes of rituximab-free ABOiKT through comparison with the earlier era of our ABOiKT program where rituximab use was universal (ABOiKT + R), and a contemporaneous cohort of living donor ABO compatible transplants (ABOcKT).

MATERIALS AND METHODS

Study Design and Setting

The Royal Prince Alfred Hospital is a tertiary referral center in Sydney, Australia with a kidney transplant unit servicing a large metropolitan district as well as a number of rural centers. We conducted a retrospective cohort study of ABOiKT performed from the inception of the program on 1 July 2007 until 1 June 2019. A near contemporaneous comparator cohort of ABOcKT (July 2010 and April 2017) with prospectively collected outcome data was adopted from a previously published study of donor specific antibodies in kidney transplantation [15].

This study received ethical approval from the Sydney Local Health District Human Research Ethics Committee (reference X17-0083 and LNR/17/RPAH/124).

Desensitization Protocol for ABO Incompatible Kidney Transplants

Prospective ABOiKT recipients between July 2007 and July 2010 (n = 18) received rituximab 375 mg/m^2 1 month pre-operatively. After this era, rituximab was omitted from the desensitization protocol and 66 further ABOiKT were performed with no other changes to immunosuppression practices. ABOiKT recipients commenced mycophenolate mofetil 1,000 mg twice daily 14 days prior to transplantation. Anti-A or B antibody removal was achieved by immunoadsorption (Glycosorb A/B®, Glycorex Transplantation AB, Sweden). Immunoadsorption sessions were scheduled according to baseline blood group antibody titer to achieve a preoperative titer of 1:8 or less. A single dose of 500 mg/kg intravenous immunoglobulin (IVIg) was administered on the day prior to transplant. Post-operative immunoadsorption sessions were performed only in cases of antibody titer rebound to greater than 1:8 or suspected anti-blood group antibody mediated rejection.

Induction and Maintenance Immunosuppression

Routine induction immunosuppression was the same for ABOiKT in both eras and ABOcKT, consisting of two doses of intravenous (IV) basiliximab 20 mg (day zero and day 4 post-operatively) and methylprednisolone 500 mg IV on day zero and 250 mg IV on day 1. Highly sensitized recipients considered to be at significant risk of rejection received anti-thymocyte globulin instead of basiliximab as induction therapy. Standard maintenance immunosuppression in all recipients was mycophenolate mofetil 1000 mg twice daily, a calcineurin inhibitor (tacrolimus or ciclosporin) and prednisolone starting at 30 mg daily and weaning to 10 mg daily by 8 weeks post-transplant.

A protocolized kidney transplant biopsy was performed on day 10 after ABOiKT if no indication biopsy had been performed prior, and another was performed at week 12. ABOcKT recipients had a protocolized biopsy at week 12 only.

All transplant recipients received *pneumocystis jirovecii* prophylaxis with trimethoprim/sulfamethoxazole or an alternative agent indefinitely while immunosuppressed. Cytomegalovirus prophylaxis with oral valganciclovir was employed for 3–6 months depending on risk of cytomegalovirus reactivation.

Anti-blood Group Antibody Measurements and Alloantibody Detection

Anti-A and B antibody titers in ABOiKT recipients were measured by column agglutination technology using DG Gel® cards and reagent red blood cells (Grifols, Melbourne, Australia). Complement dependent cytotoxic (CDC) cross matching, flow cytometric cross matching and solid-phase Luminex assay for anti-HLA donor specific antibodies (One Lambda LABScreen Single Antigen class I and II; BMT, Mehrbusch, Germany) were performed by the Australian Red Cross New South Wales transplantation and immunogenetics lab in accordance with international guidelines [16]. The threshold for reporting anti-HLA antibody positivity was mean fluorescence intensity (MFI) >500.

Prospective ABOiKT or ABOcKT recipients with high level donor specific antibodies (DSAs) (MFI >3,000) or positive CDC T-cell cross match were directed toward alternative transplant pathways wherever possible. Low or intermediate strength DSAs with negative cross match were accepted and pre-transplant therapeutic plasma exchange was employed in selected cases.

Clinical Data Collection

Data were extracted from the clinical record and managed using REDCap electronic data capture tools hosted at Sydney Local Health District [17, 18]. Delayed graft function (DGF) was defined as requirement for dialysis within 7 days of transplant. Graft failure was defined as need to return to dialysis permanently, re-transplantation or estimated glomerular filtration rate <15 mL/min/1.73 m^2 sustained for at least 6 weeks. Rejection was defined according to Banff criteria [19]; only treated episodes of biopsy proven rejection were recorded for this analysis. Early rejection was defined as occurring within 3 months of transplant.

Statistical Analysis

Open-source statistical software R (http://r-project.org) was used for all statistical analysis. Between group comparisons were performed using Student's t-test or Wilcoxon Rank Sum test for parametric and nonparametric continuous data, respectively, and Fisher's exact test for categorical data. Acute rejection was analyzed using logistic regression. Base models included all covariates with a univariable p-value ≤0.25, and a backward elimination strategy was employed to determine the final model. Death censored graft survival and overall survival were calculated using Kaplan-Meier survival tables and compared between groups using cox proportional hazards models.

RESULTS

Patient Characteristics

Sixty-six ABOiKT were performed without B-cell depleting therapy between July 2010 and June 2019. The comparator groups comprise 18 ABOiKT performed with pre-transplant rituximab between July 2007 and July 2010 and 109 consecutive ABOcKT transplants. Median follow-up for the whole cohort was 3.1 years (IQR 1.3–5.0 years).

Baseline characteristics of the three groups are presented in **Table 1**. Recipient age, sex, race, and cause of ESKD were similarly distributed between the three groups. Donor age was older in the rituximab-free ABOiKT group (52 years) compared to the earlier ABOiKT era (46 years) and ABOcKT (49 years).

Immunological Characteristics

All combinations of blood group incompatibility were represented in the ABOiKT cohort except AB to O. Thirty-nine (59%) rituximab-free ABOiKT recipients were transplanted against anti-A antibodies compared to 13 (72%) for ABOiKT + R. The median baseline anti-blood group antibody titer was 1:16 (range 1:1–1:512) in the rituximab-free ABOiKT group and 1:16 (range 1:1–1:256) in ABOiKT + R. The median number of immunoadsorption sessions required pre-transplant was 3 (range 0–8). All ABOiKT recipients achieved a titer of 1:8 or less at the time of transplant.

The mean number of HLA A, B and DR mismatches were 3.8 (SD 1.6) amongst rituximab-free ABOiKT compared to 3.5 (SD 1.5) for ABOiKT + R and 3.0 (SD 1.8) for ABOcKT. Regarding anti-HLA antibodies, only three recipients, all in the rituximab-free ABOiKT group, had a calculated panel reactive antibody (cPRA) greater than 80% (range 85%–96%). The prevalence of pre-transplant donor specific anti-HLA antibodies was 42% in rituximab-free ABOiKT, 22% in ABOiKT + R and 31% in ABOcKT. The large majority of pre-transplant DSAs were weak with MFI<2000, further details on DSA characteristics are included in **Supplementary Table S2**.

TABLE 1 | Baseline characteristics of study participants. All numbers refer to frequency and percentage unless otherwise described.

	ABOiKT		
	Rituximab-free *n* = 66	**Rituximab *n* = 18**	**ABOcKT *n* = 109**
Age in years (mean, SD)	47.9 (13.9)	43.1 (12.9)	45.5 (14.9)
Male recipient	47 (71.2%)	13 (72.2%)	73 (67.0%)
Race			
Caucasian	50 (76.9%)	11 (61.1%)	78 (71.6%)
Indigenous/Polynesian	2 (3.1%)	0	8 (7.3%)
Asian/Indian	13 (20.0%)	6 (33.3%)	21 (19.3%)
Other	1 (1.5%)	1 (5.6%)	2 (1.8%)
Cause of end stage kidney disease			
Diabetic or renovascular	11 (16.7%)	1 (5.6%)	8 (7.3%)
Polycystic kidney disease	9 (13.6%)	5 (27.8%)	15 (13.8%)
Glomerulonephritis	35 (53.0%)	6 (33.3%)	58 (53.2%)
Other	11 (16.7%)	6 (33.3%)	28 (25.7%)
Re-transplant	7 (10.8%)	0	7 (6.4%)
Preemptive transplant	20 (31.2%)	7 (38.9%)	38 (34.9%)
Peak PRA >80%	3 (7.5%)	0	0
Pre-transplant DSA	28 (42.4%)	4 (22.2%)	34 (31.2%)
MFI of immunodominant DSA			
≥2000	4 (6.1%)	0	11 (10.1%)
<2000	24 (36.4%)	4 (22.2%)	23 (21.1%)
Blood group antibody titer pre-treatment (median, IQR)	16.0 (5.0–56.0)	16.0 (4.0–32.0)	-
Blood group antibody titer on day of transplant (median, IQR)	1.0 (1.0–2.0)	1.5 (0.0–2.0)	-
Male donor	21 (32.3%)	5 (27.8%)	46 (42.6%)
Donor age in years (mean, SD)	52.2 (11.8)	46.0 (8.6)*	48.6 (11.3)*
HLA A/B/DR mismatch (mean, SD)	3.8 (1.6)	3.5 (1.5)	3.0 (1.8)*
Delayed graft function	3 (4.5%)	1 (5.6%)	2 (1.8%)
Ischemic time in hours (mean, SD)	3.9 (1.3)	4.2 (0.9)	4.2 (1.3)
Induction			
Basiliximab	66 (100%)	18 (100%)	105 (96.3%)
Thymoglobulin	0	0	2 (1.8%)
Triple immunosuppression	66 (100%)	18 (100%)	104 (95.4%)
Desensitization	66 (100%)	18 (100%)	18 (16.5%)
Rituximab	0	18 (100%)	0
Intravenous immunoglobulin	66 (100%)	18 (100.0%)	17 (15.6%)
Plasma exchange	7 (10.6%)	1 (5.6%)	6 (5.5%)
Column immunoadsorption	51 (77.3%)	14 (77.8%)	0

*p < *0.05 for comparison to rituximab-free ABOiKT group; all other comparisons to rituximab-free ABOiKT are non-significant.*

TABLE 2 | Characteristics of first acute rejection episodes.

	ABOiKT		*p*-value[a]	ABOcKT *n* = 109	*p*-value[b]
	Rituximab-free *n* = 66	**Rituximab *n* = 18**			
Any acute rejection	30 (45.5%)	4 (22.2%)	0.11	28 (26%)	0.001
Time to first rejection, days (median, IQR)	8 (6–47)	1,048 (568–1,387)	0.04	77 (10–375)	0.01
T-cell mediated rejection	24 (36%)	4 (22.2%)	0.40	25 (23%)	0.06
Banff Score					
Borderline	12 (18.2%)	1 (5.6%)	0.66	7 (6.4%)	0.27
IA	5 (7.6%)	2 (11.1%)		11 (10.1%)	
IB	2 (3.0%)	0 (0%)		1 (0.9%)	
IIA	5 (7.6%)	1 (5.6%)		6 (5.5%)	
Antibody mediated rejection	11 (16.7%)	0	0.11	8 (7.3%)	0.08

[a]*ABOiKT with rituximab compared to rituximab-free ABOiKT.*
[b]*ABOcKT compared to rituximab-free ABOiKT.*

Rejection

Over the whole follow-up period, treated biopsy-proven rejection occurred in 30 (46%) rituximab-free ABOiKT, 4 (22%) ABOiKT + R and 28 (26%) ABOcKT recipients. Early rejection, defined as any treated episode of acute rejection within 3 months of transplant, occurred in 26 (39%) rituximab-free ABOiKT, 1

TABLE 3 | Multivariable logistic regression models of treated acute rejection episodes over the whole follow-up period and early acute rejection (within 3 months of transplant).

	Odds ratio	95% Confidence interval	*p*-value
Any acute rejection episode			
Rituximab-free ABOiKT vs. ABOcKT	2.0	1.0–3.9	0.06
Rituximab-free ABOiKT vs. ABOiKT with rituximab	2.5	0.7–8.7	0.2
Age at transplantation (per 10 years)	0.8	0.6–1.0	0.07
Sex (male)	1.8	0.9–3.9	0.1
Donor age (per 10 years)	1.5	1.1–2.1	0.02
Total mismatch at HLA A, B, DR	1.3	1.0–1.6	0.04
Pre-transplant DSA	1.1	0.6–2.3	0.7
Early rejection			
Rituximab-free ABOiKT vs. ABOcKT	2.9	1.4–6.2	0.005
Rituximab-free ABOiKT vs. ABOiKT with rituximab	8.8	1.1–73.1	0.04
Total mismatch at HLA A, B, DR	1.4	1.1–1.8	0.006
Donor age (per 10 years)	1.3	0.9–1.8	0.2
Pre-transplant DSA	1.3	0.6–2.8	0.5

(6%) ABOiKT + R and 16 (15%) ABOcKT recipients. The histological type of the first rejection episode and Banff classifications are shown in **Table 2**.

No episodes of antibody mediated rejection (AMR) were observed in the ABOiKT + R cohort compared to 11 (17%) in rituximab-free ABOiKT and 8 (7%) in ABOcKT. Six rituximab-free ABOiKT recipients experienced AMR refractory to maximal medical therapy and required splenectomy at a mean of 22 days post-transplant (range 9–55 days). All but one of these recipients had a rebound of anti-blood group antibody titer to greater than 1:8 coinciding with the diagnosis of rejection. All achieved eventual resolution of AMR without acute graft loss at last follow-up. No comparable episodes of refractory AMR occurred in the ABOcKT cohort.

Results of the univariable analysis of factors associated with rejection are shown in **Supplementary Table S1**. Results of the multivariable analysis of all rejection over the follow-up period and early rejection are shown in **Table 3**. When controlling for sex, HLA mismatch, pre-transplant DSA and donor and recipient age, there was no significant difference in acute rejection over the whole follow-up period between rituximab-free ABOiKT and ABOiKT + R (OR 2.5, 95% CI 0.7–8.7, $p = 0.2$). There was a trend toward increased risk of rejection in rituximab-free ABOiKT compared to ABOcKT (OR 2.0, 95% CI 1.0–3.9, $p = 0.06$). Older donor age (OR 1.5 for every 10 years increment in age, 95% CI 1.1–2.1, $p = 0.02$) and HLA mismatch (OR 1.3 for each additional HLA-ABDR mismatch, 95% CI 1.0–1.6, $p = 0.04$) were independent risk factors for rejection in this analysis.

Early rejection occurred in significantly more rituximab-free ABOiKT recipients compared to both ABOiKT + R (OR 8.8, 95% CI 1.1–73.1, $p = 0.04$) and ABOcKT (OR 2.9, 95% CI 1.4–6.2, $p = 0.005$), controlling for donor age, HLA mismatch and pre-transplant DSA.

Rebound of Anti-A or B antibody titer >1:8 post-transplant occurred in 13 ABOiKT recipients (16%) after a median of 7 days (IQR 3–9, range 1–15) and was strongly associated with incidence of rejection (OR 6.5, 95% CI 1.8–31.2, $p = 0.008$, see also **Supplementary Table S1**). None of the patients who received pre-transplant rituximab experienced an antibody rebound >1:8.

The presence of a pre-transplant DSA was not significantly associated with all rejection or early rejection on univariable analysis ($p = 0.26$ and 0.15 respectively). **Supplementary Table S3** shows associations between various pre-transplant DSA characteristics and rejection, none of which are statistically significant. Detection of a *de novo* DSA was significantly more common in those recipients who experienced rejection compared to those who did not (39%, $n = 22$, compared to 14%, $n = 15$, univariable $p < 0.001$).

Transplant Outcome

Two recipients, both rituximab-free ABOiKT, experienced early graft loss. A 58 year-old man with immediate graft function incurred graft loss at day six, despite treatment with methylprednisolone and immunoadsorption, caused by severe AMR (proven histologically post-nephrectomy) associated with anti-A rebound. Secondly, a 34 year-old man experienced delayed graft function then developed unexplained fevers before loss of graft perfusion was noted on ultrasound on post-operative day five. Histological examination of the graft was inconclusive as to the presence of rejection due to extensive necrosis.

Death censored graft survival (DCGS) at 1 year was 95% (95% CI 89%–100%) for the rituximab-free ABOiKT group compared to 100% in both the ABOiKT + R and ABOcKT groups. DCGS at 3 years was 90% (95% CI 80%–99%) in rituximab-free ABOiKT compared to 100% and 95% (95% CI 90%–99%) in ABOiKT + R and ABOcKT respectively, with no significant differences between groups. DCGS was strongly associated with prior rejection (HR 4.5, 95% CI 1.38–14.5, $p = 0.013$).

Patient survival was not different between groups. There were two deaths with a functioning graft in rituximab-free ABOiKT, at 1,316 days from an unknown cause and 2,105 days from post-transplant lymphoproliferative disorder; two deaths after ABOiKT + R, at 61 days from infection and 1,558 days from suicide; and one death with functioning graft 415 days after ABOcKT from infection. Three year overall patient survival

was 95% (95% CI: 88%–100%) in rituximab-free ABOiKT, 94% (95% CI: 84%–100%) in ABOiKT + R and 99% (95% CI: 97%–100%) in ABOcKT.

Infection

Data on incidence of infection requiring hospitalization was available for ABOiKT recipients only. There were 74 episodes of infection requiring hospitalization in 31 ABOiKT recipients. Fifty (68%) of these were bacterial infections, 13 (18%) viral and 6 (8%) fungal with the remainder having no organism isolated. Those who experienced treated acute rejection were more likely to have an infection requiring hospitalization (OR 2.6, 95% CI 1.0–6.5, $p = 0.04$), while receipt of rituximab was not associated with infection.

DISCUSSION

In this single-center, retrospective cohort study, ABOiKT performed without rituximab in the desensitization protocol were more likely to experience early rejection than recipients of either an ABOiKT performed with rituximab or an ABOcKT. Prominent among early rejections were six episodes of severe AMR requiring salvage splenectomy and at least one causing graft failure at day 6, all in the rituximab-free ABOiKT group. The association between rituximab-free ABOiKT and early rejection remained significant when controlling for baseline risk factors including donor age, degree of HLA mismatch and presence of a DSA pre-transplant. Patient and graft survival were not different between groups; however, this study was underpowered to detect such differences at the median follow-up of 3.1 years.

The putative benefit of B-cell depletion in ABOiKT protocols is to reduce the risk of post-transplant rebound of graft-threatening blood group antibodies [20, 21]. In support of this, we observed blood group antibody rebound only in the rituximab-free ABOiKT group and rebound was strongly associated with incidence of rejection.

There are no randomized trials examining the benefit of B-cell depleting therapy in ABOiKT thus the evidence base is reliant on observational studies. A large international registry study of ABOiKT in which splenectomy was very rare ($n = 11$), found that 3 years DCGS was significantly better in the 1,058 patients who received anti-CD20 therapy compared to the 125 who did not [22]. Conversely, in a smaller 2009 study, Montgomery et al. [23] reported equivalent outcomes for 28 patients who underwent ABOiKT with no B-cell depleting therapy at Johns Hopkins Hospital compared to 32 ABOiKT from an earlier era where rituximab or splenectomy were in use. The hitherto largest published cohort of ABOiKT performed without splenectomy or rituximab ($n = 54$) was from The Royal Melbourne Hospital, Australia [13, 24]. At 1 year follow-up they reported rejection in 19% of rituximab-free ABOiKT which was comparable to contemporaneous ABOcKT (17%) and there were no episodes of refractory AMR or graft loss. This group also published a case series of successful ABOiKT ($n = 20$) performed with neither B-cell depleting therapy nor extracorporeal antibody removal in selected recipients with low baseline blood group antibody titers [25]. Recipients with preformed HLA DSA were excluded from both cohorts, in contrast to our practice, thus it is possible that lower HLA immune risk was an important factor in their reported success with rituximab-free ABOiKT.

Excess infection risk conferred by the ABOiKT desensitization protocol remains a concern [26, 27]. Increased infection related deaths have been reported in ABOiKT compared to ABOcKT recipients in a meta-analysis of observational studies and a large multi-national registry both examining the post-splenectomy era [9, 22]. Increased rates of serious infection have been observed in standard compared to low dose rituximab in ABOiKT [28, 29] and in ABOcKT treated with rituximab for various indications [30, 31]. We did not observe an excess of infections requiring hospitalization in ABOiKT who received rituximab compared to those who did not, although the numbers available for comparison in the rituximab group are small. The factor with the strongest association with infection was incidence of rejection, which likely reflects the downstream effects of increased immunosuppression used to treat this complication.

The decision to undertake kidney transplantation across the blood group barrier ultimately depends on the timely availability of alternative transplant options. Prospective ABOiKT recipients in Australia have the option to seek an ABOc transplant through either the national paired kidney exchange program or waitlisting for a disease donor organ. Both alternatives can entail significant additional waiting time with attendant risk of morbidity and mortality due to complications of ESKD [3]. Thus, our center's ABOiKT program remains active, however, rituximab was reintroduced into the conditioning protocol from August 2019 after review of the outcomes reported herein.

The limitations of this study include the retrospective observational design and the small number in the ABOiKT + R cohort. Although immunosuppression practices did not change apart from the exclusion of rituximab, there is residual risk of confounding by era when comparing the two ABOiKT cohorts. For instance, numerically more DSA-positive ABOiKT recipients were present in the later rituximab-free cohort, which may reflect both a greater leniency in candidate selection over time and the limited donor pool for sensitized individuals in our system. The overall prevalence of DSAs in this cohort may limit the generalizability of our results. For instance, it is possible that the benefit of rituximab seen here was also due to mitigation of HLA-associated immune risk rather than solely that due to ABO incompatibility. Notably, pre-transplant DSA was not associated with rejection in this cohort, likely in part because recipients with moderate to high level antibodies were either directed toward alternate donors or offered enhanced immunosuppression. Nonetheless, rituximab-free ABOiKT remained significantly associated with early rejection while controlling for HLA mismatch and DSA positivity in the multivariable analysis. Moreover, we repeated our analyses on the subgroup with no preformed DSAs and there remained significantly more early rejection in rituximab free ABOiKT compared to ABOiKT + R and ABOcKT (**Supplementary Table S4**). Finally, the

protocolization of an allograft biopsy at day 10 in ABOiKT but not ABOcKT recipients raises the possibility of increased detection of subclinical rejection in the former. On the contrary, review of patient records indicates that there was clinical suspicion of rejection motivating 18 out of the 20 biopsies diagnosing rejection in ABOiKT within 2 weeks of transplant.

In conclusion, we report the largest published single-center cohort of ABOiKT performed without B-cell depleting therapy. Rituximab-free ABOiKT recipients experienced significantly more early acute rejection than ABOiKT performed with rituximab and ABOcKT. Ideally, a randomized controlled trial would be performed to assess the safety and utility of rituximab for ABOiKT. In the absence of such a study, best practice will rely on observational data and on this basis our findings support the inclusion of rituximab for ABOiKT desensitization protocols.

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 humans were approved by Sydney Local Health District Human Research Ethics Committee. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JB participated in data collection and analysis and wrote the first draft of the manuscript; SW and KW participated in research design, data collection, analysis and editing the manuscript; SC, TY, LA, and DG participated in research design, data collection and editing the manuscript. Q-AC, JM, and MU participated in data collection and editing the manuscript. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

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

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11567/full#supplementary-material

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ORIGINAL RESEARCH
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External Validation of Toulouse-Rangueil eGFR12 Prediction Model After Living Donor Nephrectomy

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Decreased postdonation eGFR is associated with a higher risk of ESRD after living kidney donation, even when accounting for predonation characteristics. The Toulouse-Rangueil model (TRM) estimates 12 month postdonation eGFR (eGFR12) to inform counseling of candidates for living donation. The TRM was validated in several single-center European cohorts but has not been validated in US donors. We assessed the TRM in living kidney donors in the US using SRTR data 1/2000–6/2021. We compared the 2021 CKD-EPI equation eGFR12 observed estimates to the TRM eGFR12 predictions. Median (IQR) bias was –3.4 (–9.3, 3.4) mL/min/1.73 m^2. Bias was higher for males vs. females (bias [IQR] –4.4 [–9.9, 1.8] vs. –2.9 [–8.8, 4.1]) and younger (31–40) vs. older donors (>50) (bias –4.9 [–10.6, 3.0] vs. –2.1 [–7.5, 4.0]). Bias was also larger for Black vs. White donors (bias (–6.7 [–12.1, –0.3], $p < 0.001$) vs. (–3.4 [–9.1, 3.1], $p < 0.001$)). Overall correlation was 0.71. In a sensitivity analysis using the 2009 CKD-EPI equation, results were generally consistent with exception to a higher overall bias (bias –4.2 [–9.8, 2.4]). The TRM overestimates postdonation renal function among US donors. Overestimation was greatest for those at higher risk for postdonation ESRD including male, Black, and younger donors. A new equation is needed to estimate postdonation renal function.

Keywords: external validation, predictive model, living donor renal function, kidney transplantation, chronic kidney disease

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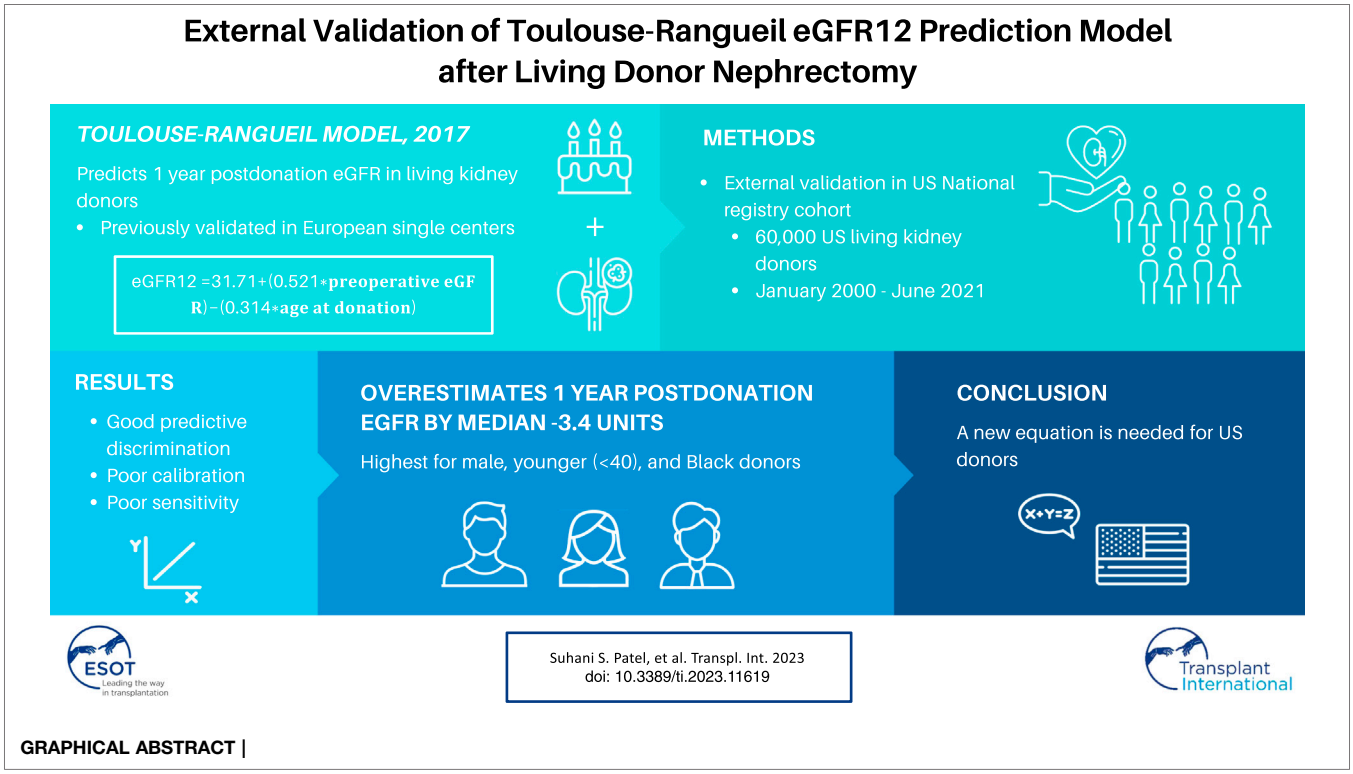
Abbreviations: AUROC, Area under the receiver operating curve; CKD, Chronic kidney disease; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR12, Postdonation 12-month estimated glomerular filtration rate; ESRD, End stage renal disease; HRSA, Health Resources and Services Administration; IQR, Interquartile range; LKD, Living kidney donor; MDRD, Modification of Diet in Renal Disease; OPTN, Organ Procurement and Transplantation Network; SRTR, Scientific Registry of Transplant Recipients; TRM, Toulouse-Rangueil model.



GRAPHICAL ABSTRACT |

INTRODUCTION

Although most living kidney donors (LKDs) do not experience renal complications, they face an increased long-term risk of end stage renal disease (ESRD) compared to healthy nondonors [1, 2]. A study of national registry data from the United States reported that estimated glomerular filtration rate (eGFR) at 6 months postdonation is associated with ESRD risk in LKDs (28% increased risk per 10 mL/min/1.73 m^2), even after accounting for predonation characteristics [3]. Male donors, Black donors, and donors with a first-degree biological relationship to the recipient are at increased risk for ESRD postdonation [4]. A model to predict postdonation eGFR as a marker for risk of ESRD can aid in predonation donor evaluation and counseling.

The Toulouse-Rangueil model (TRM), developed by Benoit et al., estimates postdonation 12 month (eGFR12) based on predonation characteristics [5]. This prediction model was created using data from 133 LKDs from 2006 to 2014 in a single-center French cohort [5]. The final model included age at donation and predonation eGFR [5]. The authors reported a Pearson correlation of 0.65 ($p < 0.001$) and an area under the receiver operating curve (AUROC) of 0.83 ($p < 0.001$) in a validation cohort [5]. Subsequent studies externally validated the TRM in single-center cohorts in France (N = 400) [6], Portugal (N = 333) [7], and Germany (N = 130) [8]. All participants in the French and Portuguese cohort were White, and the racial composition of the German cohort is unknown [6–8]. These three cohorts demonstrated similar and moderately strong Pearson correlations (0.66/0.67/0.59) and AUROCs (0.86/0.83/0.87) suggesting validity in Western European populations [6–8].

However, applicability of the TRM to donors outside of Europe is unclear. To address this knowledge gap, we conducted a retrospective study to validate the TRM using national registry data from the United States.

MATERIALS AND METHODS

Study Population

This study used data from the Scientific Registry of Transplant Recipients (SRTR). 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), U.S. Department of Health and Human Services provides oversight to the activities of the OPTN and SRTR contractors. This dataset has previously been described elsewhere [9]. This study of deidentified data was determined to be "exempt: not human subjects research" by the institutional review board of NYU Langone (ID: i22-00146).

The study population included adult (age≥18) LKDs from 1 January 2000 to 2 June 2021. To remove erroneous datapoints, individuals with a predonation creatinine level outside of the range of 0.2–1.5 (N = 276), a predonation eGFR of less than 40 (N = 3), a 12 month postdonation creatinine outside of the range of 0.2–1.9 (N = 344), or an eGFR12 greater than 120 (N = 340) were excluded. Furthermore, individuals with a creatinine lower

TABLE 1 | Kidney donor characteristics.

Donor characteristic	Entire sample (N = 60,839)
Gender, (%) Male	35.9
Age, median (IQR)	44 (34–53)
Race, (%)	
White	72.4
Black	9.1
Hispanic	13.4
Asian	3.8
Other	1.4
Predonation eGFR, (%)	
≥30–<50	0.03
≥50–<70	3.2
≥70–<90	26.0
≥90–<110	44.1
≥110–<130	25.6
≥130	1.2
eGFR12, (%)	
<30	0.005
≥30–<50	9.6
≥50–<70	52.6
≥70–<90	30.4
≥90–<110	6.6
≥110–<130	0.7
Laterality, (%)	
Left kidney	88.3
Right kidney	11.7
Procedure type, (%)	
Transabdominal	1.1
Flank (retroperitoneal)	4.3
Laparoscopic Not Hand-assisted	32.6
Laparoscopic Hand-assisted	58.7
Laparoscopic Unknown (inactive)	3.4
Natural Orifice	0.002
BMI, median (IQR)	26.6 (23.8, 29.6)
History of smoking, (%)	22.4
History of hypertension, (%)	4.0

eGFR12, Postdonation 12 month estimated glomerular filtration rate.

than or eGFR greater than their pre-donation levels were not included in the analysis. Domino and therapeutic donors were also excluded. The 12 month follow-up occurred between 9 and 18 months after donation.

Validation of TRM

We compared the TRM eGFR12 predictions to the 2021 CKD-EPI creatinine equation eGFR12 observed estimates among LKDs using the following equation for TRM: $eGFR12\ (ml/\ min\ /\ 1.73\ m^2) = 31.71 + (0.521{*}preoperative\ eGFR\ (ml/\ min)) - (0.314{*}age\ at\ donation\ (years))$ [5]. We analyzed the bias (observed - predicted) and the Pearson correlation overall and in the following subgroups: gender, age, race (White/Black/Hispanic/Asian/Other), and relationship to the recipient (biological/non biological/non directed) to assess the validity of the proposed prediction model. We compared observed vs. predicted estimates using pooled t-tests. eGFR12 was binarized as < 60 vs. ≥ 60 mL/min/1.73 m^2 to calculate the sensitivity, specificity, positive predictive value, and negative predictive value. We utilized the Hosmer-Lemeshow test to examine the model's calibration. We constructed a histogram to examine the distribution of bias (observed-predicted). To assess the agreement, we created a Bland-Altman plot.

Sensitivity Analysis

We conducted a sensitivity analysis in which we replicated the analysis using the older 2009 CKD-EPI creatinine equation, which estimates eGFR based on serum creatinine, age, sex, and race/ethnicity (coded as Black vs. non-Black) [10].

Statistical Analysis

An α of 0.05 was considered statistically significant and all tests were two-sided. All analyses were performed using SAS (v.9.4) or R Studio (v.4.0.3).

RESULTS

Study Population

The study population consisted of 60,839 LKDs from 2000 to 2021 (**Table 1**). Donors were predominantly female (64.1%) and White (72.4%) with a median age of 44 (**Table 1**). About 22.4% of LKDs have a history of smoking and 4.0% of LKDs have a history of hypertension (**Table 1**). 95.7% of donors had a predonation eGFR between 70–130 and 92.6% of donors had an eGFR12 between 30–90 (**Table 1**). The 12 month postdonation follow-up occurred between 9 and 18 months (median [IQR] 12.2 [11.8, 13.0]); 91% of follow-up occurred between 10 and 14 months.

Validation of TRM

Median bias [IQR] calculated as the difference between the observed estimate from the CKD-EPI equation and the predicted TRM eGFR12 was −3.4 [−9.3, 3.4] mL/min/ 1.73 m^2 and mean bias was −2.5 mL/min/1.73 m^2 (**Table 2**). Median bias was higher for all predicted vs. observed values. Furthermore, predicted values were statistically significantly different from observed values for all gender, age, and donor's relationship to recipient subcategories ($p < 0.001$) (**Table 2**). Bias was higher for males vs. females (bias [IQR] −4.4 [−9.9, 1.8] vs. −2.9 [−8.8, 4.1]) and younger (31–40) vs. older donors (>50) (bias −4.9 [−10.6, 3.0] vs. −2.1 [−7.5, 4.0]) (**Table 2**). Bias was larger for Black vs. White donors (bias −6.7 [−12.1, −0.3] vs. −3.4 [−9.1, 3.1]) but lower for Asian and Hispanic donors compared to White donors (bias −1.3 [−8.5, 6.1], −1.4 [−8.1, 6.4] vs. −3.4 [-9.1, 3.1]) (**Table 2**).

The overall correlation between TRM predicted and observed values was 0.71 (**Table 2**; **Figure 1B**). Moderately strong correlations exist among gender and donor's relationship to recipient subcategories (correlation (corr.) range 0.70–0.72) (**Table 2**; **Figure 2B**). Correlations by age ranged from 0.58 to 0.61; the lowest correlation among all subgroups was donors aged 18–30 (**Table 2**; **Figure 3B**). Lower age was associated with larger overestimation of eGFR12 (**Figure 3**). Asian and Hispanic donors had marginally

TABLE 2 | Median (IQR) bias and correlation overall and by subgroups.

Donor characteristic	Observed	Predicted	Bias	r	N	*p*-value
Overall	65.5 (56.7, 75.8)	69.5 (62.1, 77.5)	**−3.4 (−9.3, 3.4)**	0.71	60,839	<0.001
Gender						
Female	65.8 (57.0, 76.3)	69.5 (61.9, 77.2)	**−2.9 (−8.8, 4.1)**	0.71	38,992	<0.001
Male	64.8 (56.2, 75.0)	69.6 (62.5, 77.7)	**−4.4 (−9.9, 1.8)**	0.72	21,847	<0.001
Age						
18–30	77.7 (69.3, 88.2)	84.4 (76.5, 88.2)	**−4.0 (−11.0, 3.3)**	0.58	10,028	<0.001
31–40	70.3 (61.7, 79.9)	76.2 (69.5, 81.1)	**−4.9 (−10.6, 3.0)**	0.61	14,714	<0.001
41–50	63.5 (56.4, 71.9)	68.5 (62.8, 74.2)	**−3.7 (−9.5, 3.2)**	0.60	17,238	<0.001
>50	58.2 (51.3, 65.8)	61.3 (55.6, 66.5)	**−2.1 (−7.5, 4.0)**	0.61	18,859	<0.001
Race						
White	64.2 (55.8, 74.0)	68.2 (60.9, 75.6)	**−3.4 (−9.1, 3.1)**	0.70	44,016	<0.001
Black	62.4 (54.1, 72.3)	69.6 (62.1, 77.3)	**−6.7 (−12.1, −0.3)**	0.70	5,516	<0.001
Hispanic	73.6 (63.9, 85.3)	76.2 (68.7, 83.0)	−1.4 (−8.1, 6.4)	0.67	8,130	0.26
Asian	71.2 (61.7, 82.1)	73.4 (66.3, 80.7)	−1.3 (−8.5, 6.1)	0.66	2,328	0.16
Other	67.2 (58.5, 77.2)	72.2 (64.4, 79.7)	**−4.4 (−10.7, 2.9)**	0.66	849	<0.001
Relationship to recipient						
Biological	66.9 (57.8, 77.7)	71.2 (63.4, 79.0)	**−3.6 (−9.6, 3.5)**	0.70	28,708	<0.001
Non-biological	64.2 (55.9, 74.4)	68.2 (61.0, 75.6)	**−3.3 (−9.0, 3.4)**	0.71	26,052	<0.001
Non-directed	64.3 (55.8, 74.4)	68.3 (61.1, 76.0)	**−3.4 (−9.1, 3.2)**	0.72	6,078	<0.001

r, correlation.

Overall bias (observed-predicted) −3.4 mL/min/1.73 m^2 and correlation 0.71. All predicted values are statistically significantly different from observed with exception to Hispanic and Asian donors.

Bold values indicate statistical significance defined as p < *0.05.*

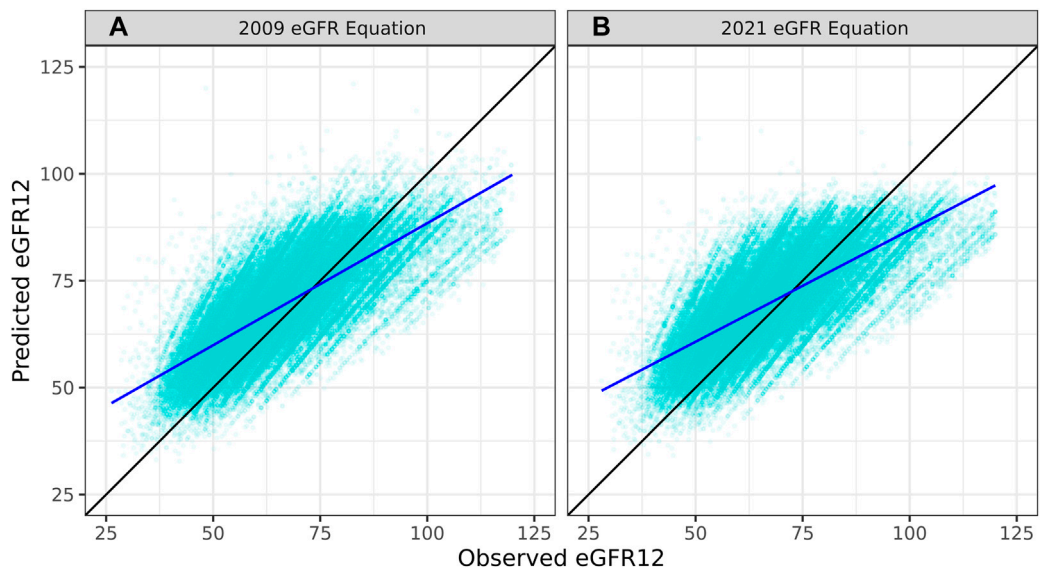


FIGURE 1 | Pearson correlation between predicted eGFR12 and observed eGFR12 by CKD-EPI equation (eGFR: mL/min/1.73 m^2) **(A)** using the 2009 eGFR equation (r = 0.71, p < 0.001) **(B)** using the 2021 eGFR equation (B: r = 0.74, p < 0.001) *eGFR12, Postdonation 12 month estimated glomerular filtration rate.

lower correlations vs. White donors (corr. 0.66, 0.67 vs. 0.70) (**Table 2**; **Figure 4B**).

Although the specificity was large (0.94), the sensitivity was low (0.45) demonstrating a poor ability to estimate LKDs with <60 eGFR (**Table 3**). Among donors predicted to have ≥60 eGFR12, 77% had an observed eGFR12 ≥60; among donors predicted to have a <60 eGFR12, 80% had an observed eGFR12 <60 (**Table 3**). According to the Hosmer-Lemeshow test, the model had good fit (p = 0.07) (**Table 4**). The mean bias (observed-predicted) is lower than the median bias (−2.51 vs. −3.44) (**Figure 5**). According to the Bland-Altman plot, the 95% limit of agreement is −22.51/17.48 (**Figure 6**).

Sensitivity Analysis

In a sensitivity analysis using the 2009 CKD-EPI eGFR equation, results were generally consistent with our main analysis. 2009 CKD-EPI estimates of predonation eGFR ≥90 and

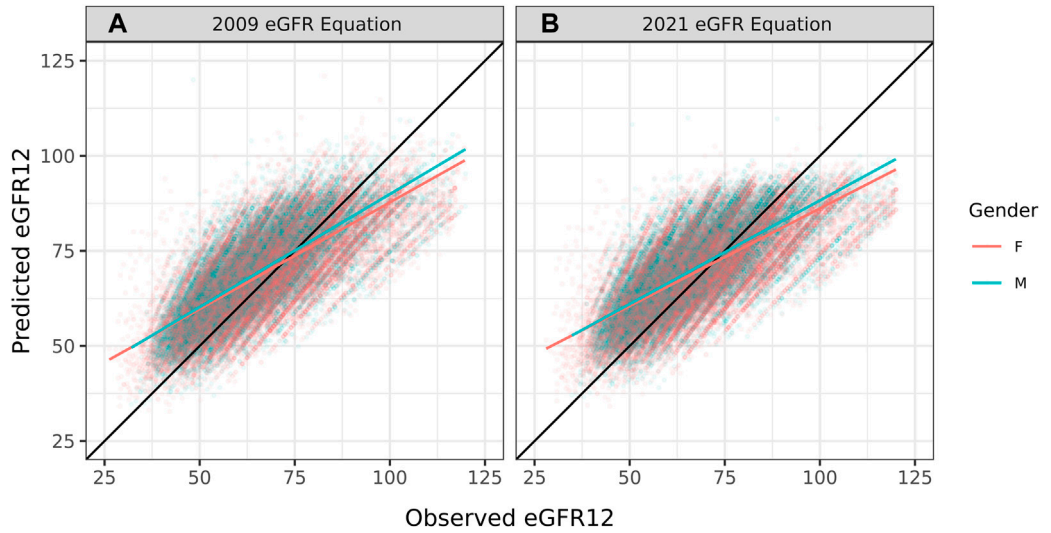


FIGURE 2 | Pearson correlation between predicted eGFR12 and observed eGFR12 by CKD-EPI equation (eGFR: mL/min/1.73 m^2). **(A)** using the 2009 eGFR equation, stratified by gender **(B)** using the 2021 eGFR equation, stratified by gender *eGFR12, Postdonation 12 month estimated glomerular filtration rate.

