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TRANSPLANT TRIAL WATCH
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Transplant Trial Watch

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Keywords: rituximab, IVIG, FSGS recurrence, systematic review, trials

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

Comparison of High-Dose IVIG and Rituximab Versus Rituximab as a Preemptive Therapy for *De Novo* Donor-Specific Antibodies in Kidney Transplant Patients.

by Kim, H. W., et al. Scientific Reports 2023; 13(1): 7682.

Aims

This study aimed to evaluate the efficacy of pre-emptive treatment of *De Novo* DSA (dnDSA) using combination of high-dose intravenous immunoglobulin and rituximab (IVIG+) or rituximab alone (IVIG-) in reduction of dnDSA titre at 3 and 12 months after treatment compared to retrospective controls.

Interventions

Both groups received rituximab (375 mg/m^2) on day 0, and the IVIG+ group additionally received high-dose IVIG (2 g/kg) after rituximab infusion.

Participants

50 adult kidney recipients with functioning graft (eGFR > 20 mL/min/1.73 m^2) and subclinical class II dnDSA with mean fluorescent intensity (MFI) ≥ 1000 of the DR or DQ DSA.

Outcomes

The primary outcome measure was dnDSA titre at 3 and 12 months. Secondary outcomes were changes in eGFR and incidence of anti-body mediated rejection.

Follow-Up

Participants were followed-up for 12 months.

Transplant Trial Watch

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Knight SR (2023) Transplant Trial Watch. Transpl Int 36:11816. doi: 10.3389/ti.2023.11816

CET Conclusion

The investigators found both groups IVIG+ and IVIG− were associated with dnDSA MFI, but that the addition of IVIG to rituximab had no added benefit for dnDSA reduction at either 3 or 12 months. This reduction is significant when they were compared with a matched group of retrospective controls. Between the two groups they also found no difference in their secondary outcome measures of eGFR at 3 or 12 months, with no episodes of anti-body mediated rejection (ABMR) in the study cohort. They also reported no difference in protein-creatinine ratio.

However, then generalisability of the study is somewhat limited due to its small sample size and possibility of selection bias given that nearly all the participants were living-related kidney transplant recipients. No episodes of ABMR occurred, which due to the size of the trial is not entirely surprising but given a core part of pre-emptive dnDSA reduction is to hopefully reduce the incidence and severity of ABMR it is hard to assess the potential importance of the interventions. There is also no mention or inclusion of the ABMR rate in their retrospectively matched control cohort which may have been of interest. The study contributes to baseline data on the potential benefit of pre-emptive treatment of dnDSA, however, a larger randomised trial of rituximab vs. placebo in an adult population would be of benefit.

Jadad Score

2.

Data Analysis

Per protocol.

Allocation Concealment

No.

Trial Registration

ClinicalTrials.gov—NCT04033276.

Funding Source

Industry funded.

SYSTEMATIC REVIEW

Incidence and Risk Factors for Recurrent Focal Segmental Glomerulosclerosis After Kidney Transplantation: A Meta-Analysis.

by Bai, J., et al. Renal Failure 2023; 45(1): 2201341.

Aims

This study aimed to investigate the incidence and risk factors associated with focal segmental glomerulosclerosis (FSGS) following kidney transplantation.

Interventions

A literature search was conducted on PubMed, Cochrane Library, Medline, Embase, Web of Science, CNKI, CBMdisc, Wanfang, and Weipu (VIP). Study selection and data extraction were performed by two independent authors. The methodological quality of the included studies were assessed using the Newcastle–Ottawa Scale (NOS).

Participants

22 studies were included in the review.

Outcomes

FSGS recurrence rate posttransplantation and risk factors of FSGS.

Follow-Up

N/A.

CET Conclusion

This is a well-conducted systematic review that searched multiple databases and included data from 966 renal transplant patients with FSGS (38% recurrence after transplantation). A review protocol was recorded in advance and the literature search and data extraction was completed in duplicate. Significant heterogeneity was identified between studies and was not explored by the authors with sensitivity analysis. This identified one study as a key source of heterogeneity, that was then later removed from statistical analysis. Publication bias was also checked statistically and was only present for one risk factor analysis (age at transplantation); correcting for this had no effect on the pooled estimate.

In summary, this study showed that the overall recurrence risk of FSGS after renal transplantation is high. Age at transplant, age at onset, time from diagnosis to kidney failure, proteinuria prior to transplant, related donor and native nephrectomy were all associated with a higher risk of FSGS recurrence. Multiple other risk factors were examined and not found to be associated with risk of recurrence of FSGS: HLA mismatch, duration of dialysis, sex, living donor, tacrolimus and previous transplant.

Trial Registration

PROSPERO—CRD42022315448.

Funding Source

None.

CLINICAL IMPACT SUMMARY

Whilst transplantation is the treatment of choice for renal failure due to focal segmental glomerulosclerosis (FSGS), it is one of the few

indications for transplantation with a known risk of recurrent disease in the transplant kidney that can affect graft survival post-transplant. Treatments such as pre-emptive plasmapheresis with or without rituximab have been used to prevent or treat post-transplant recurrence, but the evidence for effectiveness is limited [1].

A number of publications have attempted to correlate demographic and clinical features with risk of recurrence post-transplant. In a recent systematic review and meta-analysis, Bai et al. have attempted to summarise and synthesise this literature [2]. They identified 22 studies with 966 patients, showing an overall rate of FSGS recurrence of 38%. Risk factors for recurrence were identified as younger age at transplant, older age of disease onset, shorter time from diagnosis to kidney failure, higher levels of proteinuria prior to transplant, a related living donor transplant and native nephrectomy.

The review methodology was sound, with searches in multiple databases, multiple reviewers screening the literature and an evaluation of risk of bias. As might be expected when exploring retrospective cohort studies, there was heterogeneity seen in some outcomes, in particular age at transplant and pre-transplant proteinuria. Most underlying studies included in the meta-analysis explored risks in univariate analysis, without correction for confounding, and there is no way in meta-analysis to explore the interactions between risks. Limited data are available on the distinction between primary and secondary FSGS, and the impact of testing for genetic mutations and risk of recurrence [3].

Despite the limitations, the review still provides a useful guide when assessing patients with FSGS for transplantation. The findings allow us to stratify risk of recurrence and set realistic expectations during the consent process. Whilst most of the risk factors identified are non-modifiable, it would seem reasonable to avoid related living donors and prior bilateral nephrectomy where not otherwise indicated.

Clinical Impact

3/5.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has 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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POINT OF VIEW
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Chronic Pancreas Allograft Rejection Followed by Successful HLA-Incompatible Islet Alloautotransplantation: A Novel Strategy?

Denise M. J. Veltkamp[1,2†], Michiel F. Nijhoff[1,2,3†], Dennis A. J. van den Broek[1,2†], Maren Buntinx[4†], Jesper Kers[2,5†], Marten A. Engelse[1,2†], Volkert A. L. Huurman[2,6†], Dave L. Roelen[2,7†], Sebastiaan Heidt[2,7†], Ian P. J. Alwayn[2,6†], Eelco J. P. de Koning[1,2,3†] and Aiko P. J. de Vries[1,2†]*

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The purpose of pancreas or islet transplantation is to restore glycemic control in order to mitigate diabetes-related complications and prevent severe hypoglycemia. Complications from chronic pancreas allograft rejection may lead to transplantectomy, even when the endocrine function remains preserved. We present first evidence of a successful HLA incompatible islet re-transplantation with islets isolated from a rejecting pancreas allograft after simultaneous kidney pancreas transplantation. The pancreas allograft was removed because of progressively painful pancreatic panniculitis from clinically uncontrolled chronic rejection. The endocrine function was preserved. Induction treatment for this "islet alloautotransplantation" consisted of plasmapheresis, IVIg and alemtuzumab. At 1 year, the patient retained islet graft function with good glycemic control and absence of severe hypoglycemia, despite persistent low-grade HLA donor-specific antibodies. His panniculitis had resolved completely. In our point of view, islet alloautotransplantation derived from a chronically rejecting pancreas allograft is a potential option to salvage (partial) islet function, despite preformed donor-specific antibodies, in order to maintain stable glycemic control. Thereby it protects against severe hypoglycemia, and it potentially mitigates kidney graft dysfunction and other diabetes-related complications in patients with continued need for immunosuppression and who are otherwise difficult to retransplant.

Keywords: antibody-mediated rejection, donor specific antigen (DSA), islet transplantation, re-transplantation, simultaneous kidney pancreas transplantation

Point of View

OPEN ACCESS

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Abbreviations: ABMR, Antibody-mediated rejection; CMV, Cytomegalovirus; C-peptide, Connecting peptide; DSA, Donor-specific antibody; HLA, Human leukocyte antigen; IEQ, Islet equivalent; IVIg, Intravenous immunoglobulin; MFI, Median fluorescence intensity; MMTT, Mixed-meal tolerance test; SPK, Simultaneous pancreas and kidney transplantation; T1D, Type 1 diabetes; TCMR, T cell-mediated rejection.

INTRODUCTION

The purpose of pancreas or islet transplantation is to restore glycemic control in order to mitigate diabetes-related complications and prevent severe hypoglycemia. Whole pancreas transplantation has become a successful strategy since the 1960s in patients with type 1 diabetes in need of a kidney transplantation, or who otherwise have life-threatening glycemic control to warrant the impact of maintenance immunosuppression [1].

However, long-term outcomes after simultaneous pancreas-kidney (SPK) transplantation have not improved evidently in past decades, amongst others due to chronic rejection [2, 3]. Antibody-mediated rejection (ABMR) is increasingly recognized as a cause of chronic rejection in the setting of pancreas transplantation as well [3–5]. Chronic rejection is often refractory to anti-rejection therapy resulting in complications such as bleeding, duodenal perforation, and fistula/abscess formation. This may necessitate pancreatectomy despite preserved endocrine function, as pancreatic rejection is often more targeted to exocrine tissue, (micro)vasculature, or duodenum [6, 7]. Re-transplantation following sensitization becomes increasingly difficult, as pancreas allocation in the Eurotransplant area is not primarily based on HLA matching and patients are often no longer eligible (e.g., age >50–55 years, comorbidity), by the time a suitable offer becomes available, leaving them with labile diabetes again. Since the turn of the century, islet transplantation has become a less invasive alternative for whole pancreas transplantation with clinical merit to prevent severe hypoglycemia in labile diabetes as well as improved metabolic control compared to an optimized insulin regimen, especially in patients who are already on maintenance immunosuppression [8]. We previously described the option of alloautotransplantation, a strategy to preserve endogenous insulin production by isolating and re-transplanting the islets after an allograft pancreatectomy [7]. However, evidence for HLA incompatible islet alloautotransplantation from a chronically rejecting pancreas transplant has not been described before to our knowledge.

Here we present the 1 year results of an HLA incompatible islet re-transplantation after graft pancreatectomy in a patient with progressively painful panniculitis from a clinically uncontrolled chronic rejection of the pancreas (and kidney) allograft with preserved endocrine function.

EVIDENCE

The evidence concerns a non-sensitized (virtual panel-reactive antibody; vPRA 0%) 50 year-old cytomegalovirus (CMV) seronegative male with a medical history of poorly controlled type 1b diabetes (T1D) and end-stage renal disease. The patient was diagnosed with type 1 diabetes at 8 years of age after a presentation of ketoacidosis. He was negative for anti-GAD, anti-IA-2 and anti-ZnT8 at time of referral and had undetectable C-peptide since childhood (<0.01 nmol/L). With a flexible four times daily insulin regimen (on average 0.8 units/kg daily), he suffered from recurrent hypoglycemia and poor glycemic control (HbA1c 90.3 mmol/mol Hb). The patient underwent SPK transplantation from an HLA-mismatched (2-2-2) CMV seronegative donor after cardiac death. The patient provided written consent for publication.

At SPK transplantation his immunosuppressive regimen consisted of alemtuzumab induction 30 mg s.c. and methylprednisolone 500 mg once, followed by tacrolimus 5 mg b.i.d., mycophenolate mofetil 750 mg b.i.d., and prednisolone 10 mg q.d. The postoperative course (**Figure 1**) was complicated, amongst others, by delayed renal allograft function and an unexpected primary CMV infection for which valganciclovir was initiated. Due to development of leukopenia and later BK-viremia, mycophenolate mofetil was discontinued and tacrolimus tapered.

In the period from eight to 11 months posttransplant, two clinical episodes of pancreas rejection occurred each with right lower quadrant abdominal tenderness, graft pancreatitis on radiological imaging, a concomitant increase in serum amylase/lipase, and *de-novo* HLA donor-specific antibodies (DSAs) (immunodominant DQB1*05:02-DQA1*01:02 with low median fluorescence intensity (MFI) of 997 at 8 months and 943 at 11 months, and anti-DP4 with MFI 1800 at 11 months), measured with Luminex single antigen bead assays from LifeCodes (Immucor). Alternative causes of pancreatitis such as constipation, CMV relapse, and re-occurrence of T1D auto-antibodies were ruled out. A pancreas or, alternatively, a kidney allograft biopsy was not performed at first, as pancreas biopsy is not routine practice at our center due to experienced risk of complications, and kidney graft function initially remained stable. However, a kidney graft biopsy at 11 months, performed because of *de-novo* proteinuria (2.90 g/24 h), showed mixed-type chronic active T cell-mediated rejection (TCMR) and ABMR rich in plasma cells with negative staining for CMV, BK-virus (SV40), and C4d (**Figure 2A**). Both clinical rejection episodes were treated with methylprednisolone 1 g q.d. for 3 days only. However, second-line therapy (lymphocyte depleting antibodies) was withheld because of contraindications at that time (leukopenia and active viral complications: BK-viremia (8 months), and COVID-19 (11 months)). Pancreatectomy was considered but a watchful waiting strategy was chosen since endocrine function remained intact and no other complications were immediately evident. At 8 months, everolimus 0.75 mg b.i.d. was started next to tacrolimus to consolidate the first-line rejection treatment by pulsed steroids to control chronic rejection in the context of BK-virus reactivation and leukopenia. At 11 months, everolimus was substituted by mycophenolate mofetil because of proteinuria, which then improved. As complication of high-dose corticosteroids, vertebral compression fractures of the thoracic spine occurred.