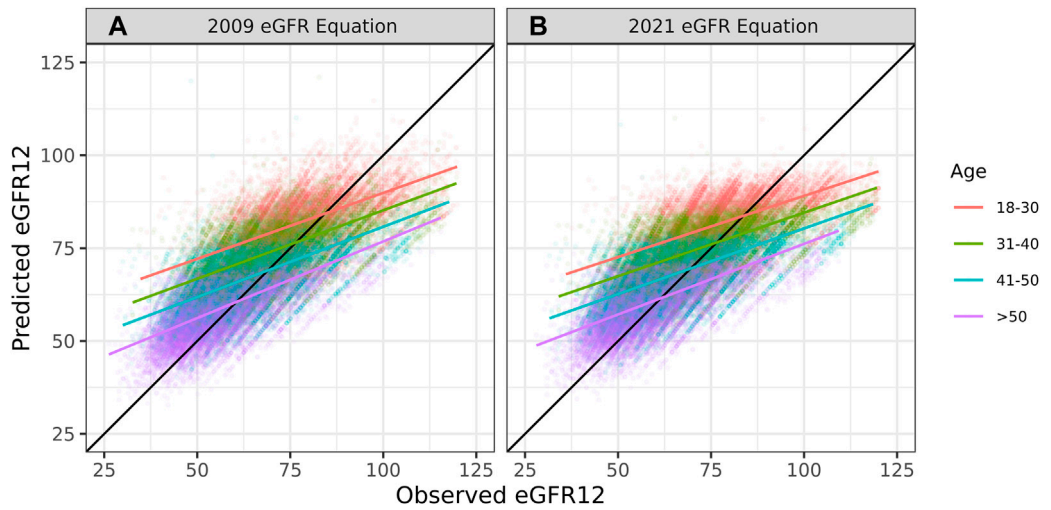


FIGURE 3 | Pearson correlation between predicted eGFR12 and observed eGFR12 by CKD-EPI equation (eGFR: mL/min/1.73 m^2). **(A)** using the 2009 eGFR equation, stratified by age **(B)** using the 2021 eGFR equation, stratified by age *eGFR12, Postdonation 12 month estimated glomerular filtration rate.

eGFR12 ≥ 70 were slightly lower than 2021 CKD-EPI estimates (**Supplementary Table S1**; **Table 1**). Compared to the 2021 CKD-EPI based TRM predictions, the 2009 CKD-EPI overall median bias was higher (median [IQR] −4.2 [−9.8, 2.4] vs. −3.4 [−9.3, 3.4]) (**Supplementary Table S2**; **Table 2**). Median bias was higher for younger (31–40) vs. older donors (>50) (median −5.4 [−11.1, 2.2] vs. −2.9 [−8.1, 2.8]), and males vs. females (median −5.2 [−10.5, 0.7] vs. −3.6 [−9.4, 3.2]) (**Supplementary Table S2**). While bias was still higher for Black vs. White donors (median −5.6 [−11.7, 1.4] vs. −4.3 [−9.7, 2.0]), bias based on the 2009 CKD-EPI for Black donors was slightly lower than the bias based on the 2021 CKD-EPI (median −5.6 [−11.7, 1.4] vs. −6.7 [−12.1, −0.3]). The overall correlation based on the 2009 CKD-EPI estimates was slightly larger than the 2021 CKD-EPI based correlation (0.74 vs. 0.71) (**Supplementary Table S2**; **Table 2**; **Figure 1A**). The specificity was the same (0.94) and the 2009 CKD-EPI based sensitivity was slightly larger but comparable to the 2021 CKD-EPI based sensitivity (0.50 vs. 0.45) (**Supplementary Table S3**; **Table 3**). While the 2009 CKD-EPI based TRM estimates failed the Hosmer-Lemeshow test for model fit ($p < 0.001$), the 2021 CKD-EPI based TRM estimates passed the Hosmer-Lemeshow test ($p = 0.07$) (**Supplementary Table S4**; **Table 4**).

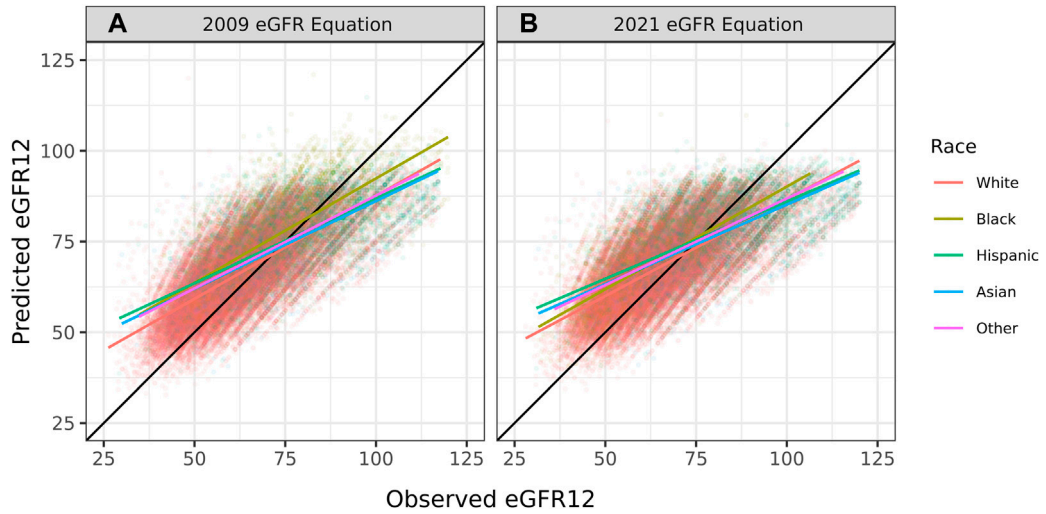


FIGURE 4 | Pearson correlation between predicted eGFR12 and observed eGFR12 by CKD-EPI equation (eGFR: mL/min/1.73 m^2). **(A)** using the 2009 eGFR , stratified by race **(B)** using the 2021 eGFR equation, stratified by race *eGFR12, Postdonation 12 month estimated glomerular filtration rate.

TABLE 3 | Contingency table to summarize the relationship between predicted and observed eGFR12 < 60 mL/min/1.73 m^2.

		Observed eGFR12		Total
		<60	≥60	
Predicted eGFR12	<60, n (%)	9,286 (44.7)	2,258 (5.6)	11,544
	≥60, n (%)	11,509 (55.3)	37,786 (94.4)	49,295
Total		20,795	40,044	60,839

eGFR12, Postdonation 12 month estimated glomerular filtration rate.
Sensitivity 0.45, specificity 0.94, positive predictive value 0.80, negative predictive value 0.77.

TABLE 4 | Hosmer-Lemeshow test for goodness of fit, $p = 0.07$.

Group	Total	eGFR12 < 60		eGFR12 ≥ 60	
		Obs	Exp	Obs	Exp
1	6,049	76	90.9	5,973	5,958.1
2	6,080	234	257.8	5,846	5,822.2
3	6,111	540	514.0	5,571	5,597.0
4	6,083	842	868.8	5,241	5,214.2
5	6,040	1,333	1,327.5	4,707	4,712.5
6	6,077	1,995	1914.4	4,082	4,162.6
7	6,095	2,607	2,632.0	3,488	3,463.0
8	6,024	3,391	3,414.5	2,633	2,609.5
9	6,103	4,389	4,360.7	1,714	1,742.3
10	6,177	5,388	5,414.7	789	762.3
χ^2	14.7				
p-value	0.07				

Obs, observed; Exp, expected; eGFR12, Postdonation 12 month estimated glomerular filtration rate.

The median bias (observed-predicted) is larger than the mean bias (−4.16 vs. −3.33) (**Supplementary Figure S1**). The 95% limit of agreement is -22.72/16.06 according to the Bland-Altman plot (**Supplementary Figure S2**).

DISCUSSION

In this external validation study, the TRM had good discrimination but poor calibration in predicting eGFR12 postdonation in a national registry cohort from the United States. Correlation between observed and predicted eGFR12 in the US cohort was moderately strong with a correlation coefficient of 0.71; higher than in previous external validation cohorts in France, Portugal, and Germany [6–8]. However, the TRM demonstrated bias, overestimating eGFR12 by median 3.4 units; the bias was more pronounced for male donors, younger donors (<40), and Black donors, populations at higher long-term risk for ESRD [4]. Moreover, the TRM performed poorly in predicting the binary outcome of eGFR12<60; specificity was high at 94%, but sensitivity was only 45%. As such, the TRM will fail to identify many donors at risk of poor postdonation renal function. Therefore, we recommend that the TRM not be used for evaluation of candidates for living kidney donation in the United States. Moreover, the TRM should be used with caution outside of Europe, and may be inappropriate for younger donor candidates or nonwhite donor candidates in Europe.

While there is currently a lack of global consensus on a universal eGFR equation, serum creatinine based eGFR equations are the most widely used [11]. The TRM model was developed and externally validated in France [6] and Portugal [7] using the serum creatinine based 2009 eGFR CKD-EPI equation although the German external validation paper [8] uses the Modification of Diet in Renal Disease (MDRD) eGFR equation. A retrospective analysis found that the 2009 CKD-EPI eGFR equation has higher accuracy than the MDRD equation when compared to the gold standard of GFR measured through the clearance of exogenous filtration markers [12]. Since the creation of the TRM, a new race-free 2021 CKD-EPI equation has been developed. This equation is recommended by the

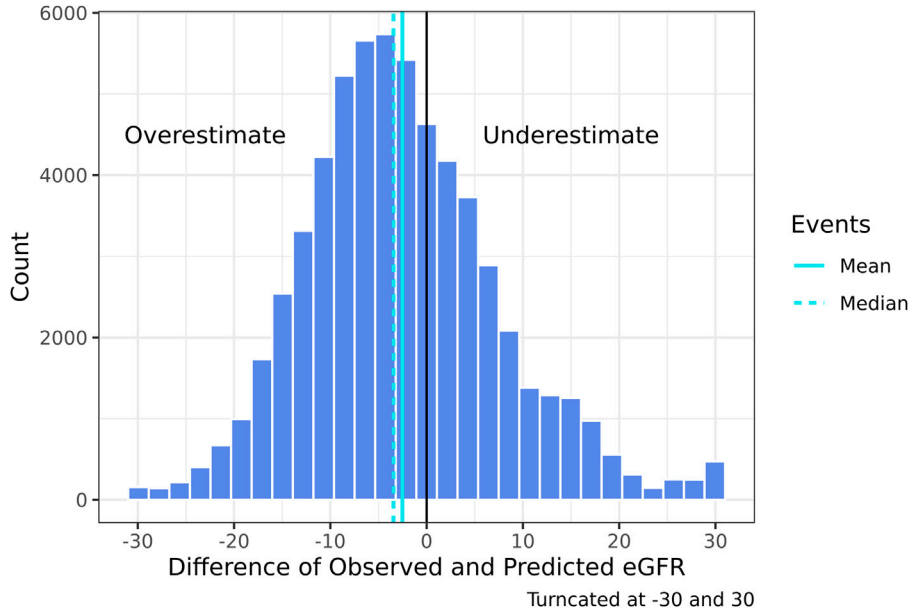


FIGURE 5 | Histogram of the difference of 2021 CKD-EPI observed—predicted eGFR12 (mean: −2.51) (median: −3.44) (eGFR: mL/min/1.73 m^2) *eGFR12, Postdonation 12 month estimated glomerular filtration rate.

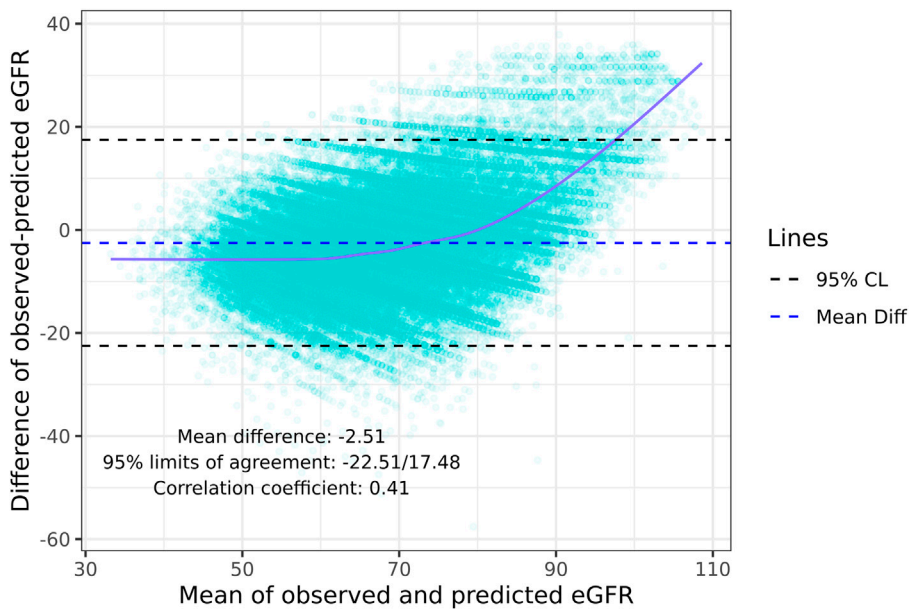


FIGURE 6 | Bland-Altman plot: Agreement and correlation coefficient between the difference and mean of the predicted eGFR12 and observed 2021 CKD-EPI eGFR12 (eGFR: mL/min/1.73 m^2). *eGFR12, Postdonation 12 month estimated glomerular filtration rate.

National Kidney Foundation and is widely utilized by US clinicians. However, according to Husain et al., the 2021 race-free CKD-EPI eGFR equation increases estimates overall by 2.1 mL/min/1.73 m^2 (IQR 0.0–3.3) and decreases estimates by 12.9 mL/min/1.73 m^2 (IQR 17.2–9.8) among Black donors [13]. Augustine et al. similarly found that among Black donors, the 2021 CKD-EPI equation underestimates eGFR but that the cystatin C based 2021 equation performed better [14]. Our postdonation estimation may be improved with the cystatin C based 2021 equation although the SRTR does not collect this metric. We chose to focus this study on the 2021 CKD-EPI equation eGFR estimates due to the availability of serum creatinine data and because it is the current standard of practice in pre-donation donor evaluation in the US.

According to our sensitivity analysis, the 2009 CKD-EPI equation based TRM predictions demonstrated a higher overall median bias compared to the 2021 CKD-EPI equation (2009 CKD-EPI: −4.2 vs. 2021 CKD-EPI: −3.4). Additionally, the Hosmer-Lemeshow test for model fit failed based on the 2009 CKD-EPI estimates but passed based on 2021 CKD-EPI estimates (2009 CKD-EPI: $p < 0.001$ vs. 2021 CKD-EPI: $p = 0.07$). Median bias was higher for Black vs. White donors (bias −5.6 vs. −4.3), younger (31–40) vs. older (>50) donors (bias −5.4 vs. −2.9), and male vs. female donors (bias −5.2 vs. −3.6). Overall, the TRM predictions based on the 2009 CKD-EPI eGFR estimates performed similarly and, in some cases, worse than the 2021 CKD-EPI eGFR based predictions. Irrespective of which equation is utilized, the TRM's performance remains questionable and potentially problematic for the estimation of eGFR12 in US cohorts due to concerns over calibration and disparities in Black, younger, and male donors.

Although renal failure is rare among LKDs, there are two prominent studies that have indicated an association between living donor nephrectomy and ESRD compared to healthy nondonors [1, 2]. Because ESRD is an uncommon outcome, a proxy may aid in identifying candidate donors at higher risk. An analysis of 71,468 US LKDs reported a 28% increased chance of ESRD per 10 mL/min/1.73 m^2 decrease in 6 month postdonation eGFR (eGFR6) after adjusting for age, race, sex, body mass index, and biological relationship [3]. There are several studies that indicate an association between predonation eGFR and postdonation ESRD risk [15, 16]. Prior research indicates that eGFR6 may fully mediate the association between predonation eGFR and ESRD.

Importantly, while early postdonation eGFR is a potential marker of long-term ESRD risk, it is only one component of full assessment of function of the remaining kidney following living donor nephrectomy. A prior registry study of living kidney donors in the United States reported that at the time of donation 3.2% of donors had hypertension and 0.05% of donors had diabetes [17]. One-year postdonation, incidence of *de novo* hypertension was 162/10,000 donors while incidence of diabetes was 6/10,000 [17]. Blood pressure, diabetes risk, and proteinuria should be carefully monitored in living kidney donors to ensure long-term renal health.

Our findings provide additional context to prior studies from single-center French, Portuguese, and German cohorts. The mean difference between observed-predicted (95% limit of agreement) was −2.5 (−22.5/17.5) compared to −2.4 (−23.1/18.3) in the French cohort [6] and +2.3 (−21.4/26.1) in the Portuguese cohort [7]. However, in our study, performance of the equation was worse for clinically important subgroups of younger donors and Black donors. Interestingly, the correlation between observed and predicted values was higher in our cohort (0.71) compared to these prior studies (0.66/0.67/0.59) [6–8]. While the French cohort demonstrated a higher sensitivity in predicting eGFR<60 (0.59 in the French cohort vs. 0.45 in our cohort) but lower specificity (0.89 in the French cohort vs. 0.94 in our cohort) [6], the Portuguese validation study reported a comparable sensitivity (0.47 in the Portuguese cohort vs. 0.45 in our cohort) and specificity (0.93 in the Portuguese cohort vs. 0.94 in our cohort) [7]. While our study population was 28% non-White, all donors in the French and Portuguese population were White. The lack of racial diversity in previous external validation studies necessitates the study of the TRM in more diverse European populations. Since our study population was larger and more heterogeneous than prior cohorts, caution may be warranted when interpreting the TRM even in European settings, particularly for younger donor candidates or racial/ethnic minorities, for whom the TRM had the highest bias in our study.

As noted in commentary by Wang and Gard, the original TRM risks bias from deriving the model from LKDs vs. candidates for LKD [18]. This bias affects our study as well, and is inherent to any study of postdonation renal function since postdonation renal function can only be assessed in individuals who actually undergo donor nephrectomy. Wang and Gard also noted that eGFR12 is an imperfect indicator of future ESRD risk [3, 18], although prior research has shown an association between early postdonation renal function and long-term ESRD risk [3]. If anything, these two concerns further weaken the case for clinical use of the TRM for evaluating LKD candidates in the United States.

Our findings must be interpreted in the context of the limitations of our study. Approximately 44% of living donors who were otherwise eligible for inclusion in our study did not have serum creatinine assessed at 12 months postdonation, and so were excluded from the analysis. However, we have no reason to think that the TRM would perform differently among donors who were lost to followup by the transplant center. Our study follow-up was not at exactly 12 months, but rather between 9 and 18 months postdonation. Having said that, 91% of samples were collected within 2 months of the 12 month follow-up date. Further, our larger sample size allowed us to conduct subgroup analysis, revealing varying levels of bias across racial, gender, and age subcategories. Future studies of the TRM in European cohorts should investigate potential bias within important demographic subgroups.

Taken as a whole, while the TRM had good predictive discrimination in an American cohort, it systematically overestimated postdonation renal function in this cohort. Notably, overestimation was greatest for those at higher risk for postdonation ESRD including male, Black, and younger donors. A new equation is needed to estimate postdonation renal function in LKDs in the United States. The TRM should be used with caution outside of Europe, and with younger donor candidates or nonwhite ethnic/racial minority candidates in Europe.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://www.srtr.org/requesting-srtr-data/data-requests/.

ETHICS STATEMENT

This study of deidentified data was determined to be "exempt: not human subjects research" by the institutional review board of NYU

Langone (ID: i22-00146). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

AM and TP-YC: research design. AM and SP: data analysis and paper writing. BL, FA, DS, AM, SP, and TP-YC: paper editing. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

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The data reported here have been supplied by the Hennepin Healthcare Research Institute (HHRI) as the contractor for the Scientific Registry of Transplant Recipients (SRTR). The interpretation and reporting of these data are the responsibility of the author(s) and in no way should be seen as an official policy of or interpretation by the SRTR or the U.S. Government.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11619/full#supplementary-material

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Pancreatic Allograft Thrombosis: Implementation of the CPAT-Grading System in a Retrospective Series of Simultaneous Pancreas-Kidney Transplantation

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Pancreatic graft thrombosis (PAT) is a major surgical complication, potentially leading to graft loss. The recently proposed Cambridge Pancreas Allograft Thrombosis (CPAT) grading system provides diagnostic, prognostic and therapeutic recommendations. The aim of the present study was to retrospectively assess computed tomography angiography (CTA) examinations performed routinely in simultaneous pancreas-kidney (SPK) recipients to implement the CPAT grading system and to study its association with the recipients' outcomes. We retrospectively studied 319 SPK transplant recipients, who underwent a routine CTA within the first 7 postoperative days. Analysis of the CTA scans revealed PAT in 215 patients (106 grade 1, 85 grade 2, 24 grade 3), while 104 showed no signs. Demographic data of the patients with and without PAT (thrombosis and non-thrombosis group) were not significantly different, except for the higher number of male donors in the thrombosis group. Pancreatic graft survival was significantly shorter in the thrombosis group. Graft loss due to PAT was significantly associated with grade 2 and 3 thrombosis, while it did not differ for recipients with grade 0 or grade 1 thrombosis. In conclusion, the CPAT grading system was successfully implemented in a large series of SPK transplant recipients and proved applicable in clinical practice.

Keywords: simultaneous pancreas kidney transplantation, pancreas allograft thrombosis, Cambridge pancreas allograft thrombosis (CPAT) grading system, computed tomography angiography, outcome predictors

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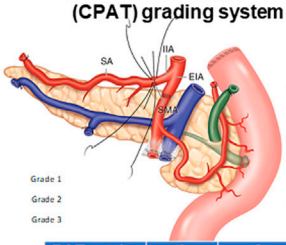
Abbreviations: BMI, body mass index; CPAT, Cambridge pancreas allograft thrombosis; CTA, computed tomography angiography; ICU, intensive care unit; IV, intravenous; LMWH, low molecular weight heparin; PAT, pancreas allograft thrombosis; SPK, simultaneous pancreas kidney; VKA, vitamin K antagonist.

Pancreatic allograft thrombosis: implementation of the CPAT-grading system in a retrospective series of simultaneous pancreas-kidney transplantation

The aim of the present study was to assess retrospectively CTA scans performed routinely in SPK recipients to correlate the CPAT grading system with the recipients' outcomes.

Cambridge Pancreas Allograft Thrombosis (CPAT) grading system



Grade 0 = no thrombosis
Grade 1= peripheral thrombosis
Grade 2 = intermediate non-occlusive thrombosis
Grade 3 = central occlusive thrombosis

PAT grade	1	2	3
Arterial	61	24	1
Venous	20	22	9
Mixed	25	39	14
Total	106	85	24

319 SPK transplant recipients, who underwent a routine CTA within the first 7 postoperative days, were retrospectively studied. Analysis of the CTA scans revealed PAT in 215 patients, while 104 showed no signs (grade 0). Demographic data of the patients with and without PAT were not significantly different, except for the higher number of male donors in the thrombosis group.

In the first 30 post-operative days pancreas graft loss was significantly higher in the thrombosis group (pancreas loss 14.4% in the thrombosis group *vs* 2.9% in the non-thrombosis group, p<0.002).
The risk of pancreatic graft loss during the follow-up was higher in the thrombosis group (27.9% *vs* 18.3% in the non-thrombosis group, p<0.052)

The association between graft loss due to PAT and grade of thrombosis proved highly significant (17/25 graft losses occurred in patients who developed grade 3 thrombosis *vs* 7/25 who developed grade 2 thrombosis in the first 30 post-operative days, test used- chi square, p=0.0000). The risk of graft loss was not increased in recipients with PAT grade 0 or 1.
Although, the indications for anticoagulation remain to be studied, we suggest to treat patients with grade 2 thrombosis with LMWH and VKA for 3-6 months but not to introduce specific treatment (VKA) for grade 1, although a treatment only with LMWH could be recommendable. Despite the low success rate, a surgical/endovascular management has to be considered in grade 3 thrombosis (followed by VKA).

Our study is the first to implement the CPAT grading system in clinical practice, and highlights the usefulness of this system, which allows to grade the thrombosis and to improve the early management of PAT. Moreover, it suggests the utility to perform an early protocol CTA to detect PAT.

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GRAPHICAL ABSTRACT |

INTRODUCTION

Pancreatic graft thrombosis (PAT) remains one of the major surgical complications and causes of graft loss in pancreatic transplantation. The reported incidence ranges from 1% to 40% [1–3] as the entity of thrombosis, ranging from partial to complete, and its extension, diagnosis, and treatment are still not well defined. In addition, partial thromboses are often underestimated, even though they are potential precursors of complete thrombosis [4, 5]. In this case, their early detection could be essential to prevent graft failure. Ultrasound and/or computed tomography angiography (CTA) are usually used to detect PAT, either routinely or when clinical symptoms develop [5–8]. The usefulness of systematic PAT detection using CTA is still debated [7–9]. Hakeem et al [10] recently proposed the Cambridge Pancreas Allograft Thrombosis (CPAT) grading system (**Figure 1**), which provides prognostic and therapeutic recommendations. The authors reported their experience of PAT in 103 patients who received pancreas transplantation between 2014 and 2017. In this study, CTA was performed only for biochemical/clinical reasons but not routinely. PAT was retrospectively graded on the basis of CTA to identify the risk of graft loss and outline a management algorithm through a retrospective review of these cases.

The aim of the present study was to retrospectively assess CTA examinations performed routinely in simultaneous pancreas kidney (SPK) transplantation recipients to implement the CPAT grading system [10] and to study its association with the recipients' outcomes.

PATIENTS AND METHODS

Design of the Study

This retrospective study included 344 patients who received for the first time a SPK transplantation between September 2005 and December 2019 at a single center.

In order to detect thrombosis at an early stage, 319 of the 344 patients who received SPK transplantation during the study period underwent a routine CTA of the abdomen and pelvis within the first 7 postoperative days. CTA was not performed in 25 patients because of graft loss intraoperatively or within the first hours after the transplantation (21 patients, 12 of whom lost their graft due to PAT) or because of poor renal function (four patients; **Figure 2**).

All CTA examinations were then retrospectively reviewed by a radiologist and a surgeon working in consensus. They assessed the presence of PAT and, when present, graded it using the classification suggested by Hakeem et al [10]:

Grade 0 = no thrombosis
Grade 1 = peripheral thrombosis
Grade 2 = intermediate non-occlusive thrombosis
Grade 3 = central occlusive thrombosis

The outcomes recorded were the incidence of PAT and its grade, its association with graft and patient survival, postoperative complications, and length of postoperative hospital stay.

Pancreas graft failure was defined as a return to insulin therapy and kidney graft failure as a return to dialysis or kidney re-

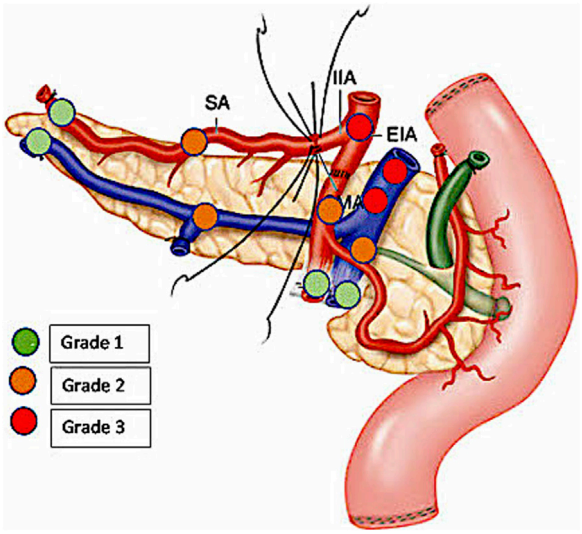

FIGURE 1 | Schema showing the localization of arterial and venous allograft thrombosis (grades 1–3) on the basis of the Cambridge Pancreas Allograft Thrombosis (CPAT) grading system [10].

transplantation. Death with a functioning graft was not considered graft failure.

CTA Imaging Protocol

Unenhanced imaging of the abdomen was first performed, followed by an arterial phase and portal-phase contrast-enhanced acquisitions of the abdomen and the pelvis. Image analysis and data were recorded.

Study Population

The 344 subjects included in this study had undergone SPK transplantation for the first time. They comprised patients diagnosed with type 1 diabetes mellitus since a median time of 26 years (range: 2–50 years) and end-stage renal disease, and 212 of them were on dialysis. There were 155 women and 189 men; their median age was 39 years (range: 22–58 years) and the median body mass index (BMI) was 22.5 (range: 15.8–31.2).

All the donors were brain-dead; they included 102 women and 242 men, with a median age of 31 years (range: 8–49 years). Donor cause of death was traumatic brain injury (30.5%), other trauma (21.2%), stroke (31.4%), and anoxia (12.8%). Cardiac arrest occurred in 19.8% of the donors, who spent a median time of 2.0 days (0–14) in the intensive care unit (ICU). The grafts were preserved in IGL-1 solution (80.9% of cases), Celsior solution (9.1%), University Wisconsin solution (6.9%), or Scott solution (3.1%).

Surgical Procedure and Post-Operative Treatment

Back-bench preparation of the pancreatic graft involved removal of the spleen, ligation of all distal mesenteric vessels, and anastomosis of a donor iliac Y-graft to the graft superior mesenteric and splenic arteries in 95.3% of the donors. Portal vein lengthening was performed in 28.5% of cases.

The pancreas was placed intraperitoneally through a midline incision in the right or the left iliac fossa in 91.5% and 8.5% of recipients, respectively. Anastomosis of the portal vein was performed to the inferior vena cava or to the common iliac vein in 90.4% of the recipients, respectively. The donor iliac artery Y-graft was anastomosed to the recipients' common iliac artery (82.3%), the external iliac artery (10.0%), or the internal iliac artery (6.8%). Exocrine drainage was performed by duodenoenterostomy (latero-lateral in 94.8% of recipients, and a Roux-en-Y duodenoenterostomy in 5.2% of them). The median cold ischemia time was 625 min (range: 330–1,162). The median anastomosis time was 31 min (range: 13–63).

The standard immunosuppression protocol included induction with antithymocyte globulins (5 mg/kg over 5 days). Maintenance immunosuppression included steroids (1 mg/kg for 3 days, progressively tapered to 5 mg/d), tacrolimus 0.05 mg/kg twice daily (trough concentration 8–12 ng/mL), and mycophenolate mofetil 1,000 mg twice daily, starting at day 0.

The patients did not receive prophylaxis with low molecular weight heparin (LMWH) but were treated with heparin (150 U/kg/d by intravenous heparin, IV) 6 h after the transplantation, before undergoing CTA. Thereafter, the patients with peripheral thrombosis received subcutaneous low-dose calcium heparin for 6 weeks post-transplantation. Intermediate PAT, not involving the arterial and/or venous donor vessels used for the reconstruction, was treated by increasing the dose of IV heparin followed by oral anticoagulant therapy (a vitamin K antagonist, VKA) for a period of 3–6 months. Complete thrombosis was treated with thrombectomy in 14 patients, followed by anticoagulant treatment, or, in 8 patients, by transplantectomy. Antiplatelet treatment (aspirin) was prescribed to all recipients (**Figure 2**).

The median follow-up time of the patient cohort was 5.3 years.

Statistical Analysis

Differences between patients with or without CTA signs of PAT were assessed through the Student's t-test for the continuous variables or the chi-square test for the categorical variables (in the latter case, when the expected values were below five, the Fisher's exact test was used).

Univariate survival analysis was carried out through Kaplan-Meier analysis. Comparisons between survival curves were made with the log-rank test.

Multivariate survival analysis was performed with the Cox proportional hazards regression analysis. Time from transplantation to graft loss was the dependent variable. The donor's age, gender, and BMI and the recipient's age, gender, BMI, duration of diabetes, dialysis before transplantation, and thrombosis grade (CTA signs of PTA) were the independent variables. The independent variables that were not significantly associated and without confounding effects were removed from the model; the final model included only variables significantly associated (p-value <0.05).

Differences among patients who developed PAT intraoperatively or during the first post-operative hours, patients who developed PAT after the first post-operative

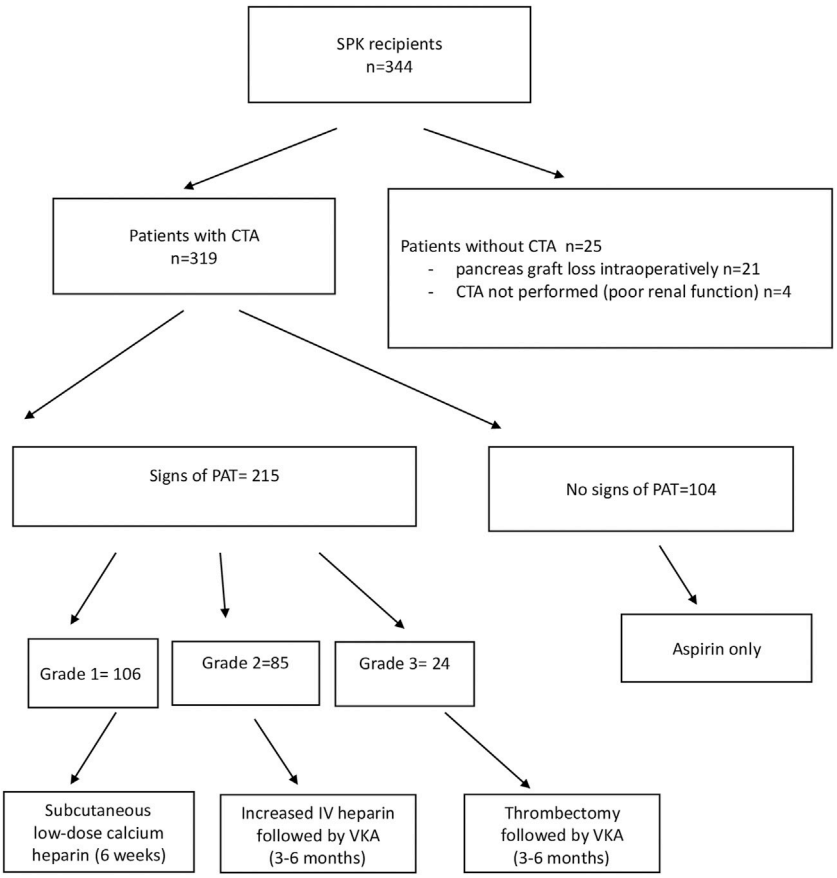

FIGURE 2 | Flowchart of the study. LMWH, low molecular weight heparin; VKA, vitamin K antagonist.

hours but within 30 days, and patients who did not develop PAT were explored through the chi-square (for categorical variables) or the Kruskal-Wallis tests (for continuous variables with non-Gaussian distribution); in the latter case, *post hoc* analysis was performed with the Mann-Whitney test.

Analyses were performed using the SPSS V 28 software.

RESULTS

In total, 344 consecutive first-time SPK transplantations were performed during the study period (from February 2005 to December 2019).

At the retrospective reading by the two readers, 215 of the 319 patients had PAT on CTA, with a thrombosis grade of 1, 2, or 3 in 106, 85, and 24 patients, respectively. The 104 remaining patients had no sign of PTA (grade 0). The thromboses were diagnosed as arterial in 86 patients, venous in 51 patients, or mixed in 78 patients (**Table 1**).

CTA signs of PAT were found in 215 patients, while 104 did not show such signs. These two groups of patients were compared. The patients who did not undergo CTA were not included in this comparative study (**Figure 2**).

There was no difference between the patients with or without PAT on CTA in terms of the donor's age, BMI, cause of death, anoxia brain damage, cardiac arrest, and period spent in the ICU (**Table 2**). The only statistically significant difference was a larger proportion of donor men in the thrombosis- vs. the non-thrombosis group (73.5% vs. 62.1%; $p = 0.039$).

There was no difference between the two groups in terms of the recipient's gender, age, BMI, duration of diabetes, dialysis status, and number of HLA mismatches (**Table 2**); preservation solution; cold ischemia time; anastomosis time; and operative procedures (**Table 3**).

As shown in **Table 4**, graft survival in the first 30 post-operative days was significantly lower in the thrombosis group (pancreas loss was 14.4% in the thrombosis group vs. 2.9% in the non-thrombosis group, $p < 0.002$). The association between graft loss due to PAT and grade of thrombosis proved highly significant (17/25 graft losses occurred in patients with grade 3 thrombosis vs. 7/25 with grade 2 thrombosis in the first 30 post-operative days, $p = 0.0000$ with the chi-square test). Whatever the cause of pancreatic graft loss, it was significantly correlated to the grade of thrombosis (**Figure 3**). Graft losses due to PAT occurred within the first 5 post-transplant days.

TABLE 1 | Grades and types of PAT.

PAT grade	1	2	3
Arterial	61	24	1
Venous	20	22	9
Mixed	25	39	14
Total	106	85	24

During the first 30 post-operative days, there was no difference between the two groups in the number of kidney graft losses, deaths, and ICU and hospitalization days (**Table 4**).

There was a statistically significant difference in the number of thrombotic complications and re-interventions between the groups, (11.2% in the thrombosis group vs. 1.9% in the non-thrombosis group, $p < 0.001$ and 14.4% vs. 1%, $p < 0.001$, respectively), and a significantly higher number of transplantectomies in the thrombosis group (13% in the thrombosis group vs. 0% in the non-thrombosis group, $p < 0.001$) (**Table 5**). There was no difference between the two groups regarding the number of other surgical complications (35.6% in the non-thrombosis group vs. 34.9% in the thrombosis group) or surgical re-exploration (34% in the non-thrombosis group vs. 34.4% in the thrombosis group). There was no significant difference between the incidence of surgical complications or surgical re-explorations or graft loss due to bleeding between the thrombosis group, which received anticoagulation, and the non-thrombosis group (**Table 5**).

Two patients in the non-thrombosis group suffered from peritonitis which prompted re-intervention and PAT detection.

As shown in **Table 5**, the risk of pancreatic graft loss during the follow-up was higher in the thrombosis group (27.9% vs. 18.3% in the non-thrombosis group, $p < 0.052$). This result was also confirmed by the Kaplan-Meier analysis (**Figure 4**).

The main cause of pancreatic graft loss was thrombosis (13% in the thrombosis group vs. 1% in the non-thrombosis group, $p < 0.001$); other causes included bleeding, peritonitis, acute and chronic rejection, and diabetes recurrence, with no significant difference between the two groups (**Table 5**).

Patient survival was not correlated to pancreatic graft thrombosis (**Figure 5**).

The multivariate analysis (Cox proportional hazard model) showed that the risk of pancreatic graft loss was significantly associated with the recipient's age, the development of hyperglycemia, hemorrhage, abdominal pain, and thrombosis grade 2 or 3, while there was no increase in the risk of graft loss in recipients with PAT grade 0 or 1 (**Table 6**).

Only in the 12 patients who developed PAT intraoperatively or during the first post-operative hours (they were not included in the thrombosis group) there was a correlation between PAT occurrence and donors' age and the recipients' duration of diabetes. The Kruskal-Wallis analysis showed that the donor's age was significantly higher in patients who developed PAT intraoperatively than in those who developed it after the first post-operative days but within 30 post-transplantation days (41 vs. 32 years, $p = 0.02$) or in the patients who did not develop PAT (41 vs. 29 years, $p = 0.01$). Similarly, the duration of the recipient's diabetes was significantly higher in the patients who developed PAT intraoperatively than in those who developed PAT after the

TABLE 2 | Donor and recipient characteristics in the two groups of patients who showed (Thrombosis group) or did not show (Non-thrombosis group) CTA signs of PAT.

Donors	Non-thrombosis group	Thrombosis group	*p*-value
Gender (M/F)	64/39	158/57	*0.039*
Age (years, median)	30.0	32.0	0.558
BMI (median)	22.5	22.8	0.794
Cause of death			
Trauma	51 (49%)	115 (53.5%)	0.456
Stroke	31 (29.8)	67 (31.2%)	0.806
Anoxia	16 (15.4%)	25 (11.6%)	0.347
Suicide	17 (16.3%)	34 (15.8%)	0.903
Cardiac arrest	22 (21.2%)	43 (20%)	0.810
Days in ICU (median)	2.0	2.0	0.524
Recipients			
Gender (M/F)	56/48	123/92	0.570
Age (years, median)	38.0	40.0	0.202
BMI median	22.2	22.5	0.962
Duration of diabetes (years, median)	24.0	26.0	0.164
Dialysis	65 (62.5%)	134 (62.6%)	0.984
DSA before transplantation	5 (4.8%)	16 (7.4%)	0.374
Total HLA mismatches 1	1 (1.0%)	0 (0.0%)	0.326
Total HLA mismatches 2	3 (2.9%)	12 (5.6%)	0.401
Total HLA mismatches 3	9 (8.7%)	35 (16.3%)	0.064
Total HLA mismatches 4	30 (28.8%)	66 (30.7%)	0.735
Total HLA mismatches 5	39 (37.5%)	71 (33.0%)	0.430
Total HLA mismatches 6	22 (21.2%)	31 (14.4%)	0.130

ICU, intensive care unit.

Italic value represents the unique significant difference between the groups.

TABLE 3 | Procurement and operative procedures in the two groups of patients who showed (Thrombosis group) or not (Non-thrombosis group) CTA signs of PAT.

	Non-thrombosis group	Thrombosis group	*p*-value
Preservation solution			
IGL-1	77 (80.2%)	167 (81.9%)	0.732
Celsior	7 (7.3%)	20 (9.8%)	0.478
Belzer (UW)	10 (10.4%)	12 (5.9%)	0.160
Scott	2 (2.1%)	5 (2.5%)	0.844
Vessel reconstruction			
Donor iliac-Y graft	97 (93.3%)	207 (96.3%)	0.234
Splenic artery onto MSA[a]	5 (4.8%)	7 (3.3%)	0.536
Other	2 (1.9%)	1 (0.5%)	0.249
Portal vein reconstruction	29 (8.2%)	57 (26.9%)	0.813
Site of arterial anastomosis			
Common iliac artery	83 (80.6%)	177 (83.9%)	0.466
External iliac artery	12 (11.7%)	19 (9.0%)	0.461
Internal iliac artery	6 (5.8%)	14 (6.6%)	0.783
Iliac bifurcation	2 (1.9%)	1 (0.5%)	0.209
Site of venous anastomosis			
Inferior vena cava	62 (93.9%)	130 (87.8%)	0.175
Common iliac vein	3 (4.5%)	13 (8.8%)	0.256
External iliac vein	1 (1.5%)	4 (2.7%)	1.00
Superior mesenteric vein	0 (0.0%)	1 (0.7%)	1.00
Enteric drainage			
Latero-lateral	95 (99%)	194 (93.2%)	0.043
Roux-en-Y	1 (1.0%)	14 (6.8%)	0.043
Cold ischemia time (min, median)	625.0	625.5	0.785
Anastomosis time (min, median)	32.0	31.0	0.580

[a]*MSA, mesenteric superior artery.*

TABLE 4 | Recipient outcomes in the first 30 post-operative days in the patients who showed (Thrombosis group) or did not show (Non-thrombosis group) CTA signs of PAT.

	Non-thrombosis group	Thrombosis group	*p*-value
Pancreas loss - any cause	3 (2.9%)	31 (14.4%)	0.002
Pancreas loss - thrombosis	1 (1.0%)	24 (11.2%)	0.001
Kidney loss - any cause	2 (2.0%)	4 (1.9%)	1.000
Death	1 (1.0%)	1 (0.5%)	0.546
Days in ICU (median)	3.0	3.0	0.587
Hospitalization days (median)	23.0	22.0	0.699

first post-operative hours but within 30 days (32 vs. 26 years, $p = 0.01$) or in the patients who did not develop PAT (32 vs. 24 years, $p = 0.01$).

DISCUSSION

This study addressed PAT that occurred following SPK transplantation in a large series of patients who underwent transplants at a single center. The study included only patients with type 1 diabetes and end-stage renal disease who received a SPK transplantation for the first time, in order to exclude additional risks of PAT.

To our knowledge, this is the first study that implemented the CPAT grading system in clinical practice after Simonis SA et al [11] assessed the applicability and the reproducibility of this system.

The large majority of the recipients (319/344, i.e., 93%) underwent systematic CTA to detect early signs of thrombosis. CTA was not performed in 25 recipients, 21 of whom had lost their pancreatic allograft intraoperatively or within the first hours after the transplantation, and 4 of whom had shown poor renal function recovery.

Although there is no consensus on when systematic CTA should be performed [8–10], we decided to perform it within the first 7 post-operative days or sooner when the patients presented signs of complications (i.e., hyperglycemia). CTA was chosen for its high specificity and sensitivity, and non-operator dependence [8, 12–14]. It was well tolerated without a significant decrease in renal function [9–11]. It was not performed only in a few patients to avoid further kidney injury. PAT was detected by CTA except in 12 patients who developed it in the operating room. Moreover, it was not diagnosed by protocol CTA in two patients, in whom PAT was detected in the operating theater during a re-operation for other causes.

In the present study, the incidence of PAT was high because all the recipients underwent CTA and all grades of thrombosis were

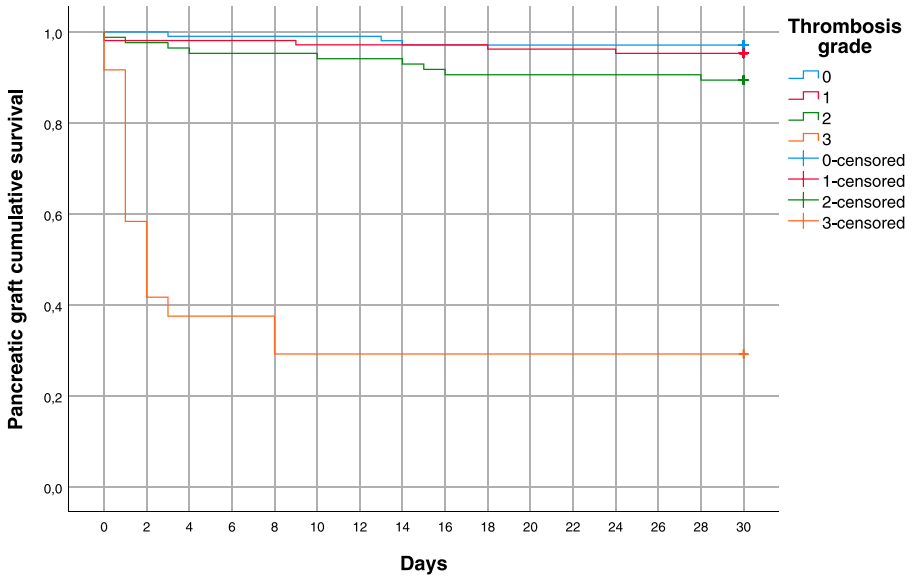

FIGURE 3 | Pancreas survival within the first 30 days in patients with PAT grades 0, 1, 2, or 3, considering all causes of graft loss.

TABLE 5 | Surgical complications and re-explorations, graft loss, and patient death during the follow-up (median follow-up 5.3 years) in the patients who showed (Thrombosis group) or did not show (Non-thrombosis group) CTA signs of PAT.

	Non-thrombosis group	Thrombosis group	*p*-value
Surgical complications	38 (36.5%)	75 (34.9%)	0.772
Thrombosis	2 (1.9%)	32 (14.9%)	<0.001
Bleeding	17 (16.3%)	24 (11.2%)	0.195
Enteric leak	8 (7.7%)	8 (3.7%)	0.128
Peritonitis	3 (2.9%)	7 (3.3%)	1.000
Small bowel obstruction	1 (1.0%)	7 (3.3%)	0.282
Eventration	3 (2.9%)	4 (1.9%)	0.687
Surgical re-exploration	35 (34.0%)	74 (44.4%)	0.939
Thrombosis	1 (1.0%)	31 (14.4%)	<0.001
Bleeding	15 (14.6%)	22 (10.2%)	0.260
Small bowel obstruction	3 (2.9%)	4 (1.9%)	0.687
Enteric leak	7 (6.8%)	10 (4.7%)	0.426
Eventration	3 (2.9%)	5 (2.3%)	0.717
Transplantectomy	0 (0.0%)	28 (13.0%)	<0.001
Peritonitis	4 (3.9%)	5 (2.3%)	0.478
Other	6 (5.8%)	4 (1.9%)	0.083
Pancreas loss	19 (18.3%)	60 (27.9%)	0.062
Thrombosis	1 (1.0%)	28 (13.0%)	<0.001
Bleeding	2 (1.9%)	2 (0.9%)	0.599
Peritonitis	5 (4.8%)	8 (3.7%)	0.764
Acute rejection	3 (2.9%)	3 (1.4%)	0.396
Chronic rejection	2 (1.9%)	5 (2.3%)	1.00
Diabetes recurrence	2 (1.9%)	5 (2.3%)	1.00
Kidney loss	10 (9.8%)	25 (11.9%)	0.581
Death	6 (5.8%)	18 (8.4%)	0.409

considered, contrasting with the majority of studies where CT scans were not performed routinely in all recipients but merely in those showing graft dysfunction, or following the appearance of symptoms [8–10]. Moreover, in the study of Simonis SA et al [11], 80%–90% of the re-analyzed CT scans showed signs of thrombosis.

In the present study, the retrospective analysis of CTA showed 106 grade 1, 85 grade 2, and 24 grade 3 thromboses, which were all included in our analysis, while grade 1 thromboses were not considered in the majority of the previous studies [8–10].

The demographic data of the two groups (thrombosis and non-thrombosis) did not show significant differences, except for the

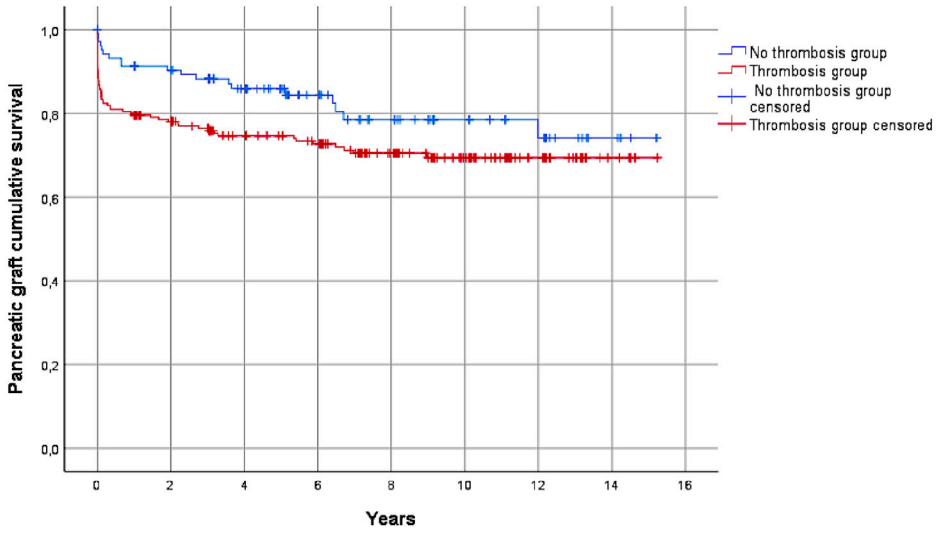

FIGURE 4 | Pancreas graft survival in the thrombosis and the non-thrombosis groups during the follow-up, considering all causes of graft loss (p = 0.052).

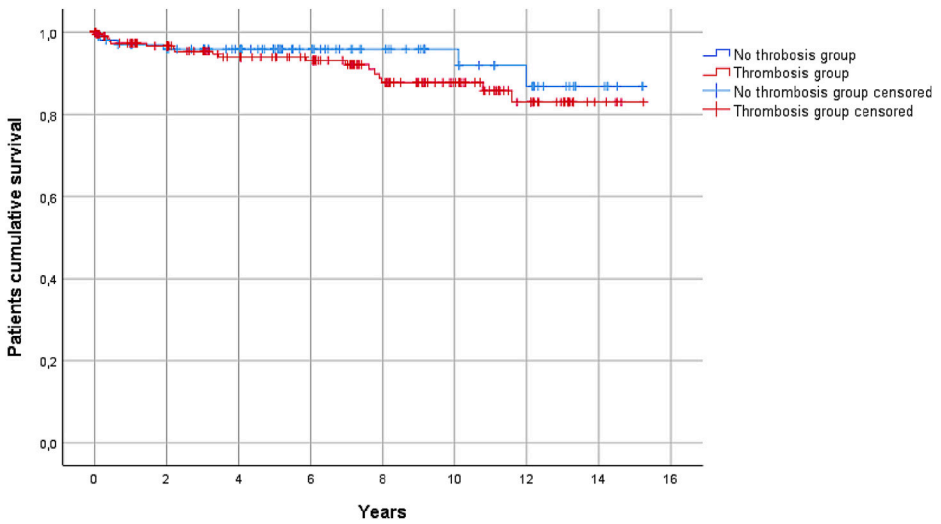

FIGURE 5 | Patient survival during follow-up did not differ significantly between the thrombosis and the non-thrombosis groups (p = 0.347).

TABLE 6 | COX proportional hazard final model (including only significant associated variables).

	Hazard ratio	95% confidence intervals	*p*-value
Recipient age (years)	1.05	1.00–1.09	0.04
Hyperglycemia (yes vs. no)	2.72	1.13–6.52	0.03
Hemorrhage (yes vs. no)	2.82	1.13–7.02	0.03
Abdominal pain (yes vs. no)	4.23	1.74–10.33	0.00
Thrombosis grade 1 vs. grade 0	1.94	0.46–8.15	0.37
Thrombosis grade 2 vs. grade 0	5.18	1.37–19.63	0.02
Thrombosis grade 3 vs. grade 0	44.29	12.14–161.53	<0.01

Dependent variables: time from transplantation to graft loss during the first post-transplant 30 days. Independent variables: all the factors which can induce graft loss. All potential confounding factors were taken into account.

higher proportion of male donors in the thrombosis group (73.5% in the thrombosis- vs. 62.1% in the non-thrombosis group) and a higher incidence of thrombosis in patients with Roux-en-Y enteric drainage, but the number of these patients is too small to be considered informative. Shahrestani S et al [15] also found that the risk of thrombosis increased by 25.6-fold in the case of male donors. Interestingly, the donor's age and the duration of the recipient's diabetes were significantly associated with the risk of developing PAT only in the 12 patients who developed it intraoperatively or during the first post-operative hours. These risk factors have been reported in many studies [6, 16–19], but in our study, we found a significant association between them and the occurrence of PAT only in these 12 patients.

The majority of the grade 1 thromboses were arterial (81.1%), while the thromboses graded 2 or 3 were either venous or mixed (77.1%).

In the present study, patients with grade 1 thrombosis had a favorable course (none of them lost their graft of PAT). Indeed, the survival analysis showed that the risk of graft loss was the same in recipients with grade 0 or grade 1 thrombosis; conversely, patients with PAT grades 2 or 3 were at a significantly higher risk of graft loss due to PAT (7/25 and 17/25, respectively) compared to patients with grades 0 or 1. Moreover, even though there was no significant difference between the two groups in the number of surgical complications, whatever the cause of pancreatic graft loss (including bleeding and pancreatitis), the risk was significantly associated with PAT grades 2 and 3.

Currently, no standard protocol exists that is able to consistently prevent thrombosis of the arterial or venous anastomosis sites or within the extension grafts following transplantation [9, 10, 20, 21]. In the present study, the patients did not receive prophylaxis with LMWH but were treated for a few days with IV heparin before undergoing the protocol CTA [20–23].

The retrospective review of the CTA scans and the use of the grading system allowed us to grade the thromboses and address the management of PAT. Indeed, only 7/85 (8.2%) of the patients who developed grade 2 thrombosis and 17/24 (70.8%) of those who developed grade 3 thrombosis lost their pancreatic graft. Although the indications for anticoagulation remain to be studied [7, 10, 17, 23–25], we suggest treating patients with grade 2 thrombosis with LMWH and VKA for 3–6 months but not introducing specific treatment (VKA) for grade 1. However, treatment only with LMWH in grade 1 could be recommendable. Despite the low success rate, surgical/endovascular management has to be considered in grade 3 thrombosis [26, 27] followed by VKA. Moreover, careful donor selection and prophylaxis with LMWH in preventing thrombosis could be useful. In the present study, 12 patients lost their pancreatic graft in the immediate post-operative period before performing CTA and starting any anticoagulation treatment. This group of patients is important, rendering necessary a better knowledge of donor and recipient characteristics (i.e., thrombophilia abnormalities) to identify the high-risk patients before transplantation (i.e., in the group of patients not on dialysis).

Our study has some limitations. Firstly, the study is retrospective and includes patients who underwent transplants over a long period of time by different surgeons with different experience and we have to consider that some cases of PAT might be associated with the surgical procedure. Moreover, some difficulties were experienced in the implementation of the CPAT grading system, particularly in the differentiation between grades 0 and 1, already experienced by Simonis SA et al [11].

In conclusion, the CPAT grading system was successfully implemented in a large series of SPK transplantations and showed its applicability in clinical practice. We suggest an early protocol CTA to detect PAT and a large prospective study introducing subgrouping in the CPAT system to better establish clear indications for PAT prophylaxis and treatment (28).

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

AUTHOR CONTRIBUTIONS

PP and HY have to be considered as first author as both designed the study and collected data. XM helped with data collection and patient management. FB managed the patients during follow-up. JC-C was responsible for patient management in ICU. LB contributed to study design and performed many of the transplantations. EM contributed to study design and patient management. HY was also the surgeon dedicated to CTA lecture while OR was the radiologist dedicated to it. CS performed statistical analysis of the study. JK contributed to study design and manuscript preparation. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

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

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ORIGINAL RESEARCH
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Improved Quality of Life Among Chronic Pancreatitis Patients Undergoing Total Pancreatectomy With Islet Autotransplantation—Single Center Experience With Large Cohort of Patients

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Total pancreatectomy with islet autotransplantation (TPIAT) is the treatment of choice to preserve pancreatic endocrine function, alleviate pain, and improve quality of life (QoL) when other strategies are ineffective for chronic pancreatitis (CP) patients. This study utilized pancreatic disease-specific surveys developed by the European Organisation for Research and Treatment of Cancer (EORTC) to conduct a comprehensive, single-center examination of a large cohort of patients to gain understanding of QoL post-TPIAT. Two validated QoL surveys of the EORTC—QLQ-C30 and QLQ-PAN26—were administered in a prospective cohort of CP patients during pre-and post-operative scheduled visits. A total of 116 patients responded to the preoperative survey and were included in this study. The global health scale of QLQ-C30 was significantly improved after TPIAT when compared to baseline with delta scores of 24.26, 20.54, and 26.7 at 1, 2, and 3 years post-TPIAT ($p < 0.001$). The EORTC-PAN26 revealed significant improvements in symptom scales for pancreatic pain, bloating, digestive symptoms, taste, indigestion, weight loss, body image, and future worries. The comprehensive surveys in such a large cohort expands the QoL criterion in CP patients and indicates significant improvement in QoL post-TPIAT, further validating TPIAT as a treatment option for refractory CP.

Keywords: pancreatitis, islet autograft, quality of life, pain, diabetes mellitus

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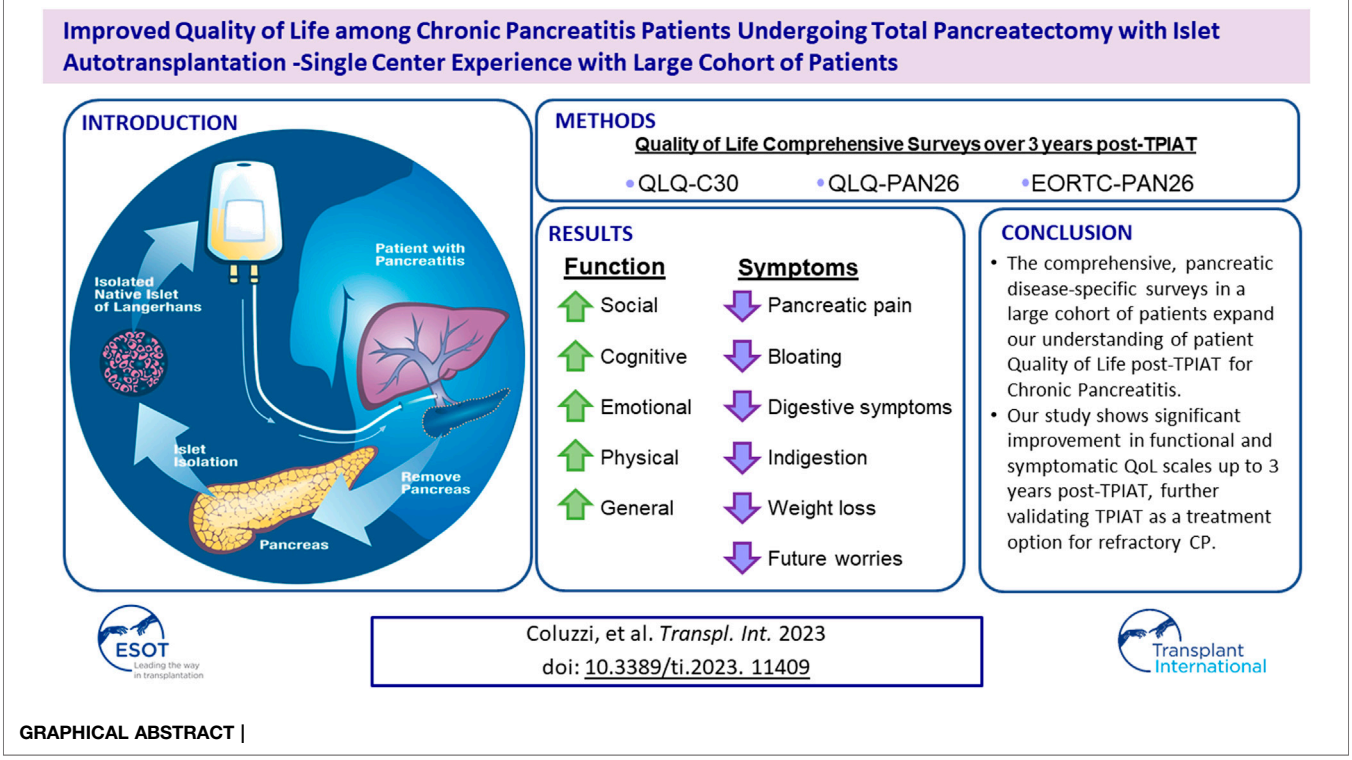
Abbreviations: CP, chronic pancreatitis; EORTC, European organisation for research and treatment of cancer; MME, morphine milligram equivalents; QoL, quality of life; SD, standard deviation; TPIAT, total pancreatectomy followed by islet autotransplantation; VAS, visual analog scale.



GRAPHICAL ABSTRACT |

INTRODUCTION

Chronic pancreatitis (CP) is an irreversible inflammatory and fibrotic disease of the pancreas leading to varying degrees of exocrine and endocrine dysfunction. In severe cases, CP can lead to permanent loss of exocrine and endocrine function [1]. Furthermore, 75% of CP patients experience abdominal pain which can become debilitating [2]. CP patients report recurrent hospitalizations and numerous treatments to relieve pain and restore some semblance of normality in their quality of life (QoL). Initial medical management for CP may include but is not limited to narcotic analgesics, replacement of pancreatic enzymes, and radiological endoscopic procedures [3–5]. Patients with progressive symptoms in which medication and endoscopic intervention fails may be candidates for surgery [6]. Surgical techniques such as Puestow, Frey, Beger, and Whipple procedures are performed to achieve pain relief in CP patients. However, there is no evidence that these procedures lead to stable maintenance of endocrine function [7].

Total pancreatectomy followed by islet autotransplantation (TPIAT) is a preferred technique to preserve endocrine function and alleviate pain when other strategies are ineffective [8]. The first human TPIAT was performed by Dr. David Sutherland at the University of Minnesota in 1977. The rationale for this procedure is by removing the source of pain and disease exacerbations, this will improve a very poor QoL, reduce or eliminate chronic narcotic use, and facilitate return to work and self-care [9].

During the last 30 years, numerous collaborations between various North American centers, including ours, have developed the TPIAT program and documented metabolic outcomes. Many studies have reported achieving the main objective, improvement of QoL, through the SF-36 questionnaire, which evaluates health-related QoL [10–15]. In the current study, we evaluated QoL in patients who received TPIAT for CP at Baylor University Medical Center (Dallas, TX, United States) using the European Organisation for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire (QLQ)-C30 survey combined with the QLQ-PAN26, designed specifically for patients with pancreatic disease [16].

PATIENTS AND METHODS

Study Participants

This prospective observational study assessed the patient-oriented outcomes of QoL in CP patients who underwent TPIAT at Baylor University Medical Center. All patients were evaluated by a multidisciplinary team and had multiple indications for TPIAT. Patient eligibility for TPIAT includes intractable pain despite previous medical treatment, detectable endogenous insulin secretion capacity evident by serum C-peptide, and the capacity to consent to the treatment. Pregnant women were not eligible for the surgical procedure. We obtained consent for the intervention and study enrollment from all participants after they had been adequately informed of the risks. This study was conducted after approval of the institutional review board of Baylor Scott and White Research Institute (IRB#009-271).

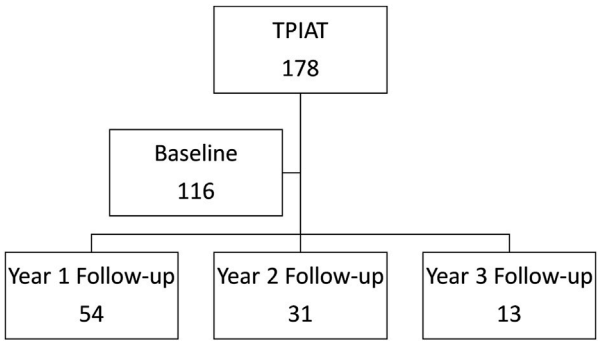


FIGURE 1 | Consortium diagram of patient participation in the two surveys from baseline to 3 years follow-up.

Data Collection

Patients were asked to answer two QoL surveys of the EORTC—QLQ-C30 and QLQ-PAN26—before TPIAT and completed the survey during follow-up or by mail at 1, 2, and 3 years after transplantation. The EORTC QLQ-C30 and QLQ-PAN26 instruments were selected because they have been validated and used in trials to evaluate other pancreatic procedures [17–19]. The QLQ-C30 consists of 30 questions. The first section examines functioning: physical functioning, role functioning, emotional functioning, cognitive functioning, and social functioning in addition to a single item of global health. The second section addresses nine symptoms: nausea and vomiting, pain, fatigue, insomnia, loss of appetite, constipation, diarrhea, dyspnea, and financial difficulties. The QLQ-PAN26 assesses functioning and pancreatic-specific symptoms. It has 26 questions that evaluate pancreatic pain, bloating, digestive symptoms, taste, indigestion, flatulence, weight loss, dry mouth, hepatic symptoms, altered bowel habit, body image, trouble with side effects, future worries, and planning of activities in addition to healthcare satisfaction and sexuality [17–19]. All scales range from 0 to 100. QLQ-C30 high scores indicate healthier status or improved QoL for global health. High scores on the symptom scales correlate with a poor QoL. High scores on the QLQ-PAN26 indicate a poor QoL, except for healthcare satisfaction and sexuality.

The patients completed these surveys during their regularly scheduled follow-up appointments in electronic or hard copy format. We confirmed the validity of the electronic format. Surveys were completed via telephone or mail for 2 and 3 years post-TPIAT if the patients were unable to attend their clinic visits. Subjects with reduced ability to understand the questionnaires were excluded from the study. Details on the patient participation in these surveys is shown in **Figure 1**.

All clinical data were recorded for each patient in a prospectively maintained database. Preoperative and postoperative clinical data in this study included levels of hemoglobin A1c, serum C-peptide, exogenous insulin requirement, pain score based on visual analog scale (VAS), and morphine equivalent dose. The VAS for pain ranged from 0 (*no pain*) to 10 (*most severe pain*). The daily dose of opioids was converted into morphine milligram equivalents (MME).

TPIAT Procedure

All patients underwent total pancreatectomy with the surgical technique described previously [20–23], with or without splenectomy based on surgeon decision. The distal common bile duct was removed, and the pancreatic blood supply was preserved during surgery as long as possible to minimize islet cell ischemia. On the back table, the spleen (if removed) and duodenum were detached from the pancreas, the pancreatic duct was cannulated, and the pancreas was placed in a container with cold preservation solution. Subsequently, the pancreas was transferred to a current good tissue practice (cGTP) facility for islet isolation processing.

Liberase MTF with Thermolysin MTF (Roche, Basel, Switzerland) or Collagenase NB with neutral proteases (SERVA Electrophoresis GMbH, Heidelberg, Germany) was infused into the pancreatic duct for digestion. Islets were isolated by the modified Ricordi method, which has been previously described [24, 25]. When the tissue volume (mL) exceeded 0.25 times body weight (kg), islets were purified with a COBE 2991 cell processor (Caridian BCT Inc., Lakewood, CO) using a density-adjusted iodixanol-based continuous density gradient. Endotoxin testing, gram staining, and bacterial and fungal cultures were performed on the final products as indicators of sterility. Isolated islets were infused into the portal vein via the superior mesenteric vein with heparin (70 unit/kg body weight) while the patient was under general anesthesia. The portal vein pressure was regularly monitored during the islet infusion.

Statistical Analysis

Data were presented as numbers and percentages for binary and categorical variables or as median and interquartile range (IQR) or as mean with standard deviations (SD) for continuous variables. The primary outcomes in this study were independent trends over time of the various scales and items of the EORTC QLQ-C30 and QLQ-PAN26. The surveys were administered at four time points: baseline, 1, 2, and 3 years. Raw scores measured at baseline and at years 1 and 2 were analyzed in longitudinal repeated measures analyses.

Generalized least square models without random effects, fitted by restricted maximum likelihood (REML) were used to examine if there was a differential effect across time (baseline to follow-up) in score measurements. The analysis focused on longitudinal single group analyses, where a single homogeneous population was followed over time. To account for the correlation in repeated measurements on the same subject, using various correlation structures with constant variance were considered. The correlation structure was selected based on AIC [29].

Due to the small sample size of respondents, scores measured at year 3 were not considered in longitudinal analyses. Additionally, a generalized least square model without random effects with restricted maximum likelihood (REML) was used instead of ordinary maximum likelihood estimation (MLE) [26, 27].

TABLE 1 | Characteristics of study participants.

Characteristics	Overall (*n* = 116)
Age (years): median (*IQR*)	41.1 (*30.4*, *49.0*)
Male: *n* (%)	41 (35%)
Body mass index (kg/m^2): median (*IQR*)	26.3 (*21.5*, *29.8*)
Epidemiology: *n* (%)	
Alcoholic	9 (7.8%)
Autoimmune	7 (6.0%)
Hereditary	19 (16%)
Idiopathic	55 (47%)
Other	26 (22%)
Pancreatic duct stent insertion or EST: *n* (%)	82 (71%)
Past history of pancreas operation: *n* (%)	18 (16%)
Duration of symptoms (years): median (*IQR*)	5.0 (*3.0*, *10.0*)
Dose (×10^3 IEQ/kg patient body weight): median (*IQR*)	5.07 (*2.93*, *7.15*)

EST, endoscopic sphincterotomy; IEQ, islet equivalent. IQR values are in parentheses and italicized.

The constant variance assumption was checked using typical residual plots. The univariate normality assumption was checked using typical Q-Q plots on residuals. For checking the correlation pattern, variograms based on estimating correlations of all possible pairs of residuals at different time points were used [26–30].

Delta, defined as a difference over time from baseline, was assessed to estimate clinical significance in the EORTC questionnaires, according to recommendations by Osoba, where a difference in health-related QoL score of 10 points or more is regarded as clinically significant [31, 32]. The radar charts depict patients' scores for the EORTC QLQ-C30 and EORTC QLQ-PAN26 scales for each domain as observed marginal means at different time points. Each domain is represented on separate axes (scaled from 0 to 100). All statistical analyses were conducted using R Statistical Software (version 4.1.2; R Foundation for Statistical Computing, Vienna, Austria). For the longitudinal analyses, the gls function from the nlme: Linear and Non-linear Mixed Effects Models, R package version 3.1-162, and the rms: Regression Modeling Strategies, R package version 6.7-0, developed by Harrell [28] were used.

RESULTS

Participant Characteristics

Between 31 March 2011, and 1 April 2021, 178 consecutive patients underwent TPIAT at our center. Among that group, 116 patients agreed to answer the two QoL surveys before transplant (65% participation rate) and were included in this study. The demographic and clinical characteristics of the study participants are presented in **Table 1**. Participants' median age at TPIAT was 41.1 (*30.4–49.0*) years, 35% were male, and the median body mass index was 26.3 (21.5–29.8) kg/m^2. To better understand our patient cohort disease progression, we looked at prior pancreatic interventions. Prior endoscopic stent management failed for 82 patients (71%). 18 patients (16%) had previous pancreatic surgery before TPIAT. Within this cohort, the median duration of diagnosed pancreatitis was 5.0 (3.0, 10.0) years. Post-TPIAT data revealed a median transplanted islet equivalent dose was 5.1 (2.9–7.2) × 10^3 IEQ/kg patient body weight. The median follow-up was 78.8 months (range 9.4–125.5 months) and at 1, 2, and 3 year follow-up, 2, 6, and 9 patients had died, respectively.

Metabolic Outcomes and Pain Control Status

12.1% of patients had diabetes before TPIAT, and 78%, 73%, and 71% were insulin-dependent at 1, 2, and 3 years after TPIAT, respectively. Glycemic outcomes pre- and post-TPIAT are outlined in **Table 2**. Daily morphine requirements and pain scores significantly decreased over time after TPIAT (p < 0.001) (**Figure 2**). There was notable decrease in mean MME dose with 118 (±*137*) mg before TPIAT and 44 (±*93*), 42 (±*68*), and 35 (±*65*) mg at years 1, 2, and 3, respectively. Pain scores evaluated with VAS also decreased after TPIAT: 5.7 (±*2.1*) at baseline, 2.2 (±*2.9*) at year 1, 2.1 (±*2.8*) at year 2, and 1.9 (±*2.6*) at year 3.

EORTC QLQ-30 and QLQ-PAN26 Surveys

Initially, 116 patients responded to the preoperative survey and respondents decreased at yearly follow-up. 54 patients completed the survey at year 1, 31 patients at year 2, and 13 patients at year 3. Radar charts visually display each domain of the EORTC QLQ-C30 functional scales and symptom scales of EORTC QLQ-C30 and EORTC QLQ-PAN26 (**Figure 3**). We displayed domains with a statistically significant trends over time as indicated by generalized least square models for repeated measures.

EORTC QLQ-30 survey functional scores increase with improved QoL after 1, 2, and 3 years (**Figure 3A**). In our patient cohort, the generalized least square models for repeated measures of functioning scales demonstrated that global health QoL, physical functioning, role functioning, emotional functioning, cognitive functioning, and social functioning significantly increased over 2 years post-TPIAT (p < 0.001, <0.001, <0.001, <0.001, = 0.007, and <0.001, respectively). Delta mean scores outlined in **Table 3** indicate the change from baseline (pre-TPIAT) to each follow-up year. In each functional scale domain of EORTC QLQ-C30 the score was ≥10 points, indicating a clinically relevant improvement from baseline.

Lower symptom scores in the EORTC QLQ-C30 indicate better QoL (**Figure 3B**). The generalized least square model of the symptom scales revealed that fatigue, nausea and vomiting, pain, insomnia, appetite loss, and constipation were significantly reduced post-TPIAT (p < 0.001, <0.001, <0.001, <0.001, = 0.001, and <0.001, respectively). Moreover, the corresponding delta scores showed changes of ≥20 points, indicating that the reduction in these symptoms was also clinically meaningful (**Table 3**).

EORTC QLQ-PAN26 surveyed symptom scales pre- and post-TPIAT in which lower scores indicate better QoL (**Figure 3C**). Again, the generalized least square model demonstrated that

TABLE 2 | Metabolic and pain outcomes at baseline and after total pancreatectomy followed by islet autotransplantation.

Variables	Baseline (*n* = 116)	Follow-up		
		Year 1 (*n* = 79)	Year 2 (*n* = 40)	Year 3 (*n* = 27)
Endocrine outcomes				
Hemoglobin A1c (%)	6.0 (*1.1*)	7.3 (*2.0*)	7.3 (*2.4*)	7.0 (*1.4*)
Serum C-peptide (ng/dL)	1.8 (*1.3*)	1.2 (*1.2*)	1.4 (*1.5*)	1.1 (*1.3*)
Fasting blood glucose (mg/dL)	102 (*29*)	152 (*94*)	151 (*65*)	124 (*54*)
Exogenous insulin amount (unit/day)	2.2 (*8.0*)	14.7 (*15.0*)	15.5 (*15.9*)	14.4 (*17.5*)
Pain control				
Pain score[a]	5.7 (*2.1*)	2.2 (*2.9*)	2.1 (*2.8*)	1.9 (*2.6*)
Morphine equivalent dose (mg/day)	118 (*137*)	44 (*93*)	42 (*68*)	35 (*65*)

[a]*Evaluated with the visual analog scale, ranging from 0 (no pain) to 10 (the most severe pain). SD values are in parentheses and italicized.*

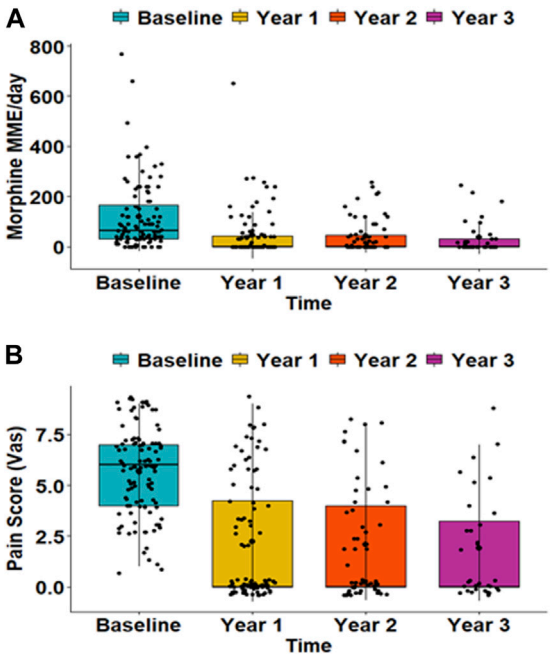


FIGURE 2 | (A) Morphine equivalent dose and **(B)** pain scores at baseline and at 1, 2, and 3 years after total pancreatectomy followed by islet autotransplantation. The morphine dose is presented as daily milligrams morphine equivalent (MME). Pain scores were evaluated with a visual analog scale (VAS) that ranged from 0 (no pain) to 10 (the most severe). Both daily morphine dose and pain score were significantly reduced over time ($p < 0.001$).

pancreatic pain, bloating, digestive symptoms, taste, indigestion, weight loss, body image, and future worries had a statistically significant trend of reduction over time ($p <$ 0.001, <0.001, <0.001, = 0.009, = 0.001, <0.001, = 0.003, and = 0.009, respectively). The corresponding delta scores indicated clinically meaningful reductions in symptoms in all domains except flatulence, hepatic symptoms, and trouble with side effects (**Table 4**). Functional scales in QLQ-PAN26 related to satisfaction with healthcare and sexuality were also significantly ameliorated after TPIAT (p = 0.004 and <0.001, respectively).

To safeguard against potential bias in study findings, an analysis was conducted to examine whether study patients who completed at least a baseline questionnaire and excluded patients that never participated in the study, displayed distinct characteristics worth exploring (**Table 5**). Moreover, a comparison between patients who responded at baseline only with participants who responses at follow-up surveys was conducted follow-up and shown in **Table 6**. Pearson's Chi-squared test; Wilcoxon rank sum test; Fisher's exact test were used for group comparisons. Both study participants and excluded patients exhibited similar characteristics unlikely to lead to potential bias with a strong impact on the study findings. The results showed that participants who did not return follow-up surveys had a significantly higher body mass index (BMI) (p = 0.036) with a median of 27.7, (IQR 23.6–32.0), compared to participants with follow-up measures, who had a median of 25.2 (IQR: 20.7–28.9).

DISCUSSION

In North America, multiple centers perform TPIAT and have demonstrated an improvement in health-related QoL in patients with CP and recurrent acute pancreatitis. The objective means used to ascertain an improvement in QoL were the SF-36 and SF-12 questionnaires [10–15, 33]. These studies evaluated QoL by reporting scores for body pain, mental composite, physical composite, and social functioning, and results showed persistent improvement for up to 5–10 years follow-up [10, 13]. By implementing pancreatic disease-specific surveys EORTC QLQ-30 and QLQ-PAN16, we were able to gain a better understanding of the QoL of our TPIAT patients by including more specific criterion in the surveys. These surveys were originally used to evaluate patients with pancreatic cancer and were validated in 2005 for evaluation of pancreatic surgery for CP [16, 18, 34, 35]. EORTC QLQ-C30 global general health scores increased by delta score of 26.70 (77% increase) which further validates that TPIAT improves patient general health.

Management of pain is a major objective of TPIAT and thus another critical metric for success. Nonetheless, pancreatic pain can be misinterpreted or reported generically through the use of

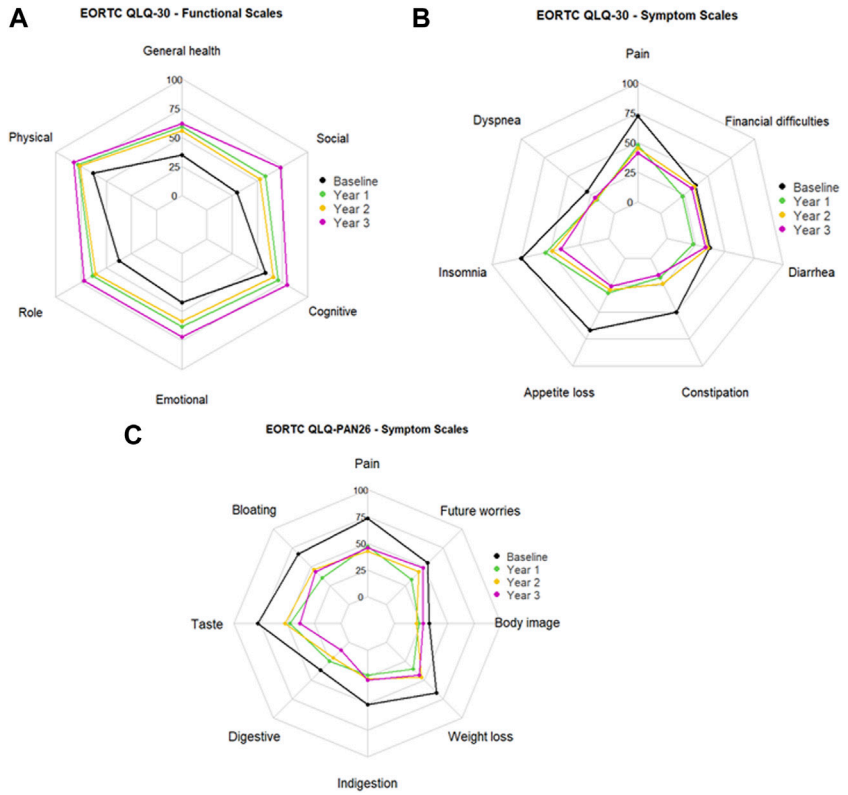


FIGURE 3 | Mean scores at baseline and 1, 2, and 3 years after total pancreatectomy followed by islet autotransplantation for **(A)** EORTC QLQ-C30 functioning scale, **(B)** EORTC QLQ-C30 symptom scale, and **(C)** EORTC QLQ-PAN26 symptom scale.

TABLE 3 | Responses on the EORTC QLQ-C30 survey before and after total pancreatectomy followed by islet autotransplantation.

Domain	Baseline (*n* = 116)	Follow-up			Delta			*p*-value
		1 year (*n* = 54)	2 years (*n* = 31)	3 years (*n* = 13)	Delta 1 year	Delta 2 years	Delta 3 years	
Global health	34.84 (*23.79*)	59.10 (*27.82*)	55.38 (*27.01*)	61.54 (*26.69*)	24.26	20.54	26.7	<0.001
Functional scales								
Physical functioning	62.82 (*24.14*)	78.27 (*22.92*)	76.13 (*21.83*)	82.05 (*24.70*)	15.45	13.31	19.23	<0.001
Role functioning	37.21 (*30.72*)	63.58 (*36.78*)	60.75 (*38.38*)	71.79 (*32.19*)	26.37	23.54	34.58	<0.001
Emotional functioning	42.17 (*26.36*)	62.81 (*31.75*)	58.06 (*30.99*)	71.79 (*20.84*)	20.64	15.89	29.62	<0.001
Cognitive functioning	55.26 (*29.94*)	70.37 (*31.67*)	65.59 (*27.87*)	79.49 (*18.20*)	15.11	10.33	24.23	0.007
Social functioning	30.32 (*32.65*)	58.33 (*36.02*)	52.69 (*36.03*)	73.08 (*33.71*)	28.01	22.37	42.76	<0.001
Symptom scales								
Fatigue	72.41 (*26.39*)	47.59 (*31.61*)	45.16 (*24.83*)	41.03 (*20.98*)	−24.82	−27.25	−31.38	<0.001
Nausea and vomiting	57.90 (*33.08*)	28.70 (*31.79*)	35.48 (*35.42*)	24.36 (*29.36*)	−29.2	−22.42	−33.54	<0.001
Pain	72.41 (*26.39*)	47.59 (*31.61*)	45.16 (*24.83*)	41.03 (*20.98*)	−24.82	−27.25	−31.38	<0.001
Dyspnea	29.02 (*31.25*)	18.52 (*27.22*)	19.35 (*25.49*)	20.51 (*28.99*)	−10.5	−9.67	−8.51	0.0697
Insomnia	75.00 (*30.10*)	54.32 (*36.22*)	48.39 (*32.02*)	41.03 (*33.76*)	−20.68	−26.61	−33.97	<0.001
Appetite loss	66.95 (*34.19*)	32.10 (*35.44*)	29.03 (*31.90*)	25.64 (*33.76*)	−34.85	−37.92	−41.31	<0.001
Constipation	50.00 (*37.94*)	17.90 (*26.47*)	23.66 (*27.48*)	15.38 (*22.01*)	−32.1	−26.34	−34.62	<0.001
Diarrhea	37.07 (*35.10*)	22.84 (*31.61*)	35.48 (*38.43*)	33.33 (*38.49*)	−14.23	−1.59	−3.74	0.061
Financial difficulties	37.07 (*35.10*)	22.84 (*31.61*)	35.48 (*38.43*)	33.33 (*38.49*)	−14.23	−1.59	−3.74	0.061

SD values are in parentheses and italicized.

the VAS score and the *Body Pain* scale in the SF-36. We utilized surveys with more specific measures to more accurately present statistical and clinical evidence of persistent pain reduction for up to 3 years. Pancreatic pain evaluated with the QLQ-PAN26 revealed clinical and statistical improvement with a delta score of 27.90. It is noteworthy that 71% of patients had a previous

TABLE 4 | Responses on the EORTC QLQ-PAN26 survey before and after total pancreatectomy followed by islet autotransplantation.

Domain	Baseline (*n* = 116)	Follow-up			Delta			*p*-value
		1 year (*n* = 54)	2 years (*n* = 31)	3 years (*n* = 13)	Delta 1 year	Delta 2 years	Delta 3 years	
Symptom scales								
Pancreatic pain	73.41 (*24.95*)	47.07 (*30.98*)	42.74 (*23.25*)	45.51 (*26.27*)	−26.34	−30.67	−27.90	<0.001
Bloating	66.67 (*30.46*)	35.19 (*32.64*)	46.24 (*32.97*)	43.59 (*31.58*)	−31.48	−20.43	−23.08	<0.001
Digestive symptoms	77.97 (*28.66*)	47.53 (*33.71*)	52.15 (*34.09*)	38.46 (*32.19*)	−30.44	−25.82	−39.51	<0.001
Taste	37.07 (*33.99*)	25.31 (*33.61*)	20.43 (*28.12*)	10.26 (*21.01*)	−11.76	−16.64	−26.81	0.009
Indigestion	50.86 (*36.11*)	23.46 (*27.19*)	26.88 (*29.08*)	28.21 (*35.61*)	−27.40	−23.98	−22.65	<0.001
Flatulence	47.41 (*34.09*)	48.15 (*37.01*)	50.54 (*38.37*)	64.10 (*34.59*)	0.74	3.13	16.69	−0.868
Weight loss	66.67 (*30.46*)	35.19 (*32.64*)	46.24 (*32.97*)	43.59 (*31.58*)	−31.48	−20.43	−23.08	<0.001
Dry mouth	42.53 (*34.78*)	30.25 (*30.56*)	38.71 (*39.53*)	20.51 (*25.60*)	−12.28	−3.82	−22.02	0.057
Hepatic symptoms	17.98 (*19.35*)	16.98 (*23.01*)	17.20 (*22.56*)	17.95 (*19.79*)	−1.00	−0.78	−0.03	−0.9524
Altered bowel habit	37.36 (*30.03*)	41.67 (*28.18*)	47.31 (*35.25*)	50.00 (*34.02*)	4.31	9.95	12.64	0.2427
Body image	33.05 (*22.84*)	22.84 (*24.29*)	20.97 (*18.24*)	26.92 (*31.58*)	−10.21	−12.08	−6.13	0.004
Troubled with side effects	7.76 (*22.14*)	9.26 (*20.90*)	8.60 (*22.72*)	15.38 (*32.25*)	1.50	0.84	7.62	−0.914
Future worries	54.89 (*37.37*)	33.33 (*31.72*)	43.01 (*30.05*)	48.72 (*39.94*)	−21.56	−11.88	−6.17	<0.001
Planning of activities	56.03 (*34.50*)	41.36 (*32.33*)	48.39 (*34.25*)	51.28 (*32.25*)	−14.67	−7.64	−4.75	0.03
Functional scale								
Satisfaction with healthcare	18.25 (*23.97*)	29.94 (*31.62*)	22.58 (*23.78*)	30.77 (*28.74*)	11.69	4.33	12.52	0.004
Sexuality	78.26 (*24.50*)	51.23 (*33.93*)	57.53 (*30.98*)	44.87 (*32.90*)	−27.03	−20.73	−33.39	<0.001

SD values are in parentheses and italicized.