At 20 months post SPK transplantation, progressively painful subcutaneous nodules on both lower legs developed (**Figure 2C**) necessitating opioid maintenance. A punch skin biopsy showed pancreatic panniculitis (**Figure 2B**), likely as complication of ongoing chronic pancreas allograft rejection, which has been described incidentally before in transplant recipients [9]. Since

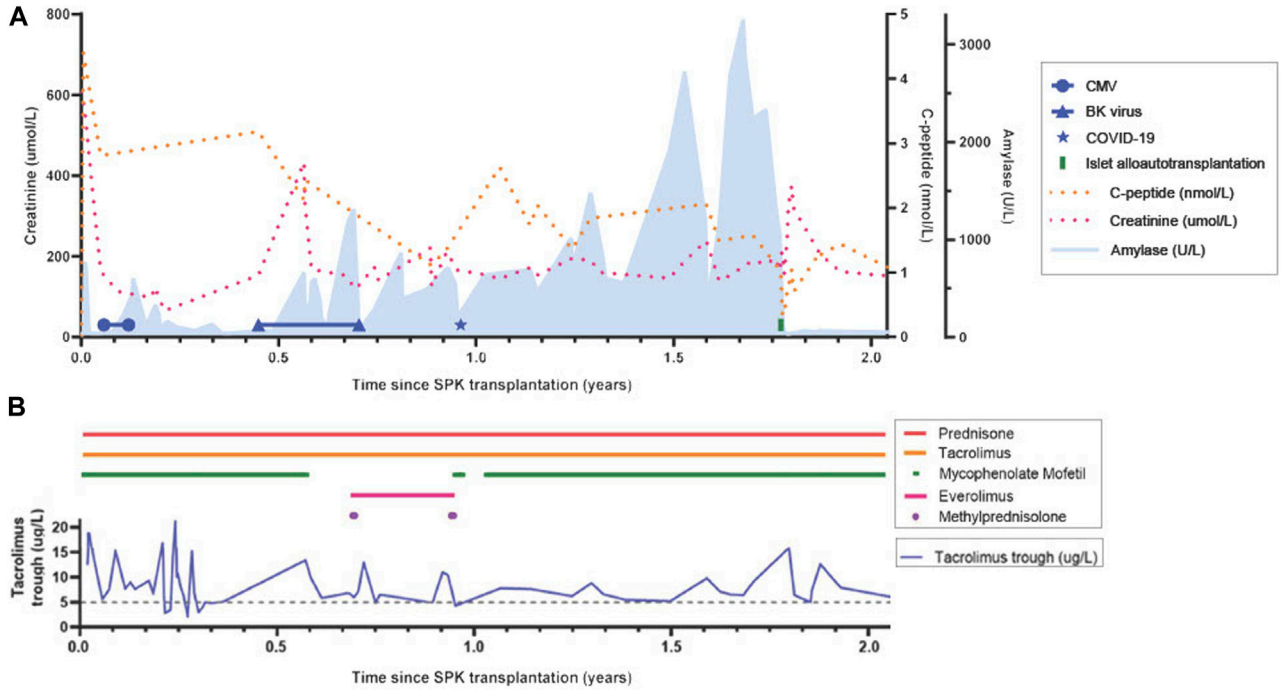

FIGURE 1 | Timeline from simultaneous pancreas-kidney transplantation to 3 months post-islet alloautotransplantation. **(A)** Lab results: C-peptide, creatinine, amylase, BK-viremia, Cytomegaloviremia, COVID-19 infection. MFI DQB1*05:02-DQA1*01:02 (months since SPK): 997 (8); 943 (11); 871 (12); 841 (15); 1180 (24). Type 1 diabetes auto-antibodies remained negative. **(B)** Medication exposure: prednisone, tacrolimus and tacrolimus trough, mycophenolate mofetil, everolimus, methylprednisolone (1,000 mg, 3 days). Target tacrolimus trough level: 10–15 ng/mL within first 6 weeks, 5–8 ng/mL beyond 6 weeks; Target 12h-auc mycophenolate mofetil: 30–45 mg*h/L. CMV, Cytomegalovirus; SPK, simultaneous pancreas-kidney transplantation.

efficacy of second-line therapy is questionable for long-standing chronic (or mixed-type) rejection and because of progressive complications, pancreatectomy was deemed favorable over a third round of methylprednisolone or escalation with second-line therapy now that contraindications were absent [6]. Nonetheless, the pancreas' endocrine function remained intact (HbA1c 38.5 mmol/mol, random C-peptide 1.6 nmol/L, glucose 5.4 mmol/L, without insulin therapy). As the patient was adamant to preserve his endocrine function 21 months after SPK transplantation, an islet alloautotransplantation was performed after pancreatectomy despite presence of (low-grade) DSAs. Before islet transplantation, a single round of plasma exchange was performed followed by intravenous immunoglobulin (IVIg) (1 g/kg). Alemtuzumab (15 mg s.c.) induction, methylprednisolone (500 mg day −1, 250 mg day 0, 125 mg day +1) and etanercept (50 mg s.c. day 0, 25 mg s.c. days 2/6/10) were prescribed following standard practice for allogeneic islet transplantation according to local protocol. At the operating room, directly after pancreatectomy the pancreas was flushed on the backtable using Ringer's acetate solution supplemented with 5 mmol/L calcium at pH 7.35 prior to static cold storage transport to the islet isolation laboratory. Islet isolation was performed according to previously published protocols [10–14]. Macroscopically, the pancreas looked edematous but non-necrotic. With iodine and penicillin-streptomycin solution the pancreas was decontaminated. The pancreas was infused with 1 vial (2533 IU) collagenase solution combined with one vial of neutral protease solution (200 IU) (both Serva NB-1® (Serva Electrophoresis GmbH, Heidelberg, Germany) in Ringer's acetate solution supplemented with 5 mmol/L calcium at pH 7.35. Gentamycin, ciprofloxacin, vancomycin and amphotericin B were added to all fluids in the isolation's procedure. In the final product, both the gram staining and the endotoxin test were negative. The islets were re-transplanted intraportally directly after the isolation procedure. The alloautograft contained 313000 Islet equivalents (IEQ) (4013 IEQ/kg patient) in a volume of 12 mL with a purity of 6%. Strict glycemic control was maintained by continuous intravenous insulin (glucose target 4–7 mmol/L) for 48 h, after which s.c. insulin was recommenced to maintain the tight glycemic target.

At 15 weeks after this islet alloautotransplantation, the patient was treated with 8 units of long-acting insulin once daily. Time-in-range of glucose concentrations was 96% (3.9–10 mmol/L; as measured by Flash Glucose Monitoring) with an HbA1c of 42.7 mmol/mol. Maximum C-peptide was 2.32 nmol/L (maximum glucose 12.4 mmol/L) during a 2 h mixed-meal tolerance test (MMTT). One year after the islet alloautotransplantation the patient retained islet graft function with absence of severe hypoglycemia. At that time, he used metformin/dapagliflozin 850mg b.i.d. combined with 20 units of long-acting insulin a day, with which he had a time-in-range of 80%–90% and an estimated HbA1c of 48 mmol/mol. Baseline C-peptide was 0.30 nmol/L with glucose of 4.1 mmol/L, and rose to 1.01 nmol/L with a maximum glucose of 9.1 mmol/L during the

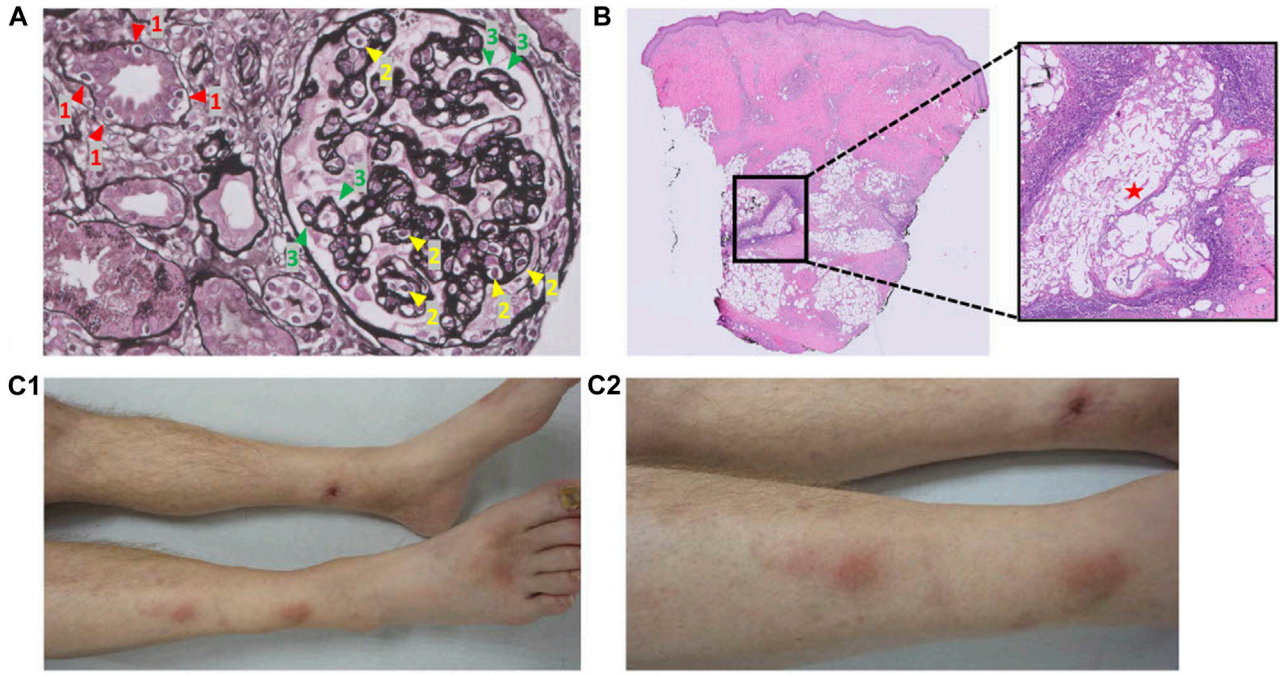

FIGURE 2 | Pathology pictures of the kidney allograft biopsy and skin biopsy, and clinical pictures of the pancreatic panniculitis. **(A)** Kidney allograft biopsy: mixed-type chronic active T cell-mediated rejection and antibody-mediated rejection rich in plasma cells. 1 (red) = tubulitis; 2 (yellow) = glomerulitis; 3 (green) = double contours of the glomerular basement membrane. Banff: g2, i0, t1, v0, ptc1, mm0, cg2, i-IFTA2, t-IFTA2, ci2, ct2, cv1, ah1. **(B)** Skin biopsy: pancreatic panniculitis. Adipocytes have been saponified by the process of enzymatic fat necrosis, accompanied by neutrophilic inflammation. **(C)** Pancreatic panniculitis on both lower legs. **(C1)**: anterior view. **(C2)**: Lateral (right)/anterior view.

MMTT. Together, this amounts to clinical treatment success as defined by a "good" function according to the Igls criteria, a beta score of 5, and a BETA-2 score of 13.55 [15]. Anti-GAD, anti-IA-2 and anti-ZnT8 remained negative and kidney function clinically stable (creatinine 134 umol/L with an albumin/creatinine ratio of 30.3 mg/mmol). The panniculitis recovered completely. The preformed immunodominant DSA DQB1*05:02-DQA1*01: 02 remained present at 1 year with a MFI of 1180. Anti-DP4 remained undetectable after the islet alloautotransplantation.

DISCUSSION

This report describes 1 year evidence of a successful (according to the Igls criteria) islet alloautotransplantation in a patient with preformed HLA-DSAs after allograft pancreatectomy for likely chronic rejection of the pancreas. Although we did not obtain histological evidence, the fact that a clinical syndrome of pancreatitis occurred twice (localized pain, rise in pancreatic enzymes, and edematous pancreas at imaging) after a period of immunosuppressive underexposure with development of a dnDSA made a clinical diagnosis of pancreas rejection likely. Non-alloimmune causes of pancreatitis were excluded albeit that the unexpected primary CMV infection may have contributed since CMV has been associated with heterologous immunity [16]. After reconfirming seronegativity of donor and recipient, it remains unknown how the CMV infection occurred. A kidney biopsy during the second episode of pancreatitis showed mixed chronic-active TCMR and ABMR further corroborating the likelihood of rejection.

Although some discordancy for rejection (12.5% kidney-only rejections) has been observed by Parajuli et al., that study did not stratify for clinical context such as simultaneous pancreas dysfunction and presence of DSA [17]. A study by Uva et al. showed that a positive renal biopsy for rejection correctly predicts pancreatic rejection in 86% of cases with concurrent pancreatic dysfunction, albeit they also did not stratify by presence of DSA [18]. Furthermore, there is debate whether discordancy truly exists or whether it's merely a matter of time [19, 20]. Our report illustrates the timely development from a single episode of clinical pancreatitis with DSA formation to recurrent pancreatitis, to eventually kidney transplant dysfunction with biopsy proven chronic mixed type ABMR and a biopsy-proven panniculitis to underscore the likelihood of chronic pancreas rejection. At the time, active viral complications and leukopenia contraindicated escalation to second-line therapy, such as ATG or alemtuzumab, to have improved rejection control [21].Notably, the pancreas graft's endocrine capacity appeared relatively unaffected by the pancreatitis episodes perhaps also questioning the likelihood of rejection. However, distinctive rejection patterns of pancreatic endocrine and exocrine cells have been described before, yet the pathophysiological mechanism is not completely clear. It might be reflective of differential HLA expression [22]. Exocrine tissue and the pancreas (micro)vasculature are often the primary targets of rejection, whereas islets remain relatively unaffected. Pancreatitis

from chronic (acinar) rejection might subsist by ongoing release of inflammatory danger signals, such as proteases, from continued pancreatitis. The persistent acinar damage and chronic vascular injury might trigger a progressive fibrogenic reaction that could eventually impair β-cell function [22]. The painful complication of the progressive pancreatic panniculitis, first described by Chiari in 1883, is not completely understood, but is thought to be subcutaneous fat necrosis from circulating proteases and enzymes released from an inflamed pancreas, which necessitated the use of opioids in our case [9, 23]. A pancreas transplantectomy and re-transplanting the islets, when endocrine function remains largely preserved, could theoretically break the vicious cycle. From our point of view, the precise etiology of the pancreatitis does not diminish the observation that it is possible to perform an islet alloautotransplantation in the context of a low-grade DSA in patients with limited access to transplantation. The patient's vPRA had increased to 87% making him an unlikely candidate for pancreas re-transplantation at his age or for HLA compatible islet transplantation. Islets are thought not to express HLA class II, except under conditions of stress/inflammation [3]. We therefore removed present antibodies using plasma exchange and IVIg before islet alloautotransplantation in order to create a "window of opportunity before DSA recurrence" to infuse islets that could have upregulated HLA class II expression due to stress of isolation and culture, instant blood-mediated inflammatory reaction, or hypoxia. Islets are relatively protected from DSAs once vascularized due to endothelial chimerism and vascular sequestration of DSAs [24]. Notably, a recent single-center study, published after our case occurred, showed favorable outcomes of allogeneic islet transplantation in the presence of preformed DSAs, questioning in hindsight the necessity for pretransplant plasma exchange and IVIg [25]. Low-grade DSA convey excellent outcome [26].

Before deciding to perform the islet alloautotransplantation, we discussed whether to treat the likely pancreas allograft rejection with a third round of methylprednisolone and a postponed second-line treatment for chronic (or mixed-type) ABMR, which consists of alemtuzumab, plasma exchange, and/or IVIg at our center. Viral complications (BK-viremia, COVID-19) had prohibited escalation before. However, we felt that efficacy of such therapy for longer-standing chronic active perhaps antibody-mediated pancreas rejection would be questionable and that, given the progressive complications (panniculitis) necessitating chronic use of opioids, a more definite solution was warranted. The persistent immunodominant HLA-DSA DQB1*05:02-DQA1*01:02 at 1 year post-islet alloautotransplantation suggests indeed that second-line treatment would not have made the DSA disappear. Although speculative, we also hypothesized that taking away the subsisting inflammatory stimulus (exocrine tissue rejection/pancreatitis) would likely increase the chance of a more sustained response to novel induction treatment and better preserve his kidney function considering his highly-immunized status [27]. A limitation of this point of view is that an auto-immune etiology of his type-1 diabetes could not be established (no detectable auto-antibodies), which might have contributed to a more favorable outcome of the islet transplantation.

CONCLUSION

Successful islet alloautotransplantation from a likely chronically rejecting pancreas with preserved endocrine function is possible to salvage (partial) β-cell function after pancreatectomy. Preformed low-grade DSAs are not necessarily a contraindication. This strategy may be considered to prevent relapse of inadequate glycemic control with recurrent severe hypoglycemic events and have improved metabolic control with better preserved kidney transplant function, when there is ongoing need for immunosuppression and the patient is an unlikely candidate for pancreas re-transplantation.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

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

DV: concept/design and drafting article; MN: critical review of the article and supervision; DB: critical review of the article; MB: critical review of the article concerning the panniculitis and providing clinical pictures of the panniculitis; JK: critical review of the article and providing pathology pictures; ME: critical review of the article; VH: critical review of the article; DR: critical review of the article and performing immunoassays; SH: critical review of the article and performing immunoassays; IA: critical review of the article; EK: critical review of the article and supervision; AV: concept/design, critical review of the article and supervision. 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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REVIEW
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Healthcare System Impact on Deceased Organ Donation and Transplantation: A Comparison Between the Top 10 Organ Donor Countries With 4 Countries in Southeast Asia

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The need for organ donation is constantly increasing. Some countries have made improvements, while others, such as countries in Southeast Asia (SEA), have some of the lowest rates of deceased donors (pmp). This review aims to compare 14 countries with regards to many variables related to healthcare systems. Countries leading in deceased organ donation spend more on health and education, which is associated with increased potential for deceased organ donation. Out-of-pocket expenditure, is also associated with a decrease in deceased organ donation. Countries in SEA are lacking in healthcare resources such as workforce and materials, which are both necessary for a successful transplant program. Most countries in SEA have an excellent foundation for successful organ donation systems, including proper legislation, government support, and brain death laws along with an overall acceptance of brain death diagnosis. Priorities should include improving coordination, donor identification, and healthcare worker education. Countries in SEA have a lot of potential to increase deceased organ donation, especially by investing in healthcare and education. There is no one size fits all for organ donation programs and countries in SEA should focus on their strengths and take cultural differences into consideration when planning interventions.