TABLE 5 | Characteristics of study participants and non-participants.

Characteristics	Study participants (*n* = 116)	Excluded participants (*n* = 62)	*p*-value
Age (years): median (*IQR*)	41.1 (*30.4*, *49.0*)	39.2 (*28.6*, *49.6*)	0.680
Male: *n* (%)	41 (35%)	25 (40%)	0.510
Body mass index (kg/m^2): median (*IQR*)	26.3 (*21.5*, *29.8*)	25.1 (*21.6*, *29.6*)	0.620
Epidemiology: *n* (%)			0.087
Alcoholic	9 (7.8%)	3 (4.8%)	
Autoimmune	7 (6.0%)	1 (1.6%)	
Hereditary	19 (16.4%)	21 (33.9%)	
Idiopathic	55 (47.4%)	23 (37.1%)	
Other	26 (22.4%)	14 (22.6%)	
Pancreatic duct stent insertion or EST: *n* (%)	82 (71%)	42 (69%)	0.820
Past history of pancreas operation: *n* (%)	15 (13%)	10 (16%)	0.560
Duration of symptoms (years): median (*IQR*)	5.0 (*4.0*, *8.0*)	5.0 (*3.0*, *10.0*)	0.820
Dose (×10^3 IEQ/kg patient body weight): median (*IQR*)	5.07 (*2.93*, *7.15*)	4.47 (*2.88*, *6.12*)	0.440

EST, endoscopic sphincterotomy; IEQ, islet equivalent. IQR values are in parentheses and italicized.

TABLE 6 | Characteristics of study participants by follow-up group.

Characteristics	With follow-up (*n* = 55)	No follow-up (*n* = 61)	*p*-value
Age (years): median (*IQR*)	37.7 (*30.8*, *48.4*)	42.7 (*30.4*, *49.0*)	0.580
Male: *n* (%)	18 (33%)	23 (38%)	0.420
Body mass index (kg/m^2): median (*IQR*)	27.8 (*23.6*, *32.0*)	25.5 (*20.7*, *28.9*)	0.036
Epidemiology: *n* (%)			0.087
Alcoholic	1 (1.8%)	8 (13%)	
Autoimmune	2 (3.6%)	5 (8.2%)	
Hereditary	12 (22%)	7 (11%)	
Idiopathic	26 (47%)	29 (48%)	
Other	14 (25%)	12 (25%)	
Pancreatic duct stent insertion or EST: *n* (%)	39 (71%)	43 (70%)	0.960
Past history of pancreas operation: *n* (%)	11 (20%)	7 (11%)	0.210
Duration of symptoms (years): median (*IQR*)	5.0 (*4.0*, *10.0*)	5.0 (*3.0*, *10.0*)	0.380
Dose (×10^3 IEQ/kg patient body weight): median (*IQR*)	5.43 (*3.76*, *6.95*)	4.63 (*2.74*, *7.34*)	0.440

EST, endoscopic sphincterotomy; IEQ, islet equivalent. IQR values are in parentheses and italicized.

pancreatic duct stent inserted and 16% had a previous pancreas operation with neither resolving pain maintenance. The survey showed an average percent reduction in morphine dose of approximately 37%, 35%, and 30% at 1, 2, and 3 years, respectively, with mean daily MME decreasing from 118 (±*137*) mg at baseline to 35 (±*65*) mg at 3 years. Our results are consistent with the international consensus guideline, where opioid doses were reduced by 71%, 69%, and 67% at 1, 2, and 5 years [36]. Our study supports improved pain management and metabolic functioning even in patients with a history of pancreatic surgery.

In addition to the pain reduction of TPIAT, we observed a significant reduction of other symptoms of CP including nausea, vomiting, weight loss, and digestive disturbances. Our study found no statistical variation in diarrhea symptoms, but there was an overall decreasing trend. This is similar to Crosby et al. report in which more than 60% of patients still reported diarrhea after TPIAT, adding that enzyme non-adherence was not a major contributor [37]. Our surveys showed no improvement in flatulence and altered bowel habits. These symptoms may be related to exocrine insufficiency, intestinal resection with reconstruction in TPIAT, and new intestinal motility after the reduction of narcotic drugs [38, 39]. As the TPIAT procedure involves infusion of pancreatic islets into the portal vein of the liver, it was important for us to document any changes in hepatic symptoms which were not present in our cohort.

A limitation of this study was reduced sample size in follow-up years as patient participation waned. We have observed reports of similar instances in other centers [10, 13, 28, 40, 41]. However, we were able to evaluate the same patient sample over time and have statistically significant data that allows us to highlight new aspects of QoL in TPIAT.

In conclusion, we found that pancreatic disease-specific surveys allow us to gain a deeper understanding of patient QoL post-TPIAT for CP. We observed significant improvements in QoL after TPIAT, and expanded our knowledge in the functional and symptom scales for CP patients up to 3 years post-transplant. Our study strongly supports the benefits of TPIAT as a treatment option for refractory CP.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by Baylor Scott & White Institutional Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MC and BN contributed to study concept and design. MC and GS performed the statistical analysis. MC, GS, and BN drafted the manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

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Development of a Radiomics-Based Model to Predict Graft Fibrosis in Liver Transplant Recipients: A Pilot Study

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Liver Transplantation is complicated by recurrent fibrosis in 40% of recipients. We evaluated the ability of clinical and radiomic features to flag patients at risk of developing future graft fibrosis. CT scans of 254 patients at 3–6 months post-liver transplant were retrospectively analyzed. Volumetric radiomic features were extracted from the portal phase using an Artificial Intelligence-based tool (PyRadiomics). The primary endpoint was clinically significant (≥F2) graft fibrosis. A 10-fold cross-validated LASSO model using clinical and radiomic features was developed. In total, 75 patients (29.5%) developed ≥F2 fibrosis by a median of 19 (4.3–121.8) months. The maximum liver attenuation at the venous phase (a radiomic feature reflecting venous perfusion), primary etiology, donor/recipient age, recurrence of disease, brain-dead donor, tacrolimus use at 3 months, and APRI score at 3 months were predictive of ≥F2 fibrosis. The combination of radiomics and the clinical features increased the AUC to 0.811 from 0.793 for the clinical-only model ($p = 0.008$) and from 0.664 for the radiomics-only model ($p < 0.001$) to predict future ≥F2 fibrosis. This pilot study exploring the role of radiomics demonstrates that the addition of radiomic features in a clinical model increased the model's performance. Further studies are required to investigate the generalizability of this experimental tool.

Keywords: CT scan, imaging biomarkers, machine learning, artificial intelligence, prognostic model

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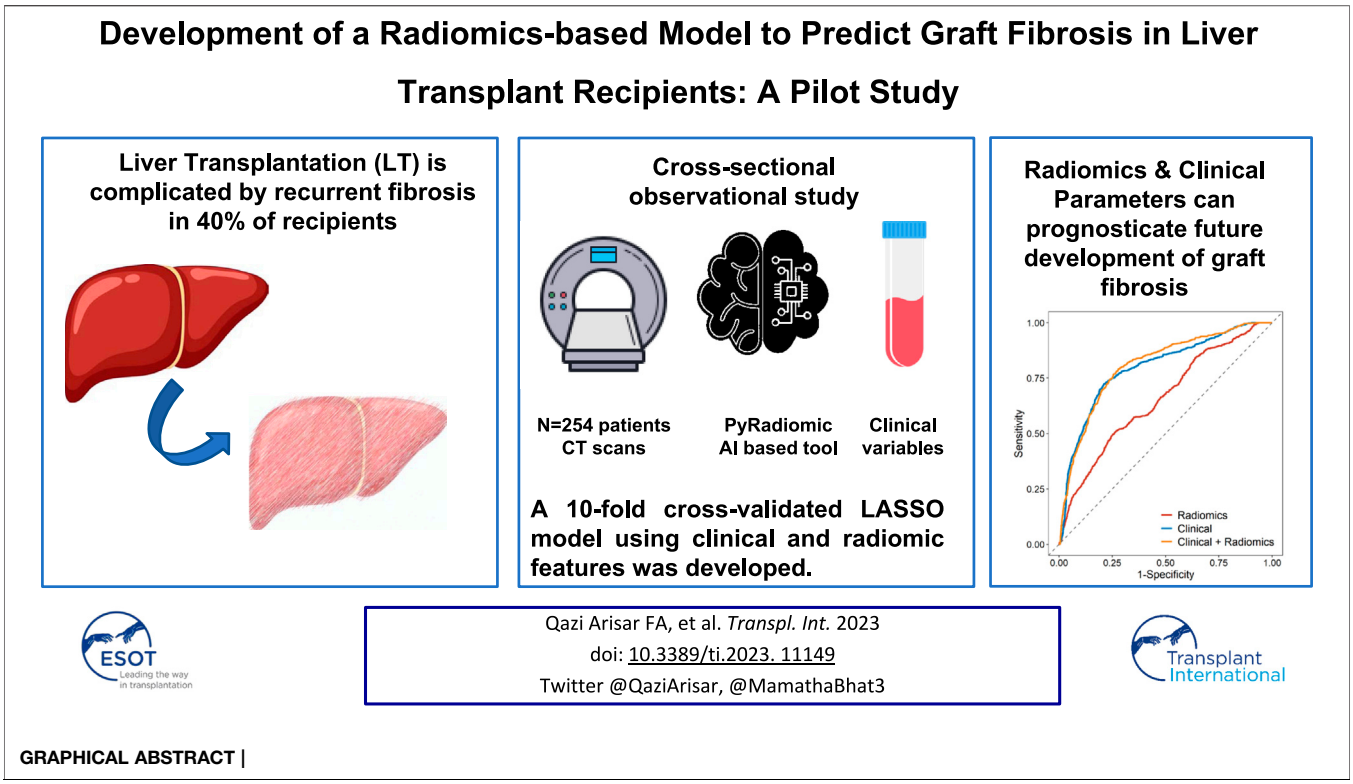
INTRODUCTION

Short-term survival rates after liver transplant (LT) have continued to improve over time, with advances in immunosuppression and post-transplant care [1]. However, this has not been matched by gains in long-term survival rates [1–3]. Recurrent fibrosis following LT continues to be a significant factor impacting long-term graft and patient survival. Advanced graft fibrosis occurs in approximately 37%–43% of LT recipients [4, 5]. Development of Stage 2 graft fibrosis within the first-year post-transplant is associated with reduced graft and patient survival [6, 7].

Graft fibrosis may occur due to repeated episodes of rejection, recurrence of primary disease, or recurrent and *de novo* non-alcoholic steatohepatitis (NASH) [8]. Liver enzymes give unreliable



GRAPHICAL ABSTRACT |

information to assess progressive graft fibrosis over time when preventive interventions are possible. Furthermore, repeated liver biopsies for screening and monitoring in LT patients are not practically feasible given the potential risks associated with an invasive procedure and expense [9, 10]. Longitudinal serum biomarkers and transient elastography are helpful in identifying patients who have developed advanced liver fibrosis [4, 5, 11]. However, more robust non-invasive tools are required to identify those at the highest risk of developing advanced graft fibrosis in the long term.

Radiomics is a method of converting medical images into high-dimensional, mineable quantitative data, followed by subsequent data analysis for decision support [12]. Radiomics has been used successfully to assess liver fibrosis on CT images in chronic liver disease [13, 14], while for LT patients it has been mainly focused on predicting early recurrence of hepatocellular carcinoma (HCC) post-transplant using pretransplant CT images [15, 16]. To our knowledge, there have been no studies to date that explore the utility of radiomic features on post-transplant images in predicting graft fibrosis in solid organ transplant recipients.

In this study, we aimed to develop and validate a radiomics-based model to predict the onset of >F2 graft fibrosis in the long term post-LT. **Figure 1** represents the schematic presentation of our aim. We opted for F2 or more fibrosis as it is categorized as clinically significant fibrosis [17]. It is important to identify patients at risk of clinically significant fibrosis in the long term. Earlier identification of such higher-risk patients will enable the implementation of preventive measures that could save the graft. We hypothesized that radiomic features such as subtle perfusion, and biliary and parenchymal changes early post-LT could provide insight into the long-term life span of the graft, beyond the longitudinal clinical and laboratory information available.

MATERIALS AND METHODS

Patient Population

This retrospective multi-center study was done at University Health Network and Mount Sinai Hospital, Toronto, and included all adult patients who underwent LT between January 2009 and December 2018 and had post-transplant contrast-enhanced computed tomography (CT) scan available, including a venous phase with/without an arterial phase, at 3–6 months after LT. This period for CT scans was selected in order to give time for the post-surgical changes to reverse, which takes a few weeks [18]. Missing clinical characteristics data were multiply imputed ten times using five iterations of multiple imputation by chained equations. The model coefficients and performance measures were pooled using Rubin's rules. The study flowchart is depicted in **Figure 2**.

We collected data on demographics (date and type of LT; recipient and donor age; recipient sex, height, weight, and body mass index (BMI); primary indication for LT; comorbidities such as diabetes, hypertension, dyslipidemia, cardiovascular disease, dialysis status, smoking, and alcohol consumption; recurrence of primary etiology (any time post LT); recurrence of hepatocellular carcinoma (HCC) or cholangiocarcinoma; development of fibrosis; re-transplantation; and death post-LT), laboratory

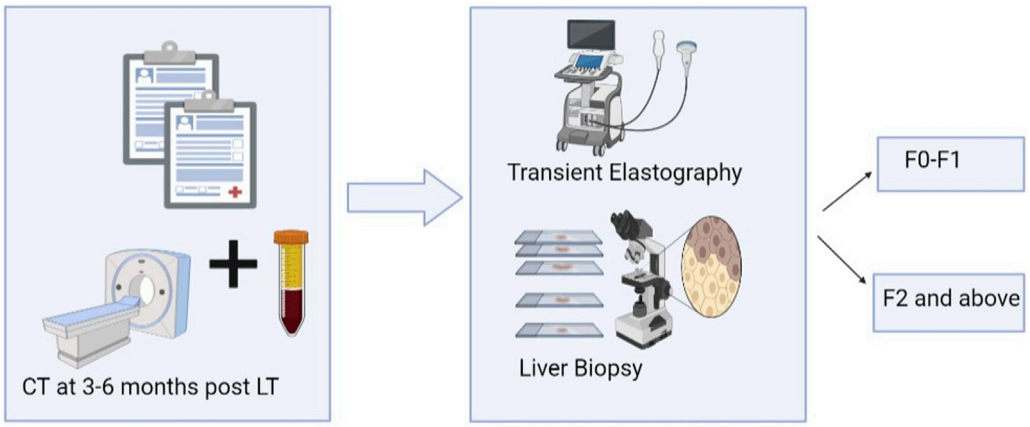


FIGURE 1 | Schematic presentation of aim and methods.

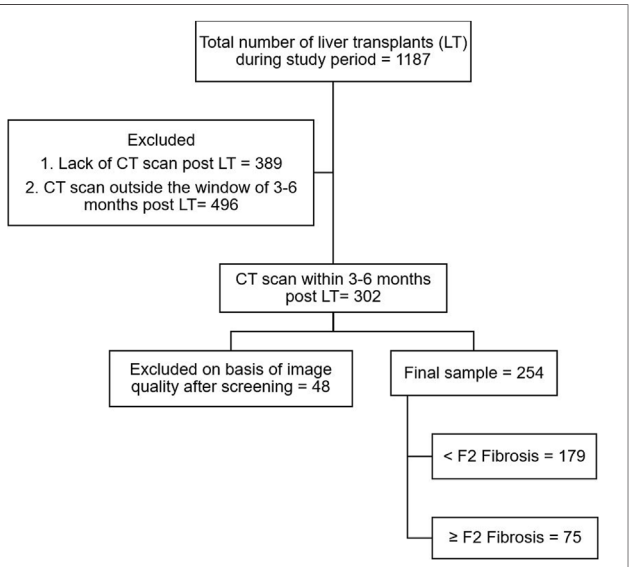


FIGURE 2 | Study flowchart.

tests at various intervals post-transplant (platelets, total bilirubin, AST, ALT, ALP, INR, sodium, creatinine, eGFR, APRI, Fib-4), and the immunosuppression regimen.

The study's primary endpoint was Fibrosis stage F2 or greater (≥F2) quantified by either transient elastography (TE) or liver biopsy. Liver biopsy was indicated either as a prerequisite of hepatitis C treatment in the interferon era or on a need basis such as for elevated liver enzymes. Since the availability of TE (2018), all patients at our center underwent routine TE annually. TE was not available for many patients due to the wide range of the study period; hence we used both TE and liver biopsy whichever was available, given their comparable performance in staging liver fibrosis, even in post-liver transplant patients [5, 19, 20]. The protocol was approved by our institutional Research Ethics Board (REB # CAPCR ID: 19-6159).

Liver biopsy samples were considered adequate if they were at least 15 mm long and carried at least 6 complete portal tracts, and were read by an expert liver pathologist [21]. Fibrosis stages in biopsy samples were scaled based on the METAVIR score, from F0 to F4 (F0: No fibrosis–F1: Portal fibrosis without septa–F2-Portal fibrosis with few septa–F3: fibrosis with numerous bridging septa–and F4: cirrhosis) [22].

Transient elastography was done using the Fibroscan device (Echosense, Paris) with standard M or XL (for obese patients, as guided by the device) probes. Liver stiffness measurement (LSM) expressed in kilopascals (kPa) identified graft fibrosis severity. LSM ≥7.4 was considered significant graft fibrosis (F2 and above) based on the results of a recent prospective study that showed a sensitivity of 0.9 for this cutoff in LT recipients with different underlying pathologies. Only examinations with at least 10 measurements and a successful rate >60%, with an interquartile range <30% of the median value were considered reliable for the study [23].

CT Feature Extraction

One radiologist (ES) manually contoured a 30 mm diameter spherical volume of interest (VOI) in the posterior aspect of the right liver lobe (segment V or VI) in the arterial and portal phase of each patient. The portal branches and hepatic veins were excluded from segmentation. A radiologist with more than 20 years of experience in abdominal radiology (MH) confirmed the contours. 3D Slicer v4.11.2 [1], an open segmentation software was used. Feature extraction was performed with PyRadiomics version 3.0, an image biomarker standardization initiative compliant analytic library [24]. CT

[1]https://www.slicer.org/

images with the region of interest in the right liver lobe are depicted as a **Supplementary Figure S1**. Typical CT parameters and hyperparameters used for analysis are listed in **Supplementary Tables S1–S3**. In total, 116 non-filtered features were extracted.

Statistical Analysis

Baseline variables were compared between cohorts using the Mann-Whitney U test and Fisher's Exact test for continuous and categorical variables, respectively. The association of the clinical variables and the radiomic features with ≥F2 was assessed by using univariable and multivariable generalized logistic regression models. Clinical features with a skewed distribution were log transformed.

Three models, radiomics only, clinical only, and radiomics + clinical, were developed to predict ≥ F2 on the liver graft. Radiomic features were standardized using Z-transformation and features with zero variance were removed. Following this, radiomic features that were significant ($p < 0.05$) in the fitted univariable logistic regression models were retained. These features were introduced in the Least Absolute Shrinkage and Selection Operator (LASSO) to generate the final radiomic model and were validated using 10-fold cross-validation. The clinical-only model was developed using a similar methodology. All the clinical features that were statistically significant ($p < 0.05$) in the univariable model were retained and then incorporated into a 10-fold cross-validated LASSO model to generate a final list of clinical features. The clinical and radiomics model included all features from the clinical-only and radiomics-only models. All models were internally validated using 10-fold cross-validation repeated 10 times. At the end, model performance was tested on patients with liver biopsy-determined fibrosis by excluding patients with fibroscan-determined fibrosis.

The mean area (AUC) under the receiver operator characteristic curve (ROC) was used to assess the discrimination of the radiomics and the clinical models. 95% confidence intervals (CI) were calculated based on 1,000 bootstrap replicates. Model calibration was visually assessed using calibration curves and quantified using average absolute calibration error. The mean ROC curve was plotted for each model. DeLong's test was used to formally compare differences in AUCs across models. Time to ≥F2 fibrosis was estimated using cumulative incidence functions; death without fibrosis was considered a competing risk. Patients who did not die or develop fibrosis were censored at the date of the last follow-up. Cumulative incidence function curves were stratified by radiomic features and differences in curves were evaluated using Gray's test.

To assess confounding between each selected clinical characteristic and the selected radiomics features when predicting ≥F2 fibrosis, separate multivariable logistic regression models incorporating each feature and the selected radiomics features were fit. A difference of 10% between the univariable and adjusted odds ratio was considered to be indicative of confounding.

All statistical tests were two-tailed, and $p < 0.05$ was considered statistically significant. Statistics were performed using R v4.0.0 (R project for statistical computing) [25]. Methods and results were reported according to the Transparent Reporting of Multivariable Prediction Model for Individual Prognosis or Diagnosis (TRIPOD) statement [26].

RESULTS

Out of 1,188 patients who underwent liver transplants during the study period, a total of 254 patients met the inclusion criteria, specifically due to the need for CT scans at 3–6 months post-LT. Patients were mostly male (76%), with a mean age of 56.3 ± 10.2 years at transplant. The most common etiology of the underlying liver disease was viral (54%). Of those included, 204 (80.3%) patients had HCC and/or cholangiocarcinoma before transplant and 75% of patients underwent deceased donor liver transplants. The median duration of follow-up was 6.7 (1.1–12.4) years. **Table 1** summarizes the demographic and laboratory variables.

In total, 75 (29.5%) patients developed ≥F2 fibrosis. The median time from transplant to ≥F2 fibrosis was 19 (4.3–121.8) months, while the time from CT scan was 14.1 (0–116) months. Recurrence of primary etiology was noted in 93 (37%) patients, while 41 (16%) had a recurrence of HCC/cholangiocarcinoma in the long term. Patients who developed ≥ F2 fibrosis in the long term had more deceased cardiac donor (DCD) LTs (17% vs. 7%, $p = 0.0079$), younger age at transplant (54 ± 9.8 vs. 57.2 ± 10.2, $p < 0.001$), higher rate of primary disease recurrence (67% vs. 24%, $p < 0.001$), elevated liver enzymes at 3 months post-LT, and less frequent use of tacrolimus at 3 months post-LT (49% vs. 82% $p < 0.001$) as described in **Table 1**.

The LASSO algorithm selected two radiomic features, original first-order maximum and original first-order root mean squared. The two were highly correlated with a Spearman correlation coefficient of 0.86, and therefore only the first-order maximum (maximum liver attenuation) was selected for the radiomics model (OR: 0.52 [95% CI: 0.38–0.71], $p < 0.001$). The results from the univariable logistic regression models for all radiomic features are presented in **Supplementary Table S4**.

Association of Radiomics-Score and Clinical Variables With Graft-Fibrosis

In the multivariable generalized regression analysis, primary etiology of alcohol (OR 5.49, 95% CI 1.60–18.80, $p = 0.007$), donor age (OR 1.04, 95% CI 1.01–1.07, $p = 0.002$), recipient age at transplant (OR 0.95, 95% CI 0.91–0.98, $p = 0.004$), recurrence of primary etiology (OR 6.31, 95% CI 2.46–16.16, $p < 0.001$), brain-dead donor (OR 0.16, 95% CI 0.05–0.48, $p = 0.001$), tacrolimus use at 3 months post-LT (OR 0.27, 95% CI 0.11–0.65, $p = 0.004$), and APRI score at 3 months post-LT (OR 1.93, 95% CI 1.26–2.95, $p = 0.003$) were the clinical variables significantly associated with ≥F2 fibrosis (**Table 2**). The discriminatory performance of the clinical model

TABLE 1 | Demographic and clinicopathological characteristics.

Variable		Full sample (*n* = 254)	<F2 fibrosis (*n* = 179)	≥F2 fibrosis (*n* = 75)	*p*-value*
Primary diagnosis	n (%)				0.74
Viral		136 (54)	91 (51)	45 (60)	
Alcohol		38 (15)	29 (16)	9 (12)	
Autoimmune liver diseases		31 (12)	22 (12)	9 (12)	
NASH		22 (9)	16 (9)	6 (8)	
Other		27 (11)	21 (12)	6 (8)	
Liver malignancy pre-LT	n (%)				0.59
Cholangiocarcinoma		3 (1)	3 (1.7)	0 (0)	
HCC		196 (77)	140 (78.2)	56 (74.7)	
HCC + Cholangiocarcinoma		4 (1.5)	4 (2.2)	0 (0)	
HCC + Gall bladder carcinoma		1 (0.4)	1 (0.6)	0 (0)	
None		50 (19.6)	31 (17.3)	19 (25.3)	
Transplant Type	n (%)				**0.0079**
Deceased cardiac donor		26 (10)	13 (7)	13 (17)	
Living donor		62 (25)	39 (22)	23 (31)	
Deceased brain-dead donor		164 (65)	125 (71)	39 (52)	
Age at transplant (years)	Mean (SD)	56.3 (10.2)	57.2 (10.2)	54.0 (9.8)	<**0.001**
Sex	n (%)				0.87
Female		59 (23)	41 (23)	18 (24)	
Male		195 (77)	138 (77)	57 (76)	
BMI (Kg/m^2)	Mean (SD)	27.1 (5.1)	27.2 (5.0)	27.0 (5.3)	0.66
BMI Category	n (%)				0.43
<30		186 (74)	133 (76)	53 (71)	
≥30		65 (26)	43 (24)	22 (29)	
Missing		3	3	0	
Donor Age (Years)	Mean (SD)	44.5 (16.5)	44.0 (17.1)	45.6 (15.2)	0.40
	Missing	2	2	0	
Diabetes Pre LT	n (%)	80 (31)	58 (32)	22 (29)	0.66
Hypertension pre-LT	n (%)	85 (33)	63 (35)	22 (29)	0.39
Dyslipidemia pre-LT	n (%)	33 (13)	26 (15)	7 (9)	0.51
Cardiovascular disease pre-LT	n (%)	20 (8)	15 (8)	5 (7)	0.80
Smoking pre-LT	n (%)	136 (54)	98 (55)	38 (51)	0.58
Dialysis pre-LT	n (%)	2 (1)	0 (0)	2 (3)	0.086
Diabetes post-LT	n (%)	125 (49)	93 (52)	32 (43)	0.16
Hypertension post-LT	n (%)	155 (61)	104 (58)	51 (68)	0.16
Dyslipidemia post-LT	n (%)	69 (27)	49 (28)	20 (27)	1
Cardiovascular disease post-LT	n (%)	33 (13)	21 (12)	12 (16)	0.41
Dialysis post-LT	n (%)	29 (11)	19 (11)	10 (13)	0.52
Smoking post-LT	n (%)	20 (8)	17 (9)	3 (4)	0.2
Alcohol consumption post-LT	n (%)	9 (4)	6 (3)	3 (4)	0.73
HCC/Cholangiocarcinoma Recurrence	n (%)	41 (16)	28 (16)	13 (17)	0.71
Recurrence of the Primary diagnosis	n (%)	93 (37)	43 (24)	50 (67)	<**0.001**
Platelet at Transplant (x10^9/L)	Median (Min, Max)	164 (29, 782)	169 (38, 782)	158 (29, 584)	0.43
Platelets at 3 months (x10^9/L)	Median (Min, Max)	157 (15, 532)	162 (39, 532)	148.5 (15, 446)	0.044
AST at Transplant	Median (Min, Max)	1040.5 (96.0, 10300.0)	1,006 (96, 8,209)	1,155 (144, 10,300)	0.48
AST at 3 months (IU/L)	Median (Min, Max)	28 (9, 358)	26 (9, 358)	42 (14, 268)	<**0.001**
	Missing	1	1	0	
ALT at Transplant (IU/L)	Median (Min, Max)	747.5 (55.0, 7509.0)	721 (55, 7,509)	770 (128, 5,229)	0.71
ALT at 3 months (IU/L)	Median (Min, Max)	35 (3, 522)	30 (3, 522)	53 (7, 493)	<**0.001**
	Missing	2	2	0	
ALP at Transplant (IU/L)	Median (Min, Max)	103.5 (37.0, 1791.0)	103 (37, 1,791)	105 (44, 1,279)	0.91
ALP 3 months (IU/L)	Median (Min, Max)	118 (39, 2,197)	108 (39, 565)	131 (49, 2,197)	**0.0041**
	Missing	2	2	0	
Total Bilirubin at Transplant (µmol/L)	Median (Min, Max)	60 (6, 613)	58 (6, 613)	65.5 (7.0, 512.0)	0.11
Total Bilirubin 3 months (µmol/L)	Median (Min, Max)	10 (3, 169)	9 (3, 169)	13 (3, 73)	<**0.001**
	Missing	2	1	1	
INR at LT	Median (Min, Max)	1.8 (0.8, 5.2)	1.8 (0.8, 4.0)	1.8 (1.0, 5.2)	0.77
INR 3 months	Median (Min, Max)	1.0 (0.9, 3.0)	1.0 (0.9, 3.0)	1.0 (0.9, 1.9)	0.87
	Missing	5	5	0	
Serum Creatinine at Transplant (µmol/L)	Median (Min, Max)	84 (43, 359)	84 (48, 307)	84 (43, 359)	0.61
Serum Creatinine 3M (µmol/L)	Median (Min, Max)	91 (28, 541)	91.5 (28.0, 159.0)	87 (44, 541)	0.32
	Missing	1	1	0	
Serum Sodium at Transplant (mmol/L)	Mean (SD)	140.3 (4.5)	139.9 (4.3)	141.1 (4.9)	**0.026**
Serum Sodium 3 months (mmol/L)	Mean (SD)	139.9 (3.1)	139.9 (3.3)	139.9 (2.7)	0.69
	Missing	1	1	0	

(Continued on following page)

TABLE 1 | (*Continued*) Demographic and clinicopathological characteristics.

Variable		Full sample (*n* = 254)	<F2 fibrosis (*n* = 179)	≥F2 fibrosis (*n* = 75)	*p*-value*
Immunosuppressant 3 months	n (%)				**<0.001**
Cyclosporine		63 (25)	27 (15)	36 (48)	
Sirolimus		7 (3)	5 (3)	2 (3)	
Tacrolimus		184 (72)	147 (82)	37 (49)	
APRI at 3 months	Median (Min, Max)	0.5 (0.1, 25.0)	0.5 (0.1, 8.7)	0.8 (0.1, 25.0)	**<0.001**
	Missing	7	6	1	
Fib-4 at 3 months	Median (Min, Max)	1.7 (0.2, 37.7)	1.5 (0.2, 11.9)	2.2 (0.2, 37.7)	**<0.001**
	Missing	8	7	1	
Duration of Follow-up (Years)	Median (Min, Max)	6.7 (1.1, 12.4)	6.6 (1.1, 12.4)	7.4 (1.1, 12.1)	0.79

*Notes: * Mann-Whitney U test for continuous covariates, and Fisher's Exact test for categorical covariates.*
Abbreviations: ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; HCC, hepatocellular carcinoma; INR, international normalized ratio; LT, liver transplant; NASH, non-alcoholic steatohepatitis; SD, standard deviation.
Bold values represents that the p *value < 0.05.*

TABLE 2 | Multivariate regression analysis of clinical and radiomics variables.

Statistic/Predictor		Clinical only	Radiomics only	Clinical + radiomics
Mean AUC (95% CI)		0.793 (0.657, 0.917)	0.664 (0.539, 0.775)	0.811 (0.670, 0.921)
Mean Absolute Calibration Error (95% CI)		0.290 (0.225, 0.343)	0.393 (0.320, 0.464)	0.284 (0.221, 0.344)
Venous Original First-Order Maximum			0.52 (0.38, 0.71) $p < 0.001$	0.61 (0.41, 0.92) $p = 0.019$
Primary Diagnosis (ref = Viral)	Autoimmune hepatitis	1.88 (0.52, 6.76) $p = 0.334$		2.15 (0.58, 8.02) $p = 0.255$
	Alcohol	5.49 (1.60, 18.8) $p = 0.007$		4.57 (1.32, 15.90) $p = 0.018$
	NASH	3.12 (0.78, 12.50) $p = 0.109$		2.54 (0.63, 10.20) $p = 0.191$
	Other	2.48 (0.57, 10.83) $p = 0.228$		2.92 (0.65, 13.01) $p = 0.162$
Age at Transplant		0.95 (0.91, 0.98) $p = 0.004$		0.95 (0.92, 0.99) $p = 0.011$
BMI (ref <30)	≥30	1.87 (0.83, 4.22) $p = 0.134$		1.67 (0.73, 3.83) $p = 0.228$
Donor Age		1.04 (1.01, 1.07) $p = 0.002$		1.04 (1.01, 1.06) $p = 0.006$
Post-LT Diabetes (ref = No)	Yes	0.59 (0.29, 1.22) $p = 0.158$		0.60 (0.29, 1.25) $p = 0.172$
Recurrence of Primary Diagnosis (ref = No)	Yes	6.31 (2.46, 16.16) $p < 0.001$		5.01 (1.92, 13.08) $p = 0.001$
Transplant Type (ref = Deceased cardiac donor)	Living donor	0.47 (0.14, 1.55) $p = 0.214$		0.40 (0.12, 1.34) $p = 0.138$
	Deceased brain-dead donor	0.16 (0.05, 0.48) $p = 0.001$		0.15 (0.05, 0.46) $p = 0.001$
Immunosuppressant (ref = Cyclosporine)	Sirolimus	2.05 (0.20, 20.71) $p = 0.545$		1.99 (0.20, 19.62) $p = 0.555$
	Tacrolimus	0.27 (0.11, 0.65) $p = 0.004$		0.27 (0.11, 0.66) $p = 0.005$
Log APRI 3M		1.93 (1.26, 2.95) $p = 0.003$		2.02 (1.31, 3.13) $p = 0.002$

p-*Values comparing AUC performance.*
DeLong's test was used to compare the AUC, for the following models:
1. Radiomics vs. clinical: p < *0.001.*
2. Radiomics vs. clinical + radiomics: p < *0.001.*
3. Clinical vs. clinical + radiomics: p = *0.006.*

for ≥F2 fibrosis prediction was 0.793 (95% CI 0.657–0.917) with a mean absolute calibration error of 0.290 (95% CI 0.225–0.343). The performance of our clinical model was better than the APRI score (at 3 months post-LT) alone to predict ≥F2 fibrosis (AUC 0.705; 95% CI 0.632–0.777, $p < 0.001$).

Among the radiomic features, portal venous phase maximum liver attenuation remains significantly associated with the outcome on multivariate analysis (OR 0.52, 95% CI 0.38–0.71, $p < 0.001$). Using the median value (−0.012) as the cutoff, venous perfusion maximum liver attenuation was significantly associated with a cumulative incidence of ≥F2 fibrosis ($p = 0.015$) as shown in **Figure 3A**. The combination radiomics and the clinical model increased the AUC to 0.811 (95% CI 0.670–0.921) from 0.793 (95% CI 0.657–0.917) for the clinical-only model ($p = 0.008$) and from 0.664 (95% CI 0.539–0.775) for the radiomics-only model ($p < 0.001$). The mean ROC curves for each model are presented in **Figure 3B**. **Supplementary Figure S2** shows the calibration plots.

Cofounding factor analysis showed a possibility of a small amount of cofounding of radiomics with the primary diagnosis, BMI, recurrence of primary disease, immunosuppression, and type of LT, while no interaction was found with recipient age, donor age, post-LT diabetes, and APRI at 3 months as shown in **Table 3** and **Supplementary Figure S3**.

We performed the analysis with biopsy-determined endpoints. In total, 11 patients who had their fibrosis detected using a Fibroscan were excluded from the analysis. Minor differences in model performance were observed. In the radiomics-only, clinical-only, and radiomics + clinical models, the mean AUCs were 0.633, 0.787, and 0.793 for the biopsy-only group as compared to 0.664, 0.793, and 0.811 for the full group, respectively (**Supplementary Tables S5, S6**).

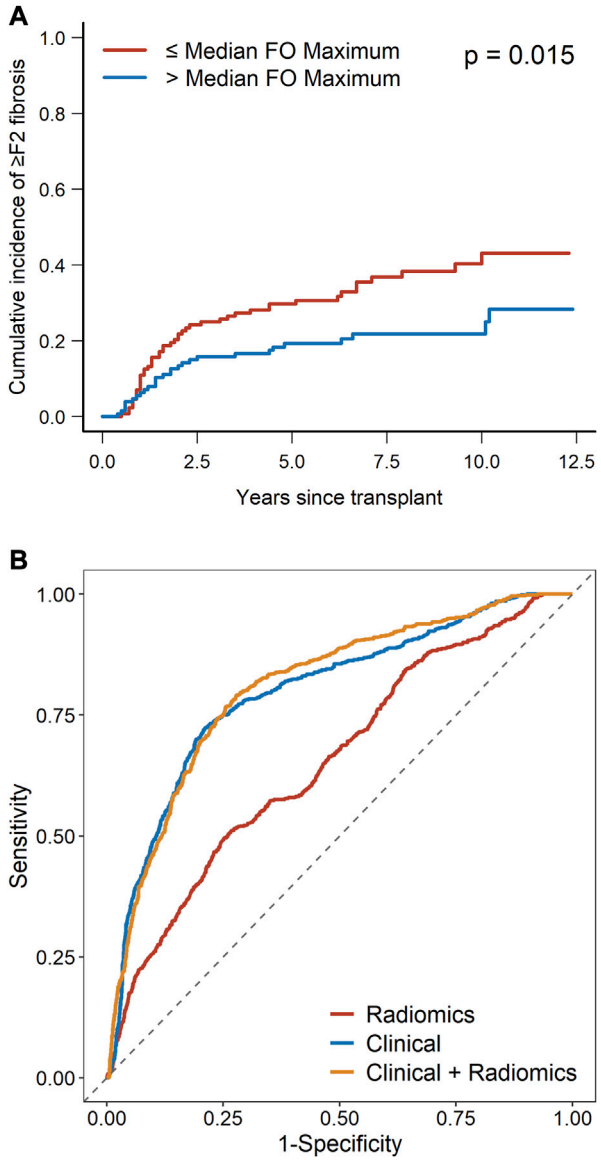


FIGURE 3 | (A) Cumulative incidence of ≥F2 fibrosis as stratified by radiomics feature (median venous perfusion original first-order maximum). **(B)** ROC curves for the radiomics model, clinical model, and radiomics plus clinical model.

DISCUSSION

Radiomics is an emerging but promising imaging-based tool for quantitative analysis of radiological data. Radiomics-based models have been used to detect cirrhosis in the pre-liver transplant setting [14, 27, 28] and have been extensively studied in the cancer setting [29]. In the transplant setting, its application is so far limited to the prediction of recurrent HCC based on pre-transplant images [30]. In a first-of-its-kind study, we evaluated the feasibility of applying radiomic imaging biomarkers in post-transplant CT scans combined with laboratory and clinical data to predict the future development of clinically significant graft fibrosis (Stage 2 or greater) after LT. We appreciate that F4 fibrosis is an important endpoint, however, limiting to F4 only would have dropped the sample size to get a meaningful result. Nonetheless, we believe that identifying patients at risk of developing F2 fibrosis will help us implement measures clinically to prevent its onset.

Radiomic CT data were used to develop a model that would serve to predict graft fibrosis in post-LT patients. The addition of radiomic features to the full clinical model further improved the mean AUC significantly. The maximum liver attenuation value on CT in a representative portion of the right lobe of the liver calculated at the portal venous phase was heavily correlated with the onset of graft fibrosis. As CT enhancement is related to perfusion, greater portal perfusion of the graft may be associated with a lower risk of long-term fibrosis. Previous studies have found that hypoxia, which could arise from low perfusion, is linked to the development of fibrosis [31–33], by upregulating HIF-1α and NF-κB expression, which activates hepatic stellate cells (HSCs), induces epithelial-mesenchymal transition, and increases inflammation. HSCs activation leads to abnormal extracellular matrix deposition, promoting the development of fibrosis. This in turn can lead to vascular resistance, further decreasing the blood flow/liver perfusion. Additionally, activated HSCs also cause sinusoidal vasoconstriction, leading to further hypoxia [31–33]. This negative cycle of events, whereby fibrosis leads to hypoxia which exacerbates fibrosis, suggests the importance of assessing venous perfusion early on to prevent or delay the fibrosis post-transplant.

The analysis of radiomics features was limited in scope to predicting fibrosis. In our exploratory analysis consisting of univariable logistic regression models, we observed that many venous and arterial first-order features were associated with the outcome, specifically, higher values of the feature were associated with decreased odds of fibrosis. However, these features were highly correlated with one another, and therefore only one was selected for the final model to prevent multicollinearity. Beyond these first-order features, no other types of features achieved statistical significance in univariable analysis.

We showed a positive correlation of fibrosis with both the donor's and recipient's age, as reported previously in the literature [34, 35]. Increasing donor age was associated with an accelerated rate of fibrosis progression, with a greater fibrosis score both at 4 and 12 months post-transplant [34]. The enhanced fibrotic response observed in older donors could be explained by age-dependent changes in the liver extracellular matrix [35, 36].

Ideally, the model should have included only variables measured closer to the CT scan. However, we anticipated that post-LT diabetes and recurrence of primary disease would have an impact on the incidence of graft fibrosis as supported by the previous literature. Hence these were included in the model. The primary etiology for the transplant and diabetes were among the top 23 ranked features impacting the incidence of graft fibrosis in a recent study based on a deep learning framework [37]. Patients with viral etiology (HBV and HCV) were less likely to develop fibrosis. This could be due to the advent of potent direct-acting antivirals (DAAs) against HBV and HCV in the recent era. This contrasts with the previous literature from the pre-DAA era, which was

TABLE 3 | Univariable and multivariable logistic regression models predicting ≥F2 fibrosis after adjustment for maximum liver attenuation.

Covariate		Unadjusted OR (95% CI)	*p*-value	Adjusted OR (95% CI)	*p*-value
Primary diagnosis (ref = Viral)	AIH	0.83 (0.35, 1.94)	0.66	0.87 (0.36, 2.12)	0.762
	ETOH	0.63 (0.27, 1.44)	0.27	0.51 (0.22, 1.21)	0.129
	NASH	0.76 (0.28, 2.07)	0.59	0.55 (0.19, 1.57)	0.268
	Other	0.58 (0.22, 1.53)	0.27	0.65 (0.23, 1.80)	0.408
	Maximum Liver Attenuation			0.50 (0.37, 0.69)	<0.001
BMI (ref <30)	≥30	1.3 (0.71, 2.39)	0.39	1.00 (0.53, 1.90)	0.999
	Maximum Liver Attenuation			0.52 (0.38, 0.72)	<0.001
Post LT DM (ref = No)	Yes	0.69 (0.4, 1.19)	0.18	0.69 (0.39, 1.22)	0.201
	Maximum Liver Attenuation			0.52 (0.38, 0.71)	<0.001
Recurrent primary disease (ref = No)	Yes	6.23 (3.45, 11.24)	<0.001	5.45 (2.98, 9.98)	<0.001
	Maximum Liver Attenuation			0.58 (0.42, 0.81)	0.0011
Age at Transplant	Age	0.97 (0.95, 1.00)	0.023	0.97 (0.95, 1.00)	0.039
	Maximum Liver Attenuation			0.53 (0.39, 0.72)	<0.001
Donor age	Age	1.01 (0.99, 1.02)	0.45	1.01 (0.99, 1.02)	0.558
	Maximum Liver Attenuation			0.52 (0.38, 0.72)	<0.001
APRI	log APRI 3M	2.12 (1.56, 2.89)	<0.001	2.10 (1.53, 2.87)	<0.001
	Maximum Liver Attenuation			0.51 (0.37, 0.72)	<0.001
Immunosuppressant (ref = Cyclosporine)	Sirolimus	0.50 (0.08, 3.20)	0.47	0.62 (0.09, 4.22)	0.622
	Tacrolimus	0.19 (0.10, 0.34)	<0.001	0.19 (0.10, 0.36)	<0.001
	Maximum Liver Attenuation			0.52 (0.37, 0.73)	<0.001
Transplant type (ref = Deceased cardiac donor)	Living donor	0.59 (0.23, 1.49)	0.27	0.46 (0.17, 1.22)	0.121
	Deceased brain-dead donor	0.31 (0.13, 0.72)	0.007	0.25 (0.10, 0.62)	0.003
	Maximum Liver Attenuation			0.50 (0.37, 0.69)	<0.001

suggestive of a high rate of fibrosis post-LT in HCV patients [38]. As shown in previous literature, alcohol etiology was related to the highest odds of developing clinically significant fibrosis [39]. We also showed that the recurrence of primary disease was significantly associated with ≥F2 fibrosis post-transplant. In patients with viral infection-related diagnoses, their immunocompromised state post-transplant is further worsened by an increased viral load and an accelerated progression of the disease [34]. Primary sclerosing cholangitis is also known to recur in around 20%–25% of patients over a 10 years period after LT. Given the lack of established treatment, it can rapidly progress leading to graft failure and the need for re-transplantation [40].