Keywords: transplantation, organ donation, deceased donation, Southeast Asia (SEA), healthcare systems

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Abbreviations: DCD, donation after circulatory death; EXT, external health expenditure; GDP, gross domestic product; GGHE-D, domestic general government health expenditure; ICU, intensive care unit; IMR, infant mortality rate; MMR, maternal mortality ratio; OOPS, out-of-pocket spending; pmp, per million population; PVT, private health expenditure; RTA, road traffic accident; SEA, Southeast Asia.

INTRODUCTION

Around the world, the need for organ transplantation is constantly growing due to an increase in non-communicable diseases and aging populations. Medical advances and expanding health coverage in the past few decades have allowed people to live much longer with their chronic illnesses, but an organ transplant remains the most cost-effective and long-lasting option in many cases [1]. Although organ donation has been steadily increasing in the last couple of decades, there remains great inequalities between different regions around the world. Europe and North America are far ahead of the other regions, with Spain and the US having 49.61 and 36.88 actual deceased organ donors per million population (pmp), respectively in 2019 [2]. In comparison, nations in SEA had some of the lowest rates of deceased organ donors in the world [3], with 3.66 pmp in Thailand and only 0.53 pmp in Malaysia [2]. This gap highlights the importance of establishing a solid framework for organ donation in SEA, which will rely on changes in legislation, education, and healthcare [3]. A lot of research has been done on the reasons why countries in SEA have such low rates of deceased organ donors, but a comparison of healthcare systems between the countries with the highest rates of deceased organ donors and countries in SEA with extremely low rates has never been done. The main purpose of this research is to highlight the similarities and differences between the healthcare systems of countries leading in deceased organ donation and countries in SEA. Furthermore, the authors wanted to identify strengths and weaknesses of each country in order to suggest interventions to increase deceased organ donation.

Healthcare systems worldwide are extremely varied and unique. A combination of resources, population needs, and organizational capacity leads to differences in access and utilization. Variation in deceased organ donation between countries has been proven to be unrelated to medical need [4, 5], but instead correlated with the availability of healthcare resources, a country's GDP *per capita*, and health expenditure (percentage of GDP spent on healthcare) [4–7]. Intuitively, higher income *per capita* allows for higher health spending and better access to advanced medical technology required for transplantation [5]. Another reason for differences in healthcare system may be due to having different healthcare related priorities due to cultural and social values [8]. Therefore,

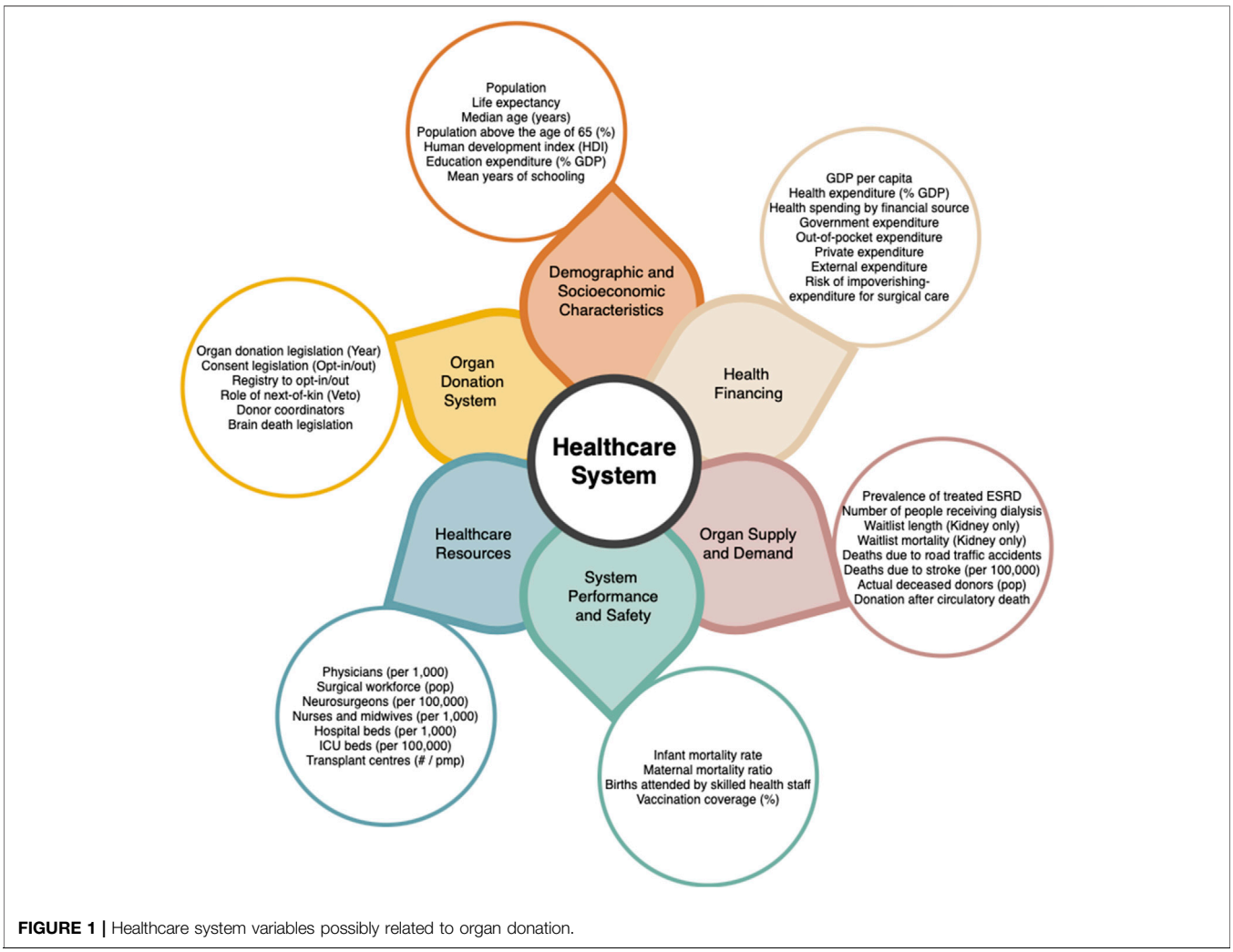

FIGURE 1 | Healthcare system variables possibly related to organ donation.

when comparing countries with different demographics, it is essential to remain aware of the circumstantial differences of each country [8]. A healthcare system is a dynamic and constantly growing mechanism. There are many different aspects that have immense impacts on efficiency and outcomes, and no one healthcare system looks the same. **Figure 1** shows the variables chosen to be explored in this research.

The countries chosen for this analysis include the ten countries with the highest rates of deceased donors per million population according to IRODaT 2019, which are Spain, United States, Croatia, Portugal, France, Belgium, Czechia, Finland, Belarus, and Malta [2]. No countries were excluded based on population size or systemic or legislative requirements. The four remaining countries were chosen due to their geographic location (being in SEA) and due to being part of the Organ Donation Initiative Strategies for Southeast Asia (ODISSeA) consortium. ODISSeA's main objective is to design and implement an academic postgraduate program in organ donation in eight universities across Malaysia, Myanmar, Philippines, and Thailand [3].

CURRENT STATUS OF ORGAN DONATION IN SOUTHEAST ASIA

SEA continues to experience low rates of deceased organ donors despite seeing a steady increase in economic growth. Inadequate organ donation legislation has led to struggles with organ trafficking and transplant tourism [9], leading to demands towards government officials to make changes regarding healthcare financing, legislation, and medical technology diffusion [10]. The Istanbul declaration of 2008 aimed to decrease illegal practices in organ transplantation, but previous higher rates of donation, which were partially due to transplant tourism, decreased dramatically and have not been able to recover [10]. Below are brief summaries of the status of organ donation in the four countries in SEA studied.

Malaysia

The healthcare services for a population of 33 million in Malaysia are delivered through public and private providers. Malaysia does not have a national insurance program; however, all citizens get treatments including transplants through centrally funded and administered government health facilities at very low cost [11]. The first organ transplant was performed in 1975 with a living-related kidney transplant and the first deceased kidney transplant was performed the following year [12]. Facilities for kidney, liver, heart, and lung transplants are available in seven public and private hospitals, all located around the capital city. Only public and university hospitals carry out transplants from deceased donors. The National Transplantation Programme is governed by the National Transplantation Council under the Malaysian ministry of health. The National Transplant Resource Centre was established in 1997 to coordinate deceased organ and tissue donation at the national level and is supported by Tissue Organ Procurement teams, which are available in regional hospitals [13]. The practice of deceased donation is legalised by the Human Tissues Act (1974) [14] and supported by the National Fatwa (1970) [15]. Despite efforts to increase organ donation, deceased donation rates remained below 1.0 donor pmp. Living donations make up the majority the organ transplantation [16].

Thailand

The country of approximately 69.6 million performed its first transplant in 1972 [17]. Thailand now performs kidney, liver, heart and lung transplants in 28 transplant centers across the country [18]. The Organ Donation Center, established in 1994 under the authority of the Thai Red Cross Society, is responsible for overseeing the transplant practice, recovery and distribution of deceased organs, public relations, fundraising, and legal issues [17]. Except for the basic principles set by the Medical Council and the Red Cross, Thailand has no laws specific to organ donation [19]. Three government health coverage schemes, namely, the Civil Servant Medical Beneficiary System, the Social Security Organization, and the Universal Health Coverage Scheme (UCS), cover the entire population. In 2008, the cost of surgery, including post-operative care and immunosuppressive medication, became reimbursable for all citizens following the launch of universal renal replacement therapy program under the UCS [20]. Deceased donation rate improved remarkably from 0.7 in 2005 to 4.8 pmp in 2020 and is now the highest in SEA [2]. The number of kidney transplant from deceased donors exceeds the number of transplants from living donors since 2011 [18]. Unlike Malaysia, both public and private hospitals perform transplant from deceased donors [18]. Organ donation rates have been on the rise thanks to public organ donation campaigns supported by the Thai Royal family; however, shortage of organs still limits the rate of transplantation [18].

Philippines

The Philippines, with a population of 108.1 million population, recorded only 26 deceased donations between 2017 and 2019 [2]. Philippines has an administratively decentralized public health system, where local governments have full policy and fiscal freedom [21]. The Department of Health (DOH) is the national health agency that develops and regulates national policies and provide tertiary and specialized hospital services [21]. Social health insurance was introduced in 1995 and administered by the Philippine Health Insurance Corporation (PhilHealth) to enhance the nation's financial risk protection, however it only contributes to a small portion of total health expenses [21]. The Passage of Organ Donation Act of 1991 legalized deceased donation for treatment, research, or medical education by will of the deceased or consent from family members [22]. Philippine Network for Organ Sharing (PhilNOS), which was established in 2010 by the DOH, is the central coordinating body that regulates transplant activities including deceased donation, organ allocation, and maintaining the national registry [9]. Organ Procurement Organizations (OPO) operate under donor service areas designated by PhilNOS responsible for brain death certification, acquiring consent, donor maintenance, retrieval organ and tissues from deceased donors for transplantation [23]. There were 18 accredited transplant centers distributed in different regions of the Philippines [24].

TABLE 1 | Healthcare system comparison variables results.

Country	A. Demographic and socioeconomic characteristics								B. Health financing and health spending			C. Health spending by financial source *per capita* in US$ (% total) (2018)			
	Population 2019 (millions)	Life expectancy	Median age	65+ (%)	HDI	Education expenditure (% of GDP)	Mean years of school	Medical schools (pmp)	GDP *per capita* 2019 (USD)	Health expenditure (% GDP) 2019	Risk of impoverishing expenditure for surgical care (% of people at risk)	GGHE-D	OOPS	PVT-D-OOPS	EXT
Spain	47.13	83.49	44.9	19.6	0.90	4.21	10.3	0.91	29,564.7	9.0	0.1	1,926 (70.4%)	606 (22.1%)	204 (7.5%)	0 (0.0%)
United States	328.24	78.79	38.3	16.2	0.93	4.96	13.4	0.59	65,297.5	16.9	0.2	5,356 (50.4%)	1,148 (10.8%)	4,120 (38.8%)	0 (0.0%)
Croatia	4.07	78.42	44.3	20.9	0.85	3.92	11.4	0.98	14,944.4	6.8	0.1	844 (83.2%)	106 (10.5%)	64 (6.3%)	0 (0.0%)
Portugal	10.29	80.68	46.2	22.4	0.86	5.02	9.3	0.78	23,214.0	9.4	0.3	1,361 (61.4%)	654 (29.5%)	198 (8.9%)	2 (0.1%)
France	67.06	82.56	42.3	20.4	0.90	5.45	11.5	0.57	40,496.4	11.3	0	3,441 (73.4%)	434 (9.3%)	815 (17.4%)	0 (0.0%)
Belgium	11.50	81.75	41.9	19.0	0.93	6.41	12.1	0.61	46,345.4	10.3	0	3,723 (75.8)	936 (19.1%)	254 (5.2%)	0 (0.0%)
Czechia	10.67	79.13	43.2	19.8	0.90	3.85	12.7	0.84	23,489.8	7.6	0	1,460 (82.7%)	251 (14.2%)	54 (3.1%)	0 (0.0%)
Finland	5.52	81.79	43.1	22.1	0.94	6.38	12.8	0.91	48,771.4	9.0	0	3,547 (78.6%)	832 (18.4%)	136 (3.0%)	0 (0.0%)
Belarus	9.42	74.23	40.3	15.2	0.82	4.79	12.3	0.42	6,698.0	5.6	0.1	251 (70.5%)	89 (25.0%)	15 (4.2%)	1 (0.3%)
Malta	0.50	82.60	42.6	20.8	0.90	4.82	11.3	4.00	29,737.3	9.0	0	1,748 (63.5%)	944 (34.3%)	61 (2.2%)	0 (0.0%)
Mean	49.44	80.34	42.71	19.64	0.89	4.98	11.71	1.06	32,855.88	9.50	0.08	2,365.7 (71.0%)	600 (19.3%)	592.1 (9.65%)	0.3 (0.04%)
Thailand	69.63	77.15	40.1	12.4	0.78	4.12	7.9	0.33	7,806.7	3.8	4.7	210 (76.1%)	30 (10.9%)	35 (12.7%)	1 (0.4%)
Malaysia	31.95	76.16	30.3	6.9	0.81	4.16	10.4	1.00	11,414.2	3.8	3.5	219 (51.2%)	150 (35.0%)	59 (13.8%)	0 (0.0%)
Philippines	108.12	71.23	25.7	5.3	0.72	2.54	9.4	0.41	3,485.1	4.4	18.6	45 (32.8%)	74 (54.0%)	17 (12.4%)	1 (0.7%)
Myanmar	54.05	67.13	29.0	6.0	0.58	1.93	5.0	0.11	1,407.8	4.8	—	9 (15.3)	45 (76.3%)	0 (0.0%)	5 (8.5%)
Mean	65.94	72.92	31.28	7.65	0.72	3.19	8.18	0.46	6,028.45	4.20	8.93	120.75 (43.8%)	74.75 (44.1%)	27.75 (9.7%)	1.75 (2.4%)

Country	D. Organ demand and supply								E. System performance and safety				G. Organ donation system					
	Prevalence of treated ESRD (pmp)	Dialysis (pmp)	Waitlist active[a] (pmp)	WaitlistMortality[b]	RTA mortality (pmp)	Stroke mortality (pmp)	Actual deceased donors (ppm)	DCD (pmp)	Infant mortality rate	Maternal mortality ratio	Births attended by skilled health staff	Immunization coverage (%)	Consent	Year	Registry	Next-of-kin can veto decision	In-hospital donor coordinator	Brain death legislation
Spain	1234[c]	587[c]	83.45	—	0.39	0.79	49.61	16.06	2.6	4	—	96.67	Opt-out	1979	No	NA	Yes	Yes
United States	2,354	1,699	184.52	3.88%	1.27	0.58	36.88	8.26	5.6	19	99.1	91.67	Opt-in	1967	Yes	No	Yes	Yes
Croatia	—	610[d]	58.29	2.72%	0.79	1.86	34.63	0	4.1	3	99.9	93.33	Opt-out	1988	Yes	Yes	Yes	Yes
Portugal	2,014	1,265	195.24	0.86%	0.82	1.62	33.8	2.6	3.1	10	98.7	98.67	Opt-out	1993	Yes	—	Yes	Yes
France	1,349	731	128.87	2.05%	0.51	0.67	33.25	6.97	3.8	4	98.1	92.33	Opt-out	1997	Yes	Yes	Yes	Yes
Belgium	1,290[c]	1,481[c]	79.48	2.01%	0.58	0.81	30.3	10.52	2.7	5	99.3	97.00	Opt-out	1986	Yes	No	—	Yes
Czechia	1,128	656	49.02[e]	5.38%[e]	0.59	1.08	27.14	1.79	2.5	1	99.8	95.33	Opt-out	2002	Yes	No	Yes	Yes
Finland	926	367	66.85	0.95%	0.39	1.01	26.23	0	1.9	8	100	93.50	Opt-out	2001	Yes	No	Yes	Yes
Belarus	248[c]	151[c]	19.85	6.32%	0.76	1.80	26.2	0	2.4	1	99.8	97.67	Opt-out	1997	Yes	Yes	—	Yes
Malta	—	600[d]	178.22	5.00%	0.41	0.66	25	0	6.1	0	99.7	97.33	Opt-in	2016	Yes	—	Yes	Yes

(Continued on following page)

TABLE 1 | (*Continued*) Healthcare system comparison variables results.

Country	D. Organ demand and supply								E. System performance and safety					G. Organ donation system					
	Prevalence of treated ESRD (pmp)	Dialysis (pmp)	Waitlist active[a] (pmp)	WaitlistMortality[b]	RTA mortality (pmp)	Stroke mortality (pmp)	Actual deceased donors (ppm)	DCD (pmp)	Infant mortality rate	Maternal mortality ratio	Births attended by skilled health staff	Immunization coverage (%)	Consent	Year	Registry	Next-of-kin can veto decision	In-hospital donor coordinator	Brain death legislation
Mean	1510.08	943.60	106.76	3.24%	0.65	1.09	32.30	4.62	3.48	5.50	99.38	95.35						
Thailand	2,028	1,885	92.16[f]	—	3.22	0.73	3.66	0	7.7	24	99.1	96.67	Opt-in	None	Yes	Yes	No	Yes
Malaysia	1,412	1,357	161.10[e]	8.92%[e]	2.25	0.62	0.53	0	7.3	23	99.6	97.33	Opt-in	1974	Yes	Yes	No[h]	No
Philippines	224[g]	607[f]	64.74[f]	—	1.20	0.67	0.09	0	21.6	206	84.4	65.67	Opt-in	1992	—	Yes	No	Yes
Myanmar	—	75[f]	—	—	2.04	1.53	0	0	35.8	244	60.2	88.00	n/a	2004	No	NA	No	No
Mean	1221.33	981.06	106.00	—	2.18	0.89	1.07	0.00	18.10	124.25	85.83	86.92						

[a]*Number of people on the waitlist at the end of 2019.*

[b]*Number of people who died while on the waitlist over the total number of people who were on the waitlist in 2019.*

[c]*Data from 2016 instead of 2018.*

[d]*Data from 2019, not 2018.*

[e]*Data from 2018 instead of 2019.*

[f]*Year of data unknown but published recently.*

[g]*Data from 2013.*

[h]*Malaysia now has a few hospitals with donor coordinators since 2020. Data in table is based on 2019, to reflect rated of actual deceased organ donors.*

Myanmar

Myanmar has a shorter history of organ transplantation, having started with kidney transplants in 1995 and liver transplants in 2004 [25, 26]. Currently, transplant for kidney and liver are available in nine hospitals. Myanmar, with a population of 54 million, has universal health coverage through public facilities but national health insurance system is not available [26]. It is an under-resourced country with key challenges in organ transplantation including shortage of immunology transplant laboratories, trained medical personnel, medication, and financial support. Before 2010, there was an average of 4–5 kidney transplants per year. With the help of international experts through joint operations, on-site medical knowledge sharing, and fellowship training programmes, the number increased substantially over the next 10 years. There were 78 kidney transplants performed in 2018, the highest number ever recorded since the launch of the program. Between 2004 and 2021, 56 liver transplants including two from deceased donor were performed [27]. Despite the improvement in transplantation, a deceased donor program has not been established in Myanmar. The Body Organ Donation Law enacted in 2004 and revised in 2015 allows deceased organ donations with the will of the deceased or consent from the relative, but most transplants are nevertheless from living and non-related donors.

HEALTHCARE SYSTEM COMPARISON

Demographic and Socioeconomic Characteristics

Life expectancy is on average lower in SEA than in countries leading in deceased organ donation, though there are some exceptions, such as Thailand and Malaysia having a higher life expectancy than Belarus. The Human Development Index (HDI) is associated with deceased donation rate, suggesting that a country needs to have a minimum socioeconomic level to set up and support a deceased donor program [9, 10]. Malaysia is classified as having a very high human development along with other countries leading in deceased organ donation. This reflects the country's high potential to develop efficient deceased donor activities. Thailand and Philippines have high human development, while Myanmar falls under the medium human development category [11]. Finally, countries in SEA have much younger populations compared to countries leading in deceased organ donation; less than 10% of the population in Malaysia, Philippines and Myanmar are aged 65 years and above (See **Table 1**: Section A).

Countries in SEA spend less on education and individuals in Thailand and Myanmar receive on average less years of schooling. However, Malaysia does have the greatest number of medical schools pmp after Malta (See **Table 1**: Section A). Government education expenditure is positively associated with deceased kidney transplant rates and the percentage of the population with higher education significantly associated with higher rates of organ donation [4, 7]. Educational attainment is also significantly associated with willingness to donate [1, 28]. Overall, education is

a vital aspect of an efficient organ donation system. Increased spending on education could increase the knowledge about organ donation in the general population and improve the quality of education available to healthcare workers interested in the field of organ donation. The concept of health literacy may also be important, especially since healthcare systems have been becoming more complex and more difficult to navigate [29].

Another vital impact on organ donation are cultural and religious beliefs. In Malaysia, many cite religion to be a reason why they would refuse to become organ donors. However, some of the more common reasons for not wanting to become an organ donor was related to a lack of trust in the healthcare system to use their body in an appropriate manner and a lack of understanding of what organ donation was and why it was such a necessity. Some cultural beliefs such as wanting their body to remain intact after death was also a common response [30]. Strong beliefs surrounding familial involvement in the decision may also be a reason why people do not give consent for donation before death [31].

A study done in Germany comparing organ donation as it relates to Christians, Muslims, Jews, Hindus, and Buddhists showed that most view organ donation as an altruistic and heroic act, as long as certain rules are respected. All except Buddhism had a universal acceptance of the concept of brain death and believed both the donor and family members had the right to decide for the donor. Despite this, many in the study had still not signed a card saying that they accepted to be organ donors. This was largely due to misconceptions or misunderstandings of religious doctrines and a fear of doing something wrong [32].

The countless studies on organ donation, culture, and religion shows the importance of education and campaigns with a highlight on religious acceptance of them. Encouraging individuals to discuss organ donation with friends and family should also be encouraged since familial decision making is so important.

Health Financing

One of the most important aspects when determining the strength of a healthcare system is undeniably related to money. Countries leading in deceased organ donation have on average 5.5 times higher GDP *per capita* than countries in SEA and spend around 2.25 times more of their GDP on health (health expenditure) (See **Table 1**: Section B). Countries leading in organ donation spend on average 9.5% of their GDP on health, ranging from 5.6% in Belarus to 16.9% in the United States. Countries from our SEA group spend on average 4.2% of the GDP on health, ranging from 3.8% in Thailand and Malaysia, to 4.8% in Myanmar. We also need to consider the difference in raw GDP, meaning the low percentage is exponentially lower in actual amount of money spent. Increased health expenditure is associated with increased quality of critical care, which is essential for organ donation [33]. Furthermore, individuals living in SEA are much more at risk of impoverishing expenditure due to need of surgical care, a risk that does not exist in countries leading in organ donation.

Health Spending

To better understand health financing, we need to look at the sources of financing, namely, government, external sources, out-of-pocket (household spending), and other private sources such as insurance (See **Table 1**: Section C; **Figure 2A**). Government contribution in SEA is fairly low, especially in the Philippines and Myanmar. However, the government in Thailand contributes on average 76%, which is more than any other SEA country and even surpasses some countries leading in organ donation. Percent share of OOPS is much higher in SEA, although the United States has the highest crude OOPS by far, it only accounts for 10.8% of all health financing. This could be due to differences in cost of care in different countries [34]; individuals in the United States pay more for health services, but the government and private sources also contribute more (See **Figure 2B**). The United States has the highest crude and proportion of spending coming from other private sources due to its notable privatized insurance system. The proportion of financing coming from private sources is much higher in SEA, except Myanmar, which instead has a notable source of funding coming externally.

Higher government spending (%) and lower OOPS (%) is associated with higher rates of deceased organ transplantation, whereas private health expenditure had no impact on rates of deceased organ transplantation (See **Figure 3**). By decreasing out-of-pocket costs by either increasing government spending or by increasing access to equitable and efficient private insurance, deceased organ donation capacity may be greatly increased in SEA.

Organ Demand and Supply

The incidence and prevalence of end-stage-renal disease (ESRD) is increasing globally. This is also leading to an increase in need for dialysis and transplantation. In this 14-country comparison, there is not a big difference in ESRD prevalence between the two groups (See **Table 1**: Section D). Malaysia and Thailand have higher rates of dialysis than the average for countries leading in organ donation (943.60). Philippines and Myanmar, however, are below that average, possibly due to high out-of-pocket costs for dialysis [34]. Dialysis is a very expensive, long-term treatment, costing generally twice as much as a renal transplant when looking at a time frame of more than 1 year [35]. In countries with government reimbursement for dialysis, such as Thailand and Malaysia, increasing deceased organ donation should be a government goal due to cost-effectiveness.

Waitlist length is difficult to interpret because a low number could represent either a low need for transplantation, an unused waitlist system, or an effective transplant system. Waitlist mortality, represented as the percentage of people who died while waiting for an organ (Waitlist includes total for kidney, liver, heart, lungs, pancreas, and small bowel) out of everyone who was ever on the waitlist in that year, is a better indicator of unmet needs for organ donation. Malaysia has a waitlist mortality of 8.92%, nearly three times larger than the average for countries leading in organ donation. Data for the other three countries in SEA could unfortunately not be found.

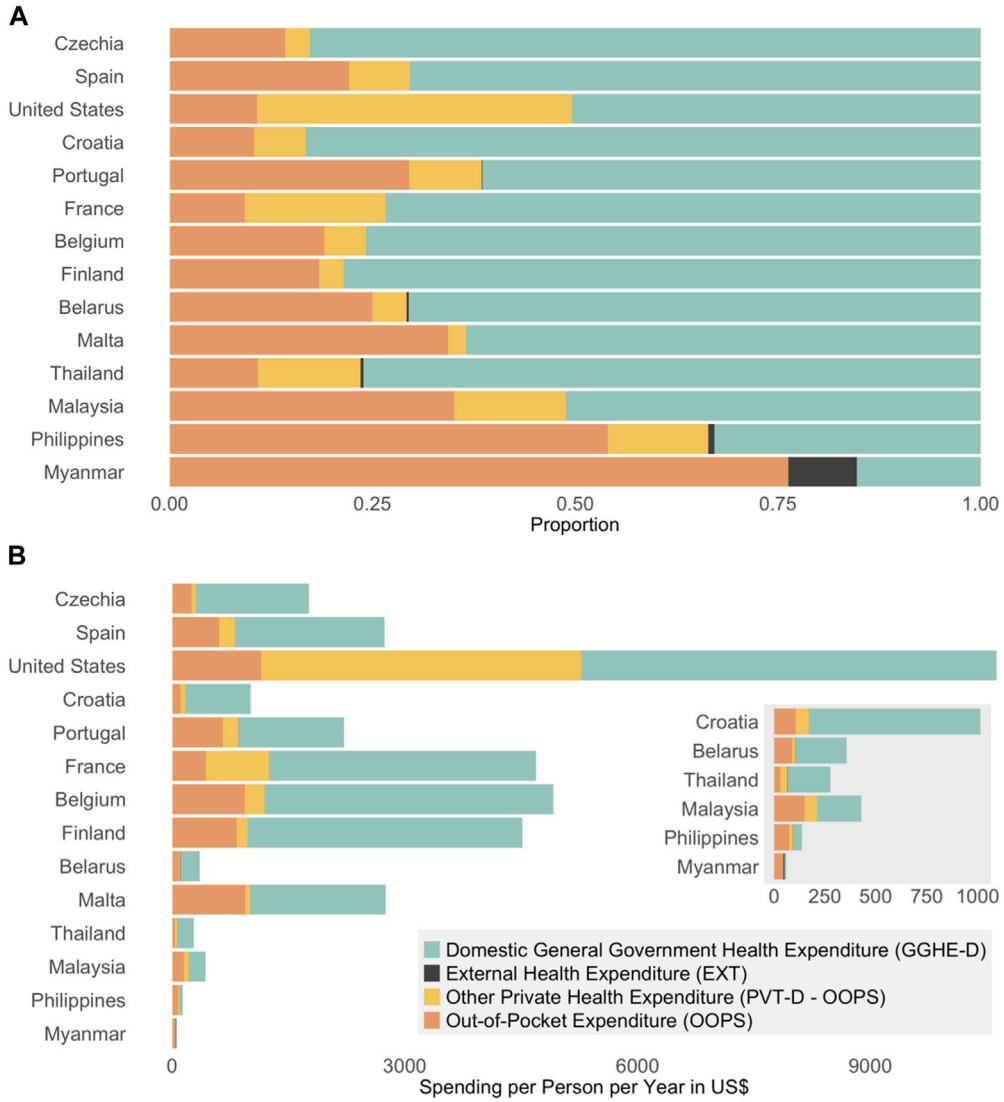

FIGURE 2 | (A) proportion of health spending by financial source. **(B)** Health spending by financial source *per capita* in US$.