The type of LT donor also contributed to the likelihood of developing clinically significant fibrosis post-LT. Recipients from a donor of circulatory death (DCD) were at significantly greater risk of developing severe fibrosis post-LT than those from a neurologically determined dead (NDD) donor or a living donor. Though, an earlier study reported an insignificant difference in fibrosis between DCD and NDD groups [41]. However, the improved prognosis in fibrosis for those with living donors has been previously reported, although mostly with an HCV population, and may be explained by the younger age and shorter cold ischemic times of living donor livers [42, 43].

The immunosuppression regimen was also linked to fibrosis occurrence post-LT, with the use of sirolimus linked to a higher risk for the development of ≥F2 fibrosis and the use of tacrolimus associated with a lower risk when compared to cyclosporin. This was in concordance with previous larger UNOS/SRTR data-based studies showing the superiority of tacrolimus over cyclosporin and sirolimus [44].

While many studies have tested the accuracy of APRI and FIB4 tests in predicting fibrosis in patients with liver diseases, few have investigated their accuracy in the post-LT population [4, 5, 11]. APRI and FIB-4 tests successfully detected fibrosis in post-LT patients with AUCs of 0.87 and 0.78, respectively [45]. In another study, APRI and FIB-4 significantly corresponded with F2 fibrosis on liver biopsy in a post-LT setting (p = 0.009 and 0.022, respectively) with sensitivities of 63.4% and 57.7% and specificities of 66.7% and 69.6%, respectively for APRI and Fib-4 [46]. In our cohort, a univariable logistic regression model with APRI at 3 months post-LT obtained an AUC of 0.705 to predict future fibrosis, while a full clinical model, with the removal of correlated variables, returned a mean AUC of 0.803, suggesting the need for a more robust prediction model of fibrosis for post-LT populations.

Clinical Significance

Recurrent fibrosis following liver transplantation negatively impacts long-term graft and patient survival, increasing the need for re-transplantation. Radiomic features early post-transplant can offer additive prognostic value and insight into the development of significant graft fibrosis in the long term. Due to the lack of correlation between liver enzymes and histology, and the rapid progression of fibrosis in post-transplant patients, there is a need for more robust tools to predict and implement appropriate preventive and therapeutic measures. Based on the current model using clinical and radiomic features, clinicians may consider closer monitoring with Fibroscan in those patients who have high-risk radiomic features and clinically predictive features (therefore higher risk of future F2 fibrosis).

Limitations

We acknowledge the limitations of the smaller sample size and lack of external validation cohort; however, this was a first-of-its-kind proof of principle study. We also acknowledge the component of ascertainment bias as the number of HCC patients was higher (80%) than usual (40%) in our cohort. This could be due to the retrospective study design and the selection criterion of CT scan done between 3–6 months which is often done for HCC surveillance and not available for non-HCC patients. However, we believe that this would not have impacted the model's capacity to predict future graft fibrosis as HCC patients were equally distributed in the two groups, and both groups were followed for an equal period. Further, the CT technology changes over the last decade could add some bias. However, limiting the timeframe to more recent dates would reduce the sample size and the follow-up duration. We acknowledge that performing an interobserver variability analysis would have been ideal. However, prior studies have shown that the first-order features found to be significant in this study are amongst the most stable radiomics features with intraclass correlation coefficient (ICC) > 0.9 [47]. Thus, it is reasonable to assume good ICC for this particular radiomics feature. Future work will include further analysis with ICC in particular to assess the usability of second-order features. Moreover, the indications of liver biopsy and other donor factors such as comorbidities, steatosis, liver enzymes, and cold ischemia time were not analyzed, as the major goal of this study was to assess the predictability of radiomic features for graft fibrosis rather than identifying clinical factors affecting graft fibrosis. Furthermore, there was a small amount of confounding for a few clinical variables with radiomic features, hence limiting the increment in AUCs after the addition of radiomics in the clinical model.

CONCLUSION

Clinical parameters early post-transplant can prognosticate the future development of clinically significant graft fibrosis. This pilot study exploring the role of radiomics demonstrates that the addition of radiomic features in a clinical model significantly increased the model's performance. Further studies would be required to investigate the generalizability of this experimental tool.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because the data are not publicly available due to privacy or ethical restrictions. Requests to access the datasets should be directed to the corresponding authors.

ETHICS STATEMENT

The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments. It was reviewed and approved by University Health Network Research Evaluation Board (CAPCR ID: 19-6159). Given the retrospective chart review, written informed consent to participate in this study was not required as per REB policy.

AUTHOR CONTRIBUTIONS

Conception and design of the work: FQ, ES, MH, and MB. Acquisition, analysis, or interpretation of data: FQ, ES, KL, CC, AA, GH, DD, MH, and MB. Initial draft: FQ, ES, and KL. Revision of manuscript: FQ, KL, MH, and MB. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

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

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11149/full#supplementary-material

Supplementary Figure 1 | The left panel shows an axial CT image in the portal venous phase with a spherical (green circle) region of interest (ROI) in the periphery of the liver avoiding all blood vessels. The right panels are coronal **(upper right)** and sagittal **(lower right)** reconstructions showing the placement of ROI. The only radiomic feature of interest was the maximum Hounsfield unit value within the ROI in the portal venous phase. The arterial phase radiomic features were not significant.

Supplementary Figure 2 | Calibration plots for clinical model and clinical + radiomics model. Dashed lines represent the 95% confidence intervals.

Supplementary Figure 3 | Scatterplots **(A–C)** for age at transplant, donor age, APRI score and boxplots **(D–I)** for primary diagnosis, BMI, post LT diabetes, recurrence of primary disease, Transplant type, and immunosuppression type comparing maximum liver attenuation in patients with or without ≥F2 fibrosis.

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Liver Inclusion Appears to Be Protective Against Graft Loss-Due-to Chronic But Not Acute Rejection Following Intestinal Transplantation

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In intestinal transplantation, while other centers have shown that liver-including allografts have significantly more favorable graft survival and graft loss-due-to chronic rejection (CHR) rates, our center has consistently shown that modified multivisceral (MMV) and full multivisceral (MV) allografts have significantly more favorable acute cellular rejection (ACR) and severe ACR rates compared with isolated intestine (I) and liver-intestine (LI) allografts. In the attempt to resolve this apparent discrepancy, we performed stepwise Cox multivariable analyses of the hazard rates of developing graft loss-due-to acute rejection (AR) vs. CHR among 350 consecutive intestinal transplants at our center with long-term follow-up (median: 13.5 years post-transplant). Observed percentages developing graft loss-due-to AR and CHR were 14.3% (50/350) and 6.6% (23/350), respectively. Only one baseline variable was selected into the Cox model indicating a significantly lower hazard rate of developing graft loss-due-to AR: Transplant Type MMV or MV ($p < 0.000001$). Conversely, two baseline variables were selected into the Cox model indicating a significantly lower hazard rate of developing graft loss-due-to CHR: Received Donor Liver (LI or MV) ($p = 0.002$) and Received Induction ($p = 0.007$). In summary, while MMV/MV transplants (who receive extensive native lymphoid tissue removal) offered protection against graft loss-due-to AR, liver-containing grafts appeared to offer protection against graft loss-due-to CHR, supporting the results of other studies.

Keywords: intestinal transplantation, graft loss-due-to acute rejection, graft loss-due-to chronic rejection, prognostic factors, long-term results CHR, chronic rejection

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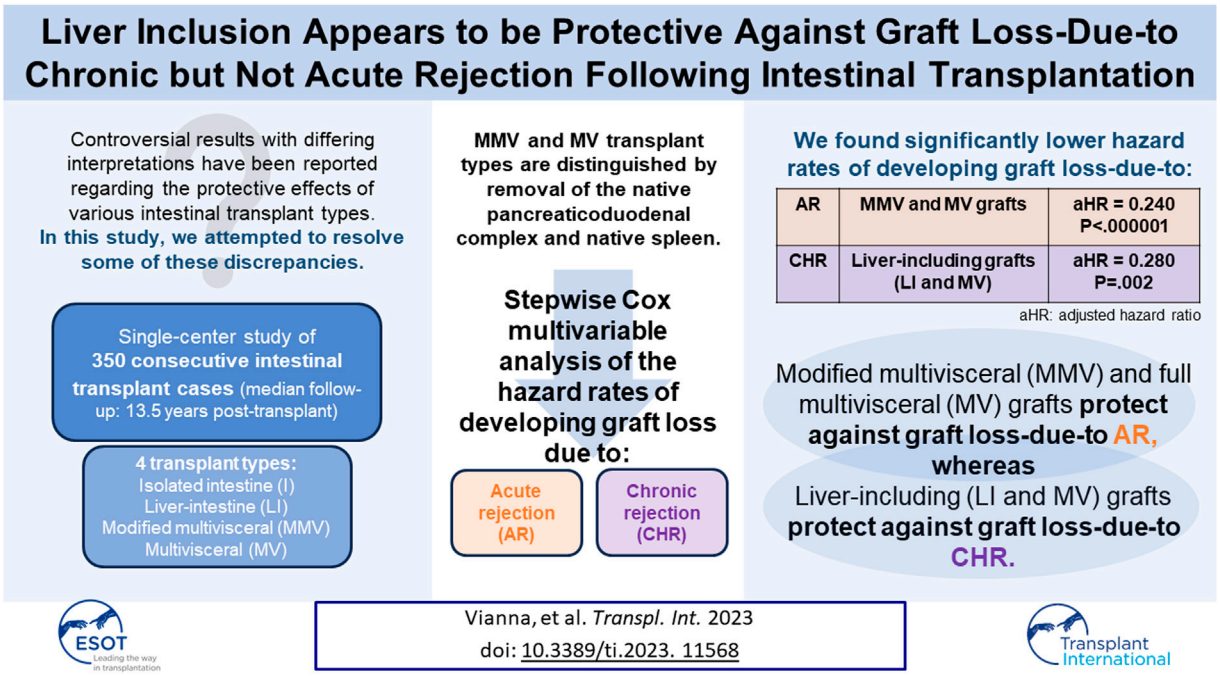
Abbreviations: ACR, acute cellular rejection; AMR, antibody-mediated rejection; AR, acute rejection; CHR, chronic rejection; DSA, donor specific antibody; I, isolated intestine; LI, liver-intestine; MMV, modified multivisceral; MV, multivisceral; mo, months; rATG, rabbit antithymocyte globulin; SE, standard error; TAC, tacrolimus; wk, week.



GRAPHICAL ABSTRACT |

INTRODUCTION

In intestinal transplantation controversial results with differing interpretations on the protective effects of various transplant types have been reported, with liver-including grafts being shown in some studies to have significantly more favorable graft survival [1–4] and lower graft loss-due-to rejection [5–7] rates. However, other studies, have shown that modified multivisceral (MMV) and full multivisceral (MV) transplant recipients have significantly more favorable freedom from acute cellular rejection (ACR) [8], freedom from severe ACR [8–12], and lower graft loss-due-to rejection [8, 12–14] rates in comparison with isolated intestine (I) and liver-intestine (LI) transplant recipients. The latter results suggest that there is a protective effect of MMV and MV which is likely explained by more extensive native lymphoid tissue removal.

In our recent report of 445 consecutive intestinal transplant cases [8], 76.8% (53/69) of the observed graft losses-due-to rejection (during the first 60 months post-transplant) were due to acute rejection (AR), with 23.2% (16/69) being due to chronic rejection (CHR). In contrast, among the 101 observed graft losses-due-to rejection (out of a total of 500 intestinal transplant cases) reported by the University of Pittsburgh [6], only 25.7% (26/101) were due to AR, whereas 74.3% (75/101) were due to CHR. Reported follow-up was much longer in the latter study. In addition, it was clearly reported in Abu-Elmagd et al [6] as well as in an earlier University of Pittsburgh report [15] that the hazard rate of graft loss-due-to CHR was highly significantly lower among recipients of liver-containing (LI and MV) grafts in comparison with liver-free (I and MMV) grafts.

In a separate Abu-Elmagd et al study [16], the hazard rate of developing chronic (but not acute cellular) rejection was significantly higher among patients 1) with preformed donor specific antibodies (DSAs) that persisted over time post-transplant or 2) who developed *de novo* DSAs post-transplant. Patients with liver-containing grafts were significantly less likely to develop either persistent or *de novo* DSAs in that study [16]. In addition, Wu et al [17] showed that the presence of DSAs was associated with a significantly higher risk of the patient developing acute antibody mediated rejection (AMR), and liver-containing allografts offered significant protection against the development of acute AMR.

We recently reported the results of a rather comprehensive multivariable analysis of prognostic factors for the hazard rates of developing a 1st ACR, a severe ACR, and graft loss-due-to rejection (AR or CHR) during the first 60 months post-transplant (among 445 consecutive intestinal transplant cases at our center between 1994–2017); however, separate analyses of predictors of the hazard rates of graft loss-due-to AR vs. CHR had not been performed [8]. In the attempt to resolve some of the previously reported discrepant results between our center and those of other centers, we wanted to analyze multivariable predictors of the hazard rates of graft loss-due-to AR vs. CHR in our cohort with follow-up longer than 60 months post-transplant. We therefore analyzed all consecutive intestinal transplants performed at our institution between 1994 and 2012 (350 cases), with a date of last follow-up of 15 March

2019 (thus, a planned minimum follow-up of over 6 years post-transplant). Results of this observational study are presented here.

MATERIALS AND METHODS

Patients and Immunosuppression

Our historical cohort of 350 consecutive intestinal transplant cases (308 primary recipients and 42 retransplants) at the Miami Transplant Institute during 1994–2012 were followed prospectively through 15 March 2019—the same last follow-up date as in our recent reports [8, 18]. In order to allow for a sufficiently long minimum follow-up of all patients, our more recent group who were transplanted at our center since 2013 were excluded here. Over the years the center institutional review board approved each immunosuppression protocol used for these patients; all patients gave written informed consent before enrollment. In addition, all clinical and research activities adhered to the ethical principles (as revised in 2013) of the Helsinki Declaration.

As in our previous reports [8–12, 18, 19], recipients were divided into four transplant types: isolated intestine (I), liver-intestine (LI), modified multivisceral (MMV), and multivisceral (MV). While the donor pancreas was sometimes transplanted into I and LI recipients, the native pancreaticoduodenal complex was always left intact along with the native spleen (in the great majority of cases). Conversely, MMV and MV transplants were defined by removal of the native pancreaticoduodenal complex and native stomach, along with performing a native splenectomy (in the great majority of cases). In addition, the intent with MMV and MV transplants was to orthotopically transplant *en bloc* the donor stomach, donor pancreaticoduodenal complex, and donor intestine into the recipient [8–12, 18, 19]. Since a near-total removal of the gastrointestinal tract (except for a segment of large intestine), including native splenectomy, is performed in MMV and MV recipients, a much more complete lymphadenectomy is achieved compared with I and LI grafts, where splenic, celiac, and gastric lymph nodes are left *in situ* [8–12, 18, 19].

Recipients were divided into four induction groups [8]. Group 1 (1994–1997) comprised 44 recipients who received no/old induction therapy (high-dose corticosteroids only in 34, OKT3 in 7, and cyclophosphamide in 3). Among primary cases, OKT3 was used first (8/94–1/95), followed by cyclophosphamide (4/95–6/95). Once their use was abandoned, high-dose corticosteroids only were used (7/95–12/97). Group 2 (1998–2011) comprised 159 recipients who received an anti-CD25 monoclonal antibody (daclizumab in 156, and basilixmab in 3). Daclizumab (2 mg/kg) was given on postoperative days 0, 7, and 14, and then every 2 weeks during the first 3 months post-transplant; thereafter, daclizumab dose was reduced to 1 mg/kg every 2 weeks for the following 3 months and then stopped. Basiliximab (10 mg) was given on postoperative days 0 and 4, as the three recipients were small children (<35 kg). Group 3 (2001–2011) comprised 113 recipients who received alemtuzumab, with two different schedules being used: 0.3 mg/kg ×4 (pre-operatively, immediately post-transplant, and on postoperative days 3 and 7); and 30 mg ×2 (on postoperative days 1 and 4). Group 4 (2006–2012) comprised 34 recipients who were scheduled to receive 3 rATG doses (total planned rATG dose: 5 mg/kg, with 2.0 mg/kg being given on postoperative day 0, and 1.5 mg/kg being given on postoperative days 2 and 4). However, the actual number of rATG doses that these patients received was uneven: 12/34 received only the first dose, 3/34 patients received only two doses, and 19/34 patients received all 3 doses.

Of note, daclizumab was the only induction agent that was used during the 3 year period from 1998 to 2000. Thus, prior to 2001, the various induction approaches were tried sequentially. In 2001, alemtuzumab was introduced as a tolerance induction protocol; however, due to its initially poor results in young children, starting in August 2002, its use was limited to patients 4 years of age or older at the time of transplant [11]. Since August, 2002, most of the patients who received daclizumab induction (Group 2) were children, whereas most of the patients who received alemtuzumab induction (Group 3) were adults. In total, the percentage of adults in Groups 2 and 3 was 15.1% (24/159) vs. 74.3% (84/113), respectively. In addition, only 3/159 of Group 2 patients were transplanted since 2009 (3 young children who received basiliximab); thus, most of the children transplanted during 2009–2011 belonged to Group 4.

Maintenance immunosuppression consisted of TAC and corticosteroids (tapered off by 6–9 months post-transplant) except in patients who received alemtuzumab induction (Group 3), where TAC alone was planned to be used. Target TAC trough levels during the first 3 months and beyond 3 months post-transplant were 15–20 ng/mL and 10–15 ng/mL for patients transplanted during 1994–1997, and 12–16 ng/mL and 8–12 ng/mL for patients transplanted during 1998–2012.

Clinical Outcomes

Schedules for monitoring, diagnosis, and treatment of ACR episodes and non-immunosuppressive prophylactic therapy have been described elsewhere [8]. Of note, once an ACR was clinically suspected, an immediate endoscopy and biopsy were performed. All ACR episodes were clinically suspected, pathologically diagnosed [20, 21], and treated; ACR grade (mild, moderate, or severe) was determined as the maximum pathologic grade observed during that episode [8, 12]. High-dose corticosteroids (via intravenous bolus injections) were used to treat mild ACR episodes. Antilymphocyte therapy was used in treating steroid-resistant and moderate-to-severe ACR episodes. Graft dysfunction due to resistant rejection was treated with graft removal and listing for re-transplantation.

Graft loss was defined as the date of intestinal graft failure (graft removal) or death, whichever occurred first, with the underlying cause of (triggering event leading to) graft loss being determined in each case [8, 9, 13]. CHR was determined at the time of graft explant based upon conventional pathological criteria [20, 22]. Thus, in contrast to the determination of ACR episodes (as described above), CHR was only determined at the time of graft explant.

TABLE 1 | Distributions of selected baseline variables (*N* = 350).

Baseline variable	Mean ± SE if continuous; percentage with characteristic if categorical
Date of Transplant	Median = 4/1/03; Interquartile Range: 8/1/00–12/15/06
Recipient Age (years)	16.4 ± 1.0 (*N* = 350) Median = 6.9; Interquartile Range: 0.3–65.6
Recipient Age (years):	
<5	46.9% (164/350)
5–17	13.7% (48/350)
≥18	39.4% (138/350)
Recipient Gender:	
Female	49.7% (174/350)
Male	50.3% (176/350)
Recipient Race/Ethnicity	
White (non-Hispanic)	68.0% (238/350)
Black (non-Hispanic)	16.9% (59/350)
Hispanic	13.4% (47/350)
Asian	1.7% (6/350)
CMV Status	
D-/R-	28.0% (98/350)
D-/R+	19.4% (68/350)
D+/R-	24.9% (87/350)
D+/R+	27.7% (97/350)
Donor Age (yr)	10.2 ± 0.7 (*N* = 329) Median: 5.0; Interquartile Range: 0.8–17.0
Intestinal Transplant Status	
Primary	88.0% (308/350)
Retransplant	12.0% (42/350)
Transplant Type:	
Isolated Intestine (I)	27.4% (96/350)
Liver-Intestine (LI)	10.9% (38/350)
Modified Multivisceral (MMV)	9.7% (34/350)
Multivisceral (MV)	52.0% (182/350)
Underwent Native Splenectomy	
No	38.0% (133/350)
Yes	62.0% (217/350)
Native Pancreaticoduodenal Complex Removed	
No	38.3% (134/350)
Yes	61.7% (216/350)
Received a Kidney:	
No	90.9% (318/350)
Yes	9.1% (32/350)
Received a Large Bowel:	
No	53.1% (186/350)
Yes	46.9% (164/350)
Received a Liver:	
No	37.1% (130/350)
Yes	62.9% (220/350)
Received a Pancreas:	
No	32.3% (113/350)
Yes	67.7% (237/350)
Received a Spleen:	
No	74.9% (262/350)
Yes	25.1% (88/350)
Received a Stomach:	
No	39.1% (137/350)
Yes	60.9% (213/350)
In Hospital (vs. at Home) Prior to Transplant	
No	54.9% (180/328)
Yes	45.1% (148/328)
Induction Type:	
Received No/Old Induction[a]	12.6% (44/350)
Received Anti-CD25	45.4% (159/350)
Received Alemtuzumab	32.3% (113/350)
Received rATG (pre-2013)	9.7% (34/350)

Abbreviations: anti-CD25, anti-Interleukin-2 receptor alpha chain (Daclizumab or Basiliximab); rATG, rabbit anti-thymocyte globulin (Thymoglobulin).

[a]*In this subgroup of 44 recipients, 7/44 recieved induction with OKT3, 3/44 received induction with cyclophosphamide, and 34/44 received only high-dose corticosteroids.*

Statistics

Frequency distributions were determined for baseline categorical variables; the mean along with standard error (SE) (as well as the median and interquartile range) were calculated for baseline continuous variables. Tests of association among baseline variables were performed using Pearson (uncorrected) chi-squared tests and ordinary (two sided) t-tests.

Two distinct clinical outcomes were analyzed in this study: graft loss-due-to AR and graft loss-due-to CHR. Differences in freedom from occurrence of each clinical outcome were compared by the log-rank test, with actuarial estimates and time-to-cause-specific failure curves generated using the Kaplan-Meier method. Patients were censored at the time of graft loss from other causes (or at the time of being lost to follow-up, if it occurred). p-values ≤ 0.05 were considered to be statistically significant.

Stepwise Cox regression was utilized to identify significant multivariable predictors for each of the two primary outcomes: the hazard rate of developing graft loss-due-to AR, and the hazard rate of developing graft loss-due-to CHR. Again, in performing each analysis, any competing events (i.e., graft losses) occurring other than the cause of interest were treated as censored observations. Baseline variables that were considered for their prognostic value included demographics, transplant-related information, and type of induction received (see **Table 1**). For two baseline variables in which a small subset of patients had a missing value, the observed mean was imputed for missing values in the multivariable analyses [23]. Testing the validity of the Cox model proportional hazards assumption was performed by considering the inclusion of time by covariate interaction effects.

RESULTS

Baseline Characteristics

Baseline characteristics are shown in **Table 1**. Median date of transplant was 1 April 2003 (interquartile range: 1 August 2000–15 December 2006). Mean age at transplant was 16.4 years (median age: 6.9 years), with African-Americans and Hispanics comprising 16.9% (59/350) and 13.4% (47/350), respectively; retransplant cases comprised 12.0% (42/350). The percentage of recipients who received isolated intestine (I), liver-intestine (LI), modified multivisceral (MMV), and full multivisceral (MV) allografts was 27.4% (96/350), 10.9% (38/350), 9.7% (34/350), and 52.0% (182/350), respectively. Thus, only 28.4% (38/134) of I/LI grafts vs. 84.3% (182/216) of MMV/MV grafts were liver-containing ($p < 0.000001$).

Crosstabulations of transplant type with the removal of native organs/receiving donor organs are shown in **Table 2**. The native pancreaticoduodenal complex was removed in 0.0% (0/134) of I/LI vs. 100% (216/216) of MMV/MV cases ($p < 0.000001$). Similarly, native splenectomy was performed in only 3.0% (4/134) of I/LI recipients vs. in 98.6% (213/216) of MMV/MV recipients ($p < 0.000001$). Of note, in 2 I cases with a native splenectomy, these two cases were retransplants of previously failed MV grafts (i.e., native splenectomy was performed during the primary MV transplant). Thus, removal of the native pancreaticoduodenal complex and native splenectomy were jointly performed in only 3.0% (4/134) of the I/LI cases vs. 98.6% (213/216) of the MMV/MV cases (nearly a complete one-to-one relationship). Lastly, the donor spleen was transplanted in no I/LI cases vs. 40.7% (88/216) of MMV/MV cases ($p < 0.000001$). Of note, while extremely rare, 1.6% (3/182) of the MV cases did not receive a donor stomach (documented poor quality in one case).

Selected associations among the major baseline characteristics are presented in **Table 3**. The distribution of transplant type by induction type and by transplant date (before vs. after 1/1/01) shows that LI was much more commonly performed prior to 1 January 2001, whereas MV transplants were more commonly performed since that time ($p < 0.000001$). However, the percentage of patients having liver inclusion (LI or MV) has not changed over time ($p = 0.32$), nor has the percentage of transplanted adults changed over time ($p = 0.50$). Lastly, the distribution of recipient age by induction type shows that anti-CD25 and rATG induction were used mostly in children, whereas alemtuzumab was used mostly in adults ($p < 0.000001$).

Graft Loss-Due-to AR vs. CHR

As of the last follow-up date (15 March 2019), the observed incidence of graft loss-due-to any cause was 77.4% (271/350), with the underlying cause of graft loss being due to AR, CHR, infection, and other causes in 14.3% (50/350), 6.6% (23/350), 23.1% (81/350), and 33.4% (117/350) of cases, respectively. Thus, among the transplanted cases who experienced graft loss-due-to rejection, 68.5% (50/73) vs. 31.5% (23/73) were due to AR vs. CHR. The observed percentages of graft loss-due-to AR and graft loss-due-to CHR cases who previously experienced a severe ACR episode were 94.0% (47/50) and 56.5% (13/23), respectively. Median time to graft loss-due-to AR and median time to graft loss-due-to CHR (among the 50 and 23 patients who experienced those events) were 2.3 (range: 0.3–97.8) and 52.9 (range: 3.1–188.3) months post-transplant, respectively. Median follow-up among 79 transplant cases who were still alive with a functioning graft as of last follow-up was 161.1 (range: 79.7–286.5) months post-transplant. Lastly, the total risk set of this 350-patient cohort who were being followed beyond 1, 3, 6, 9, 12, and 15 years post-transplant was 195, 148, 112, 88, 67, and 37, respectively.

Freedom from graft loss-due-to AR curves by transplant type in **Figures 1A, B** show that the hazard rate of graft loss-due-to AR was significantly higher among I and LI transplant cases in comparison with MMV and MV cases ($p < 0.000001$), with essentially identical outcomes for I vs. LI recipients as well as for MMV vs. MV recipients, respectively. Conversely, the freedom from graft loss-due-to CHR curves by transplant type in **Figure 2A** suggest that liver-containing (LI and MV) grafts had a more favorable outcome in comparison with liver-free (I and MMV) grafts ($p = 0.002$). **Figure 2B** shows that freedom from graft loss-due-to CHR was also less favorable for transplant recipients who received no/old induction in comparison with the other three induction groups combined ($p = 0.02$). Lastly, freedom from graft loss-due-to CHR curves by induction type (no/old vs. other) and transplant type (I/MMV vs. LI/MV) in

TABLE 2 | Cross-tabulations of transplant type with removal of native organs (No/Yes) and receiving donor organs (No/Yes).

Organ-specific surgery	Transplant type			
	I	LI	MMV	MV
Native PC Removed	0.0% (0/96)	0.0% (0/38)	100.0% (34/34)	100.0% (182/182)
Native Splenectomy	2.1% (2/96)	5.3% (2/38)	94.1% (32/34)	99.5% (181/182)
Received a Kidney	3.1% (3/96)	2.6% (1/38)	11.8% (4/34)	13.2% (24/182)
Received a Large Bowel	35.4% (34/96)	21.1% (8/38)	52.9% (18/34)	57.1% (104/182)
Received a Liver	0.0% (0/96)	100.0% (38/38)	0.0% (0/34)	100.0% (182/182)
Received a Pancreas	1.0% (1/96)	52.6% (20/38)	100.0% (34/34)	100.0% (182/182)
Received a Spleen	0.0% (0/96)	0.0% (0/38)	41.2% (14/34)	40.7% (74/182)
Received a Stomach	0.0% (0/96)	0.0% (0/38)	100.0% (34/34)	98.4% (179/182)

Abbreviations: I, isolated intestine; LI, liver-intestine; MMV, modified multivisceral; MV, multivisceral; PC, pancreaticoduodenal complex.

TABLE 3 | Selected associations among the major baseline characteristics.

A) Cross-tabulation of transplant type by induction type

Transplant Type	Received type No/Old induction	Received Anti-CD25	Received Alemtuzumab	Received rATG (pre-2013)	*p*-value
I	22.7% (10/44)	20.8% (33/159)	40.7% (46/113)	20.6% (7/34)	
LI	34.1% (15/44)	12.6% (20/159)	2.7% (3/113)	0.0% (0/34)	
MMV	0.0% (0/44)	3.8% (6/159)	22.1% (25/113)	8.8% (3/34)	
MV	43.2% (19/44)	62.9% (100/159)	34.5% (39/113)	70.6% (24/34)	
Total	44	159	113	34	< 0.000001

B) Cross-tabulation of transplant type by date of transplant

Transplant Type	DOT < 1/1/01	DOT ≥ 1/1/01	*p*-value
I	28.9% (28/97)	26.9% (68/253)	
LI	29.9% (29/97)	3.7% (9/253)	
MMV	4.1% (4/97)	11.9% (30/253)	
MV	37.1% (36/97)	57.7% (146/253)	
Total	97	253	<0.000001

C) Cross-tabulation of liver inclusion by date of transplant

Liver Inclusion	DOT <1/1/01	DOT ≥ 1/1/01	*p*-value
No	33.0% (32/97)	38.7% (98/253)	
Yes	67.0% (65/97)	61.3% (155/253)	
Total	97	253	0.32

D) Cross-tabulation of recipient age by date of transplant

Recipient Age (yr)	DOT < 1/1/01	DOT ≥ 1/1/01	*p*-value
<18	57.7% (56/97)	61.7% (156/253)	
≥18	42.3% (41/97)	38.3% (97/253)	
Total	97	253	0.50

E) Cross-tabulation of Recipient Age by Induction Type

Recipient Age (year)	Received No/Old Induction	Received Anti-CD25	Received Alemtuzumab	Received rATG (pre-2013)	*p*-value
<18	59.1% (26/44)	84.9% (135/159)	25.7% (29/113)	64.7% (22/34)	
≥18	40.9% (18/44)	15.1% (24/159)	74.3% (84/113)	35.3% (12/34)	
Total	44	159	113	34	<0.000001

Figure 2C clearly show a significantly more favorable outcome for liver-containing grafts once induction type was controlled (p = 0.01 in the no/old induction stratum; p = 0.04 in the other induction stratum; and p = 0.003 by the stratified log-rank test).

Using stepwise Cox regression, only one baseline variable was selected into the Cox model indicating a significantly lower hazard rate of developing graft loss-due-to AR (**Table 4**): Transplant Type MMV or MV (p < 0.000001). The estimated hazard ratio (HR) and 95% Confidence Interval (CI) for effect of Transplant Type MMV or MV was 0.240 [0.131–0.440]. Once this variable was controlled, none of other baseline variables offered additional prognostic value (p > 0.05). For instance, while

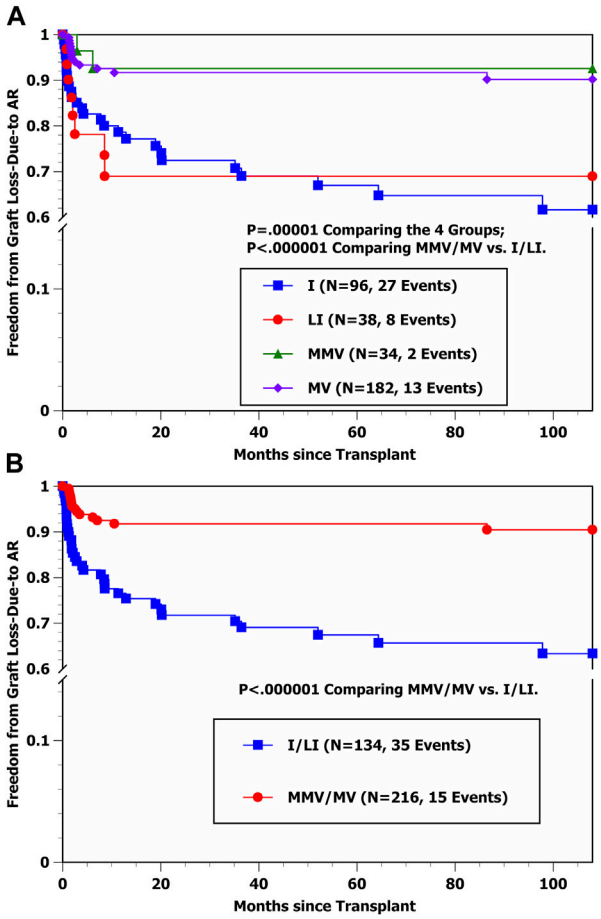


FIGURE 1 | (A) Kaplan-Meier Freedom from graft loss-due-to AR by four transplant types (I, LI, MMV, and MV). **(B)** Kaplan-Meier freedom from graft loss-due-to AR by transplant type (I/LI combined vs. MMV/MV combined).

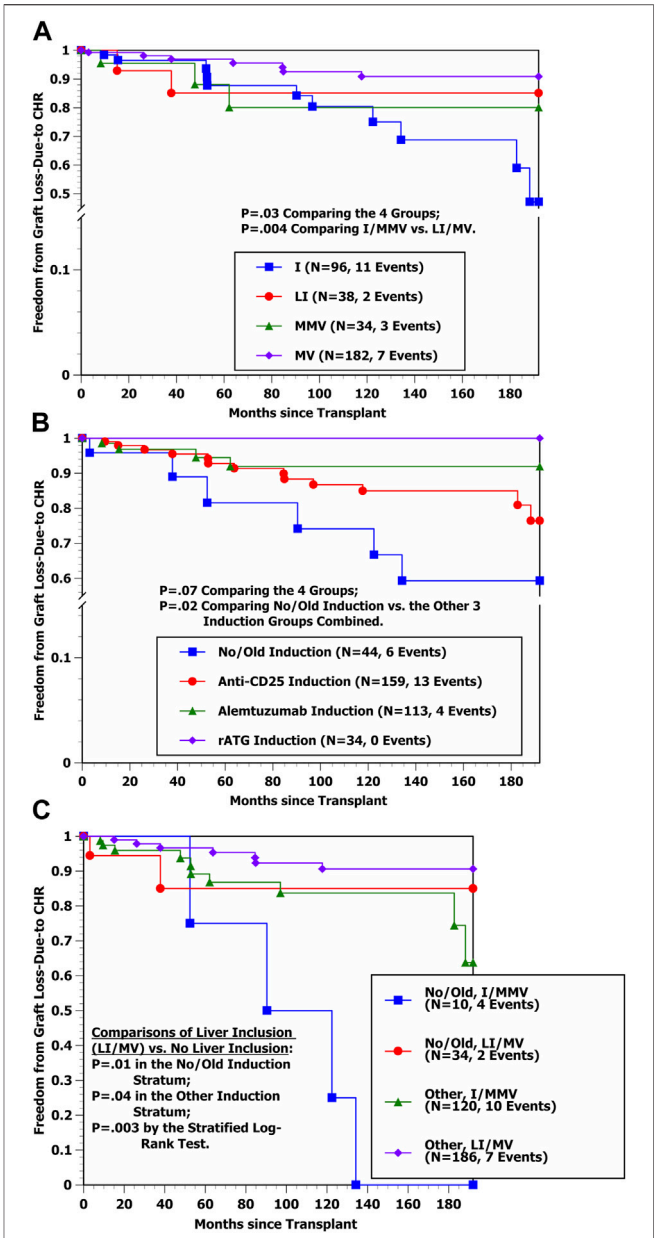


FIGURE 2 | (A) Kaplan-Meier freedom from graft loss-due-to CHR by four transplant types (I, LI, MMV, and MV). **(B)** Kaplan-Meier Freedom from graft loss-due-to CHR by four induction groups (No/Old, anti-CD25, Alemtuzumab, and rATG). **(C)** Kaplan-Meier Freedom from graft loss-due-to CHR by induction group (No/Old vs. Other) and liver inclusion (No vs. Yes) (i.e., I/MMV vs. LI/MV).

Table 4 shows that Received Native Splenectomy, Received Donor Spleen, and Received Donor Liver (LI or MV) were each associated in univariable analysis with a significantly lower hazard rate of developing graft loss-due-to AR (p = 0.000002, 0.0006, and 0.003, respectively), due to their significant positive associations with Receiving Transplant Type MMV or MV, once this latter variable was controlled in the Cox model, multivariable tests to include Received Native Splenectomy, Received Donor Spleen, and Received Donor Liver (LI or MV) were non-significant (p = 0.91, 0.07, and 0.97, respectively).

Using stepwise Cox regression, two baseline variables were selected into the Cox model indicating a significantly lower hazard rate of developing graft loss-due-to CHR (**Table 5**) (shown by order of selection): Received Donor Liver (LI or MV) (p = 0.002) and Received Induction Other than No/Old (p = 0.007). Estimated HRs and 95% CIs for the effects of Received Donor Liver (LI or MV) and Received No/Old Induction were 0.280 [0.119–0.661] and 3.379 [1.316–8.674], respectively. Once these two variables were controlled, none of the other baseline variables offered additional prognostic value ($p > 0.05$). **Table 5** shows that while Transplant Type MMV or MV was associated in univariable analysis with a significantly lower hazard rate of developing graft loss-due-to CHR (p = 0.02), once the two selected variables were controlled, the multivariable test to include this variable yielded p = 0.94. Thus, the stepwise Cox model results in **Tables 4**, **5** match closely with the Kaplan-Meier comparisons shown in **Figures 1A–2C**.

TABLE 4 | Cox model for the hazard rate of developing graft loss-due-to AR (50 events).

Selected Cox model via stepwise regression				
Baseline variable[a]	**Univariable *p*-value**	**Multivariable *p*-value**	**Model Coeff ± SE**	**Estimated HR [95% CI]**
Recipient Age	0.84			
Recipient Age ≥18 years	0.80			
Male Recipient	0.05			
Black (Non-Hispanic) Recipient	0.78			
Hispanic Recipient	0.55			
Intestinal Retransplant	0.70			
CMV Antibody Status: D+/R-	0.84			
Donor Age	0.09			
Transplant Type I	0.00003			
Transplant Type LI	0.06			
Transplant Type MMV	0.15			
Transplant Type MV	0.00007			
Transplant Type MMV or MV	<0.0000001	(√) <0.000001	−1.426 ± 0.309	0.240 [0.131–0.440]
Received Donor Liver (LI or MV)	0.003			
Received Donor Spleen	0.0006			
Received Donor Large Bowel	0.19			
Received Native Splenectomy	0.000002			
In Hospital Pretransplant	0.10			
Received No/Old Induction	0.03			
Received anti-CD25 Induction	0.18			
Received Alemtuzumab Induction	0.60			
Received rATG Induction	0.36			

Abbreviations: AR, acute rejection; Coeff, Coefficient; HR, hazard ratio; CI, confidence interval.

Note: (√) represents selection into the Cox model.

[a]Variables included in the Cox model were defined as follows: Transplant Type MMV or MV = {1 if Transplant Type = MMV or MV, 0 otherwise}. Once Transplant Type MMV or MV was controlled, none of the other baseline variables offered additional prognostic (p > 0.05).

TABLE 5 | Cox model for the hazard rate of developing graft loss-due-to CHR (23 events).

Selected Cox model via stepwise regression				
Baseline variable[a]	**Univariable *p*-value**	**Multivariable *p*-value**	**Model Coeff ± SE**	**Estimated HR [95% CI]**
Recipient Age	0.19			
Recipient Age ≥18 years	0.42			
Male Recipient	0.42			
Black (Non-Hispanic) Recipient	0.49			
Hispanic Recipient	0.40			
Intestinal Retransplant	0.22			
CMV Antibody Status: D+/R-	0.90			
Donor Age	0.09			
Transplant Type I	0.009			
Transplant Type LI	0.99			
Transplant Type MMV	0.45			
Transplant Type MV	0.006			
Transplant Type MMV or MV	0.02			
Received Donor Liver (LI or MV)	0.004	(√) 0.002	−1.272 ± 0.438	0.280 [0.119–0.661]
Received Donor Spleen	0.45			
Received Donor Large Bowel	0.95			
Received Native Splenectomy	0.05			
In Hospital Pretransplant	0.97			
Received No/Old Induction	0.02	(√) 0.007	1.218 ± 0.481	3.379 [1.316–8.674]
Received anti-CD25 Induction	0.85			
Received Alemtuzumab Induction	0.21			
Received rATG Induction	0.27			

Abbreviations: CHR, chronic rejection; Coeff, Coefficient; HR, hazard ratio; CI, confidence interval.

Note: (√) represents selection into the Cox model.

[a]Variables included in the Cox model were defined as follows: Received Donor Liver (LI or MV) = {1 if Transplant Type = LI or MV, 0 otherwise}; and Received No/Old Induction = {1 if Recipient received No/Old Induction, 0 otherwise}. The order of selection for the two baseline variables selected into the Cox model via stepwise regression were as follows: Received Donor Liver (LI or MV), and Received No/Old Induction. Once the two selected variables were controlled, none of the other baseline variables offered additional prognostic (p > 0.05).

DISCUSSION

The results of this observational study with a median follow-up of nearly 13 ½ years post-transplant demonstrate three findings: 1) Transplant Type MMV or MV is the single factor that clearly protects against graft loss-due-to AR, whereas this combination of transplant types did not independently protect against graft loss-due-to CHR, 2) Once the favorable influence of Transplant Type MMV or MV on the hazard rate of graft loss-due-to AR was controlled, Liver Inclusion (Transplant Type LI or MV) showed no protective effect against graft loss-due-to AR, and 3) Liver Inclusion appears to independently and significantly protect against graft loss-due-to CHR. These results are consistent with our most recent report [8] (as well as with our earlier reports [9–12]) showing that Transplant Type MMV or MV but not Liver Inclusion protects against the development of a first ACR (of any grade) [8] as well as against the development of a severe ACR [8–12]. At our center, Transplant Type MMV or MV is nearly completely distinguished from Transplant Type I or LI by the joint removal of the native pancreaticoduodenal complex and native spleen; thus, the extensive removal of native lymphoid tissue (i.e., spleen, mesenteric lymph nodes, and intestinal mucosal lymphoid tissue) would appear to explain the more favorable freedom from ACR, severe ACR, and graft loss-due-to AR outcomes that we have observed over the years for Transplant Type MMV or MV.

Such a scenario was also shown in a cardiac allograft animal model with indefinite immunological tolerance after removal of secondary lymphoid organs [24]. Conversely, a separate cardiac allograft animal study from the University of Pittsburgh showed that while liver inclusion did not protect against subsequent ACR incidence, it provided clear protection against the development of CHR [25]. Previous intestinal transplant results by the University of Pittsburgh have also demonstrated a clear protective effect of Liver Inclusion against the development of CHR [6, 15]; thus, the CHR results reported here are, in fact, consistent with the University of Pittsburgh findings. It should also be noted that in none of their earlier studies [2, 5, 6] (to our knowledge) were any multivariable analyses of the hazard rates of developing a first ACR, severe ACR, or graft loss-due-to AR ever reported.

The vast vascular (sinusoidal) endothelial surface of the liver uniquely enables it to absorb circulating DSAs, thereby offering protection against potential acute and chronic damage caused by their presence [26]. This type of protection is similarly offered in both liver-alone and liver-combined-with other organ transplants (e.g., liver-kidney, liver-heart) [26]. In kidney-alone transplants, it is well-known that the presence of DSAs are associated with significantly higher rates of developing hyperacute rejection, ACR, and acute AMR [27–31], and studies of simultaneous liver-kidney transplantation have clearly demonstrated protection by liver inclusion against these types of rejection [32]. In intestinal transplantation, liver inclusion has been shown to be helpful in clearing preformed DSAs [16] as well as to offer protection against the development of *de novo* DSAs [16, 33–35]. Abu-Elmagd et al [16] also showed that while persistent performed and *de novo* DSAs were significantly associated with a much higher hazard rate of developing CHR, no significant associations of these types of DSAs with the hazard rate of developing ACR were observed. In fact, the hazard rates of developing ACR and CHR were not noticeably different between recipients having preformed DSAs that cleared after transplant vs. those who remained free of DSAs both before and after transplant [16]. Kubal et al [33] also appeared to show associations between the presence of *de novo* DSAs and higher rates of developing acute AMR and CHR but without a concomitantly higher rate of developing ACR (note: a clear distinction was made in that study between acute AMR presence vs. strictly ACR occurrence). Other previous studies have reported an association between the presence of *de novo* DSAs and a higher incidence of ACR development, but without a clear separation of acute AMR presence vs. strictly ACR occurrence being made [36, 37]. Thus, while it is still unclear as to what extent liver inclusion offers protection against the potential damage of circulating DSAs in intestinal transplantation, the results presented to date do indicate a clear protection of its inclusion against CHR development.

Since DSA and humoral rejection data were not available in most of our patients transplanted prior to 2013, no attempt to analyze such results was made here, which is a clear study limitation. In addition, while Wu et al [17] showed that the presence of DSAs were associated with a significantly higher risk of developing acute AMR, with liver-containing allografts offering significant protection against acute AMR development, no standardized definition of acute AMR has yet to be made in intestinal transplantation.

Another clear study limitation was the fact that this study spans over several years, and variables such as immunosuppression, indications, and surgical techniques have changed. This makes interpretation of the results rather difficult. However, multivariable analysis of predictors of the hazard rate of graft loss-due-to AR found no significant effects of induction type, and multivariable analysis of predictors of the hazard rate of graft loss-due-to CHR found that only our earliest approaches ("no/old induction" during 1994–1997) were significantly less favorable. In addition, while this cohort of 350 consecutive intestinal transplant cases were prospectively followed and represents one of the largest experiences with intestinal transplantation ever reported, the liver-intestine and modified multivisceral subgroups were relatively small. Thus, generalization of our results to other centers could be limited by these relatively small subgroup sample sizes. Nonetheless, we believe that we are reporting statistically sound results regarding the significant multivariable predictors of the hazard rates of developing graft loss-due-to AR vs. CHR.

Other observed differences in clinical outcomes between two of the historically largest intestinal transplant centers are worth noting. As reported here, among the transplanted cases who experienced graft loss-due-to rejection, 68.5% (50/73) and 31.5% (23/73) were due to AR and CHR, respectively. This is in stark contrast to the University of Pittsburgh results (with similarly long patient follow-up) [6] in that only 25.7% (26/101) of their reported graft losses due-to-rejection were due to AR, whereas 74.3% (75/101) were due to CHR. In terms of absolute numbers, the observed percentages who developed graft loss-due-to AR and

CHR in this study were 14.3% (50/350) and 6.6% (23/350), respectively, versus 5.2% (26/500) and 15.0% (75/500) in the Abu-Elmagd et al study [6], similar in value when the two outcomes are combined. However, severe ACR usually occurs much earlier post-transplant in comparison with CHR occurrence. Since nearly all patients at our center who experienced graft loss-due-to AR had previously experienced a severe ACR [8–12], is it possible that the (unreported) incidence rate of severe ACR was concomitantly lower among University of Pittsburgh patients who received a preconditioning anti-lymphocyte induction regimen [6] with either rATG (thymoglobulin) or alemtuzumab (in comparison with our historical cohort of 350 patients)? Is it possible that their preconditioning strategy to give most (or all) of their anti-lymphocyte induction prior to reperfusion [38–40] helps to alleviate severe ACR risk? These questions are still left unanswered.

We also recently reported more favorable graft survival outcomes using our newer, more intensive induction strategy (since 2013) of combining a larger total dose (post-reperfusion) of rATG (10 mg/kg, 2 mg/kg ×5) with 1 standard rituximab dose given during the first 8 days post-transplant (with longer prophylactic care as well) [8, 41]. In our most recent report [8], fewer ACR (of any grade) and severe ACR episodes were observed among the 95 patients who received this more intensive rATG/rituximab induction strategy, with the observed percentages developing graft loss-due-to AR and CHR during the first 60 months post-transplant being 7.4% (7/95) and 3.2% (3/95), respectively. It will therefore be of interest to recalculate these percentages with more patients and after longer post-transplant follow-up has accrued.

In summary, while the results reported here are based on an historical cohort of intestinal transplant cases who were transplanted at our center between 1994–2012 and received varying older induction immunosuppression protocols, we believe this study has helped to clarify some of the previously reported discrepancies in results that have existed between our center and the University of Pittsburgh regarding predictors of graft loss-due-to AR vs. graft loss due to CHR. It is our hope that some additional clarity has been provided here in terms of distinguishing between these two important clinical outcomes following intestinal transplantation. In addition, while direct comparison of two high volume intestinal transplant programs is relevant, it begs the question of a collaborative investigation using a multi-center approach rather than independent reports.

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 humans were approved by the University of Miami Institutional Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

AUTHOR CONTRIBUTIONS

RV: Designed research/study, performed research/study, collected data, analyzed data, wrote the paper. JJG: Designed research/study, performed research/study, collected data, analyzed data, wrote the paper. GS: Designed research/study, performed research/study, collected data, analyzed data, wrote the paper. AF: Designed research/study, performed research/study, collected data, analyzed data, wrote the paper. JG: Designed research/study, performed research/study, collected data. AT: Designed research/study, performed research/study, collected data. MT: Performed research/study, wrote the paper. GC: Designed research/study, performed research/study, wrote the paper. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

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

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Application of Intestinal Barrier Molecules in the Diagnosis of Acute Cellular Rejection After Intestinal Transplantation

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Diagnosing acute rejection after intestinal transplantation currently heavily relies on histopathological analysis of graft biopsies. However, the invasive risks associated with ileoscopic examination and the inaccessibility for biopsy after ileostomy closure hinder real-time detection of rejection responses. Molecules comprising the intestinal barrier have been identified as physiological and molecular biomarkers for various bowel conditions and systemic diseases. To investigate the potential of barrier function-related molecules in diagnosing rejection after intestinal transplantation, plasma samples were collected longitudinally from transplant recipients. The samples were categorized into "indeterminate for rejection (IND)" and "acute rejection (AR)" groups based on clinical diagnoses at each time point. The longitudinal association between plasma levels of these barrier function-related molecules and acute rejection was analyzed using the generalized estimating equations (GEE) method. Logistic GEE models revealed that plasma levels of claudin-3, occludin, sIgA, and zonulin were independent variables correlated with the clinical diagnosis of acute rejection. The subsequent prediction model demonstrated moderate ability in discriminating between IND and AR samples, with a sensitivity of 76.0%, specificity of 89.2%, and accuracy of 84.6%. In conclusion, monitoring plasma levels of claudin-3, occludin, sIgA, and zonulin shows great potential in aiding the diagnosis of acute rejection after intestinal transplantation.

Keywords: biomarkers, acute rejection, intestinal transplant, intestinal barrier, noninvasive

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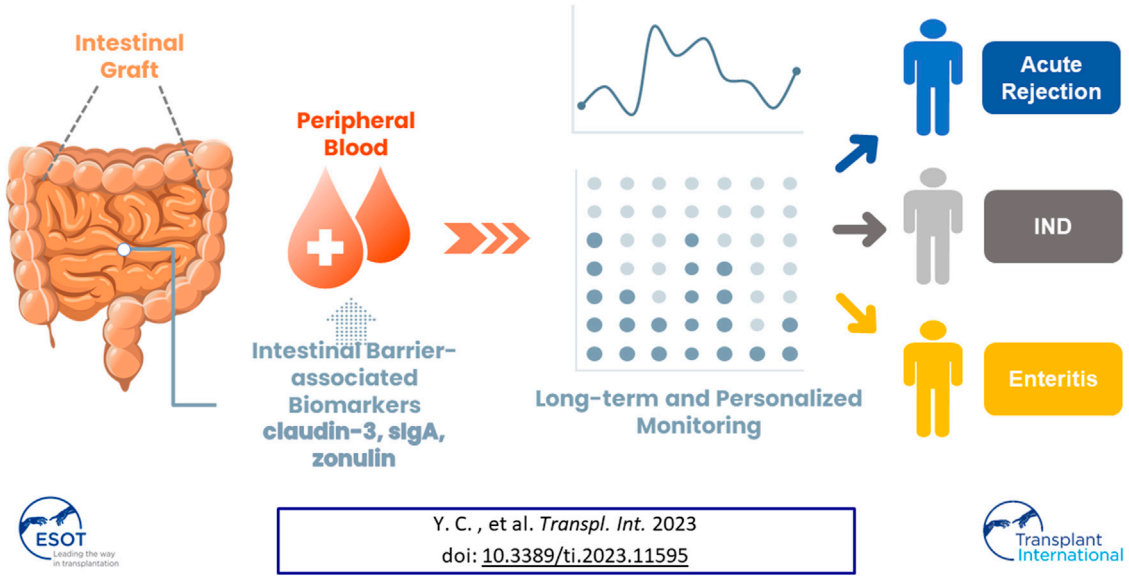
Abbreviations: AR, acute rejection; CD, Crohn's disease; IND, indeterminate for rejection; IBS, irritable bowel syndrome; IBD, inflammatory bowel disease; ITx, intestinal transplantation; GEE, generalized estimating equations; L-FABP, liver-type fatty acid-binding protein; OR, odds ratio; ROC, Receiver Operating Characteristic; sIgA, secretory immunoglobulin A; UC, ulcerative colitis; ZO-1, zonula occludens-1.



GRAPHICAL ABSTRACT |

INTRODUCTION

Intestinal transplantation (ITx) is considered the definitive treatment for patients with irreversible intestinal failure or life-threatening complications after long-term reliance on parenteral nutrition [1, 2]. The small intestine, with its abundant lymphoid tissue and diverse bacterial flora, has a higher incidence of acute rejection compared to other organ transplants [3, 4]. Approximately 50%–75% of small bowel transplantation patients experience acute rejection, ranging from mild forms with cryptic apoptosis to severe cases that result in ulcerative destruction of the epithelial mucosa, posing a challenge to graft and patient survival [3–6].

At present, the gold standard for diagnosing acute rejection following ITx depends on endoscopic observation and biopsy histology [7, 8]. However, the discontinuation of scheduled biopsies after ileostomy closure poses challenges in the early detection of acute rejection [9, 10]. Therefore, the identification of novel molecular biomarkers that can be non-invasively detected with high accuracy has been a crucial goal in aiding the clinical detection of rejection in intestinal transplantation [11–13].

The intestinal barrier plays a pivotal role in maintaining immune response homeostasis and immune tolerance by protecting the mucosal surface of the intestine [14–17]. The "microbiota-immune axis" concept has linked the intestinal barrier to various pathological conditions. Impairment of the intestinal barrier can lead to increased microbial translocation, inducing pro-inflammatory conditions in the intestine and subsequent systemic disorders [15–19]. Research has identified junctional molecules such as claudins, occludin, zonula occludens-1 (ZO-1), and regulatory proteins like secretory IgA (sIgA) and zonulin as potential biomarkers for several pathological conditions, including inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), food allergy, metabolic diseases, and leaky gut syndrome [20–23].

From 2007 to 2022, we conducted 31 ITx surgeries for 30 patients, with 5 year survival rates of 71.0% for patients and 51.6% for grafts, comparable to global figures [4, 5, 24]. To improve long-term outcomes by reducing graft loss related to acute rejection, we aimed to explore non-invasive biomarkers to enhance the accuracy and timeliness of acute rejection diagnosis. This study aimed to investigate the correlation between molecular levels of intestinal barrier components in plasma and the incidence of acute rejection, with the goal of developing a predictive model for diagnosing acute rejection.

MATERIALS AND METHODS

Study Design and Sample Collection

To establish a time-series database for monitoring the plasma levels of intestinal barrier molecules in intestinal transplant recipients, plasma sample collection commenced on the day of transplantation, prior to the operation. Subsequent plasma collections followed the blood draw schedule outlined in the post-transplant monitoring protocol (see below). Blood samples were promptly transferred into heparin-containing tubes upon collection. After undergoing standardized centrifugation at 300×g, the plasma was divided into polypropylene tubes and stored at −80°C until analysis. The listing of plasma samples was documented based on the clinical manifestations and diagnosis recorded on each respective day.

Post-Transplant Monitoring Protocol and Diagnosis of Acute Rejection

At the time of ITx surgery, a Santulli's proximal chimney ileostomy was created in each recipient for endoscopic examination and biopsy of the graft. The frequency of endoscopic examination was twice a week in the first month, once a week in the second month, once every other week in the third month, once a month in the fourth to sixth month, and whenever necessary.

The frequency of drawing blood was per day in the first week, twice a week in the second to the fourth week, once a week in the second month, once every other week in the third month, once a month in the fourth to sixth month, and whenever necessary.

The diagnosis of acute rejection was established through the pathological analysis of the biopsy, in conjunction with the identification of significant morphological changes in the graft mucosa during endoscopic examination [3, 25].

Quantification of Plasma Levels of the Intestinal Barrier Molecules

The plasma samples were thawed and vortexed before being subjected to ELISA assays. The procedure for detection and determination of their concentrations were performed according to the manufacturer's protocols. The ELISA kits used in the study included: Citrulline (CEA505Ge, Cloud-Clone Corp., Katy, TX 77494, USA), Claudin-1 (CSB-EL005490HU, Cusabio Life Science, Houston, TX 77054, USA), Claudin-2 (CSB-EL005500HU, Cusabio Life Science), claudin-3 (CSB-EL005505HU, Cusabio Life Science), Claudin-4 (CSB-E17961h, Cusabio Life Science), L-FABP (HA404-1, Hycult Biotech Inc., Wayne, PA 19087, USA), Occludin (SEC228Hu, Cloud-Clone Corp.), sIgA (SEA641Hu, Cloud-Clone Corp.), zonular occludens-1 (CSB-E13916h, Cusabio Life Science) and zonulin (K5601, Immundiagnostik AG, 64625 Bensheim, Germany).

Statistical Analysis

This study employed generalized estimating equations (GEE) models to account for the effect of repeated measures, with Patient ID serving as the subject variable to define individual subjects within the dataset. Age and the concentrations of ten barrier function-related molecules were treated as continuous variables, while gender was considered as a categorical variable. The biopsy result was used as the binary outcome variable. Binary logistic GEE analysis was utilized to calculate the regression coefficients and odds ratios for the independent variables. The predictive probability of acute rejection and the clinical incidence of acute rejection were further analyzed using ROC (Receiver Operating Characteristic) curves.

Statistical analysis was conducted using SPSS software (version 22.0, IBM Corp., Chicago, IL, USA). The statistical data are presented as mean ± SE. The significance level was indicated by p-values, with a value of $p < 0.05$ considered statistically significant for all analyses.

RESULTS

Patients and the Grouping of Plasma Samples

A total of 172 time-series plasma samples were collected from seven patients between September 2016 and June 2022, along with their corresponding medical records, including histopathological reports of graft biopsies during the same period. Plasma samples corresponding to non-rejection intestinal conditions (e.g., enteritis) and other systemic situations (e.g., sepsis) were excluded from the analysis. The basic information of the seven patients and the number of plasma samples collected are presented in **Table 1**. Next, based on clinical findings and/or biopsy reports on the day of blood collection, 143 plasma samples were categorized as IND (indeterminate for acute rejection, $n = 93$) and AR (acute rejection, $n = 50$). The mean plasma levels of ten intestinal barrier-related molecules are shown in **Table 2**.

Univariate Analysis

The association between plasma levels of intestinal barrier molecules and the diagnosis of AR was investigated by using univariate GEE analysis (**Table 3**). Among the examined variables, claudin-3 demonstrated a significant positive association with AR (coefficients = 0.013, $p < 0.001$). Conversely, citrulline demonstrated a significant negative association with acute rejection (coefficient = −0.121, $p = 0.022$). Notably, occludin and zonulin also exhibited significant negative association with acute rejection with the coefficients −0.339 ($p = 0.010$) and −0.367 ($p < 0.001$), respectively. The remaining variables, including claudin-1, claudin-2, claudin-4, L-FABP, sIgA, ZO-1, did not demonstrate statistically significant associations with AR in this univariate analysis (**Table 3**).

Multivariable Analysis

Further multivariate GEE analysis was conducted to better understand the collective impact of these molecules on the risk of acute rejection. In **Table 4**, three regression models revealed certain significant associations between plasma levels of intestinal barrier molecules and acute rejection. Claudin-3 demonstrated a consistent positive association with acute rejection across all models, with odds ratios (OR) of 1.026 (95% C.I. 1.012–1.040, $p < 0.001$) in model 1, 1.025 (95% C.I. 1.013–1.037, $p < 0.001$) in model 2, and 1.022 (95% C.I. 1.011–1.032, $p < 0.001$) in model 3. On the other hand, occludin showed consistent negative associations with acute rejection, with ORs of 0.566 (95% C.I. 0.390–0.820, $p = 0.003$) in model 1, 0.627 (95% C.I. 0.459–0.857, $p = 0.003$) in model 2, and 0.574 (95% C.I. 0.417–0.791, $p = 0.001$) in model 3. Zonulin also exhibited a significant negative association with acute rejection, with ORs of 0.743 (95% C.I. 0.582–0.947, $p = 0.016$) in model 1, 0.778 (95% C.I. 0.631–0.960, $p = 0.019$) in model 2, and 0.817 (95% C.I. 0.684–0.975, $p = 0.025$) in model 3. Additionally, sIgA demonstrated a significant negative association with acute rejection in model 1, with an OR of 0.986 (95% C.I. 0.973–0.999, $p = 0.031$). However, other

TABLE 1 | Basic characteristics of the patients whose plasma samples were used in this research.

Patient ID	Age	Gender	No. of plasma samples	Episode(s) of AR	Severity and timing of AR[a]
Pt-1	31	Female	42	4	mild (D13) severe (D30) severe (D175) severe (D234)
Pt-2	58	Male	21	2	mild (D21) mild (D82)
Pt-3	29	Female	22	0	
Pt-4	37	Male	23	2	severe (D16) mild (D73)
Pt-5	58	Female	14	1	severe (D36)
Pt-6	28	Female	10	1	mild (D20)
Pt-7	63	Male	11	0	

[a]*Timing of AR was represented as the day after transplant.*

TABLE 2 | Mean plasma levels of intestinal barrier molecules in the IND and AR groups.

	IND (Mean ± S.E.)	AR (Mean ± S.E.)	Unit
N	93	50	
citrulline	17.03 ± 0.50	16.21 ± 0.51	ng/mL
claudin-1	309.76 ± 65.01	240.09 ± 15.05	pg/mL
claudin-2	319.76 ± 38.20	276.58 ± 31.86	pg/mL
claudin-3	76.20 ± 5.07	109.45 ± 8.04	pg/mL
claudin-4	47.43 ± 5.29	50.62 ± 8.08	pg/mL
L-FABP	21.40 ± 2.30	16.52 ± 2.00	ng/mL
occludin	4.02 ± 0.43	2.34 ± 0.25	ng/mL
sIgA	120.17 ± 7.24	81.64 ± 5.26	μg/mL
ZO-1	403.13 ± 25.04	437.91 ± 30.32	pg/mL
zonulin	5.99 ± 0.32	4.02 ± 0.24	ng/mL

variables, including citrulline, claudin-1, claudin-2, claudin-4, L-FABP, and ZO-1, did not exhibit statistically significant associations with acute rejection in the multivariable analysis models.

Evaluation of the AR Prediction Models

The model performance, as assessed by the QICC (corrected quasi-likelihood under the independence model criterion), showed that GEE model 2 had the lowest value (QICC = 139.552), suggesting a better fit compared to model 1 (QICC = 150.572) and model 3 (QIC = 145.405). The diagnostic sensitivity, specificity, and accuracy of model 2 were 76.0%, 89.2%, and 84.6%, respectively (**Table 4**).

The predictive probability of acute rejection was calculated for each sample using regression model 2, and the relationship between the predictive probability and the incidence of acute rejection was analyzed using the ROC curve. The AUC was calculated as 0.862 (95% C.I. 0.794 to 0.930, $p < 0.001$), with a model probability cut-off of 0.432 being identified as the optimal threshold (**Figure 1**).

DISCUSSION

In the present study, our results demonstrated that there were significant changes of claudin-3, occludin, sIgA, and zonulin during the onset of acute rejection after intestinal transplantation. These four molecules were independent factors most related to the clinical diagnoses of acute rejection, with that the increase in claudin-3 was associated with higher probability of acute rejection while increased occludin, sIgA and zonulin were negatively associated with acute rejection.

Endoscopic examination and tissue biopsy, as the most conventional method for graft monitoring, is still holds as the most definite way of confirming the diagnoses of rejection after intestinal transplantation [8, 9]. The search for non-invasive

TABLE 3 | Longitudinal association between the plasma levels of intestinal barrier molecules and acute rejection by univariate GEE analysis.

	Regression coefficient	Standard Error	Wald	*p*-value	OR	95% C.I. for OR	
						Lower	Upper
citrulline	−0.121	0.053	5.245	0.022	0.886	0.799	0.983
claudin-1	<0.001	<0.001	0.056	0.813	1.000	0.999	1.001
claudin-2	<0.001	0.001	0.156	0.693	1.000	0.999	1.001
claudin-3	0.013	0.003	15.078	< 0.001	1.013	1.006	1.020
claudin-4	0.003	0.003	0.814	0.367	1.003	0.997	1.009
L-FABP	<0.001	<0.001	0.840	0.360	1.000	1.000	1.000
occludin	−0.339	0.132	6.601	0.010	0.712	0.550	0.923
sIgA	−0.002	0.004	0.442	0.506	0.998	0.990	1.005
ZO-1	<0.001	0.001	0.361	0.548	1.000	0.999	1.002
zonulin	−0.367	0.082	20.113	< 0.001	0.693	0.590	0.813

TABLE 4 | Multivariate GEE analyses of the association between the plasma levels of intestinal barrier molecules and acute rejection.

	Model 1[a]		Model 2[a]		Model 3[a]	
	OR[b] (95% C.I.)	*p*-value	OR (95% C.I.)	*p*-value	OR (95% C.I.)	*p*-value
claudin-3	1.026 (1.012–1.040)	<0.001	1.025 (1.013–1.037)	<0.001	1.022 (1.011–1.032)	<0.001
occludin	0.566 (0.390–0.82)	0.003	0.627 (0.459–0.857)	0.003	0.574 (0.417–0.791)	0.001
zonulin	0.743 (0.582–0.947)	0.016	0.778 (0.631–0.960)	0.019	0.817 (0.684–0.975)	0.025
sIgA	0.986 (0.967–0.999)	0.031	0.990 (0.978–1.001)	0.082		
citrulline	0.906 (0.791–1.039)	0.157				
claudin-1	1.000 (0.999–1.001)	0.972				
claudin-2	1.001 (0.999–1.004)	0.315				
claudin-4	1.007 (0.996–1.017)	0.209				
L-FABP	1.000 (1.000–1.000)	0.847				
ZO-1	0.999 (0.996–1.001)	0.258				
QICC[b]	150.572		139.552		145.405	
sensitivity	72.0%		76.0%		68.0%	
specificity	94.6%		89.2%		79.6%	
accuracy	86.7%		84.6%		75.5%	

[a]*Analyses were adjusted for gender and age.*
[b]*OR, odds ratio; QICC, corrected quasi likelihood under independence model criterion.*

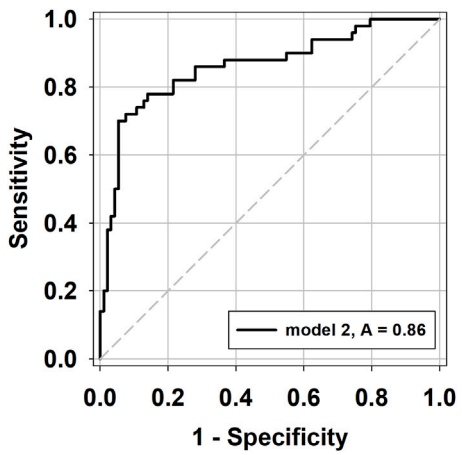


FIGURE 1 | ROC curves of the predictive probability (PP) values from GEE model 2 in the prediction of acute rejection.

biomarkers for diagnosing acute rejection had been on in the recent decade. For example, blood citrulline and stool calprotectin had been considered as potential biomarkers for this purpose. Decreased citrulline was reported to reflect reduced enterocyte mass and intestinal insufficiency during acute rejection [11, 26, 27]; increased fecal calprotectin implicated ongoing immune responses in the intestine [28]. However, the lack of diagnostic specificity had limited their application in diagnosing acute rejection [29–31].

The molecules regulating intestinal barrier function had been identified as biomarkers to evaluate intestinal permeability thus being applied in the diagnosis of inflammatory bowel diseases [20–23]. We therefore investigated the applicability of these biomarkers in the detection of acute rejection after intestinal transplantation. As the results shown, we have tracked down to four molecules with different roles in barrier functions.

Secretory IgA serves as a crucial defense effector in the intestinal barrier, playing a key role in microbial neutralization and immune exclusion It is produced by plasma cells in the epithelial lamina propria, transported across epithelial cells, and then secreted into the lumen [32, 33]. Quantifying sIgA in serum or saliva has been applied for diagnosing Crohn's disease (CD), ulcerative colitis (UC), and mucositis, with elevated levels observed in active CD and reduced levels in UC [34–36]. In our study, we found a negative association between sIgA levels and the onset of acute rejection (**Table 4**), suggesting altered sIgA production or depletion during rejection. Intestinal microbial stimulation and Th1-inhibiting/Th2-stimulating cytokines play a role in balancing sIgA levels [37]. Given that Th1-inhibiting cytokines (e.g., IL-6, IFN-γ and TNF-α) are involved in acute rejection, the downregulation of sIgA could serve as an early indicator of the acute rejection-associated Th1 immune response.

Altered expression of claudins in intestinal tissue has been extensively studied in patients with various intestinal disorders. Reduced expression of claudin-1 was observed in patients with inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) [38, 39], while an increase in claudin-2 was found in the inflamed epithelium of patients with ulcerative colitis (UC) [40, 41]. The variation in claudin-3 and claudin-4 expression in IBD remains controversial, with studies reporting both reduced and increased expression [42–44]. In our study, we found a significant association between claudin-3 and acute rejection (**Table 4**), suggesting increased levels of claudin-3 in circulation due to intestinal tissue destruction during rejection.

The expression of occludin has shown variability in intestinal biopsies of patients with Crohn's disease (CD) and ulcerative colitis (UC), suggesting inconsistent patterns in occludin expression within these studies [45, 46]. However, limited research has explored the use of plasma occludin as a marker for intestinal diseases. Interestingly, plasma occludin has gained attention in the context of blood-brain barrier damage, demonstrating fluctuating levels of occludin in different types of stroke [47].

Zonulin is an important regulator of barrier function that can disrupt the tight junctions between cells [48]. Previous research has highlighted the association between increased zonulin expression and various conditions, including inflammatory bowel disease (IBD), food allergy, diabetes, arthritis, liver disease, and aging [49–52]. In our study, we initially hypothesized that higher zonulin levels would contribute to the compromised intestinal integrity observed during acute rejection. However, contrary to our expectations, we found lower levels of zonulin in the acute rejection group. It is worth noting that the intestinal epithelial cells are a significant source of zonulin [53]. The reduction in zonulin levels in the acute rejection group could potentially be attributed to impaired or dysfunctional intestinal cells during the onset of acute rejection.

Our investigation into predictive factors for acute rejection, including claudin-3, occludin, sIgA, and zonulin, has illuminated distinct roles in maintaining intestinal barrier integrity. While claudin-3, occludin, and zonulin consistently emerged as significant factors associated with acute rejection in both univariate and multivariable analyses, sIgA demonstrated significance when other variables were considered. Excluding sIgA from model 2 led to reduced prediction sensitivity and accuracy in model 3, underscoring its crucial contribution.

ROC curves were generated to determine the optimal cutoff values for claudin-3, occludin, sIgA, and zonulin in predicting the occurrence of acute rejection. The analysis revealed that claudin-3 levels above 90.32 pg/mL ($p < 0.001$), occludin levels below 2.55 pg/mL ($p = 0.185$), sIgA levels below 63.37 μg/mL ($p < 0.001$), and zonulin levels below 2.95 ng/mL ($p < 0.001$) were indicative of the diagnosis of acute rejection, as depicted in **Supplementary Figure S1**. It is important to note, however, that the ROC analyses did not take into consideration the potential impact of repeated measurements within individual samples. Therefore, these cutoff values should not be used for clinical purposes at this time.

The quest for acute rejection-specific biomarkers is a challenging endeavor. Low specificity in differentiating acute rejection from enteritis complicates conclusive outcomes [31]. Due to the dispersed distribution of samples representing enteritis and sepsis outcomes within our patient cohort, we opted not to include these groups in our GEE analysis. However, we conducted a detailed comparison of relative changes in claudin-3, occludin, sIgA, and zonulin levels across the IND, AR, enteritis, and sepsis samples, as outlined in **Supplementary Table S1**. Noteworthy differences emerged among these sample groups, with both enteritis and sepsis-related samples displaying elevated concentrations of barrier markers compared to the IND group. In sepsis cases, we observed an exceptionally high mean level of claudin-3, spanning a wide range. This suggests the possibility that a simultaneous increase in these markers might indicate pathological conditions such as enteritis and sepsis. Importantly, this finding highlights that elevations in claudin-3 alone may not reliably indicate acute rejection, emphasizing the need for a more comprehensive diagnostic framework or a combination of markers.

Furthermore, our prediction model revealed a significant insight: the variation trends in sIgA and zonulin for patients with acute rejection were opposite to those observed in patients with other inflammatory or ulcerative intestinal diseases, both in existing literature and our own data. The mean values of sIgA and zonulin were relatively correlated with the severity of acute rejection with the AR-severe group displaying greater significance ($p = 0.011$ for sIgA; $p = 0.002$ for zonulin) than the AR-mild group ($p = 0.023$ for sIgA; $p = 0.006$ for zonulin) (**Supplementary Table S2**; **Supplementary Figure S2**). This finding holds significant potential when differential diagnoses must be made, providing a valuable advantage.

Our study, although illuminating, faces certain limitations, primarily due to a small number of patients and sample size. Enhancing the model's sensitivity, specificity, and accuracy would benefit from additional laboratory data, including white blood cell counts, immunosuppressant concentrations, liver function parameters, and renal function indicators. Another limitation stems from the restricted quantity of plasma samples, limiting the exploration of potential molecules associated with barrier function. However, the innovative aspect of our study lies in our statistical approach, acknowledging the importance of individual variations. Accounting for repeated measures within each patient enables the capture of dynamic trends in diagnostic markers, creating a more comprehensive and reliable basis for detecting rejection. This approach differentiates our study from prior research, emphasizing the need to consider nuanced variations for a more accurate diagnosis.

CONCLUSION

In conclusion, our study has identified claudin-3, sIgA, and zonulin as promising non-invasive biomarkers for diagnosing acute rejection in recipients of intestinal transplants. Notably, this is the pioneering investigation to employ GEE analysis for comparing plasma levels of intestinal barrier molecules in the rejection and non-rejection phases of intestinal transplant recipients. We anticipate that our model holds significant potential to enhance post-transplant monitoring of intestinal grafts, ultimately advancing patient care in this critical domain.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available to preserve individuals' privacy under local IRB regulation. Requests to access the datasets should be directed to yahuitsi@saturn.yzu.edu.tw.

ETHICS STATEMENT

The studies involving humans were approved by Research Ethics Review Committee of Far Eastern Memorial Hospital (New Taipei City, Taiwan) (No. 105025-F). The studies were conducted in accordance with the local legislation and

institutional requirements. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YC and C-YC designed the study; YC, Y-HT, and S-HT participated in the acquisition, analysis, and interpretation of the data, and drafted the initial manuscript; YC, Y-HT, S-HT, and C-YC revised the manuscript and approved the manuscript to be published. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11595/full#supplementary-material

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Preclinical Study of DCD and Normothermic Perfusion for Visceral Transplantation

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Considering recent clinical and experimental evidence, expectations for using DCD-derived intestines have increased considerably. However, more knowledge about DCD procedure and long-term results after intestinal transplantation (ITx) is needed. We aimed to describe in detail a DCD procedure for ITx using normothermic regional perfusion (NRP) in a preclinical model. Small bowel was obtained from pigs donors after 1 h of NRP and transplanted to the recipients. Graft Intestinal samples were obtained during the procedure and after transplantation. Ischemia-reperfusion injury (Park-Chiu score), graft rejection and transplanted intestines absorptive function were evaluated. Seven of 8 DCD procedures with NRP and ITx were successful (87.5%), with a good graft reperfusion and an excellent recovery of the recipient. The architecture of grafts was well conserved during NRP. After an initial damage of Park-chiu score of 4, all grafts recovered from ischemia-reperfusion, with no or very subtle alterations 2 days after ITx. Most recipients (71.5%) did not show signs of rejection. Only two cases demonstrated histologic signs of mild rejection 7 days after ITx. Interestingly intestinal grafts showed good absorptive capacity. The study's results support the viability of intestinal grafts from DCD using NRP, contributing more evidence for the use of DCD for ITx.

Keywords: donation after circulatory death, normothermic perfusion, intestinal transplantation, experimental DCD feasibility, intestinal graft viability

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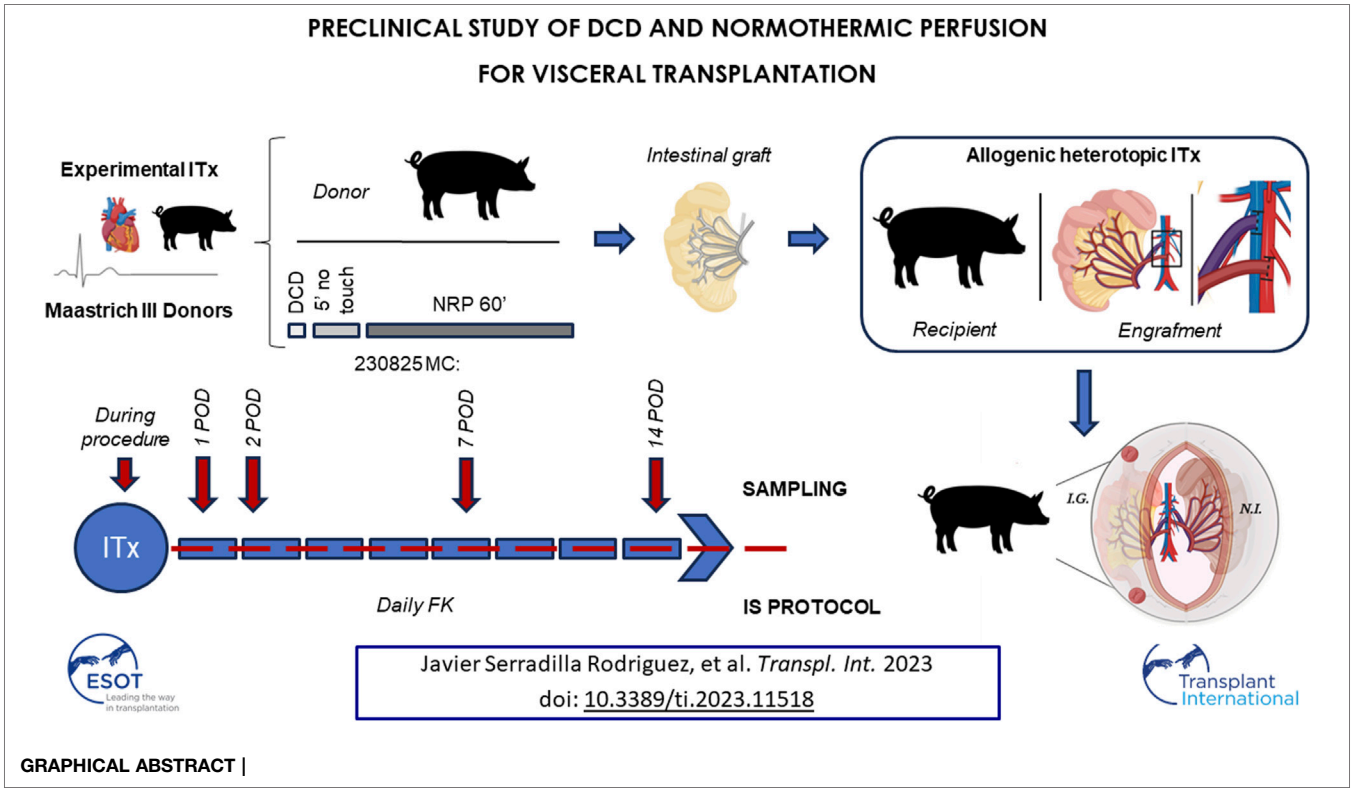
Abbreviations: CIT, cold ischemia time; Ct, threshold cycle; DCD, donation after circulatory death; ECMO, extracorporeal membrane oxygenation; EpCAM, epitelial cell adhesión molecule; fWIT, functional warm ischemic time; IEC, intestinal epithelial cells; IL, interleukin; IRI, ischemia-reperfusion injury; ITx, intestinal transplantation; LYZ, lysozyme; MLCK, myosin light chain kinase; MUC2, mucine 2; NRP, normothermic regional perfusion; PCS, Park-Chiu score; POD, postoperative day; TJP1, tight junction protein; TNF-α, tumour necrosis factor alpha; WLST, withdrawal of life support therapy.



GRAPHICAL ABSTRACT |

INTRODUCTION

The universal shortage of organs has prompted the growing use of donation after circulatory death (DCDs). Kidneys, liver, lungs, pancreas, and, more recently, the heart from DCDs have been successfully employed for transplantation in many centers worldwide [1, 2]. Most of the pitfalls and concerns regarding DCD have been addressed by strict donor selection [3], improvements in normothermic regional perfusion (NRP) [4] and *ex vivo* machine perfusion devices [5]. Although the true influence of routine DCD use is difficult to assess, this form of organ donation has been recognized to increase the pool of donors [6].

Intestinal transplantation (ITx) is used in selected cases of irreversible intestinal failure, as it has clinical and economic advantages over prolonged use of parenteral nutrition [7]. However, the use of DCD as a source of intestinal grafts has been denied through years due to concerns about ischemic susceptibility [8]. The experimental studies that support this point of view are limited and involve heterogeneous methodologies and currently, it is contrasted with updated experimental evidence that shows DCD as promising in the field of intestinal and multivisceral transplantation [9, 10]. In addition, the clinical evidence supporting the use of these grafts in the clinical setting is promising but scant [11, 12]. Recently, our work group has reported the first case of pediatric DCD and multivisceral transplantation with a good outcome to date [13].

Currently, the search for strategies that increase the availability of solid organs for transplantation remains a challenge for the medical-scientific community [14]. Among patients awaiting solid organ transplantation, ITx candidates show longer waiting periods and high morbidity and mortality rates. This is especially relevant for pediatric patients, who also suffer from other secondary complications, such as lack of growth, increased family dependency and lack of social and academic development.

Therefore, further evaluation of the potential use of DCD intestinal grafts is warranted. Taking this background into account, this study aimed to evaluate the feasibility of DCD intestinal grafts in a preclinical model of ITx.

MATERIALS AND METHODS

Animal Use and Care

Sixteen Large White pigs (25.5 ± 2.5 kg) were used for ITx (eight donors, eight recipients). They were all female, non-related, outbred, and aged 8–10 weeks to ensure uniformity in veterinary management. Prior to use, all animals underwent strict veterinary control that determined their good health and condition. The protocol was approved by the Animal Welfare Ethics Committee (PROEX 58.7/20) and complied with the EU and Spanish Directives on experimental animals (63/2010 EU, RD 53/2013). The entire donation process under DCD conditions was strictly controlled by the veterinary staff of our institution.

Circulatory Death Induction, NRP, and Donor Surgery

The animals were starved for 24 h before surgery. Premedication was performed directly in the box (12 mg/kg of ketamine intramuscularly + 0.5 mg/kg of midazolam). With the animals under inhalational anesthesia (sevoflurane, 4%), we performed catheterization of the ear veins and arteries and endotracheal intubation, which was followed by a propofol (11 mg/kg) and fentanyl (5.4 μg/kg/h) infusion. A 14-gauge angiocath was inserted in the fifth intercostal space in the mid-axillary line. Withdrawal of life support therapy was performed, and a bolus of unfractionated heparin (300 IU/kg) was injected through a central venous catheter. Manual air thoracic insufflation (100 mL/2′) was performed to induce progressive tension pneumothorax and subsequent lethal cardiovascular collapse. After 200 ± 50 mL, a persistent decline in oxygen saturation level was observed. Functional warm ischemic time (fWIT) was initiated when oxygen saturation was sustained at <80% and/or mean blood pressure was <50 mmHg, as established by the Spanish legislation for DCD in clinical practice [15]. Death was declared after cardiac arrest and a 5 min "no-touch period."

A rapid laparotomy was performed, and the aorta and inferior vena cava were cannulated (14/16 French [Fr] and 20/22/24 Fr, respectively; DLP™ Medtronic, Minneapolis, MN, United States). Thoracotomy was performed, and the descending thoracic aorta was cross-clamped to prevent perfusion of the brain and coronary arteries. Subsequently, NRP was established using a compact custom closed extracorporeal circulation circuit (Rotaflow™ RF-32 centrifugal pump; Maquet, Hirrlingen, Germany). The detailed procedure for NRP control and determinations during this 1 h period are provided as **Supplementary Material**.

During NRP, the small bowel was prepared for procurement. After 1 h of NRP, the circuit was stopped, and the same cannulas were used for cold perfusion with cold-preservation solution (Celsior^{R}, IGL). The graft comprised the small bowel from the duodenum to the terminal ileum, with a vascular pedicle formed by the superior mesenteric artery and portal vein. During this cold ischemia time (CIT), the graft was kept in cold storage using the same solution until engraftment.

Recipient Procedure

Heterotopic ITx was performed. The native bowel was shifted to the left side of the abdomen, and the infrarenal aorta and cava were exposed. Portocaval anastomosis, followed by anastomosis between the superior mesenteric artery and aorta, was completed, and reperfusion of the bowel was established. The proximal end of the bowel remained closed, and a terminal ileostomy was performed for graft sampling. A gastrostomy was performed to facilitate daily administration of immunosuppressive drugs, antibiotics and analgesics during the post-transplant monitoring of the animals.

Recipient and Graft Clinical Monitoring

After ITx, the recipients were individually isolated with controlled feed and water *ad libitum*. The animals' welfare was supervised twice a day. Analgesia was provided with a fentanyl patch every 3 days for 1 week and with ibuprofen (10 mg/kg/12 h for 7 days, by tube). Cefixime 20 mg/kg was administered every 12 h. Tacrolimus was administered daily, and the doses (0.2–1 mg/kg/24 h) were adjusted according to serum levels. All drugs were administered through gastrostomy.

Ileoscopy was performed through the ostomy using a 9 mm pediatric endoscope on postoperative days (PODs) 1, 2, 7, and 14. Four biopsies were obtained during the examination. The animals were sacrificed and sampled on POD 14 by intravenous injection of 5M KCl under general anesthesia.

Sample Collection

Graft samples were obtained at 30′ and 60′ after NRP initiation. An additional sample was obtained after flushing with the cold-preservation solution. Three intraoperative intestinal graft samples were obtained as follows: pre-reperfusion, immediately after reperfusion, and 60′ later. Samples were always taken from the ileum for uniformity. Additional intestinal samples were obtained on PODs 1, 2, 7, and 14 through the ileostomy. Blood samples were obtained at 5′, 30′, and 60′ after the beginning of NRP (donor) and on PODs 1, 2, 7, and 14 (recipient). Baseline samples were collected from each animal.

Histopathological Analysis

Histological analysis was performed on 5 μm hematoxylin–eosin-stained tissue sections. Ischemia-reperfusion injury (IRI) was evaluated using the Park-Chiu score (PCS), whereas the Wu score was used to evaluate rejection.

RNA Isolation and Gene Expression Assessment

Mucosal intestinal biopsy specimens were immediately submerged in RNAlater solution (Invitrogen, Waltham, MA, United States) and stored at 4°C for 24 h and then at −80°C. RNA was isolated using the RNeasy Mini Kit (Qiagen, Hilden, Germany) and retrotranscribed into cDNA using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Waltham, MA, United States).

Expressions of genes for tight junction protein 1 (TJP1), epithelial cell adhesion molecule (EpCAM), mucine 2 (MUC2), lysozyme (LYZ), interleukin (IL)-6, and tumor necrosis factor alpha (TNF-α) were measured using predesigned TaqMan™ Gene Expression assays (Ss03373514_m1, Ss03384752_u1, Ss03377386_u1, Ss03394856_m1, Ss03384604_u1, Ss03391318_g1) with TaqMan™ Fast Advanced Master Mix on a 7500 Fast Real-Time PCR System (Applied Biosystems). Threshold cycle (Ct) scores were calculated as the mean of the duplicates and normalized against the Ct scores of the endogenous control GAPDH (Ss03375629_u1). Relative expression was determined as $2^{-\Delta Ct}$, where ΔCt = Ct gene of interest–Ct endogenous control [16]. $2^{-\Delta Ct}$ values were normalized by logarithmic transformation, and fold-change values were calculated using the median value of native intestines as a reference.