Most deceased organ donation occurs after brain death, usually caused by road traffic accident (RTA) injury and stroke [36]. Countries in SEA have on average 3.35 times more deaths from RTA injury (pmp) than countries leading in organ donation but have on average fewer deaths due to stroke (pmp). Donation after circulatory death (DCD) is becoming increasingly common. No country in SEA performs DCD, but 6 of the top 8 countries do as of December 2020, with Croatia and Finland planning to implement legislation in the near future [37]. Finland did have its first DCD transplants in 2021 (IRODaT). Some researchers recommend expanding DCD programs to increase potential donors in countries with currently low rates of deceased organ donation [38, 39]. Unfortunately, instating legislation for DCD is complex and requires a lot of organizational and financial capacity [37]. Furthermore, the need for DCD is mostly due to the decreasing rates of traumatic brain injuries from RTA in developed countries, a problem that SEA is not yet facing [40]. For these reasons, implementing DCD should not be a priority for SEA at this time. However, due to a high number of potential donors due to elevated RTA mortality, donor identification, one of the first steps in the deceased organ donation process, should be prioritized [41]. This comes back to investing in educational programs for healthcare workers.

System Performance and Safety

Some health indicators are more often used to measure the status of a healthcare system and are widely accepted as representative of a country's overall health. These often include infant mortality (IMR) and maternal mortality (MMR) [42, 43]. Because most maternal deaths are preventable, they should be close to zero in a safe and effective system [43]. High maternal mortality is often

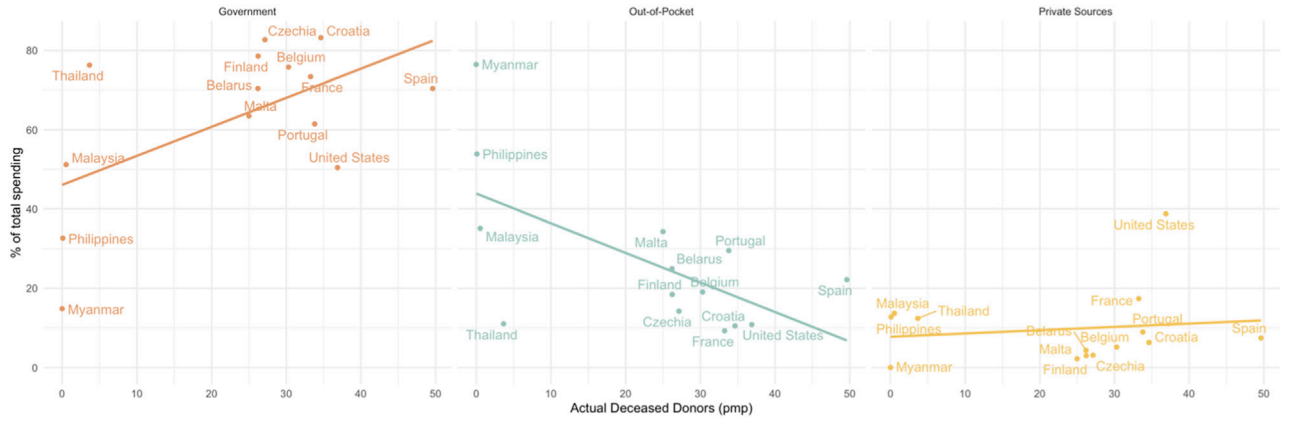

FIGURE 3 | The relationship between actual deceased donors (pmp) and financial source.

associated with scarcity of health resources and certain political issues such as government corruption [43]. The IMR in Thailand and Malaysia only about twice as high as the average IMR in countries leading in deceased organ donation. However, the IMR is 6 times greater in Philippines and 10 times greater in Myanmar compared to the top 10 countries. MMR follows the same trend, with Thailand and Malaysia being around 4 times greater than the average for countries leading in organ donation, whereas Philippines and Myanmar have a MMR 37.5 times and 44.3 times greater, respectively. Delivery by a skilled birth attendant is a measure of the progress toward eliminating maternal mortality and is commonly used as a measure of access to and safety of healthcare in a country [44]. Almost 100% of births are attended by a skilled healthcare professional in Thailand and Malaysia, like all countries leading in organ donation, whereas only 84.4% of births in Philippines and 60.2% of births in Myanmar are attended by a skilled healthcare professional. Average infant immunization rates (Hepatitis B, Measles, and DTP) are also as high in Thailand and Malaysia, but Myanmar and Philippines are still lacking in this area (See **Table 1**: Section E). The system performance between countries is very different in SEA, namely, Malaysia and Thailand appear to be far ahead of Myanmar and Philippines. Malaysia and Thailand have a lot of potential to increase deceased organ donation through slight alterations in legislation and education, whereas Myanmar and Philippines may need a few more years to catch up and organ donation may not be a priority at this time. Major issues of safety and access first need to be addressed.

Healthcare Resources

Some of the biggest barriers for obtaining organ donors include poor hospital infrastructure, missing manpower, and inability to identify and support brain dead donors [45]. On average, countries leading in organ donation have 4.1 times more physicians, 9.8 time more surgical workforce, 4.6 times more neurosurgeons, and 3.6 times more nurses and midwives than countries in SEA. Regarding materials, countries leading in organ donation have on average around 3.5 times more beds, ICU beds, and transplant centres (pmp). Data for healthcare resources can be found in **Supplementary Table S2** and are visually presented in **Figure 4**.

The availability of staff and materials has a very negative effect on the organ donation process. The "death to donation to transplantation process" suggested by Manzano in 2014 relies heavily on availability of healthcare professionals for donor identification and retrieval, consent to donation, and organ retrieval [41]. The lack of nurses and doctors in SEA severely decreases the ability of staff to fulfill organ donation related tasks on top of their regular tasks. To optimize the process, countries in SEA should focus on incentivising people to enter healthcare professions. Another option is to use non-medical professionals to carry out donor coordinator tasks, like what is done in the United States. Although donor coordinators should ideally be given enough time to carry out donor coordinator related task, a minimum requirement would be to pay them for the work they do, either per patient or per hour. This is done in most countries leading in organ donation who do not have donor coordinator only positions.

The organ donation process is also dependent on expensive materials for donor assessment, donor maintenance, and organ storage and transportation [41]. A lack of essential equipment such as hospital beds and ICU beds could be detrimental to deceased organ donation [38]. If there are insufficient beds, the hospital cannot justify keeping a bed for even just several hours to wait for a recipient of the organs. However, the use of ICU beds in the organ donation process varies greatly from country to country, meaning some countries may have a more efficient way of managing ICUs and distributing patients across different levels of care units [46].

This can be seen with the leader of deceased organ donation, Spain, having one of the lowest number of ICU beds per 100,000 population in the top 10 leading countries, having even fewer ICU beds per 100,000 population than Thailand (See **Supplementary Table S2**). This demonstrates that although a baseline ICU capacity is needed, efficient

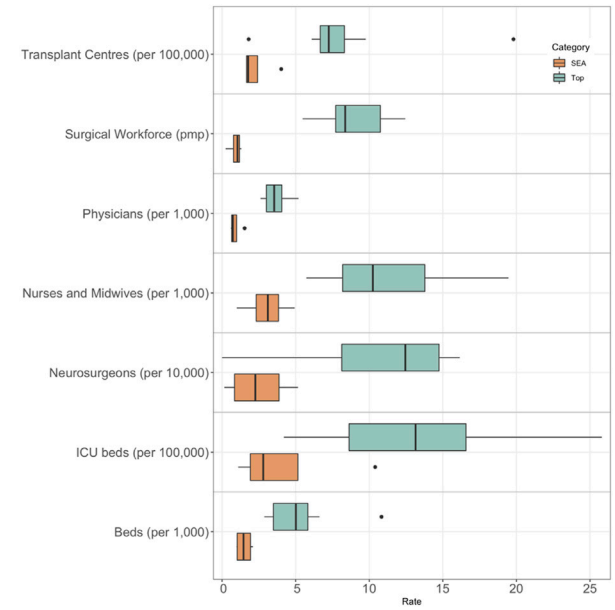

FIGURE 4 | Healthcare resources in SEA compared to countries leading in deceased organ donation.

management of assessing and treating potential donors is just as important if not more. This is due to other necessary components of an efficient transplant system such as institutional reformation, quality assurance, reimbursement schemes and comprehensive training programs [47]. The organizational components of Spain's transplant system, such as donor coordinators, may also contribute to the efficiency of their ICUs without the need for as many beds as other countries leading in organ donation. Another non-medical but closely related variable that organ donation is highly dependent on is access to efficient transport. In Spain, individuals in rural areas needing transplant can be transported by helicopter, whereas this type of rapid transport is not available in SEA. This rapid transportation system makes for an extremely efficient transplant network.

Organ Donation System

Every country has a unique combination of laws and regulations regarding practices, coordination, and consent (See **Table 1**: Section G). All countries in SEA have opt-in consent systems, except Myanmar, which lacks regulations to be considered either. Countries leading in deceased organ donation are mostly opt-out countries, except US and Malta. A lot of research has been done comparing opt-in versus opt-out countries and found that although deceased donor rates are higher in opt-out countries, the difference is not significant and is most likely not solely due to the consent legislation, but rather due to other organizational components [7, 48, 49]. There does not seem to be an association between rates of organ donation and the year of initial donation legislation, since Malaysia was one of the first to implement legislation, even before Spain. However, organ donation did not take off in Spain until the creation of the National organization of transplantation (ONT) in 1989 [50]. This suggests that merely having a legislation or law regarding organ donation is not sufficient to increase organ donation and having organizational components are mandatory for efficiency and success.

The usefulness of registries is also a topic of debate. Most countries have a registry, either to opt-in or opt-out, or in the case of Belgium, both opt-in and opt-out. Donor registries can be useful not only for identifying potential donors, but also to promote public awareness [51]. However, since Spain does not have a registry, we can confidently say that the success of an organ donation system does not depend on the presence of a registry, though this may be truer for opt-out systems. There has never been research done on the effectiveness of a registry and how many donors come from checking the registry compared to asking family for consent. Obtaining consent from family members is considered one of the essential elements of a successful organ donation system [51]. In most countries, the final decision is ultimately up to the next-of-kin, also known as soft opt-out [52]. In Belgium, however, an individual's name on either the opt-in or opt-out registry is legally binding. So even if the family knows their loved one had changed their mind, the organs cannot be retrieved. In Malaysia and Thailand, consent to donate is always asked from the next-of-kin whether the individuals' name is on the registry or not. With this, individuals who have opted-in can still become non-viable donors due to declined family consent. Some believe this "overrule" could jeopardize the trust in the donation system, since individuals will not feel like their wishes will be respected [1]. Many countries with hard opt-out legislation still use a soft opt-out approach because not following the wishes of the family leads to more negative publicity that could put organ donation in a negative light.

Another vital component of the organ donation system are donor coordinators. Spain is often cited as the poster-child of deceased organ donation, having the most successful program in the world [2]. The "Spanish Model" relies on access to higher education to support doctors and nurses working in ICUs who have high exposure to potential donors [40]. With advanced education in donor identification, brain death diagnosis, donor management, family approach, grief counselling, refusal management, and organ allocation, healthcare professionals are more familiar and have a more positive view of the organ donation process [53, 54]. In Spain, donor coordinators are often physicians familiar with the critical care unit and are highly motivated about organ donation. This maximizes efficiency since they may already have a relationship with the families, they approach to request donation consent [55]. Donor coordinators are different from transplant coordinators, who often work on dialysis units and support recipients of organs. Many countries have followed Spain's example and have implemented in-hospital donor coordinators such as Croatia [56], leading to a dramatic increase in deceased organ donation. However, Germany also attempted to implement this type of in-hospital coordinator in 2012 but did not see the same success [40]. The ODISSeA project allowed a group of physicians from SEA to attend seminars in Spain in 2019 to

help develop a post-graduate organ donation program in SEA. Some trained healthcare professionals in organ donation started working in hospitals as acting donor coordinators at the start of 2020 and, despite the negative impacts of COVID-19 on the healthcare system, Malaysia saw an increase from 0.53 pmp in 2019 to 0.9 pmp in 2020. Many hope that by increasing the availability of these programs in universities across SEA and implementing more in-hospital donor coordinators, countries could continue to see an increase in deceased donor transplantation.

Increasing organ donation relies heavily on both professional and public acceptance of brain death [46]. The lack of awareness around this concept can lead to a significant reduction in potential donors as well as a decrease in donor identification [45]. Although most countries have some laws regarding brain death diagnosis, these vary slightly between different countries [57]. Brain death legislation was introduced a lot later in most Asian countries, where cultural resistance and fear of abuse remain serious issues [39]. Brain death is legally recognized in Thailand (1989), Malaysia (2006), Philippines (1991) and Myanmar (2009), but there is no official law in Malaysia and Myanmar [58]. Brain death diagnosis requires multiple exams separated by a determined time and the presence of 2–3 doctors with varying qualifications (neurologist/neurosurgeon, anesthesiologist, intensivist, internist). These criteria are the same in countries leading in organ donation, but the availability of such specialists is a lot lower in SEA. Brain death is becoming more accepted among both health professionals and the general population in SEA. Nevertheless, religion and culture are still some of the main reasons for family objection to donation [59].

DISCUSSION

The countries in this comparison come from a variety of economic and developmental backgrounds. This makes comparison very difficult. For example, even in SEA, Thailand and Malaysia are very different from Philippines and Myanmar regarding financial and resource capacity. In the group of countries leading in deceased organ donation, countries are more homogeneous, with Belarus being a unique example. Belarus is the only upper-middle income country in the group of top ten countries in deceased organ donation. This is possible evidence that Thailand and Malaysia, which are both also upper-middle income countries, have the capacity to increase deceased organ donation through organizational changes. Due to cultural, social, and economic differences between the four SEA countries, every country has strengths and weaknesses regarding deceased organ donation capacity and should implement strategies to increase donation based on those particularities (See **Table 2**).

Thailand currently has the highest number of deceased donors pmp in SEA. They have a high HDI and the second fastest growing GDP and GDP *per capita* in SEA after the Philippines. They already have high government spending on health and therefore low out-of-pocket costs for health. Along with the highest rates of surgical workforce, hospital beds, neurosurgeons, and ICU beds in SEA, they also have the highest rates of transplant centres in SEA. With a decrease in IMR and MMR and an increase in access and safety of healthcare, Thailand is on its way to catching up to other countries leading in organ donation. Some things standing in the way of Thailand perfecting its transplant program include lower than average levels of population education, low levels of doctors and nurses, and a high prevalence of ESRD and dialysis, meaning an elevated need for organ donation. The Thai government should focus on organ donation based on cost-effectiveness; encouraging people to become organ donors after death to help the thousands of people on dialysis. They also need to address the low levels of doctors and nurses, encouraging people to enter the profession. Luckily, Thailand already has an incredible infrastructure and just needs to fine tune its organizational components to increase donor identification and referral. We recommend funding University level programs for the training of donor coordinators that could increase the efficiency of Thailand's transplant program.

Malaysia also has a lot of potential, considering its very high HDI, high GDP *per capita*, and high spending on education leading to a highly educated population and the most number of medical schools pmp. This in turn leads to Malaysia having the highest rates of physicians. Malaysia is also catching up the high-income countries leading in organ donation with its good monitoring system for disease, treatment, and organ donation activity, decreasing IMR and MMR, and increase in access and safety of healthcare. Weaknesses include high out-of-pocket costs for healthcare, a high prevalence of ESRD and dialysis, and a high waitlist mortality. Malaysia should prioritize developing an efficient organ donation system due to so many people requiring dialysis. They should focus on training physicians to be donor coordinators by making more programs available throughout the country. The government should also focus on population education through educational campaigns to raise awareness about organ donation. Finally, the Malaysian government should focus on reducing out-of-pocket spending by either increasing government spending or increasing access to private insurance.

The Philippines has a high HDI with the fastest growing GDP and GDP *per capita* in SEA. They also have the highest ratio of nurses in SEA and high levels of population education despite having a low GDP *per capita* and low education expenditure. What weakens the healthcare system is a lack of physician and hospital beds, high out-of-pocket spending for healthcare, and inadequate diseases, treatment, and organ donation activity surveillance. We recommend the Philippines to nevertheless focus on training donor coordinators but also include nurses at potential donor coordinators to compensate for the low levels of physicians. Increasing surveillance will also help in the efficiency of the transplant system. As a final comment, the Philippines has struggled with organ trafficking and transplant tourism, especially in the past, creating a threat to creating an efficient organ donation program [10]. New legislation has made it more difficult to illegally sell organs, but the population still has some negative views towards the practice in general.