TABLE 1 | Analytical monitoring during NRP.

		NRP		
	Baseline	**5 min**	**30 min**	**60 min**
AST (UI/L)	31.6 ± 11.94	38 ± 20.43	39.5 ± 15.45	49.16 ± 21.62
ALT (UI/L)	49.66 ± 14.88	36.16 ± 8.43	32.66 ± 9.97	25.83 ± 5.20
Urea (mg/dL)	24.5 ± 5.28	20.4 ± 3.55	22.66 ± 4.02	22 ± 3.95
Creatinine (mg/dL)	1.14 ± 0.14	1 ± 0.27	0.98 ± 0.27	0.83

Aspartate aminotransferase (AST), alanine transaminase (ALT), urea and creatinine were measured during DCD and NRP.

Functional Evaluation of Transplanted Intestines

Citrulline levels were measured sequentially (baseline, PODs 1, 2, 7, and 14) as a biomarker of small bowel mass and function. The absorptive function of the grafts was evaluated on POD 14, as previously reported [17]. A solution containing glucose (2 g/kg) was intraluminally administered to the transplanted intestines. The glucose level of peripheral blood (tongue) and draining veins from both the intestinal graft and native small bowel was measured (Accu-Chek blood glucose meter, Roche) immediately before and 15′, 30′, and 60′ after glucose administration to verify whether the increase was due exclusively to the absorption of the solution and not to a pre-existing blood glucose level from its native intestine.

Statistical Analysis

Differences in gene expression values between grafts and native bowels were assessed using the unpaired t-test (with the Welch correction for significantly different variances). In contrast, the paired t-test was used to compare the graft samples at different time points. Spearman rho and linear regression values were calculated for correlation analysis between gene expression values and ischemia times (fWIT or CIT). Citrulline levels were compared among the different time-points using the paired t-test. Statistical significance was set at $p < 0.05$, and GraphPad Prism (version 8.0.2) software was used for all the tests.

RESULTS

Experimental DCD Feasibility, Transplanted Recipient Outcome, and Graft Viability

Pneumothorax-induced cardiac arrest within <5′, and fWIT was 20′ ± 5′. NRP achieved good reperfusion of the abdominal organs and lactate clearance in all cases. The laboratory variables analyzed are summarized in **Table 1** and **Figure 1**. After 1 h of NRP, 8/8 intestines were successfully obtained from DCD since all eight showed a good macroscopic appearance during graft dissection and intravascular washing. After 147.8′ ± 12′ of CIT, the grafts were implanted, achieving good graft reperfusion in 7/8 procedures (87.5%). In one case, the transplanted small bowel was poorly reperfused because of venous stenosis; hence, this animal was excluded. The remaining seven recipients recovered adequately. One animal with a gastrostomy leak required an anticipated endpoint on POD 10, while the remaining six reached the scheduled POD 14.

Endoscopic Follow-Up of the Graft

The distal 40–50 cm of the graft was explored. All cases showed a mucosa with normal appearance, coloration and vascularization, without erosions, ulcers or bleeding, with a well-preserved villous pattern.

IRI and Graft Rejection

As shown in **Figures 2A–D**, in both NRP-30′ and NRP-60′ samples, the small bowel showed a well-preserved architecture. However, two NRP-60′ samples showed edema at the villus tip (PCS 1). As expected, the highest IRI was observed after 1 h of graft reperfusion, with denuded villi appearing in 3/7 evaluated samples. Interestingly, all grafts recovered 48 h after transplantation.

Mild rejection appeared on POD 7 in 2/7 (28.5%) cases; one showed full recovery, and the other showed severe acute cellular rejection on POD 14 (1/7, 14.2%). The remaining 5/7 (71.4%) recipients showed no signs of rejection at any point (**Figures 3A–D**).

Gene Expression Analysis

Relative expressions of integrin EpCAM, TJP1 (zonulin), LYZ, MUC2, the proinflammatory cytokines IL-6, and TNF-α were assessed by quantitative polymerase chain reaction in serial samples (NRP-30′, NRP-60′, pre-reperfusion, and PODs 0, 2, 7, and 14) of five animals and three additional samples of independent native bowels (**Figure 4** and **Supplementary Figure S1**). MUC2 was excluded because it was undetectable in most samples. NRP-30′ samples showed differences in the molecular graft signature of native intestines (**Figure 4**) and a dramatic 350–1600-fold increase in TNF-α levels ($p < 0.001$). IL-6 levels also increased 2–8-fold in four of the five animals ($p = 0.17$). Among genes related to epithelial integrity, zonulin showed an almost significant upregulation ($p = 0.06$), while EpCAM appeared to be downregulated in all samples (not significant because of a high dispersion). Generally, LYZ expressions were quite similar to those in the native intestines.

Next, we evaluated the possible correlation between fWIT, CIT, NRP times and gene expression values, were no significant association was observed (**Supplementary Figure S1**).

In samples taken 1 h after reperfusion, gene expression values seemed to normalize at the end of the transplantation procedure

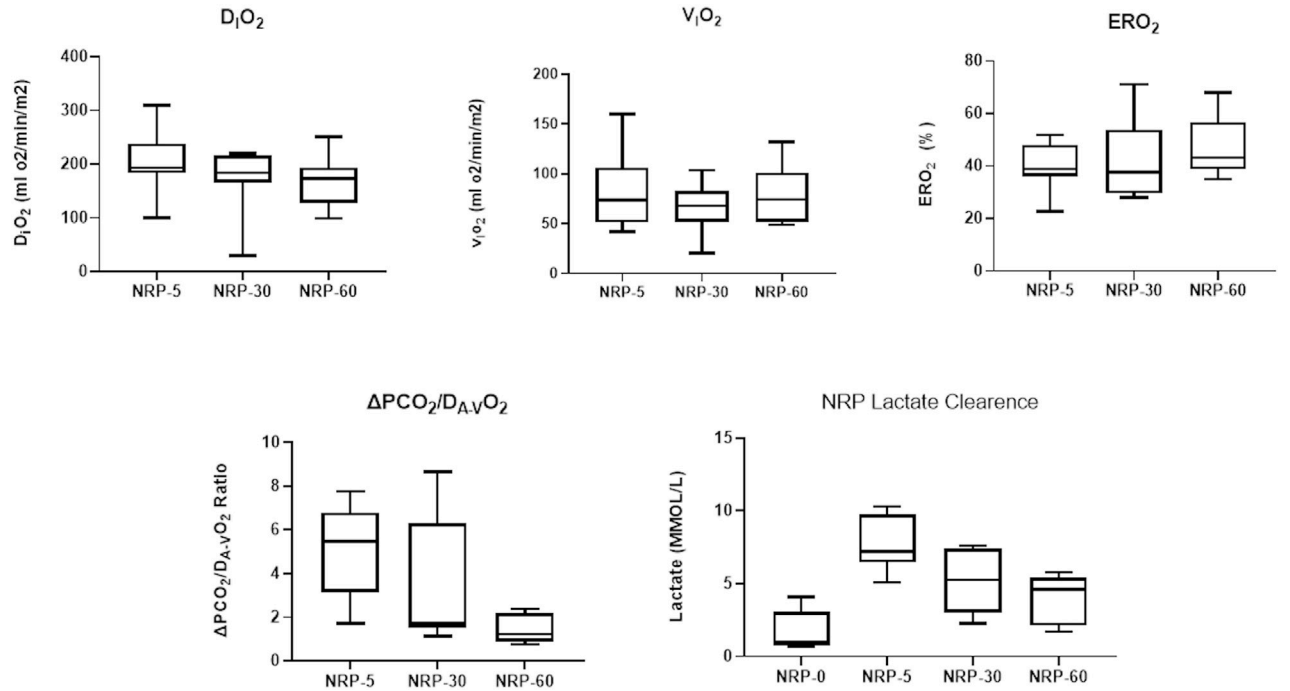


FIGURE 1 | Monitoring during NRP: Oxygen-derived parameters [indexed O_2 delivery (D_IO_2), indexed O_2 consumption (V_IO_2), O_2 extraction (ERO_2)], carbon dioxide-derived parameters (ΔpCO_2/DA-VO_2 Ratio), and lactate clearance were evaluated at different timepoints of NRP during donor procedure. NRP, normothermic regional perfusion.

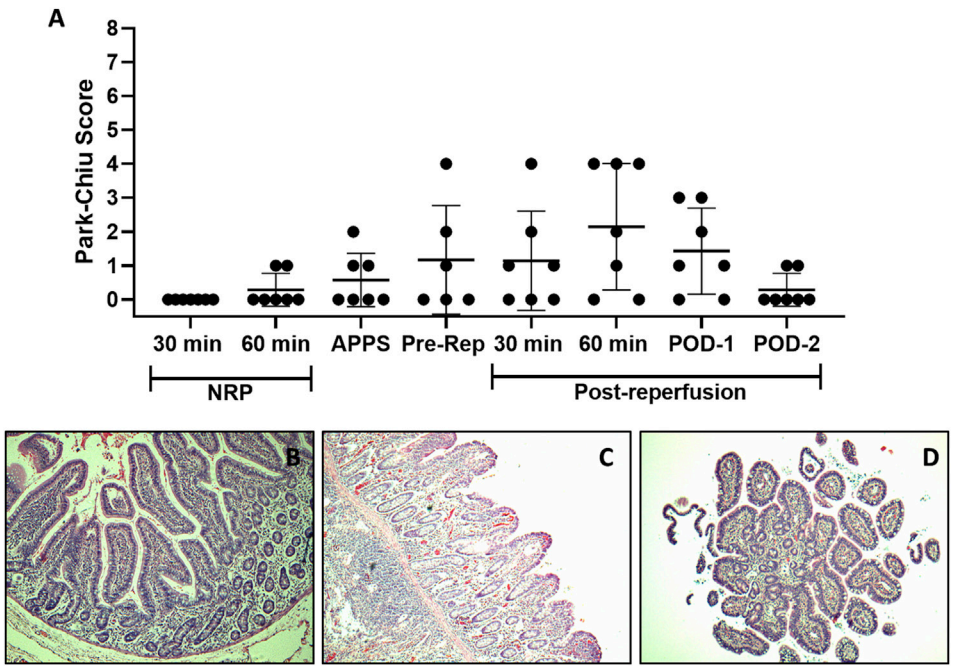


FIGURE 2 | Intestinal IRI analysis. Histological damage at different stages of the ITx procedure with DCD and NRP was studied using the PCS. Each point depicts a simple sample evaluation. **(A)** Microscopic representative images of an intestine after 60 min of NRP **(B)**, 60 min, **(C)** and 2 days after graft reperfusion **(D)** (H/E-stained samples, ×10). APPS, after perfusion with preservation solution; ITx, intestinal transplantation; DCD, donation after circulatory death; NRP, normothermic regional perfusion; H/E, hematoxylin–eosin.

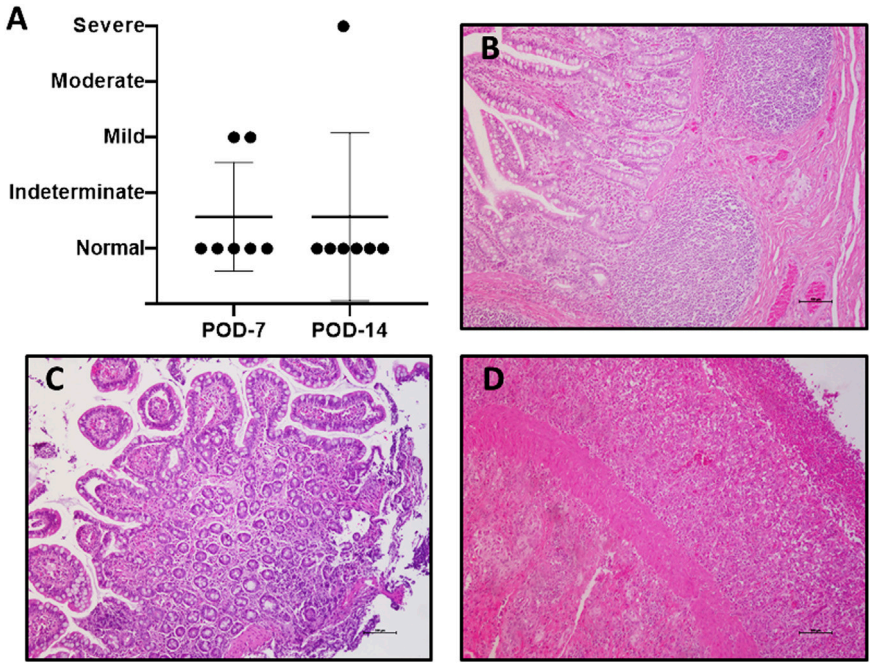


FIGURE 3 | Graft rejection. Histological study in search of acute cellular rejection was performed using the Wu scheme **(A)**. Most of the evaluated samples were normal **(B)**. A few cases present microscopic alterations, consistent with mild rejection, **(C)** and only one case of severe rejection on POD 14 was observed **(D)**. (H/E-stained samples, ×10). POD, postoperative day; H/E, hematoxylin–eosin.

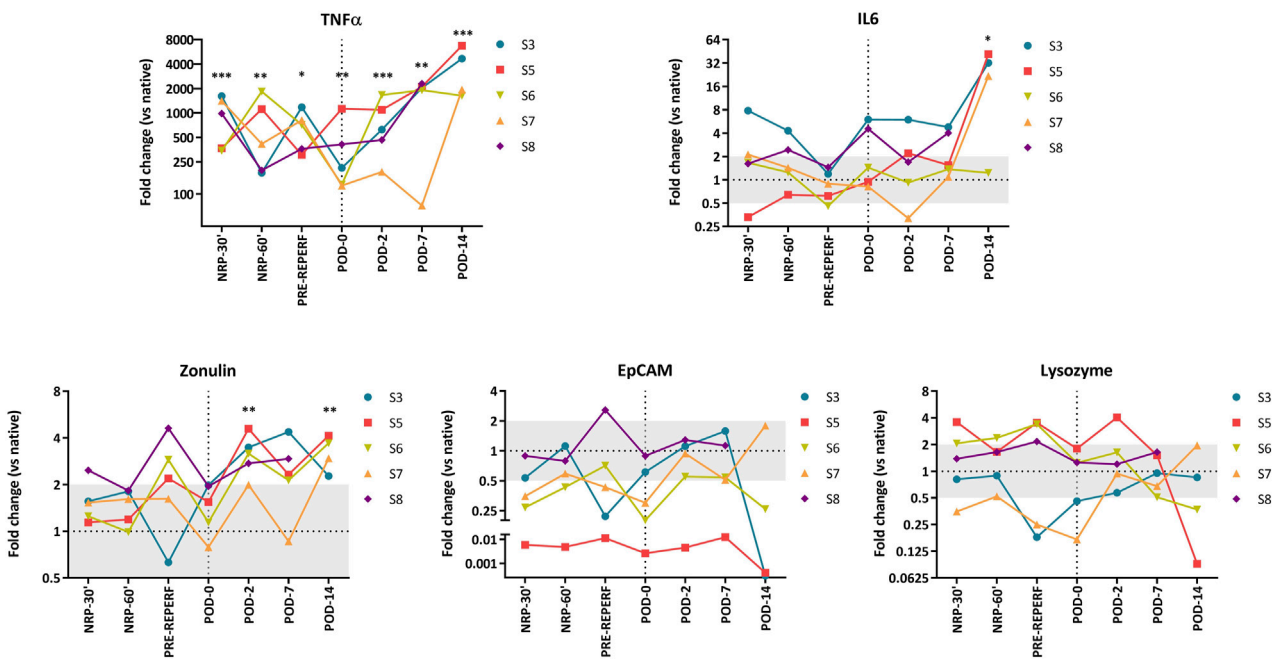


FIGURE 4 | Fold change gene expression values. Each line represents values from a single animal. Comparison between grafts and native intestines was made using an unpaired t-test. Significant *p*-values are plotted as follows: *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

(POD 0), except TNF-α, which remained high ($p = 0.002$). Interestingly, no relevant changes in gene expression were observed when compared to the pre-reperfusion samples, and IL-6 expression levels showed only a slight increase ($p = 0.055$).

During the post-transplantation period, TNF-α expression persisted above the native intestinal levels, differing significantly at every time point studied (POD 2, $p < 0.001$; POD 7, $p = 0.002$; POD 14, $p < 0.001$). Additionally, upregulation of this gene became

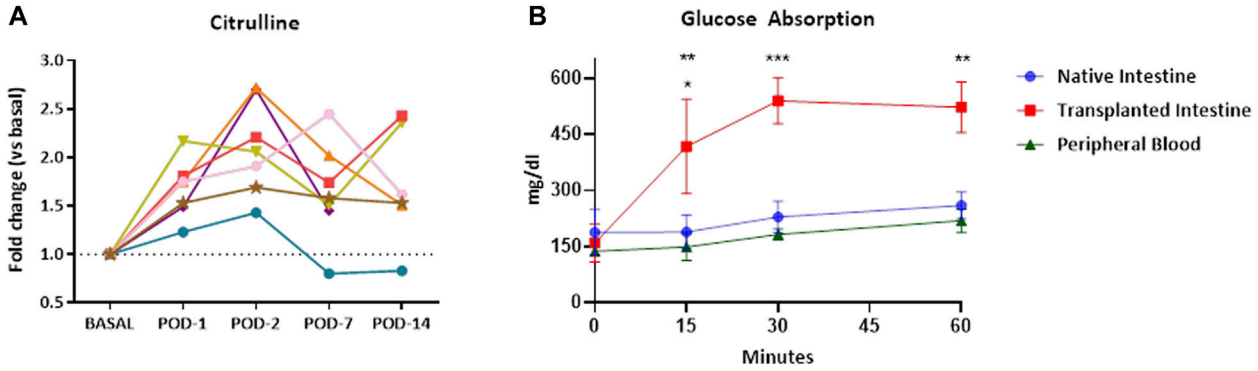


FIGURE 5 | Graft functional evaluation. Citrulline plasma levels increase after ITx. Each line represents values from a single recipient **(A)**. The glucose absorption test of the transplanted intestines from DCD procedures was performed by introducing a glucose solution into them. The transplanted intestine was observed to have a good absorptive capacity. The glucose level increases peripherally and in native intestines as it is distributed through the body **(B)**. Results are presented as the mean ± SEM. Significant *p*-values are plotted as follows: *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. ITx, intestinal transplantation; DCD, donation after circulatory death; SEM, standard error of the mean.

evident on POD 7, becoming significant on POD 14 ($p = 0.003$). The maximum expression values were also observed at this point, with 1600–6700-fold changes. A similar, albeit less prominent, dynamic was observed for IL-6, which showed stable expression until POD 7, followed by an abrupt increase (except in one animal) at the endpoint ($p = 0.037$ versus controls).

The epithelial-related genes EpCAM and zonulin showed significant upregulation on POD 2 compared to POD 0 samples ($p = 0.008$ and $p = 0.005$, respectively). On POD 7, these differences persisted ($p = 0.019$ for both) and remained until POD 14 for zonulin ($p = 0.042$). Moreover, zonulin expression remained significantly higher than that in native bowels throughout the post-transplantation period (POD 2: $p = 0.003$; POD 7: $p = 0.055$; POD 14: $p = 0.004$). LYZ expression varied among the animals, and it seemed to be lower than that in native intestines; however, it was not significantly different from the POD 0 samples.

Intestinal Graft Functional Evaluation

Citrulline blood levels increased significantly in all cases in the first 2 days post-ITx ($p = 0.0003$). Despite differences between the animals after this point, only animal 3 showed levels below the baseline, which corresponded to severe acute cellular rejection in the histological examination (**Figure 5A**). Graft-absorption capacity on POD 14 was evident 15′ after glucose administration, reaching maximum glucose levels at 30′. Elevated graft blood glucose levels were maintained throughout the study period (**Figure 5B**). In addition, blood samples from the peripheral circulation and native small bowel showed a slow and gradual increase in glucose levels, confirming that the increase in the glucose level is due to the graft's glucose absorption and its distribution to the general circulation.

DISCUSSION

Intestinal grafts from DCD are universally considered non-viable for transplantation, mainly because of the vulnerability of the intestine to IRI [18] and the relationship between graft quality and loss of barrier function. However, recent improvements in DCD and ITx have opened a new window of opportunity to increase the limited pool of donors. Our experimental study aimed to further validate the potential of DCD grafts for clinical ITx, enhancing knowledge of DCD and visceral transplantation and increasing the scientific evidence that supports the use of this type of donor. Under experimental settings, fWIT, a critical factor for graft viability, was similar to that reported in other studies [19]. The CIT was shorter than the usual time in clinical settings, only for logistic reasons, and was far from the established 9 h limit for acceptance [12].

We used large white pigs for this model. Numerous studies have confirmed this species to be the most suitable research model for human ITx because of its similarity in size, physiology, immunology, organ development, and function [20]. Our immunosuppressive treatment scheme with daily tacrolimus has already demonstrate its efficacy [21, 22].

Performing a heterotopic transplant allows the study of the viability and function of the grafts without affecting the digestive function of the animals while avoiding the possible surgical complications that could arise from an orthotopic transplant. In addition, it allows possible pathological events that may occur in the graft to be tolerated much better [23]. Therefore, in our experience this modality allows the animals to maintain a good general health status without affecting the study objectives.

Despite the importance of intestinal IRI, the effect of NRP has been scarcely evaluated. Guo et al. demonstrated that intestinal conditioning with 1 h of extracorporeal membrane oxygenation (ECMO) improved the energy status and graft viability in a pig model [24]. Nevertheless, these improvements were compromised gradually as the extracorporeal support used by Hamed et al. [25] demonstrated the viability and function of the intestine from DCD in an experimental model with *ex vivo* normothermic perfusion for 4 h. Unfortunately none of these studies included transplantation as proof of viability. Guo et al. [26] published another well-designed study overcoming this limitation. They compared graft

viability and function after 7 days in three experimental groups: living donor, DCD (rapid technique), and DCD with ECMO. Cell apoptosis and endotoxin levels were lower and intestinal absorptive function was significantly better in the ECMO group than in the DCD group. They concluded that ECMO exerted a protective effect on IRI, probably by reducing caspase-3 protein expression and apoptosis. These results are consistent with our findings since the mucosal function and structure were almost normal for 7 days, and only minor histological changes appeared after 14 days, which might be attributed to incipient rejection. Moreover, our gene expression analysis demonstrated that TNF-α, zonulin, pCAM, and LYZ expressions at NRP-60′ were similar to those before reperfusion, indicating graft integrity during NRP and adding more valuable information regarding the recovery effect of ECMO support after DCD. Thus, our findings and the previous literature suggest that NRP transforms the deleterious influence of IRI inherent to DCD into an "ischemic preconditioning" phenomenon to improve intestinal graft viability. However, Softeland et al. [27] performed a comparative study between species on the development of intestinal epithelial lesions and observed that these lesions develop more slowly in pigs. Although it is a study of non-transplanted intestinal samples, this fact should be considered when interpreting the findings.

Additionally, the molecular study showed a >250-fold-increase in TNF-α expression at the beginning of the procedure. Hypoxia could upregulate the TNF-α pathway through NF-κB activation after increasing mitochondrial reactive oxygen species in innate immune cells [28]. In addition, intestinal epithelial cells (IECs) respond to low-oxygen conditions by secreting TNF-α, which is derived from epithelial barrier disruption [29, 30]. One of the mechanisms by which TNF-α directly increases permeability is through the induction of occludin endocytosis via myosin light chain kinase activation [31]. Although the PCS for most samples showed no or mild damage in the intestinal mucosa and no clinical manifestations of barrier disruption, the molecular profile of epithelial-related genes demonstrated certain changes. Thus, a significant increase in EpCAM (an IEC integrin), and especially zonulin (a tight junction complex protein), was evident within the first week after transplantation, probably as a compensatory mechanism for this TNF-α-mediated permeability disruption. Based on these findings, Oltean et al. [32] demonstrated the negative effect of IRI and loss of tight junctions on barrier function. Since most experimental animals showed no clinical manifestations of intestinal barrier disruption and had a good absorptive function and elevated citrulline levels (except in the case of severe rejection), these changes in the molecular pattern may be a part of the regular IRI and the subsequent healing process without any pathological meaning. TNF-α, zonulin and IL-6 showed maximum levels at the endpoint. This molecular-level proinflammatory state may reflect an early rejection stage because it was correlated with an "indeterminate for rejection" diagnosis in one out of the five samples studied and a "severe rejection" diagnosis in another; however, these markers are highly non-specific, and other inflammatory or infectious events may justify these findings.

Since one of the advantages of NRP and *ex vivo* perfusion is the possibility of graft intervention, further studies should address the administration of anti-TNF-α during these procedures. The use of anti-TNF-α monoclonal antibodies as induction therapy for ITx has shown beneficial effects by reducing the IRI inflammatory response in experimental rat models and humans [22, 33, 34]. In addition to IRI prevention, other strategies directly targeting the immune system, such as the administration of rabbit anti-rat thymoglobulin and fludarabine, which were recently published, could also be facilitated by NRP or *ex vivo* perfusion [35, 36].

The glucose absorption test demonstrated that the grafts' absorptive function seems not affected. Glucose solution produced an immediate and significant increase in the glucose level from grafts' draining veins. This elevation also occurred progressively in peripheral blood and in the veins from the native intestine, demonstrating its distribution throughout the body. This test has already been employed in other studies, sometimes with components other than glucose [17, 26]. As observed in previous studies, the use of NRP seems to be crucial to maintaining this function [25].

Regarding citrulline levels, their increase was expected since the animal received a heterotopic transplant. This increase does not have to be perfectly double, since it may have a slight decrease related to transient inflammatory events (such as the transplant itself), or to the fact that the intestine is not in use [37] or is shorter (duodenum and last part of the ileum were removed). However, a pronounced decrease seems to be related to epithelial cell apoptosis in acute cellular rejection as seen in animal 3. This is consistent with the findings of our group and others [37, 38]. It is unclear if the decreases observed in some animals could be a predictive factor for rejection since the inter-individual variability makes it difficult to clarify its value.

The difficulties and limitations of extrapolating animal research to clinical practice are well known. Our study did not include randomization since only one group was included in our proof-of-concept methodology, which is a major limitation. Previous studies have covered the differences among living donor and DCD, NRP, and *ex vivo* perfusion; therefore, repeating the same control groups was not justified ethically since this was intended to be a test to prove the feasibility of the procedure. Additionally, our study has other limitations such as the lack of more complex molecular tests and a short cold ischemia time. Nevertheless, to our knowledge, this is the first study to conduct experimental transplant of intestines from DCD with such a long and detailed follow-up, considering not only IRI but acute cellular rejection. Thus, our study could be the basis for the development of new studies to perform a proper comparison between groups in the future. A new meta-analysis would undoubtedly be warranted when more literature regarding NRP becomes available [39].

In conclusion, our experimental assay showed that NRP yielded viable intestinal grafts from DCD. Considering the feasibility, histologic, functional, and molecular results, we hypothesized that NRP could revert the deleterious IRI inherent to DCD facilitating graft viability. The preclinical model developed by us and presented in this article provided the evidence to perform the first multiviceral transplant with DCD in humans worldwide, with encouraging results in terms of graft and recipient outcome [13]. Despite recent promising results in both the clinical and experimental field, more

evidence is needed to standardize the use of DCD-derived grafts in intestinal and multivisceral transplantation and reduce waiting list times.

DATA AVAILABILITY STATEMENT

Original datasets are available upon request to the corresponding author.

ETHICS STATEMENT

The animal studies were approved by the Animal Welfare Ethics Committee (PROEX 58.7/20) and complied with the EU and Spanish Directives on experimental animals (63/2010 EU, RD 53/2013). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was not obtained from the owners for the participation of their animals in this study because the animals used do not have an owner.

AUTHOR CONTRIBUTIONS

JS, AA, PaS, and FH: Design of the experiment, development of intestinal transplant procedures, data analysis, writing of the manuscript. Paper submission. PT: Analysis of gene expression, critical review of the manuscript. PB: NRP manager, data analysis. OC and MV: sample processing, histopathological analysis. FR, AB, AS, CZ, CD, OR, and PaS: performed the intestinal transplant procedures. Monitoring of transplanted animals. CL: Anesthesia and maintenance of animals during the experimental procedure. Experimental Design and Manuscript Writing. PiS, ER, GP, and ML: Monitoring of the intestinal graft, performance of endoscopies, data analysis. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

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

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11518/full#supplementary-material

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BRIEF RESEARCH REPORT
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Beyond the Concepts of Elder and Marginal in DCD Liver Transplantation: A Prospective Observational Matched-Cohort Study in the Italian Clinical Setting

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Donation after circulatory determination of death (DCD) is a valuable strategy to increase the availability of grafts for liver transplantation (LT). As the average age of populations rises, the donor pool is likely to be affected by a potential increase in DCD donor age in the near future. We conducted a prospective cohort study to evaluate post-transplantation outcomes in recipients of grafts from elderly DCD donors compared with younger DCD donors, and elderly donors after brainstem determination of death (DBD). From August 2020 to May 2022, consecutive recipients of deceased donor liver-only transplants were enrolled in the study. DCD recipients were propensity score matched 1:3 to DBD recipients. One-hundred fifty-seven patients were included, 26 of whom (16.6%) were transplanted with a DCD liver graft. After propensity score matching and stratification, three groups were obtained: 15 recipients of DCD donors ≥75 years, 11 recipients of DCD donors <75 years, and 28 recipients of DBD donors ≥75 years. Short-term outcomes, as well as 12 months graft survival rates (93.3%, 100%, and 89.3% respectively), were comparable among the groups. LT involving grafts retrieved from very elderly DCD donors was feasible and safe in an experienced high-volume center, with outcomes comparable to LTs from younger DCD donors and age-matched DBD donors.

Keywords: liver transplantation, elderly donors, donation after circulatory determination of death, donation after brainstem death, liver transplantation outcomes

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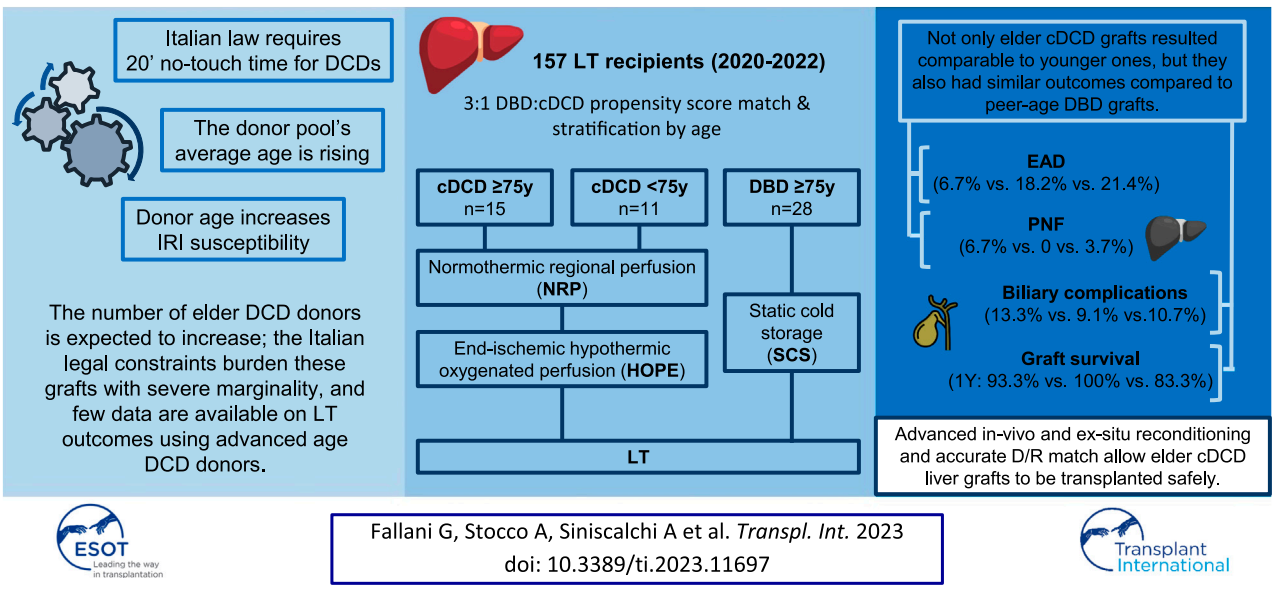
Abbreviations: aWIT, asystolic Warm Ischemia Time; CCI®, Comprehensive Complication Index; CPT, Cold Preservation Time; DBD, Donors after Brainstem Death; DCD, Donation after Circulatory determination of Death; EAD, Early Allograft Dysfunction; fWIT, functional Warm Ischemia Time; HCC, HepatoCellular Carcinoma; HOPE, Hypothermic Oxygenated PErfusion; IQR, Inter-Quartile Range; IRI, Ischaemia-Reperfusion Injury; ISP, In-Situ Perfusion; LT, Liver Transplant; MAP, Mean Arterial Pressure; MELD, Model for End Stage Liver Disease; NRP, Normothermic Regional Perfusion; OPTN, Organ Procurement and Transplantation Network; PNF, Primary Non Function; PRS, Post Reperfusion Syndrome; PSM, Propensity Score Match; SCS, Static Cold Storage; tWIT, total Warm Ischemia Time; WIT, Warm Ischemia Time.



GRAPHICAL ABSTRACT |

INTRODUCTION

Liver transplantation (LT) is considered the treatment of choice for patients with end-stage liver disease. The inclusion of extended criteria donors (ECDs) and donors after circulatory determination of death (DCD) is growing in the attempt to address the critical gap between donors and recipients. DCD donors are a valuable source of grafts, even if concerns have been raised about potentially impaired outcomes related to prolonged warm ischemia time (WIT). Nevertheless, several recent studies have described acceptable results after transplantation involving those donors [1, 2].

According to Italian law [3, 4], death can only be declared after 20 min of lack of any cardiac electrical activity. A strategy of *in-situ* normothermic regional perfusion (NRP) [5]—aimed to interrupt the prolonged ischemia and to maintain a near-physiologic environment during retrieval [6]—is considered mandatory in the Italian scenario. Moreover, to further mitigate ischemia-reperfusion injury (IRI), most Italian transplant centers also implement end-ischemic hypothermic oxygenated perfusion (HOPE) on DCD liver grafts [7].

The constant increase in the average age of the general population also implies a subsequent change in the demography of the organ donor pool. This phenomenon is most likely to affect the pool of DCD donors too, and data on LT from advanced-age DCD donors are emerging in the literature [8–10].

Even if donor age impacts the outcomes of LT [11–13], the acceptance of older DBD donor grafts could be effective in selected recipients [14]. Thus, this study aims to compare the outcomes of LTs involving elder DCD donors with both younger DCD donors and elder DBD donors in the specific context of an experienced Italian transplant center.

METHODS

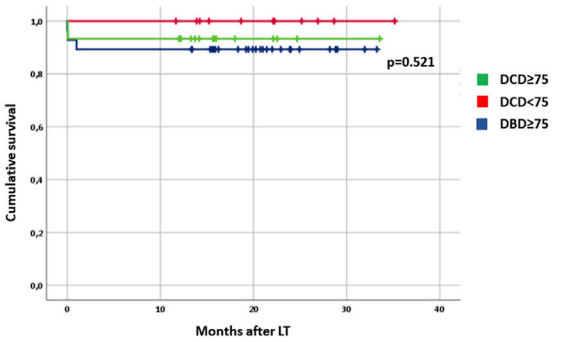
We conducted a single center prospective observational cohort study including consecutive recipients of liver-only transplantation of controlled DCD (cDCD) donors [15, 16] from August 2020 to May 2022. Based on pre-transplant donor and recipient characteristics, cDCD liver recipients underwent propensity score matching (PSM) with recipients of liver-only transplantation from DBD donors in a 1:3 DCD:DBD ratio. The study population was stratified by age to compare outcomes of grafts from younger cDCD donors (<75 years-old) and elder cDCD donors (≥75 years-old), with elder DBD donors (≥75 years-old).

Informed consent was obtained from all the recipients. The study was approved by the Institutional Review Board (Comitato Etico—Area Vasta Emilia Centro, CE-AVEC, protocol no. 895/2021/Oss/AOUBo).

Donor Management and Procurement

The technique of abdominal organ procurement in cDCD donors in Italy has been previously described in detail [17], and the timeline of events is reported in **Supplementary Figure S1**.

After circulatory arrest and no-touch time, NRP is initiated, targeting lactate clearance, pH normalization, normocapnia, and avoidance of hyperoxemia; hemoglobin concentration is



Donor type	6-months graft survival*	12-months graft survival**	24-months graft survival***
DCD donors ≥75 years	93.3%	93.3%	93.3%
DCD donors <75 years	100%	100%	100%
DND donors ≥75 years	89.3%	89.3%	89.3%

FIGURE 1 | Graft survival rates stratified by donor type and age. DCD: donation after circulatory determination of death; DBD, donation after brainstem death. * number of patients at risk: 54; ** number of patients at risk: 54; *** number of patients at risk: 14.

maintained above 8 g/dL and hyperglicemia is corrected to facilitate organ resuscitation. Since NRP starts every 30′, blood gas analysis and liver enzyme measurement are performed throughout extracorporeal perfusion. NRP is maintained for at least 60–90 min to assess the organ functional recovery; the criteria of viability include fWIT<60 min, pH normalization and stability, progressive lactate decrease, and SGOT/SGPT decrease (usually evident after 30 min of NRP). When the metabolic and perfusion profiles of the donors are considered optimal, the surgical procedure is initiated: first, liver (and kidney) biopsies are obtained, then the warm dissection phase of the procurement is performed. In our practice, liver biopsy is a cornerstone to assess organ viability, as the presence of extensive lobular necrosis represents a contraindication to further proceed with retrieval.

NRP ends with *in-situ* cold preservation (ISP): once the organs are retrieved, they are put in static cold storage (SCS) to be transported to the transplant center where bench surgery is performed, and HOPE is implemented until implantation.

Conversely, liver grafts from DBD donors were retrieved with standard technique and preserved with SCS until implantation.

Outcome Definitions and Measures

For DCD donors, total WIT (tWIT) was defined as the timeframe occurring between WLST and NRP initiation, while functional WIT (fWIT) was defined as the timeframe between hypotension (systolic arterial pressure below 50 mmHg) or desaturation (peripheral oxygen saturation below 70%)—whichever occurring first—and NRP initiation. Cold preservation time (CPT) was defined as the interval from aortic cross-clamping/ISP and portal reperfusion upon LT, thus including both SCS and HOPE duration (**Figure 1**).

Complications were defined as any event deviating from the expected postoperative course that does not imply failure to cure [18]. For each patient, the postoperative complications were graded according to the Clavien-Dindo classification [18] and summarized through the Comprehensive Complication Index (CCI®) [19]; major complications were defined as Clavien-Dindo grade ≥3A.

Post-reperfusion syndrome (PRS) was defined according to Aggarwal et al. [20] Primary non-function (PNF) of the graft was defined according to the Organ Procurement and Transplantation Network (OPTN) criteria [21], while early allograft dysfunction (EAD) was defined according to the criteria proposed by Olthoff et al. [22] Severe acute kidney injury was defined according to the Kidney Disease: Improving Global Outcomes (KDIGO) clinical practice guideline [23].

Statistical Analysis

Qualitative variables were reported as absolute values and percentages, whereas quantitative variables were reported as median values and IQR or mean ± SD as appropriate. Univariate analysis was performed using Pearson's chi-squared or Fisher's exact test for categorical variables, depending on the sample size, and with Student's t-test or Mann-Whitney U test for continuous variables, depending on their distribution.

PSM was performed through logistic regression analysis to adjust for clinically confounding factors between groups, including donor age, recipient age at the time of transplantation, MELD score, and hepatocellular carcinoma (HCC) as an indication for LT. DCD and DBD recipients were matched in a 1:3 ratio with the closest estimated propensity. Survival curves were plotted with the Kaplan-Meier estimators and compared through the log-rank test.

Differences of $p < 0.05$ were considered significant.

All the statistical analyses were performed using IBM SPSS, version 26.0 (IBM Corporation—Armonk, NY).

RESULTS

From August 2020 to May 2022, 183 liver grafts were offered for transplantation to the Department of Hepatobiliary Surgery and Transplantation of Policlinico Sant'Orsola in Bologna. Of these, 30 (16.4%) came from DCD donors: four were declined, with an acceptance rate of 86.7%. Two livers were discarded due to a combination of prolonged fWIT, and poor liver and metabolic parameters during NRP (as per our aforementioned criteria). The remaining two livers were refused as malignancy was detected during the retrieval procedure. The remaining 153 liver grafts offered for transplantation (83.6%) came from DBD donors. Of these, 12 were discarded for marginality upon senior transplant surgeon judgment, with an acceptance rate of 92.2%. Other 10 liver grafts were allocated for multiorgan transplantation or re-transplantation and thus excluded from the study.

The final population included in the study consisted of 157 recipients, 131 of which (83.4%) were transplanted with grafts from DBD donors, and 26 (16.6%) with grafts from cDCD donors.

Donor and Recipient Pre- and Post-operative Characteristics

Compared with DBD donors, DCDs had higher median age (75 years vs. 63 years, $p = 0.018$); notably, most DCD donors

TABLE 1 | Baseline preoperative recipients' characteristics and donor characteristics before and after propensity score matching.

Variables	Before PSM			After PSM		
	DCD-LT (*n* = 26)	DBD-LT (*n* = 131)	*p*	DCD-LT (*n* = 26)	DBD-LT (*n* = 78)	*p*
Recipient age in years, median [IQR]	61 [56–64]	58 [53–64]	0.214	61 [56–64]	59 [54–65]	0.435
Recipient BMI in kg/m^2, median [IQR]	23.8 [22.3–29.2]	26 [23.1–28.1]	0.340	23.8 [22.3–29.2]	25.5 [22.8–28.1]	0.538
Indication for LT						
Hepatocellular carcinoma, n (%)	18 (69.2)	54 (41.2)	**0.009**	18 (69.2)	41 (52.6)	0.137
Virus-related cirrhosis, n (%)	10 (38.5)	52 (39.7)	0.906	10 (38.5)	31 (39.7)	0.908
Alcohol-relates cirrhosis, n (%)	8 (30.8)	52 (39.7)	0.392	8 (30.8)	31 (39.7)	0.413
NAFLD, n (%)	3 (11.5)	24 (18.3)	0.572	3 (11.5)	11 (14.1)	1
Cholestatic liver disease, n (%)	3 (11.5)	17 (13)	1	3 (11.5)	12 (15.4)	0.756
Acute liver failure, n (%)	0	6 (4.6)	0.590	0	2 (2.6)	1
Other, n (%)	4 (15.4)	27 (20.6)	0.541	4 (15.4)	15 (19.2)	0.766
Previous abdominal surgery, n (%)	17 (65.4)	76 (58.5)	0.511	17 (65.4)	51 (66.2)	0.937
Previous liver resection, n (%)	5 (19.2)	10 (7.6)	0.077	5 (19.2)	8 (10.3)	0.303
TIPSS, n (%)	3 (11.5)	12 (7.9)	0.463	3 (11.5)	3 (3.8)	0.163
Portal thrombosis, n (%)	4 (15.4)	24 (18.3)	1	4 (15.4)	12 (15.4)	1
Platelet count *10^3/μL, median [IQR]	103 [63–161]	72 [46–133]	0.140	103 [63–161]	96 [61–154]	1
MELD at transplant, median [IQR]	10 [8–14]	15 [10–24]	**<0.001**	10 [8–14]	12 [9–15]	0.297
Recipient comorbidities						
Diabetes mellitus, n (%)	10 (40)	39 (29.8)	0.321	10 (40)	22 (28.2)	0.267
Cardiovascular disease, n (%)	3 (11.5)	28 (21.4)	0.250	3 (11.5)	16 (20.5)	0.390
Respiratory disease, n (%)	6 (23.1)	26 (19.8)	0.709	6 (23.1)	15 (19.2)	0.672
Renal disease, n (%)	3 (11.5)	18 (13.7)	1	3 (11.5)	7 (9)	0.708
Donor age in years, median [IQR]	75 [64–78]	63 [50–75]	**0.018**	75 [64–78]	69 [56–79]	0.367
Donor BMI in kg/m^2, median [IQR]	26.2 [23.1–29.8]	25.7[23.8–27.8]	0.797	26.2 [23.1–29.8]	25.9 [24.2–29.1]	0.901

DCD-LT, donation after circulatory determination of death liver transplantation; DBD-LT, donation after brainstem death liver transplantation; IQR, interquartile range; BMI, body mass index; NAFLD, nonalcoholic fatty liver disease; TIPSS, transjugular intrahepatic portosystemic shunt; MELD, model for end-stage liver disease.
Bold values highlight statistical significance.

were 75 years or older. Recipients of DCD liver grafts had lower MELD scores (10 vs. 15, $p < 0.001$), and were more often transplanted due to HCC (69.2% vs. 41.2%, $p = 0.009$). Full demographic and clinical characteristics of recipients and donors are summarized in **Table 1**.