Myanmar may have the lowest rates of actual deceased donors pmp but medical professionals in the country remain motivated and hopeful, participating in ODISSeA and other research contributing to finding ways to increase organ donation in the country. Unfortunately, they do have the

TABLE 2 | SWOT analysis of increasing deceased organ donation in 4 SEA countries.

	Strengths	Weaknesses	Opportunities	Threats
Thailand	- Highest actual deceased donors pmp in SEA - High HDI - Second fastest growing GDP and GDP *per capita* in SEA - High government spending (%) on health - Low out-of-pocket spending - Highest rate of RTA mortality = high potential for brain dead donors - Highest rate of surgical workforce, beds, neurosurgeons, and ICU beds in SEA - Highest rate of transplant centres in SEA - Decreasing IMR and MMR - High access and safety of healthcare	- Low level of population education - High prevalence of ESRD and dialysis = high need for transplantation - Low levels of doctors and nurses	- Focus on organ donation for cost-effectiveness, since so many people require dialysis - To address low levels of doctors and nurses, either encourage more to enter healthcare professions or use non-medical staff as donor coordinators - Infrastructure (transplant centres) is already pretty good, so just focus on organizational components to increase donor identification and referral: consider Spanish model donor coordinators	
Malaysia	- Very high HDI - High GDP *per capita* - High government spending (%) on education - Highly educated population (mean years of school) - Highest number of medical schools pmp - Highest rate of physicians in SEA - Good monitoring system for disease, treatment, and organ donation activity - Decreasing IMR and MMR - High access and safety of healthcare	- Excessive out-of-pocket costs - High prevalence of ESRD and dialysis = high need for transplantation - High waitlist mortality	- Continue training physicians to be donor coordinators by making more programs available throughout the country - Focus on population education through educational campaigns to raise awareness about organ donation - Focus on organ donation for cost-effectiveness, since so many people require dialysis - Reduce out-of-pocket spending by either increasing government spending or increasing access to private insurance	- Population level superstitions related to organ donation [28] - Slowest growing GDP in SEA
Philippines	- High HDI - Fastest growing GDP (80% 10 year increase) and GDP *per capita* (57% 10 year increase) in SEA - Highest ratio of nurses to population in SEA - Good education despite low GDP *per capita* and low education expenditure	- Lowest level of physicians and hospital beds - Inadequate diseases, treatment, and organ donation activity surveillance - High out-of-pocket spending	- Use nurses as donor coordinator to compensate for the low levels of physicians - Increase surveillance of supply and demand of transplantation along with illness to better track progress	- Issues with organ trafficking and transplant tourism [10]
Myanmar	- Relatively fast-growing GDP *per capita* - Medical professionals remain motivated and hopeful, participating in ODISSeA and other research contributing to finding ways to increase organ donation in the country - Lowest rates of actual deceased donors per population means the greatest potential to increase	- Low HDI - Low GDP *per capita* - Low education attainment - Low government health spending (15%) - High out-of-pocket spending (76%) - No private sources of health financing - Inadequate diseases, treatment, and organ donation activity surveillance	- Focus on education initiative for both the general population and healthcare professionals	- Political instability [56] - Health-seeking behaviour rooted in traditional health beliefs [56]

lowest rates on almost all indicators presented in this review and have a long way to go to catch up to the other 3 SEA countries in this review but by focusing primarily on education, both of medical professionals and the general population, they can develop their transplant program with the help of countless motivated healthcare professionals. Some threats to developing an efficient organ donation program include political instability [60] and health-seeking behaviour rooted in traditional health beliefs [60].

Limitations of the Review

This research is a very broad overview of healthcare system variables in relation to organ donation capacity. The limited number of countries makes it difficult to make conclusions regarding concrete areas in need of improvement, but hopefully the research highlights many areas of interest for future research. Another major limitation is the lack of some data, especially for the Philippines and Myanmar. These countries often do not report some disease, treatment, and organ donation data due to lack of advanced surveillance systems. Furthermore, we could not get an interview with a representative from each country and for the countries we did get further input, it was from one single expert. Finally, using globally reported variables is also problematic due to not being able to control for variation in data collection. This is especially problematic when taking variables from different sources, such as was done for ICU beds and prevalence of ESRD and dialysis.

CONCLUSION

Organ transplantation is a lifesaving practice that increases the quality of life of those lucky enough to receive one. Deceased organ donation is a very efficient way of mitigating organ waitlists. Although some countries have been able to increase efficiency and maximize their potential by using their strengths, other countries have fallen behind. Countries in SEA have a lot of unused potential which could be utilized by having government support through financial inputs in healthcare. Organ donation education for healthcare workers, such as the initiation of the ODISSeA (Organ Donation Innovative Strategies in Southeast Asia) [3] in Malaysia, Philippines, Myanmar, and Thailand, is an essential part of any developing nation regardless of their resources and limitation.

Due to cultural and economic differences, countries in SEA have different strengths and weaknesses, and should focus on these when planning interventions. There is no one-size-fits-all for organ donation systems; the priority is to find the system that works the best with what each country has to offer.

AUTHOR CONTRIBUTIONS

SC—First author, data collection, data interpretation, statistical analysis, manuscript draft, revision of manuscript, final manuscript preparation. J-KK—Corresponding author, data interpretation, manuscript draft, final manuscript preparation. S-HC—Data interpretation, statistical analysis, manuscript draft, revision of manuscript, final manuscript preparation. DS—Data interpretation, manuscript draft, final manuscript preparation. PG—Data interpretation, manuscript draft, final manuscript preparation. B-KY—Data interpretation, manuscript draft, final manuscript preparation.

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.2023.11233/full#supplementary-material

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Cytokines Removal During *Ex-Vivo* Lung Perfusion: Initial Clinical Experience

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Ex Vivo Lung Perfusion (EVLP) can be potentially used to manipulate organs and to achieve a proper reconditioning process. During EVLP pro-inflammatory cytokines have been shown to accumulate in perfusate over time and their production is correlated with poor outcomes of the graft. Aim of the present study is to investigate the feasibility and safety of cytokine adsorption during EVLP. From July 2011 to March 2020, 54 EVLP procedures have been carried out, 21 grafts treated with an adsorption system and 33 without. Comparing the grafts perfused during EVLP with or without cytokine adsorption, the use of a filter significantly decreased the levels of IL10 and GCSFat the end of the procedure. Among the 38 transplanted patients, the adsorption group experienced a significant decreased IL6, IL10, MCP1 and GCSF concentrations and deltas compared to the no-adsorption group, with a lower in-hospital mortality ($p = 0.03$) and 1-year death rate ($p = 0.01$). This interventional study is the first human experience suggesting the safety and efficacy of a porous polymer beads adsorption device in reducing the level of inflammatory mediators during EVLP. Clinical impact of cytokines reduction during EVLP must be evaluated in further studies.

Keywords: lung transplant, *ex-vivo* lung perfusion, ischemia-reperfusion, cytokines, inflammation, primary graft dysfunction

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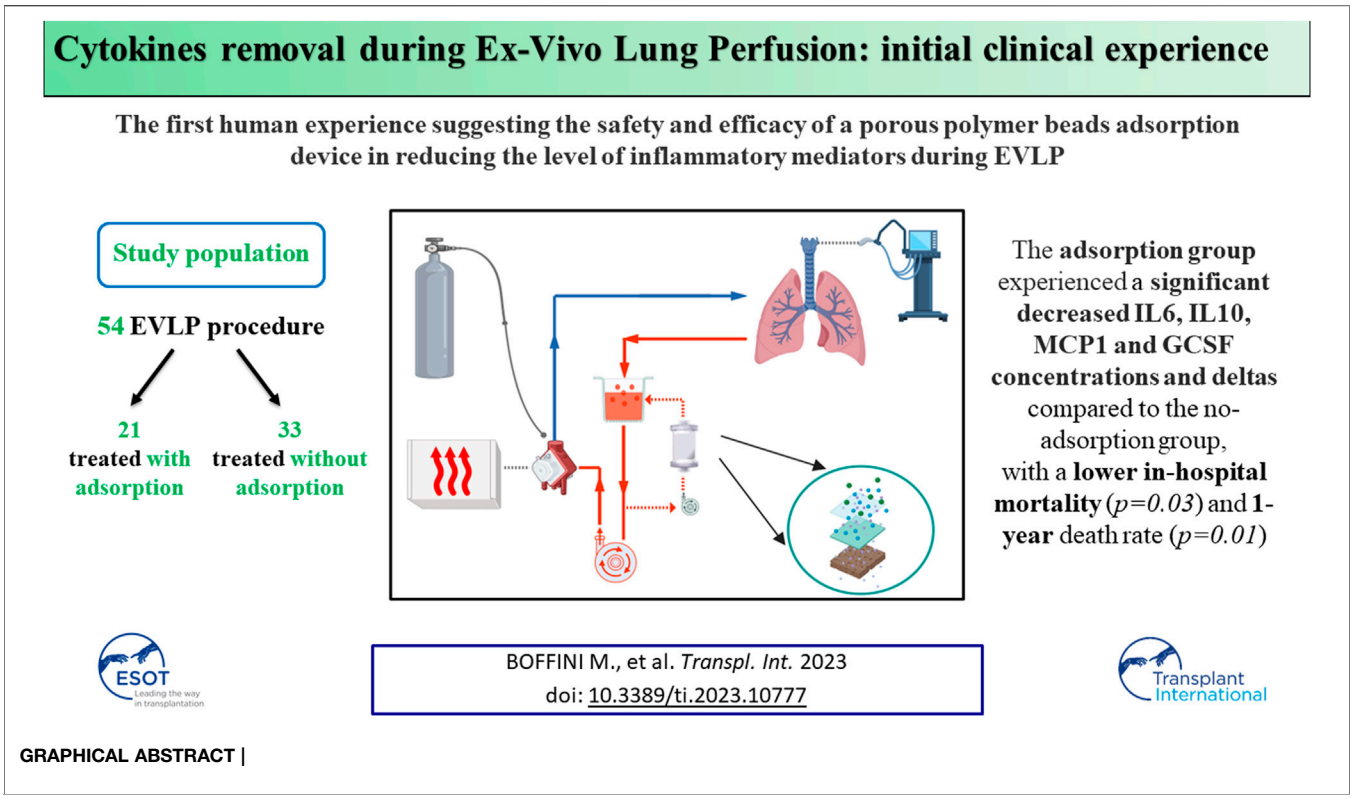
INTRODUCTION

Lung transplantation (LTx) is a well-established therapy for selected patients with various forms of end stage, progressive lung disease. Since the first lung transplant in 1963, the field of LTx has advanced in the selection of candidates, operative techniques, critical care management, immunosuppression, and long-term follow-up. During the last 10 years a significant increase of lung transplant procedures has been recorded if compared with other organs [1]. According to the 2020 International Society for Heart and Lung Transplantation (ISHLT) Registry, almost 70,000 adult lung transplant procedures have been reported since its inception [2]. However, a significant imbalance between the number of transplants performed and the clinical demand still remains.

Although nowadays LTx is a well-established treatment for patients with end-stage lung diseases, shortage of suitable lung grafts is still a major limitation for an extensive application of this therapy. Mortality of patients on the waiting list for a lung transplant is the highest if compared with other solid organ transplants and it can be estimated that up to one out of five patients on the



GRAPHICAL ABSTRACT |

lung transplant waiting list will die before a suitable organ is identified. A major challenge facing the lung transplant community is how to increase the number of usable donor lungs without compromising the success of the procedure. Lungs from donors both after brain death (BDD) and cardiac death (DCD) are subjected to several injurious mechanisms during the donation process. Attempt to transplant injured donor lungs can lead to high incidence of severe primary graft dysfunction (PGD) and associated short- and long-term poorer results [3]. Thus, it is not surprising that the majority of donor lungs are not utilized for transplantation and among the donor pool, the utilization rate of lung grafts is nearly 20%. Expansion of the donor pool has been attempted by extending the conventional donor selection criteria, by use of DCD and, lastly, with the implementation of *Ex Vivo* Lung Perfusion (EVLP) techniques. The ideal donor characteristics are the followings: age <55 years, with a smoking history <20 pack-year, no chest trauma, clear chest X-ray, PaO_2/FiO_2 (P/F) ratio >300 and absence of purulent secretions at bronchoscopy [4]. This scenario is known to correspond to less than half of the donors utilized for transplantation [5] and what have previously been regarded as "ideal" donor lung criteria by the ISHLT are becoming less representative of what is now deemed acceptable in most centers. This has raised the numbers of available donor lungs for transplant, but this may increase the complexity of clinical management of the transplanted patients [6]. EVLP has emerged as a relatively novel technique for preserving, evaluating and eventually reconditioning extended criteria donor lungs. Lung transplant activity may be increased by 15%–30% in Lung Transplant Programs adopting EVLP protocols [7, 8].

Ischemia-reperfusion (IR) injury after lung transplantation can lead to devastating complications, such as primary graft dysfunction, acute rejection, chronic graft dysfunction with a significant impact on morbidity and mortality [9, 10]. In the setting of IR injury, cytokine production plays a crucial role in mediating the inflammatory process that can leave the donor lung permanently dysfunctional. Cytokines and chemokines are small molecules that promote injury through neutrophil recruitment, capillary leakage and cell death [11]. Pro-inflammatory cytokines have been shown to accumulate progressively in perfusate over time during EVLP and cytokine increase has been correlated with poor outcomes related to PGD [12]. CytoSorb® is an absorbent filter which is highly effective in non-selective but concentration-dependent removal of mediators between the molecular weight of 10 and 50 kDa through a 850 m^2/g surface.

Aim of the present study is to investigate the feasibility and safety of the adsorbent filter CytoSorb® during EVLP.

MATERIALS AND METHODS

Study Design

In July 2011 a reconditioning program based on EVLP has been activated at the Lung Transplant Center of Città della Salute e della Scienza University Hospital in Turin, Italy. Until March 2020, 54 perfusions have been carried out on pulmonary grafts deemed unacceptable for direct transplantation at donation site.

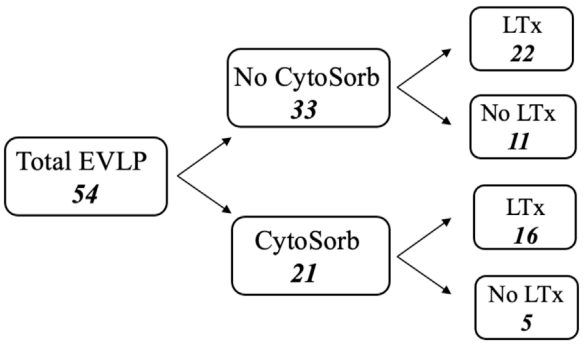


FIGURE 1 | Flow chart of total *ex-vivo* lung perfusion procedures.

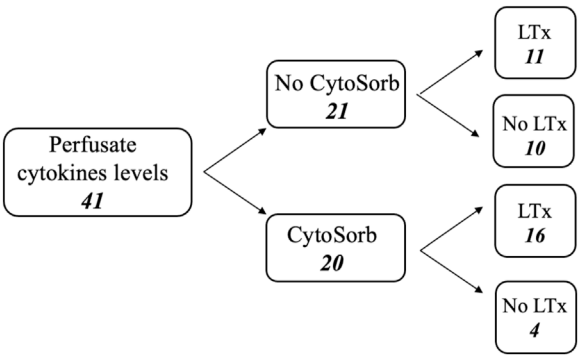


FIGURE 2 | Flow chart of perfusate analysis during *ex-vivo* lung perfusion procedures.

Among those, 38 (70.4%) grafts showed a normal function after perfusion and they have been transplanted (31 bilateral and 7 single LTx). EVLP program allowed a nearly 30% of increase of lung transplant activity and LTx using perfused grafts is the 22% of all LTx performed in Turin. The reconditioning protocol is that described by the Toronto Lung Transplant Group [3] and perfusion has been conducted for 4–6 h. Very briefly, our protocol is based on four principles: 1) use of an acellular solution (STEEN Solution), 2) closed circuit allowing a constant positive pressure in the left atrium, 3) low flow perfusion (with a target flow of 40% of theoretical cardiac output), 4) protective ventilation (tidal volume 7 mL/kg of donor's predicted body weight, respiratory rate 7/min, positive end expiratory pressure 5 cm H_2O, Fraction of Inspired Oxygen, FiO_2, 21%). Preliminary clinical results have been already published elsewhere [13].

EVLP has been accomplished using components available for every day clinical practice. An Euroset™ circuit with Admiral oxygenator, an anti-leucocyte filter (Pall LeukoGuard-6® Arterial Filter) and a Medtronic Bio-Medicus® pump have been used for perfusion and the circuit has been connected to the graft through specially designed funnel-shaped cannulas with built-in pressure probes (Vitrolife®). The circuit has been primed with a buffered extracellular solution added with an optimal colloid osmotic pressure and dextran (Steen Solution™), broad-spectrum antibiotics (imipenem/cilastatin 500 mg/500 mg), heparin (10,000 IU) and methylprednisolone (500 mg). In two cases burdened with significant pulmonary embolism, fibrinolytic agents have been added to the perfusate. From October 2016 the last 21 consecutive grafts have been perfused adding CytoSorb® filter to the circuit. CytoSorb® has been connected via a veno-venous shunt from the reservoir, filtering the perfusate which is then re-collected in the reservoir. Among these, 16 (76%) grafts have been transplanted. Out of the 33 grafts treated without CytoSorb® system, 22 (67%) have been transplanted (**Figure 1**).

The cytokines levels in the perfusate (interleukin 10/IL-10, interleukin 6/IL-6, monocyte chemotactic protein 1/MCP-1 and granulocyte colony-stimulating factor/G-CSF) at the beginning (time 0, *T0*) and at the end of the EVLP (final time, *Tf*) have been measured in 41 procedures and the results have been analyzed. **Figure 2** shows a flow chart.

Functional assessment of EVLP was performed hourly. Dynamic compliance was calculated as the ratio between tidal volume and delta pressure (= peak inspiratory pressure minus positive end-expiratory pressure); static compliance was calculated as the ratio between tidal volume and delta pressure (= plateau pressure minus positive end-expiratory pressure). Blood gas tests were performed on perfusate samples to calculate the left atrial PO_2.

The protocol was created in adherence to the Institutional Review Board of *Città della Salute e della Scienza di Torino* (IRB: 2CEI-178).

Inclusion and Exclusion Criteria for EVLP

Donors

High-risk donor lungs were defined as those meeting any of the following criteria: P/F ratio of less than 300 mmHg; multiple blood transfusions; pulmonary edema detected by chest X-ray or by bronchoscopy or surgical evaluation. Donor lungs with diagnosed pneumonia, persistent secretions on bronchoscopy, aspiration, trauma or contusion were excluded. Pulmonary embolism was not considered a contra-indication and two grafts with severe pulmonary embolism have been perfused with Steen Solution and fibrinolytic agents before their implant.

Recipients

All patients awaiting a single or bilateral lung transplant at Città della Salute e della Scienza University Hospital in Turin who have given written informed consent to transplantation with a reconditioned graft, were eligible. After the EVLP, the graft was transplanted if the following parameters were achieved: delta PaO_2 (PaO_2 in the pulmonary veins–PaO_2 in the pulmonary artery) higher than 350 mmHg; stability or improvement of organ hemodynamic parameters (pulmonary artery pressure ≤15 mmHg, pulmonary vascular resistance stable or decreased) lung dynamics (stable or increased static and dynamic compliance, stable or decreased airway pressure);

TABLE 1 | Comparison of cytokines concentration in the perfusate at the beginning (*T0*) and at the end (*Tf*) of *ex-vivo* lung perfusion with and without CytoSorb®.

	No-Cytosorb (*n* = 21)	Cytosorb (*n* = 20)	*p*-value
IL-6 $_{log10}$ T0	3.0 ± 1.7	3.5 ± 0.7	0.8708
IL-6 $_{log10}$ Tf	4.8 ± 0.2*	4.4 ± 0.4*	0.0002
IL-6 $_{log10}$ delta	1.7 ± 1.7	0.9 ± 0.6	0.1550
IL-10 $_{log10}$ T0	1.3 ± 0.7	1.1 ± 0.5	0.2382
IL-10 $_{log10}$ Tf	2.6 ± 0.4*	1.9 ± 0.4*	0.0000
IL-10 $_{log10}$ delta	1.3 ± 0.7	0.8 ± 0.7	0.0440
MCP1 $_{log10}$ T0	3.2 ± 0.6	2.6 ± 0.5	0.0020
MCP1 $_{log10}$ Tf	3.8 ± 0.3*	3.0 ± 0.4*	0.0000
MCP1 $_{log10}$ delta	0.6 ± 0.5	0.4 ± 0.5	0.1408
GCSF $_{log10}$ T0	1.6 ± 1.6	1.8 ± 0.9	0.5935
GCSF $_{log10}$ Tf	4.0 ± 0.6*	3.3 ± 0.6*	0.0015
GCSF $_{log10}$ delta	2.4 ± 1.4	1.4 ± 0.9	0.0358

List of abbreviations: IL-6, interleukin 6; IL-10, interleukin 10; MCP1, monocyte chemotactic protein 1; GCSF, granulocyte colony-stimulating factor. $_{log10}$*, natural logarithm* *p < *0.01 vs. T0.*

TABLE 2 | Comparison of cytokines concentration in the perfusate at the beginning (*T0*) and at the end (*Tf*) of *ex-vivo* lung perfusion with and without CytoSorb® in transplanted grafts.

	No-Cytosorb (*n* = 11)	Cytosorb (*n* = 16)	*p-value*
IL-6 $_{log10}$ T0	2.1 ± 1.7	3.5 ± 0.7	0.0577
IL-6 $_{log10}$ Tf	4.8 ± 0.2*	4.3 ± 0.4*	0.0002
IL-6 $_{log10}$ delta	2.8 ± 1.7	0.9 ± 0.7	0.0014
IL-10 $_{log10}$ T0	1.1 ± 0.5	1.1 ± 0.5	0.9172
IL-10 $_{log10}$ Tf	2.7 ± 0.4*	1.9 ± 0.3*	0.0000
IL-10 $_{log10}$ delta	1.6 ± 0.5	0.8 ± 0.6	0.0027
MCP1 $_{log10}$ T0	3.0 ± 0.5	2.6 ± 0.6	0.0709
MCP1 $_{log10}$ Tf	3.8 ± 0.2*	3.0 ± 0.4**	0.0000
MCP1 $_{log10}$ delta	0.8 ± 0.4	0.4 ± 0.6	0.0229
GCSF $_{log10}$ T0	0.7 ± 1.1	1.7 ± 0.9	0.0242
GCSF $_{log10}$ Tf	3.8 ± 0.6*	3.3 ± 0.6*	0.0457
GCSF $_{log10}$ delta	3.1 ± 0.8	1.4 ± 1.0	0.0002

List of abbreviations: IL-6, interleukin 6; IL-10, interleukin 10; MCP1, monocyte chemotactic protein 1; GCSF, granulocyte colony-stimulating factor; $_{log10}$*, natural logarithm;* *p < *0.01 vs.* T0; **p < *0.05 vs.* T0.

lung X-ray and bronchoscopy negative; positive clinical judgment of the transplant team.

Statistical Analysis

Data were tested for normal distribution by Shapiro-Wilk test and a test on the equality of standard deviations (variances) on every variable was performed. Data were expressed as mean and standard deviation (SD) or median with interquartile range 25–75 (IQR), as appropriate.

Base 10 logarithmic transformations on absolute cytokine's levels (IL-6, IL-10, GCSF and MCP1) were performed to reduce skewness and kurtosis.

Descriptive statistics are presented as mean, median, standard deviation and ranges for the continuous variables, and as counts and percentages for categorical variables. Differences between groups were assessed with Wilcoxon rank-sum test for independent samples and Wilcoxon matched-pairs signed-rank test for matched pairs and with t tests (paired or unpaired) on the equality of means as appropriate. Categorical variables were analyzed with Chi-squared or Fisher's exact test, as appropriate. Statistical difference has been considered significant for $p < 0.05$. All analyses were performed using Stata 16.1/SE (Stata Corp TX, United States) and SPSS 20.0 (IBM Corp., Armonk, NY, United States).

RESULTS

Cytokines levels at *T0* and *Tf* and deltas (difference between *T0* and *Tf*) are described in details in **Tables 1**, **2**.

During EVLP, cytokines' levels increase over time with a significant difference between *T0* and *Tf* both overall and in the transplanted group (**Tables 1**, **2**) despite the use of Cytosorb®. In overall perfusions, at the comparison between the Cytosorb® vs. no-Cytosorb group®, deltas are similar for IL6 and MCP1 ($p = 0.15$ and $p = 0.14$), and decreased only for IL10 ($p = 0.04$) and GCSF ($p = 0.03$). All the details are reported in **Table 1** and **Figure 3**. **Table 2** shows the results obtained from the comparison between the transplanted grafts perfused with or without the use of CytoSorb®. The two cohorts have similar levels of cytokines at *T0*, significant decreased IL6, IL10, MCP1 and GCSF concentrations and deltas in the Cytosorb® group (**Table 2** and **Figure 4**).

Among the transplanted grafts, the comparison of "physiological assessment" (based on gas exchange and lung dynamics) during EVLP stratified according to the use or not of CytoSorb® suggests no difference, but a test could not be performed (**Figure 5**).

Table 3 summarizes the comparison of donors' and recipients' characteristics of transplanted grafts with or without CytoSorb® during EVLP. Donors are similar in the two groups being the duration of mechanical ventilation, that is longer in the no-CytoSorb® group (4 ± 2 vs. 3 ± 2 days, $p = 0.02$), the only significant difference. Patients in the CytoSorb® group are older (60 [54–63] vs. 49 [35–57], $p = 0.02$), received a bilateral lung transplant and required CPB less frequently (63% vs. 95%, $p = 0.01$, 31% vs. 73%, $p = 0.01$, respectively).

Among the 38 transplanted patients, there was not enough evidence to show that the patients included in the no-CytoSorb® group had more frequently severe-grade 3 PGD (with the definition and grading by the report of the ISHLT in 2016 [14], retrospectively adopted for all the patients) if compared to the patients in the CytoSorb® group both at arrival in ICU and at 72 h after transplant [11 (50%) vs. 3 (19%) pts, and 6 (28%) vs. 1 (6%), respectively], and needed more frequently post-transplant VV-ECMO [8 (36%) vs. 4 (25%) pts]. The patients included in the no-CytoSorb® group showed a higher in-hospital mortality [5 (23%) vs. 0 pts, $p = 0.03$] and 1-year death [8 (36%) vs. 0 pts, $p = 0.01$] (**Supplementary Table S1**).

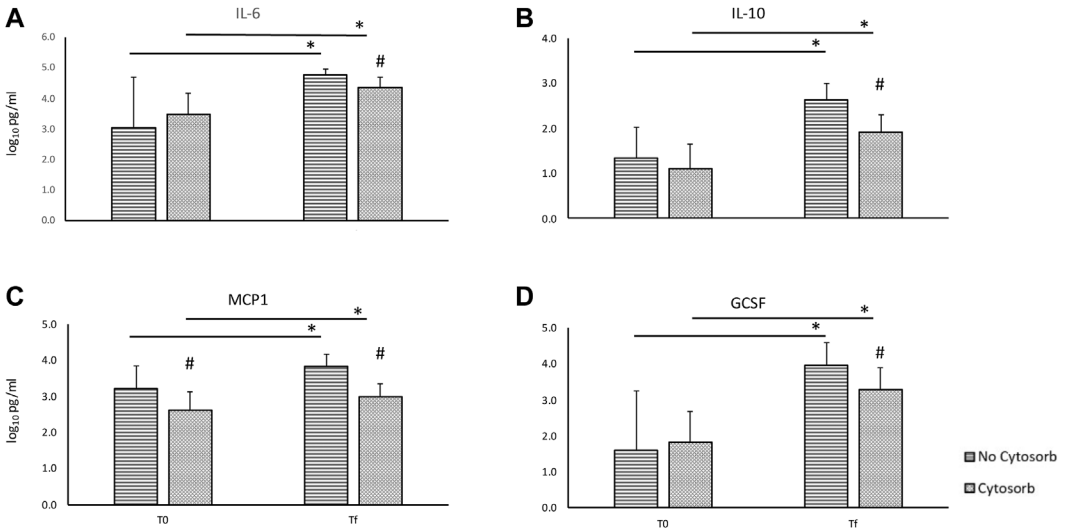


FIGURE 3 | Comparison of cytokines concentration in the perfusate at the beginning (*T0*) and at the end (*Tf*) of *ex-vivo* lung perfusion with and without CytoSorb® [base 10 log of IL6, panel **(A)**; base 10 log of IL10, panel **(B)**; base 10 log of MCP1, panel **(C)**; base 10 log of GCSF, panel **(D)**]. Values are expressed as mean ± standard deviations. $^{*}p < 0.01$ vs. T0; $^{**}p < 0.05$ vs. T0; $^{\#}p < 0.01$ vs. no-Cytosorb group; $^{\#\#}p < 0.05$ vs. no-Cytosorb group.

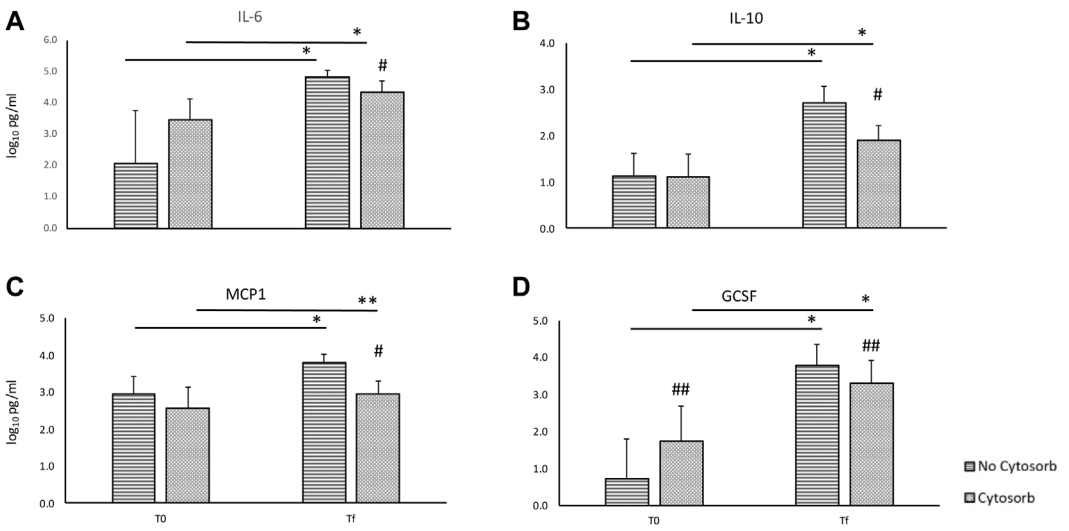


FIGURE 4 | Comparison of cytokines concentration in the perfusate at the beginning (*T0*) and at the end (*Tf*) of *ex-vivo* lung perfusion with and without CytoSorb® in transplanted grafts [base 10 log of IL6, panel **(A)**; base 10 log of IL10, panel **(B)**; base 10 log of MCP1, panel **(C)**; base 10 log of GCSF, panel **(D)**]. Values are expressed as mean ± standard deviations. $^{*}p < 0.01$ vs. T0; $^{**}p < 0.05$ vs. T0; $^{\#}p < 0.01$ vs. no-Cytosorb group; $^{\#\#}p < 0.05$ vs. no-Cytosorb group.