After PSM, the population resulted in 26 DCD donors and 78 DBD donors; the subsequent analysis demonstrated the comparability of baseline characteristics (**Table 1**).

DCD grafts had a median tWIT of 45 min and a median fWIT of 40 min, with a median timeframe of 6 min from death declaration to NRP initiation. NRP had a median duration of 209 min and end-ischemic HOPE had a median duration of 105 min. No significant differences have been observed between elder and younger DCD donors in terms of tWIT, fWIT, NRP duration, metabolic and functional parameters during NRP (pH, lactates, SGOT, SGPT), bioptic findings, or HOPE duration. These results are summarized in **Supplementary Table S1**. Grafts from DCD donors underwent shorter CPT (345 vs. 388 min, $p = 0.010$) and shorter duration of transplant (430 vs. 470 min, $p = 0.040$).

The analysis of postoperative data showed that recipients of livers from DCD and DBD donors had comparable results in terms of surgical complications, length of hospital stay, and graft function (**Table 2**).

Post-Transplant Outcomes According to Donor Type and Donor Age

After stratification by age, three subgroups were identified: 15 recipients of DCD donors ≥75 years, 11 recipients of DCD donors <75 years, and 28 recipients of DBD donors ≥75 years. No significant differences were evident in terms of CPT, surgical complications, length of hospital stay, and graft function. One recipient of an elder DCD donor experienced PNF and was successfully retransplanted. Amongst the elder DBD recipients two have been retransplanted due to PNF in one case and hepatic artery thrombosis in the other; another recipient of an elder DBD donor died a few hours after LT due to massive myocardial infarction. Altogether, six patients developed biliary complications during the follow-up period (with comparable rates between groups), all consisting of anastomotic strictures with successful endoscopic management. The results are summarized in **Table 3**. Patients were followed up for a median of 19 months [IQR: 14–24 months] without any significant difference in terms of graft survival for the three subgroups (**Figure 1**).

DISCUSSION

The average age of donors in Italy is continuously increasing, with the median age rising from 57.7 in 2012 to 60.9 years in 2021. The number of donors over the age of 80 is also increasing, representing—to date—a consistent portion of the donor pool (13.5% in 2021). In parallel, the mean age of cDCD donors increased to 67 years, with 6% of donations coming from octogenarians. In the hypothesis of a steady trend, a progressive increase in elderly cDCD donors is expected, possibly representing an additional opportunity for transplant

TABLE 2 | Procedural and outcome data after propensity score matching.

Variables	DCD-LT (n = 26)	DBD-LT (*n* = 78)	*p*
Cold preservation time in minutes, median [IQR]	345 [314–393]	388 [344–473]	**0.010**
Reperfusion syndrome, n (%)	2 (7.7)	2 (2.7)	0.260
Transplant duration in minutes, median [IQR]	430 [381–493]	470 [429–523]	**0.040**
ICU stay in days, median [IQR]	4 [3–5]	3 [2–5]	0.116
Peak SGOT (POD 1–7) in U/L, median [IQR]	347 [259–1,026]	566 [289–1,361]	0.363
Peak SGPT (POD 1–7) in U/L, median [IQR]	495 [168–854]	519 [272–1,057]	0.416
Post-operative infectious complications, n (%)	7 (26.9)	20 (25.6)	0.896
Severe acute kidney injury, n (%)	1 (4)	4 (5.1)	1
Respiratory failure, n (%)	0	2 (2.6)	1
Post-operative haemorrhage, n (%)	0	1 (1.3)	1
Reintervention, n (%)	1 (4)	8 (10.3)	0.546
Hepatic artery thrombosis, n (%)	0	1 (1.3)	1
12 months biliary complications, n (%)	3 (11.5)	7 (9.1)	0.710
30 days acute cellular rejection, n (%)	0	3 (3.9)	0.570
90 days mortality, n (%)	0	2 (2.6)	1
Early allograft dysfunction, n (%)	3 (11.5)	17 (21.8)	0.250
Primary graft non function, n (%)	1 (4)	3 (3.9)	1
Re-transplantation, n (%)	1 (4)	5 (5.3)	0.678
Major complications, n (%)	4 (15.4)	16 (20.5)	0.566
Comprehensive Complication Index®, 75th percentile	29.6	30.8	0.487
Hospital stay in days, median [IQR]	15 [13–23]	15 [11–23]	0.919

DCD-LT, donation after circulatory determination of death liver transplantation; DBD-LT, donation after neurological determination of death liver transplantation; IQR, interquartile range; NRP, normothermic regional perfusion; HOPE, hypothermic oxygenated perfusion; ICU, intensive care unit; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; POD, postoperative day.
Bold values highlight statistical significance.

TABLE 3 | Procedural and outcome data after propensity score matching and stratification by donor type and age.

Variables	DCD≥75 [group 1] (*n* = 15)	DCD<75 [group 2] (*n* = 11)	DBD≥75 [group 3] (*n* = 28)	*p* (1 vs. 2)	*p* (1 vs. 3)
Cold preservation time in minutes, median [IQR]	335 [300–390]	350 [320–400]	360 [340–405]	0.336	0.097
Reperfusion syndrome, n (%)	2 (13.3)	0	1 (3.6)	0.492	0.275
Transplant duration in minutes, median [IQR]	425 [383–486]	480 [360–505]	469 [419–503]	0.568	0.221
ICU stay in days, median [IQR]	3 [3–5]	4 [3–5]	3 [2–5]	0.576	0.467
Peak SGOT (POD 1–7) in U/L, median [IQR]	429 [298–1,010]	305 [253–1,399]	478 [248–1,218]	0.494	0.904
Peak SGPT (POD 1–7) in U/L, median [IQR]	501 [159–825]	292 [176–1,572]	555 [210–1,007]	0.913	0.775
Post-operative infectious complications, n (%)	5 (33.3)	2 (18.2)	9 (32.1)	0.679	0.938
Severe acute kidney injury, n (%)	0	1 (9.1)	0	0.440	—
Respiratory failure, n (%)	0	0	0	—	—
Post-operative haemorrhage, n (%)	0	0	0	—	—
Reintervention, n (%)	1 (6.7)	0	3 (10.7)	0.874	0.909
Hepatic artery thrombosis, n (%)	0	0	1 (3.7)	—	1
12 months biliary complications, n (%)	2 (13.3)	1 (9.1)	3 (10.7)	0.774	0.807
30 days acute cellular rejection, n (%)	0	0	2 (7.4)	—	0.530
90 days mortality, n (%)	0	0	1 (3.7)	—	1
Early allograft dysfunction, n (%)	1 (6.7)	2 (18.2)	6 (21.4)	0.556	0.391
Primary graft non function, n (%)	1 (6.7)	0	1 (3.7)	0.874	1
Re-transplantation, n (%)	1 (6.7)	0	2 (7.4)	0.874	0.956
Major complications, n (%)	3 (20)	1 (9.1)	6 (21.4)	0.614	1
Comprehensive Complication Index®, 75th percentile	29.6	29.6	30.5	0.979	0.612
Hospital stay in days, median [IQR]	15 [13–23]	13 [12–23]	17 [13–24]	0.465	0.798

DCD-LT, donation after circulatory determination of death liver transplantation; DBD-LT, donation after neurological determination of death liver transplantation; IQR, interquartile range; NRP, normothermic regional perfusion; HOPE, hypothermic oxygenated perfusion; ICU, intensive care unit; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; POD, postoperative day.

candidates, especially for those at greater risk of drop-out or death while on the waiting list.

We reported the first Italian data involving a relatively large number of elder DCD donors; in particular, as far as we know, the 75 years age cut-off that we considered is higher than any other previously published and outreaches the eldest age reported in many reports on elderly DCD donors [8–10].

This study aimed to investigate whether utilizing very elderly DCD donors allows them to achieve comparable outcomes to younger DCD donors and peer-age DBD donors.

Since DCD in the Italian scenario entails severe ischemic burden on liver grafts, these donors have long been considered "marginal". Nevertheless, recent literature demonstrated a progressive alignment of post-transplant outcomes to those of DBD donors in terms of graft function and graft and recipient survival [24–26]. These findings may suggest that an incremental volume and expertise of the transplant team play a significant role in contributing to the success of DCD liver transplantation. Furthermore, some reports showed that accepting DCD donors or elderly donors, despite their perceived marginality, can significantly improve the survival of selected recipients [27–29].

Our results appear to be consistent with this recent evidence. Although recipients of DCD liver grafts are characterized by a lower MELD score (10 vs. 15, $p < 0.001$), and a higher prevalence of HCC as a primary indication to transplant (69.2% vs. 41.2%, $p = 0.009$), post-operative outcome results were comparable after adequate minimization of existing biases.

Despite the requirement for PSM, it is important to mention that the different utilization of DCD and DBD liver grafts in our case series reflects the contemporary trends from medical literature and the higher individual transplant benefit for patients at high risk of drop-out from the waiting list [27–29]. Consistently with this evidence, we usually match DCD donors with recipients who, despite stable liver function, have a high-risk of drop-out from the waiting list due to either oncological risk (e.g., recurrent or down-staged HCC) or infectious risk (e.g., recurrent cholangitis); donor age has little influence in our allocation algorithm, given that DCD donors are preferably accepted for patients with stable liver function, which can more easily tolerate the increased ischemic burden. As a result, outcomes of LT for both DCD and DBD donors not only appeared comparable after PSM, but they also showed similar results after stratification by age and type of donation. Specifically, recipients of very elderly DCD donors had homogenous results in terms of CPT, surgical complications, hospitalization, and graft function, compared to recipients of same-age DBD and younger DCD donors. Moreover, the rate of biliary complications was acceptable in all subgroups despite donors being at high risk, conversely to the previously reported data [30–32]. In our opinion, this resulted from the extensive use of HOPE for DCD grafts pre-conditioning, and from shorter CPT (345 min vs. 388 min, $p = 0.010$).

Considering the strict Italian legislation, the tWIT liver grafts are exposed to are strongly conditioned by the requirement of a 20-minute-long standoff period before NRP initiation. The outcomes of DCD liver transplantation observed in our cohort, besides being consistent with DBD transplantation, are also the result of meticulous donor management, coupling advanced strategies of *in-situ* and *ex-situ* perfusion strategies, and accurate, tailored donor-recipient matching. The improvement in donor and recipient management to minimize tWIT and CPT, starting with routine use of end-ischemic HOPE for DCD liver grafts, showed promising results in preventing and mitigating ischemic insults, and related post-transplant ischemia-reperfusion injury, ultimately leading to satisfactory results [33–36].

We can also assume that the strict Italian legislation [3, 4] aimed to overguarantee the respect of the so-called "dead-donor-rule"—played a major role in forcing transplant teams to pursue accurate management of both DCD donors and grafts in order to overcome the imposed procedural limitations.

Limitations

This study has some limitations. First, the need for case-matching, as well as the limited number of patients in the derived subgroups, affected our ability to draw definite conclusions. Nevertheless, the relatively limited size of the study group reflects a hopefully initial experience with very elderly DCD donors. The short duration of the study implied a limited follow-up for the patients with a late enrolment, although a minimum twelve-month follow-up was provided for all the included recipients. Finally, in this study, we compared grafts from elderly cDCD donors—exposed to extensive reconditioning through NRP and end-ischemic HOPE—with ECD liver grafts (at least according to donor age) from DBD donors, which have been implanted without advanced perfusion strategies. This approach might be considered a bias, as DBD grafts can benefit from HOPE too [35, 36], but it reflects the state of the art in DCD liver transplantation in Italy. In fact, under Italian legal and procedural circumstances, this approach enabled the safe transplant of organs potentially carrying a relevant ischemic burden. Moreover, its extensive use also achieved comparable outcomes between cDCD grafts and ECD grafts, with the latter still being transplanted without HOPE in the majority of transplant centers worldwide.

Conclusion

According to the preliminary results of our single center experience, the inclusion of very elderly cDCD donors in liver transplantation programs might provide acceptable outcomes, comparable to those achieved with younger cDCD donors, and with same-age DBD donors. With donor management and graft allocation and reconditioning becoming more and more accurate as further experience and evidence accumulate, the ability of clinicians to achieve optimal utilization and to improve transplantation outcomes for DCD grafts will be enhanced, overcoming existing concerns about these donors' perceived marginality.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by Comitato Etico - Area Vasta Emilia Centro (CE-AVEC). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Research conception: GF, AlS, MC, MR. Research design: GF, AlS, AnS, LT, MM, MC, MR. Data acquisition: GF, AlS, APS, AA, EP, MA. Data analysis: GF, AlS, MC, MR. Manuscript draft: GF, AlS, EP, MA, MC, MR. Critical revision: GF, AlS, AnS, APS, AA, EP, MA, LT, MM, MC, MR.

CONFLICT OF INTEREST

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

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11697/full#supplementary-material

Supplementary Figure S1 | Timeline of events preceding and following declaration of death with cardiocirculatory criteria Maastricht category 3 controlled donors. Agonal phase and fWIT start with hypotension (mean arterial pressure dropping below 50 mmHg) or desaturation (peripheral oxygen saturation dropping below 50%), whichever occurring first; tWIT and fWIT refer to the potential donor. NRP interrupts ischemic time. CIT refers to the graft; the time the graft is treated with hope is not to be considered functionally ischemic, despite the lack of hematic perfusion, as the liver is oxygenated. Times not to scale. WLST, withdrawal of life sustaining treatments; tWIT, total warm ischemia time; fWIT, functional warm ischemia time (hypoxemic and/or hypotensive); NRP, normothermic regional perfusion; ISP, in situ-preservation; SCS, static cold storage; CPT, cold preservation time; HOPE, hypothermic oxygenated perfusion; LT, liver transplantation.

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BRIEF RESEARCH REPORT
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Association Between Pre-Transplant Oral Health and Post-Liver Transplant Complications

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Oral disease is linked with systemic inflammation and various systemic conditions, including chronic liver disease. Liver transplantation (LT) candidates often need dental infection focus eradication, and after LT, there is high risk of many inflammation-related complications. We studied whether pre-LT dental status is associated with the occurrence of post-LT complications. This study included 225 adult LT recipients whose teeth were examined and treated before LT, and 40 adult LT recipients who did not have pre-LT dental data available. Data on post-LT complications were collected from the national liver transplant registry and followed up until the end of July 2020. Worse pre-LT dental status was associated with a higher risk of acute rejection post-LT compared to patients with good dental status. Worse dental status was also associated with higher 1-year-post-LT ALT levels and lower albumin levels. In conclusion, poor pre-LT oral health seems to associate with an increased risk of post-LT acute rejection and with elevated ALT levels and decreased albumin levels, suggesting an effect on post-LT liver health. Therefore, prevention and treatment of oral and dental diseases should be promoted early in the course of liver disease.

Keywords: oral health, liver transplantation, acute rejection, infection foci, oral disease

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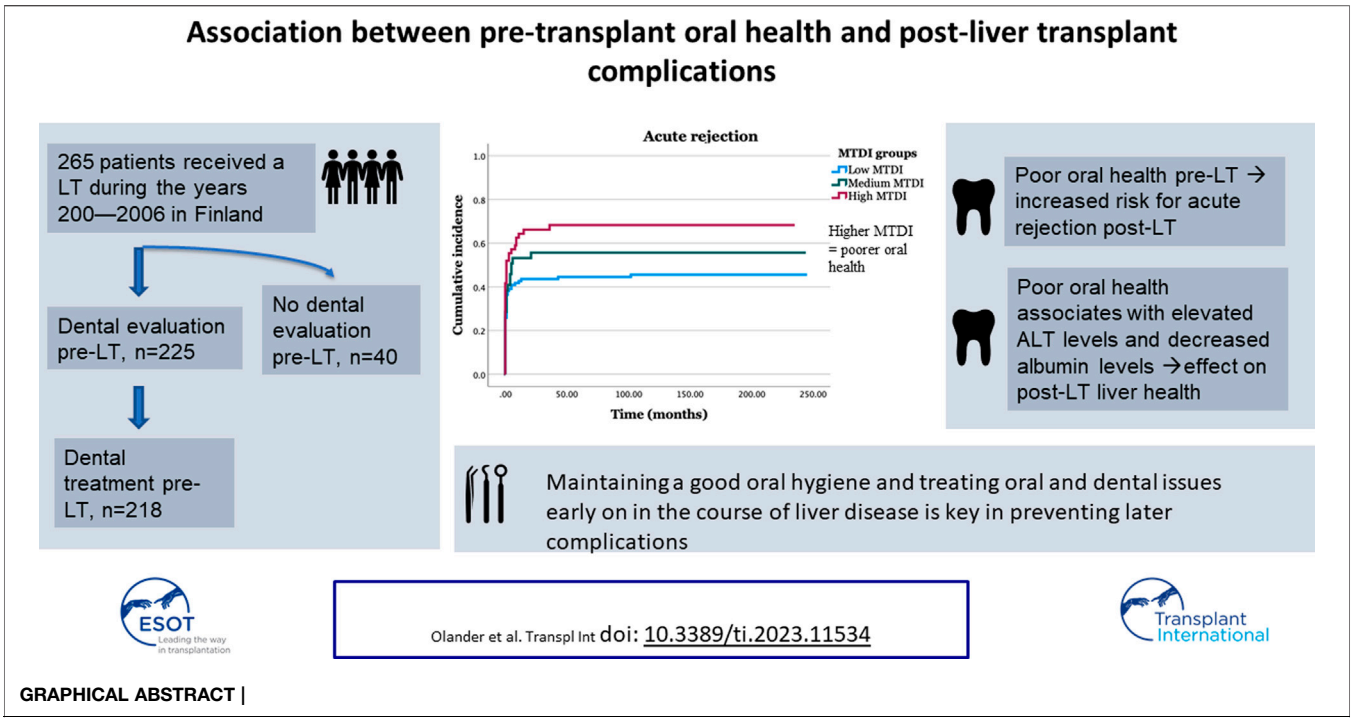
INTRODUCTION

A relationship between poor oral health and liver disease has been presented previously. The systemic inflammation caused by oral infections seems to accelerate the progression of chronic liver disease (CLD) [1–3], and CLD, in turn, seems to affect oral diseases [4, 5]. Oral infection foci need to be eliminated before liver transplantation (LT) to avoid severe systemic complications [6]. A significant link between a lack of pre-LT dental treatment and post-LT systemic infections has been suggested in patients with acute liver failure (ALF) [7]. In a study by Göbel et al. [8], dental infection foci was associated with a higher risk of pre- and post-LT bacterial infections. After transplantation, patients use immunosuppressants for the rest of their lives. During the first year post-LT in particular, but also later, patients are prone to severe, even fatal, infections [9, 10].

The need for dental and periodontal treatment has proved to be high among LT recipients, and poor oral hygiene [4, 11, 12]. Compared to healthy controls, LT candidates are shown to have a higher prevalence of apical periodontitis and other oral diseases (i.e., dental caries, periodontal and



GRAPHICAL ABSTRACT |

oral mucosal diseases) [13, 14]. Furthermore, the presence of oral diseases has been linked to a higher risk of mortality in liver cirrhosis patients [15, 16]. Although the connection between oral health and liver disease is increasingly being studied, studies about the connection between oral infection foci and post-LT complications remain scarce.

Common complications after LT include graft rejection, biliary strictures, and various infections [17]. LT recipients also have increased risks of many diseases, such as diabetes, hypertension, cardiovascular disease, and cancer [17]. The risk factors for these complications in LT recipients are only partly known.

The objective of this study was to examine the connection and impact of pre-LT oral health on post-LT complications, such as graft rejection, cardiovascular disease, infections, cancer, and mortality. We hypothesized that LT recipients with worse oral health have more post-LT complications.

PATIENTS AND METHODS

This study was performed in accordance with the Declaration of Helsinki and has been approved by the HUS ethics committee (192/13/03/02/2008. 12 August 2008).

This study included all 265 adult LT recipients who received a liver graft during the years 2000–2006 at Helsinki University Hospital (HUS), which is the only LT center in Finland. Children under 15 years were excluded from this study. Of these patients, 225 (total 233 liver transplantations) underwent a dental evaluation prior to transplantation. Forty patients either lacked sufficient dental data or did not undergo a pre-LT dental evaluation. A flowchart of the patients included in the study is shown in **Supplementary Figure S1**. The underlying indications for transplantation are presented in **Supplementary Table S1**.

Complication Data and Laboratory Values

Outcome data until the end of July 2020 were obtained from the national LT registry. The national LT registry contains follow-up data on relevant complications following LT. These data are collected during annual follow-up visits at the transplantation center and local hospitals [10]. Complications were grouped into severe infections requiring hospital care, cardiovascular disease, cancer, acute or chronic rejection, death or re-transplantation, and incidental diabetes, and were considered outcomes. For the 225 patients with dental data available, we also registered the model for end-stage liver disease (MELD) scores at transplantation, and 1 year-post-LT laboratory values for alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin, total bilirubin, gamma-glutamyl transferase (GGT), and c-reactive protein (CRP).

Dental Examination and Parameters

Dental screening for infection foci was performed for 225 patients before transplantation, and 191 of these patients underwent a clinical and radiographic (panoramic tomography x-ray) dental examination. However, 32 patients were evaluated only based on the panoramic tomography x-ray and two did not undergo either a full clinical examination or panoramic tomography x-ray because they were edentulous. Furthermore, for 218 of the 225 patients, acute infection foci were eliminated prior to transplantation. The clinical examination consisted of examining the extraoral status, the oral mucosa, the dentition

TABLE 1 | Definition and scoring of the Modified Total Dental Index (MTDI).

	Points
Caries	
No caries lesions	0
1–3 caries lesions	1
4–7 caries lesions or no teeth in mandible or maxilla	2
≥8 caries lesions or radix or no teeth	3
Periodontitis	
No alveolar bone loss	0
Alveolar bone loss in cervical third	1
Alveolar bone loss in middle third	2
Alveolar bone loss in apical third	3
Periapical lesions	
1 periapical lesion or vertical bone pocket or both	1
2 periapical lesions	2
≥3 periapical lesions	3
Pericoronitis	
Absent	0
Present	1
Maximum score	10

and occlusion, and the periodontium. The oral health of the patients was evaluated using the modified total dental index (MTDI) score [18]. The MTDI score, presented in **Table 1**, considers the amount of caries lesions, edentulous jaws, radiological alveolar bone loss, number of apical lesions, and pericoronitis. A total score was counted for each patient, with a maximum possible score of 10 points. The patients were further divided into equal tertiles based on their MTDI score: low MTDI (0–2), medium MTDI [3] and high MTDI [4–10]. The low MTDI group was considered to have a low number of dental infection foci, whilst the high MTDI group was considered to have a high number of dental infection foci. A DMFT (decayed, missing, filled teeth) score was also registered for the patients, along with the number of extracted teeth.

Data Analyses

The data analyses were performed using IBM SPSS Statistics software version 27.0 (SPSS, Inc., Armonk, NY, United States). Data are given as mean ± SD or count (%). Continuous variables were assessed for normality using the Shapiro–Wilk test and histograms. None of the continuous variables were normally distributed. Differences between groups were analyzed using the Fisher–Freeman–Halton exact test and, for nonparametric variables, using the Kruskal–Wallis test. For *post hoc* test Z-test for proportions and Dunn's test, both Bonferroni corrected, were used as appropriate. The association between MTDI score and post-LT complications were first analyzed using univariate Cox regression analysis. Variables that were found to be significant in univariate analyses were further analyzed in multivariate analyses. Dental parameters and complications were adjusted for age, sex, indication for transplantation, smoking, pre-LT diabetes, MELD score at LT, and the number of LTs. Laboratory values were adjusted for sex, age at LT and MELD score at LT. We also analyzed whether there was a difference in the occurrence of complications between patients with or without pre-LT dental data with Cox regression models. Hazard ratios (HRs) with 95% confidence intervals (CIs) are reported. Linear regression analysis was used to assess the association between MTDI score and laboratory values, and p-values <0.05 were considered statistically significant.

RESULTS

Basic Characteristics

Basic characteristics for patients with low, medium, and high MTDI scores are presented in **Table 2**. Patients in the high MTDI group were older compared to the low MTDI group ($p = 0.027$). The high MTDI group had a larger proportion of patients with acute rejection post-LT than the low MTDI group ($p = 0.018$). Analyses on dental parameters showed that patients in the low MTDI group had lower DMFT scores than patients in the medium MTDI group ($p = 0.037$) and the high MTDI group ($p = 0.008$). Patients in the low MTDI group also had more teeth before dental treatment compared to patients in the medium MTDI group ($p = 0.005$). Furthermore, patients in the high MTDI group had more teeth extracted pre-LT compared to the low MTDI group ($p < 0.001$). Moreover, patients in the low MTDI group had higher 1 year-post-LT albumin levels compared to patients in the medium MTDI group ($p = 0.013$) and the high MTDI group ($p = 0.008$).

Basic characteristics, including background, dental and outcome parameters, for patients with and without dental data are presented in **Supplementary Table S1**. The ratio between men and women differed significantly ($p < 0.001$) between the two groups; otherwise, no significant differences were observed.

Association Between Pre-LT MTDI Score and Post-LT Complications

The correlation between pre-LT MTDI score and post-LT complications was analyzed with a Cox regression. The results are shown in **Supplementary Table S2**. In univariate analysis with MTDI group (low, medium, high) as the independent variable, patients with high MTDI had a significantly higher risk of acute rejection compared to patients in the low MTDI group (HR 1.7, CI 95% 1.1–2.6, $p = 0.012$). No other significant findings between MTDI score and other complications were seen. When investigating the correlation between MTDI score and acute rejection further, we found that the results also remained significant after adjusting for age, sex, smoking, indication for LT, pre-LT diabetes, MELD score at transplantation, and re-transplant status (HR 1.9, CI 95% 1.2–3.0, $p = 0.004$). Furthermore, when adjusting for the time between dental evaluation and LT or the interaction variable between MTDI groups and the time between dental evaluation and LT, the results remained significant. Results are presented in **Figure 1**.

Moreover, the proportion of LT recipients with more than one acute rejection episode was significantly higher ($p = 0.014$) in the high MTDI group (20%) compared to the low MTDI group (6%). No significant difference was found when analyzing the correlation between MTDI group and early (<3 months post-LT) or late (>3 months post-LT) acute rejection.

TABLE 2 | Basic characteristics of the 218 LT patients who underwent elimination of dental infection foci pre-LT and the underlying indication for transplantation.

Parameter	Low MTDI	Medium MTDI	High MTDI	*p*-value
Number of patients	116	42	60	
Age[a] (years)	46.5 (±13.1)	50.9 (±11.2)	52.2 (±9.2)	**0.011**[b]
Sex[c] (male/female)	58 (50%)/58 (50%)	25 (60%)/17 (40%)	40 (67%)/20 (33%)	0.101
Indication for transplantation[c]				
Chronic liver disease	92 (79%)	34 (81%)	47 (78%)	
I) Primary sclerosing cholangitis	30 (26%)	9 (21%)	10 (17%)	
II) Primary biliary cholangitis	15 (13%)	6 (14%)	9 (15%)	
III) Alcohol cirrhosis	15 (13%)	9 (21%)	17 (28%)	
IV) Cryptogenic cirrhosis/NASH	12 (10%)	4 (10%)	7 (12%)	
V) Other cirrhosis	12 (10%)	8 (19.%)	7 (12%)	
VI) Other CLD[d]	16 (14%)	3 (7%)	2 (3%)	
Acute liver failure	12 (10%)	3 (7%)	6 (10%)	
Tumor (all)[e]	11 (10%)	5 (12%)	7 (12%)	
I) Tumor (no other CLD)[f]	4 (3%)	0 (0%)	2 (3%)	
Metabolic disease	1 (1%)	0 (0%)	0 (0%)	
Complication data[c]				
No of patients: ≥1 complication	115 (99%)	42 (100%)	60 (100%)	1.000
No. of patients: no complications	1 (1%)	0 (0%)	0 (0%)	
No. of patients: Survival				0.133
I) Retransplantation	12 (10%)	5 (12%)	2 (3%)	
II) Death	31 (27%)	13 (31%)	26 (43%)	
No. of patients: Infection	77 (66%)	31 (74%)	39 (65%)	0.601
No. of patients: cardiovascular disease	11 (10%)	7 (17%)	11 (18%)	0.186
No. of patients: Incident diabetes	33 (28%)	14 (33%)	14 (23%)	0.539
No. of patients: Hypertension	66 (57%)	24 (57%)	37 (62%)	0.823
No. of patients: Cancer	31 (27%)	10 (24%)	18 (30%)	0.878
No. of patients: Acute rejection	52 (45%)	23 (55%)	40 (67%)	**0.023**[g]
No. of patients: Chronic rejection	3 (3%)	0 (0%)	1 (2%)	0.816
Dental parameters[a]				
DMFT score	20.6 (±9.2)	24.6 (±7.3)	25.5 (±4.8)	**0.003**[h]
Number of teeth pre dental treatment	25.1 (±6.6)	18.3 (±11.9)	23.5 (±6.5)	**0.003**[i]
Number of extracted teeth pre-LT	1.4 (±2.2)	2.4 (±3.5)	7.4 (±5.1)	<**0.001**[l]
MELD score at LT[a]	18.4 (±8.6)	19.4 (±7.9)	18.0 (±7.3)	0.712
Laboratory values at 1 year post-LT[a]				
P-ALT (U/L)	32 (±23)	34 (±33)	55 (±59)	0.102
P-ALP (U/L)	144 (±113)	163 (±143)	155 (±101)	0.415
P-Bilirubin (μmol/L)	15 (±8)	14 (±7)	15 (±13)	0.386
P-Albumin (g/L)	38 (±4)	36 (±4)	36 (±4)	**0.001**[k]
P-GGT (U/L)	89 (±188.03)	88 (±140)	131 (±212)	0.117
P-CRP (mg/L)	3 (±14)	3 (±10)	4 (±15)	0.582

Patients with low (0–2), medium (3) and high (4–10) MTDI scores are compared.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; CLD, chronic liver disease, CRP, c-reactive protein; DMFT, decayed, missing, filled teeth; GGT, gamma-glutamyl transferase; LT, liver transplantation; MELD, model of end-stage liver disease; MTDI, modified total dental index; NASH, non-alcoholic steatohepatitis. Non-parametric variables were analyzed using Kruskal-Wallis test, and categorical variables using Fisher's exact test. Post-hoc testing was done using, as appropriate, Dunn's test and the Z-test for proportions, respectively, with both being Bonferroni corrected.

[a]*Data given as mean (SD).*

[b]*In pairwise comparison, patients in the high MTDI group were significantly older than in the low MTDI group (*p *= 0.027).*

[c]*Data given as* n *(%).*

[d]*Other chronic liver diseases includes Budd–Chiari disease, polycystic disease, extrahepatic biliary atresia, congenital biliary fibrosis, alpha-1 antitrypsin deficiency, choledochal cyst, Caroli disease, and cystic fibrosis.*

[e]*All patients with a tumor as the underlaying cause.*

[f]*Patients with a tumor as the only underlaying cause.*

[g]*The high MDTI group had a significantly larger proportion of patients with acute rejection compared to the low MTDI group (*p *= 0.018).*

[h]*In pairwise comparison, patients in the low MTDI, group had significantly lower DMFT scores compared to patients in the medium MTDI group (*p *= 0.037) and the high MTDI group (*p *= 0.008).*

[i]*In pairwise comparison, patients in the low MTDI group had significantly more teeth before dental treatment compared to patients in the medium MTDI group (*p *= 0.005).*

[j]*In pairwise comparison, patients in the high MTDI group had significantly more teeth extracted compared to patients in the low MTDI group (*p *< 0.001) and medium MTDI, group (*p *< 0.001).*

[k]*In pairwise comparison, patients in the medium MTDI group (*p *= 0.013) and high MTDI, group (*p *= 0.008) had significantly lower albumin values compared to patients in the low MTDI group.*

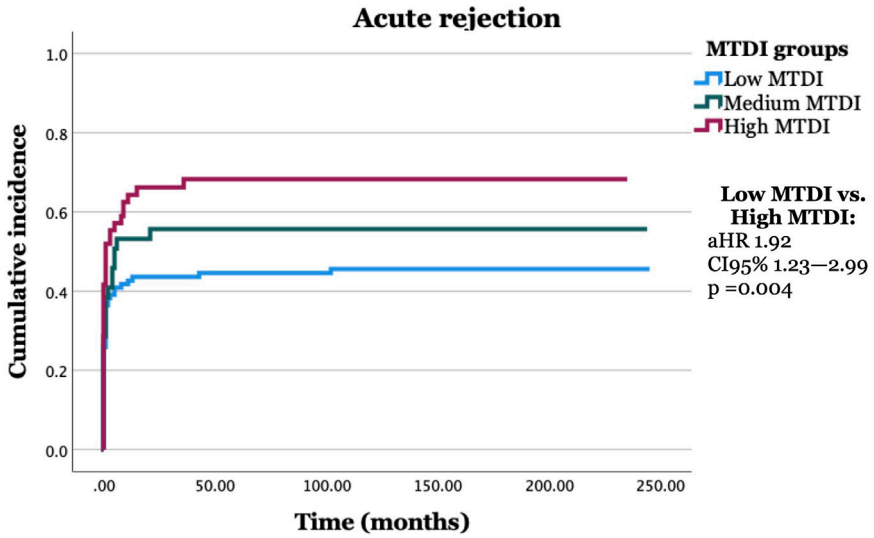


FIGURE 1 | Liver transplant (LT) recipients with a high need for dental treatment (high MTDI) and multiple infection foci pre-LT expressed significantly more acute rejection post-LT compared to LT recipients with a low or no need for dental treatment (low MTDI) pre-LT.

TABLE 3 | Univariate linear regression analysis of the connection between MTDI score and laboratory values 1 year post-transplantation.

Laboratory value	β	CI 95% for B	R^2	*p*-value
P-ALT (*U/L*)	3.97	1.20–6.74	0.038	**0.005**
P-ALP (*U/L*)	3.44	−4.87–11.75		0.415
P-Bilirubin (μmol/L)	0.09	−0.58–0.76		0.782
P-Albumin (*g/L*)	−0.48	−0.77 to −0.19	0.052	**0.001**
P-GGT (*U/L*)	12.44	−0.93–25.82		0.068
P-CRP (*mg/L*)	0.78	−0.20–1.75		0.119

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase, CRP, c-reactive protein, GGT, gamma-glutamyl transferase.
β = *regression coefficient.*
These results concern 218 patients whose teeth were examined and treated pre-transplantation.
MTDI score (0–10) analyzed as a continuous variable.

Association of Pre-LT MTDI Score and Dental Data With 1 year-post-LT Laboratory Values

In **Table 3**, the results of the linear regression analysis of the correlation between MTDI score as a continuous variable and 1 year-post-LT laboratory values for ALT, ALP, bilirubin, albumin, GGT and CRP are shown. There was a significant association between a higher MTDI score and higher ALT values (β = 4.0, CI 95% 1.2–6.7, R^2 = 0.039, p = 0.005) and with lower albumin values (β = −0.5, CI 95% −0.8 to −0.2, R^2 = 0.052, p = 0.001). The results remained significant after adjusting for sex, age at LT, and MELD score at LT (β = 4.2, CI 95% 1.3–7.1, R^2 = 0.04, p = 0.005 and β = −0.5, CI 95% −0.8–0.2 R^2 = 0.1, p = 0.002, respectively).

When further analyzing whether the pre-LT number of teeth correlates with 1 year-post-LT ALT and albumin values in separate linear regression analysis, we found that fewer teeth associated with lower albumin values (β = 0.2, CI 95% 0.04–0.2, R^2 = 0.05, p = 0.002).

DISCUSSION

The main finding in our study was that patients with high pre-LT MTDI scores, indicating a worse dental status, seemed to have a higher risk of acute post-LT rejection compared to patients with low pre-LT MTDI scores. Furthermore, a higher MTDI score seems to associate with higher 1 year-post-LT ALT levels and lower albumin levels.

To the best of the authors' knowledge, the association between poor dental status and acute rejection post-LT has not been reported in LT recipients previously. In our study, we found that a higher MTDI score (worse oral health) independently predicts acute rejection. The correlation remained significant even when adjusted for confounders. Previous studies on kidney transplant recipients show contradictory results on the matter. Zweich et al. [19] showed that poor oral hygiene was an indicator for increased risk of hospitalization and acute rejection, and found that the Community Periodontal Index of Treatment Needs correlated with acute rejections in kidney transplant recipients. However, other studies have demonstrated no correlation between pre-transplant oral health and graft rejection [20, 21]. One study showed that severe periodontitis in kidney transplant recipients was independently associated with a lower incidence of acute T-cell-mediated rejection, which was hypothesized to depend on the immunomodulatory effect of periodontitis [22].

In this study, all acute odontogenic infection foci were treated pre-LT. However, not all patients underwent periodontal treatment systematically. A potential link between

periodontitis/gingivitis and graft rejection could be a periodontitis-related, IL-6-modulated, pro-inflammatory state. IL-6 production can be induced by both pathogen-associated molecular patterns and pro-inflammatory cytokines, and it seems to have a pro-inflammatory effect on the adaptive immune response [23]. In the solid organ transplantation context, IL-6 has been shown to promote acute allograft rejection [24–26]. Periodontitis has been shown to increase levels of proinflammatory cytokines such as TNF-alfa, IL-1 and IL-6 [27]. In particular, patients with chronic periodontitis have elevated levels of proinflammatory cytokines, including IL-6, in the gingival crevicular fluid and serum compared to healthy controls [28, 29]. Periodontal treatment, in turn, is shown to lower the levels of these proinflammatory cytokines [29, 30]. Thus, we hypothesize that untreated periodontal inflammation plays a role in the development of acute rejection.

Previous publications on the association between liver enzymes and oral health are contradictory and are mainly focused on periodontitis. Some studies show no association between liver enzyme values and alveolar bone loss in patients without liver disease [31, 32], while other studies show higher liver enzyme levels in patients with periodontitis [33, 34]. Some studies hypothesize that periodontal disease might be a risk factor for the development of non-alcoholic steatohepatitis [35–37] and chronic liver disease [38]. Our results showed significantly higher ALT levels and significantly lower albumin levels in patients with worse dental health. ALT is a highly liver-specific enzyme, and it being elevated in patients with worse oral health supports our hypothesis that untreated oral or dental inflammation might also influence the liver post-LT. Poor nutrition is shown to reduce albumin production [39]. In our study, worse oral health and fewer teeth were associated with lower albumin values. Hence, worse oral health and fewer teeth may contribute to malnutrition by negatively affecting chewing capacity and mastication; this connection has previously been discussed in patients with chronic kidney disease [40].

Our study reports novel findings on the connection between oral health and post-LT complications. Strengths of our study include the long follow-up time, the utilization of data from the national LT registry, and using both clinical and laboratory data. Despite the novel findings, our study has limitations. In this study setting we could not collect detailed data on the patients' periodontal status and periodontal treatment. Furthermore, our study analyzed oral health as a whole, and we were not able to exam different oral diseases separately, due to our retrospective study setting not allowing this. Moreover, two of the patients did not undergo a clinical or radiological examination because they were edentulous, which increases the risk for error in our analyses. However, we chose to include these patients, since the examinations would not have changed their MTDI scores. Another limitation is the relatively small sample size. However, we included all eligible patients in Finland, and this country-wide setting is a strength of our study. Despite its limitations, our study provides novel results on the possible connection between poor oral health and post-LT acute rejection, and the results remain indicative despite these limitations. However, despite the link, common confounders might affect both poor oral health and acute rejection post-LT. Therefore, further studies examining confounders affecting this relationship are needed. A possible area of further research based on this study would be the impact of oral diseases on post-LT complications, especially acute rejection. Another interesting topic for further study would be to assess the impact of receiving periodontal treatment prior to transplantation, while adjusting for the stage of periodontal disease.

Conclusion

In conclusion, poor pre-LT oral health seems to be associated with an increased risk of post-LT acute rejection. Poor oral health is also associated with elevated ALT levels and decreased albumin levels, suggesting an effect on post-LT liver health. Therefore, attention should be given to treating oral and dental issues early in the course of liver disease and to highlighting the importance of maintaining good oral hygiene.

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 humans were approved by Helsinki University Hospital Ethics committee. The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from a by-product of routine care or industry. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

AO: contributed to conception and design of the study, organized the database, preformed the statistical analysis and wrote the manuscript. FÅ contributed to conception and design of the study, contributed to the statistical analysis and wrote sections of the manuscript. JH-H, AN, JS, and HR: contributed to conception and design of the study and wrote sections of the manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11534/full#supplementary-material

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LETTER TO THE EDITOR
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Considering ABO Incompatible Living Donor Kidney Transplantation Before Deceased Donor Kidney Transplantation in Children: A Letter to the Editor

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Keywords: ABO incompatible, living donation, paediatric, kidney transplantation, outcomes

Dear Editors,

Advances in neonatal and metabolic medicines have brought up a new profile of paediatric kidney transplant recipients. More children born with congenital anomalies of the kidneys and urinary tract and metabolic conditions are surviving outside of infancy. Some of those children are needing renal replacement therapy since early childhood and therefore are considered as transplant candidates with a high likelihood of needing more than one kidney transplant in their life. Recent meta-analysis states that pre-emptive transplantation has advantages in overall patient and allograft outcomes over transplantation done following a period on dialysis [1].

In the UK there are currently 101 children waiting for a kidney transplant from a deceased donor [2]; this number is even higher in the EuroTransplant region at 138 children [3]. However, the number of children receiving a deceased donor kidney each year is far lower with only 52 children receiving a deceased donor kidney in the UK in 2022 (ranging from 42–60 deceased donor transplants per year over the last decade) [2]. The average waiting time in the UK in the last year for children has been 270 days although this has ranged from 258 to 342 days in the last decade [2]. We also know that allografts coming from living donors have better outcomes than deceased donor allografts and so is the preferred transplant option [4, 5]. However, not all children have suitable living donors that are blood group compatible. In such cases, most children go on to the deceased donor waiting list and can wait a long time before a kidney becomes available, which may still lead to poorer outcomes compared to if they had a living donor [2].

Given the scarcity of deceased donor organs and increasing waiting times, there has been extensive research into ABO incompatible (ABOi) transplantation from a living donor since the 1980s, as a way of increasing donor pool and as a possible alternative to deceased donation. While the studies initially focused on adult donors and recipients, over recent years more evidence has been produced to support this practice for paediatric recipients. An analysis of the Japanese Kidney Transplant registry was published in 2018 which described the results of 102 children who had undergone ABOi kidney transplants from living donors. The outcomes of these recipients were compared to the outcomes of the children on their registry who had undergone ABO compatible living donor transplants, with no difference found in patient or allograft survival between the two groups [6]. Initially the pre-transplant protocol for ABOi transplants included recipient splenectomies prior to or at the time of transplantation [7]. This approach has further developed since then and excellent results have been achieved using protocols with medicines, such as Rituximab for so called "medical splenectomies." The largest paediatric transplant center in the UK shared their experience of a tailored desensitisation protocol where immunosuppression is

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based on the recipients' level of ABO antibody titres pre-transplant. It involves the use of Rituximab ± immunoadsorption and/or double filtration plasmapheresis [8] if titers are 1:8 or higher. Several centers in the UK reported on outcomes of ABOi kidney transplantation with a cohort of 23 children and similarly found no difference in patient or allograft survival, acute rejection or graft function compared to ABO compatible living donor transplants [8, 9]. Other centers in Sweden [10] and Japan [11] using a similar approach to desensitisation have also shared equally encouraging results. Some studies have even found that infants with low antibody titres prior to ABOi transplantation did not require any pre-transplant desensitisation to achieve excellent results [10]. In all studies where Rituximab was used, it was shown that the use of Rituximab pre-transplant was not associated with an increased risk of infection or any other complications either, confirming safety of this drug to be used in ABOi kidney transplant programmes.

Given the increasing evidence of positive outcomes following ABOi kidney transplantation in children, and evidence of better allograft survival of kidneys coming from living donors, perhaps it is time to consider ABOi living donor kidney transplantation in children before being listed for deceased donor organs. To date, there is no prospective study comparing the outcomes between those receiving ABOi living donor transplants and those receiving ABO compatible transplants from a deceased donor. However, the evidence that we do have in the literature so far, supports the idea that ABOi transplants should be considered in the paediatric population as a transplant option prior to proceeding with a transplant from a deceased donor, as it has the potential to lead to better patient and allograft outcomes.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://statistics.eurotransplant.org/; https://www.odt.nhs.uk/statistics-and-reports/annual-activity-report/.

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 to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

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

CONFLICT OF INTEREST

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

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