DISCUSSION

The present study shows our experience on consecutive unselected lung grafts treated with the EVLP technique before their assessment for transplant suitability. The analysis has been focused on the feasibility and safety of the use of an adsorbent device during EVLP.

The mechanism of action of EVLP is still not completely understood: the use of a hyper-oncotic perfusion solution in suboptimal grafts counteracts lung parenchyma fluid overload, thus allowing the recovery of an optimal pulmonary function. However, a more complex mechanism of action involving the fragile balance of inflammatory and anti-inflammatory response can also be supposed. EVLP may act as a "purification" system from potentially toxic molecules such as inflammatory mediators related to the static cold ischemic storage of lung grafts before EVLP [15–19].

During the "cold ischemic period", potentially harmful events—such as reactive oxygen species formation, sodium pump inactivation, intracellular calcium overload, iron release

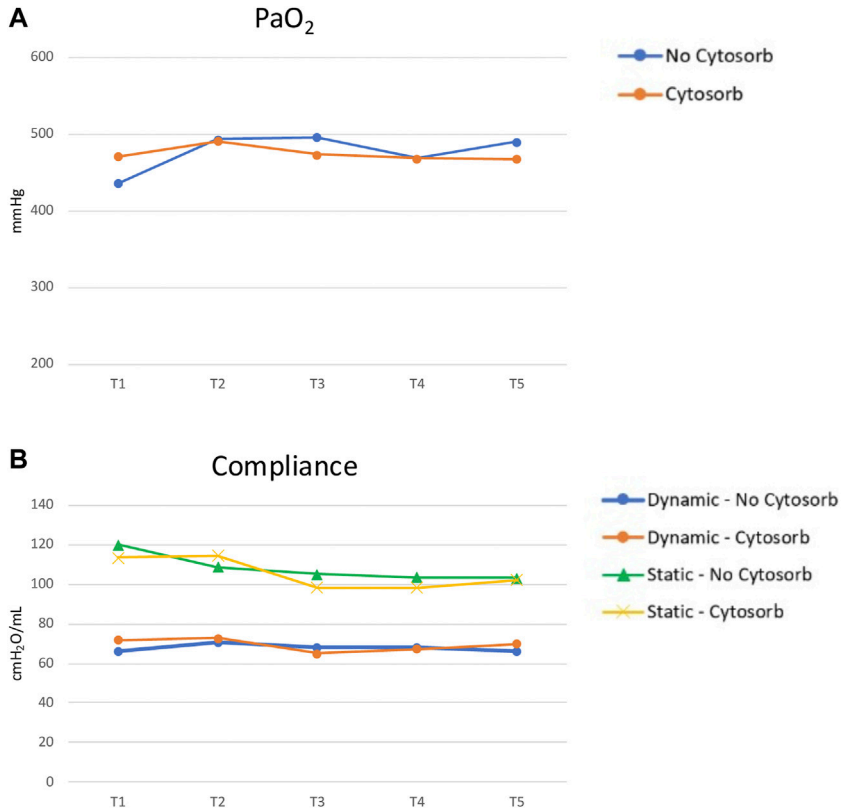


FIGURE 5 | EVLP parameters comparison between Cytosorb and no-Cytosorb group in transplanted grafts [PaO_2, panel **(A)**; static and dynamic compliance, panel **(B)**].

and cell death—may occur. These phenomena can promote the upregulation of adhesion molecules and the release of pro-inflammatory mediators with recruitment and activation of donor or recipient leukocytes after reperfusion [19]. The inflammatory response associated with the release of pro-inflammatory cytokines may play a pivotal role in the development of PGD [20]. To date, little is known whether the inflammatory response can be contrasted during EVLP.

In our series, a significant increase in cytokines levels has been found during perfusion despite the use of Cytosorb®. Same results have been registered by Sadaria et al. [21] who investigated cytokine expression profile and histologic effects in human donor lungs undergoing prolonged normothermic EVLP. Moreover, inflammatory response during EVLP has been associated both to the final result of the EVLP and to the pulmonary function after transplant [22].

The inflammatory cytokine profile expression after lung transplantation has been studied by various groups. De Perrot et al. [9] in Toronto explored cytokine expression in transplanted lungs during cold ischemia and at different timepoints during reperfusion. This study demonstrated that tumor necrosis factor (TNF)-α, interferon (IFN)-γ, IL-10, IL-12, and IL18 were elevated during ischemia, whereas IL-8 was predominantly elevated after reperfusion. In another study, given the anti-inflammatory effects of IL-10, Cypel et al. [23] tried to apply this effect during EVLP. After the delivery of an adenoviral vector encoding human IL-10 during EVLP in the airways, the authors showed significant improvement in pulmonary function in comparison with those lungs undergoing EVLP alone.

Unfortunately, the parameters commonly used for the physiological assessment during EVLP do not allow to precisely predict pulmonary function after transplantation. Inflammation burden during EVLP has been proposed to predict donor related lung injury after transplant [24] and persistence of severe-grade 3 PGD at 72 h seems to be associated with higher levels of IL 6, IL1b and MCP1 in the perfusate [25].

However, so far EVLP is more commonly used to preserve and to evaluate grafts than as an effective strategy to obtain a real reconditioning. On the other hand, EVLP represents a reliable platform to be used in order to repair injured dysfunctional grafts. Among other solid organs, the lungs are unique because of its dual access system and both bronchial or vascular route can be used for direct intervention. The EVLP phase may potentially allow the administration of the most effective therapies based on the specific causes of lung injuries avoiding systemic toxicity. In particular, the reduction of the inflammatory storm can be theoretically addressed during EVLP.

TABLE 3 | Comparison of recipient and donor characteristics in transplanted grafts after *ex-vivo* lung perfusion with and without CytoSorb®.

	Cytosorb (*n* = 16)	no-Cytosorb (*n* = 22)	*p-value*
Baseline LTx recipient characteristics			
LAS score	31.2 [30.8–31.3]	31.4 [30.7–31.6]	0.28
Age at transplant (years)	60 [54–63]	49 [35–57]	0.02
Male sex (*n*, %)	10 (63)	13 (59)	0.80
ECMO at LTx (*n*, %)	0 (0)	2 (9)	0.49
Bilateral lung transplantation (*n*, %)	10 (63)	21 (95)	0.01
CPB during LTx (*n*, %)	5 (31)	16 (73)	0.01
Ischemia time (min)	784 ± 96	781 ± 33	0.9
Lung disease			
Idiopatic fibrosis	8	7	
Cystic fibrosis	2	5	0.9
COPD	4	6	
Vascular disease	1	3	
Other	1	1	
Baseline donor characteristics			
Age (years)	50 [38–53]	48 [38–53]	0.9
Smoker (*n*, %)	9 (41%)	7 (44%)	0.2
Time on mechanical ventilation (days)	3 ± 2	4 ± 2	0.02
P/F (mmHg)	311 ± 157	337 ± 107	0.5
PaO_2 with FiO_2 0,4 (mmHg)	125 ± 52	125 ± 29	0.9
P/F > 350	6 (38%)	11 (50%)	0.5
Cause of death			
Cerebral hemorrhage	6	16	
Anoxic brain injury	3	3	0.7
Trauma	4	1	
Other	3	2	

List of abbreviations: EVLP, ex vivo *lung perfusion; ECMO, extracorporeal membrane oxygenation; LTx, lung transplantation; CPB, cardiopulmonary bypass; COPD, chronic obstructive pulmonary disease; P/F PaO_2/FiO_2 ratio; PF/100.*

In overall perfusions, despite a significant lower concentration of cytokines at the end of EVLP in the grafts perfused with Cytosorb®, deltas from T0 and Tf between Cytosorb® and no-Cytosorb® group are decreased only for IL10 and not for IL6 and MCP1. However, it must be taken into account that indication to EVLP is a poor graft gas exchange due to several reasons in which inflammation can play a major role and this process can be reversible or not. In case of not reversible cause of graft dysfunction and/or inflammatory state (i.e., misdiagnosed pneumonia or irreversible ventilatory lung injury or primary pulmonary disease) the EVLP remains useless to rehabilitate marginal or initially rejected grafts regardless the use of Cytosorb®. In these situations, the inflammatory cascade is maintained during perfusion and the cytokines removal may not be effective due to the continue production and release of inflammatory products. Moreover, the duration of EVLP is clinically driven and it is based on clinical parameters (always unrelated with inflammation) collected during the perfusion suggesting the utility or futility of the EVLP. This creates a different length of duration of the treatment. As a matter of fact, median duration of EVLP was 4 (IQR 3–4) and 5 (IQR 4–6) hours in the rejected and transplanted groups, respectively ($p < 0.01$) and IL6 concentrations higher in the rejected grafts in comparison with transplanted grafts (IL6 *@T0* 4.2 IQR 3.3–4.5 vs. 3.2 IQR 2.6–3.6, $p = 0.01$, respectively). This means that, based on clinical evaluation, the rejected grafts are more "inflamed" and less responsive to EVLP with or without Cytosorb® and the reconditioning therapy is shorter because the futility of EVLP becomes evident earlier. Conversely, the transplanted group is more homogenous for the duration of EVLP and cytokines concentrations at the beginning of perfusion. In this cohort the effect of cytokines' absorption can be more visible: same T0 concentration or even worse level in the Cytosorb® group, same duration of treatment, less cytokines concentration at the end of the perfusion and lower deltas.

The main finding of our initial clinical series is that the reduction of the level of inflammatory mediators can be effectively achieved using a porous polymer beads adsorption during EVLP with the use of CytoSorb® in the clinical setting of lung transplantation and this represents the first experience reported in man so far.

As a matter of fact, the impact of cytokines removal during EVLP has been investigated in the animal model only, both on normal or injured grafts and never in man. In 2010 the Japanese group of Kakishita and coworkers [26] tested for the first time an adsorbent membrane (Lixelle S-35) during EVLP on normal swine lungs. The EVLP was run for 12-h. The filter was laterally attached to the circuit in order to remove pro-inflammatory cytokines from perfusate. The authors showed a significant reduction of TNF-α and IL-8 levels without any impact on pulmonary function suggesting that cytokines removal is effective and safe. Iskender et al. [27] in 2017 hypothesized that cytokine filtration would improve lung function through the clearance of inflammatory mediators during prolonged EVLP. Ten pig lungs were stored at 4°C for 24 h and randomly divided into two groups according to the use or not of

the filter added to the EVLP circuit. From their analysis, continuous filtration through beads has been shown to decrease cytokines concentration with a better pulmonary function during EVLP. Moreover, the post-transplant beneficial effects [28] of perfusate adsorption during EVLP have been studied in an animal model of injured grafts showing a more preserved post-transplant graft function in those grafts treated with EVLP plus CytoSorb®.

Our study suffers from both conceptual and methodological limitations. CytoSorb® acts as a not selective filtering membrane according to the dimensions of molecules and porous beads. Mechanical removal depends on concentration and molecular weight (up to 50 KDa) of the mediators, therefore both inflammatory and anti-inflammatory cytokines are removed. It can be speculated that however, the removal of pro-inflammatory mediators overcomes the potential negative impact of anti-inflammatory cytokines removal. Moreover, the role of the ratio between pro and anti-inflammatory mediators could be investigated. From a methodological perspective, our results come from a no-randomized retrospective series and potentially confounding factors may jeopardize our clinical findings. Many factors (both related to donor and recipient) during all the phases of transplant (from organ retrieval, *ex-vivo* perfusion and implantation) may interact each other in the definitive decision-making process. Moreover, the two groups refer to a different "historical" period: the no-CytoSorb® cohort refers also to the very beginning of EVLP program with an intrinsic learning curve phase in terms of indication, management and assessment of grafts treated with EVLP. It should be noticed that the two cohorts of transplanted patients are similar but with some statistically significant differences. For example, the need of CPB was higher in the no-CytoSorb® group even if the no-CytoSorb® recipients were younger and receiving more frequently a bilateral transplant (the latter are well-known positive prognostic factors). Regarding recipients' characteristics no-CytoSorb® group received a graft from donors with a longer mechanical ventilation time although this statistical difference seems insignificant from a clinical point of view (3 vs. 4 days). Finally, the relatively small sample size does not allow a deeper statistical analysis reducing the possibility to draw robust conclusions. However, our first aim was only to evaluate the safety and efficacy of cytokines reduction in the clinical setting. Considering the mean values of cytokines levels (IL-6 log10, IL-10 log10, MCP1 log10, GCSF log10) at *Tf* between the Cytosorb® and no-Cytosorb® groups, their standard deviation and the total sample size, with an alpha error of 0.05, our study power is ≥0.95. The power of the mean difference between base 10 logarithm of IL-6, IL-10 and GCSF levels at *Tf* and *T0* in the transplanted population between the Cytosorb® and no Cytosorb® groups, considering their standard deviation and the total sample size, with an alpha error of 0.05 is ≥0.93 and 0.45 for MCP1.

The clinical impact of cytokines adsorption must be further validated in more rigorous, prospective, randomized clinical trials. However, our analysis refers to a consecutive lung transplant series in a medium-volume center and it can be considered a representative picture of daily clinical practice given the limited number of lung transplants and even fewer procedures of EVLP run worldwide. Clinical scenario of lung transplantation is changing and graft perfusion techniques play an important role.

EVLP not only represents a reliable platform to evaluate and preserve graft before transplant but it can be potentially used to manipulate organs and to achieve a proper reconditioning process. Inflammatory response has been shown to have a central role on graft function after transplant and an active treatment using removal strategies of cytokines during perfusion is very attractive.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Comitato Etico Interaziendale A.O.U. Città della Salute e della Scienza di Torino—A.O. Ordine Mauriziano—A.S.L. Città di Torino, Turin, Italy. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MB: study design, data interpretation and analysis and manuscript writing and revision. MM: study design, data collection and analysis and manuscript revision. ES: data collection and interpretation and manuscript revision. FS: data collection and interpretation, manuscript revision. AC: data collection and analysis, manuscript revision. VF: data interpretation and manuscript revision. CB: data interpretation and manuscript revision. PS: data collection and manuscript revision. LB: data interpretation and manuscript revision. MR: study design, data analysis and interpretation, manuscript writing and revision. MB is the guarantor of the paper, taking responsibility for the integrity of the work as a whole, from inception to published article. All the authors approved the final version of the manuscript to be published and agreed to be accountable for all aspects of the work.

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.2023.10777/full#supplementary-material

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ORIGINAL RESEARCH
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A Comprehensive Landscape of *De Novo* Malignancy After Double Lung Transplantation

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Although the association between post-transplant malignancy (PTM) and immunosuppressive therapy after organ transplantation has been studied, an integrated review of PTM after lung transplantation is lacking. We investigated the incidence and types of *de novo* PTM and its impact on survival following double lung transplantation (DLT). The incidence and type of PTM as well as the annual and cumulative risks of each malignancy after DLT were analyzed. The overall survival (OS) of recipients with or without PTM was compared by the Kaplan–Meier survival method and landmark analysis. There were 5,629 cases (23.52%) with 27 types of PTMs and incidences and OS varied according to the types of PTMs. The recipients with PTM showed a significantly longer OS than those without PTM ($p < 0.001$). However, while the recipients with PTM showed significantly better OS at 3, and 5 years ($p < 0.001$, $p = 0.007$), it was worse at the 10-year landmark time ($p = 0.013$). And the single PTM group showed a worse OS rate than the multiple PTM group ($p < 0.001$). This comprehensive report on PTM following DLT can help understand the risks and timing of PTM to improve the implementation of screening and treatment.

Keywords: post-transplant malignancy, *de novo* malignancy, double lung transplant, incidence, survival outcomes

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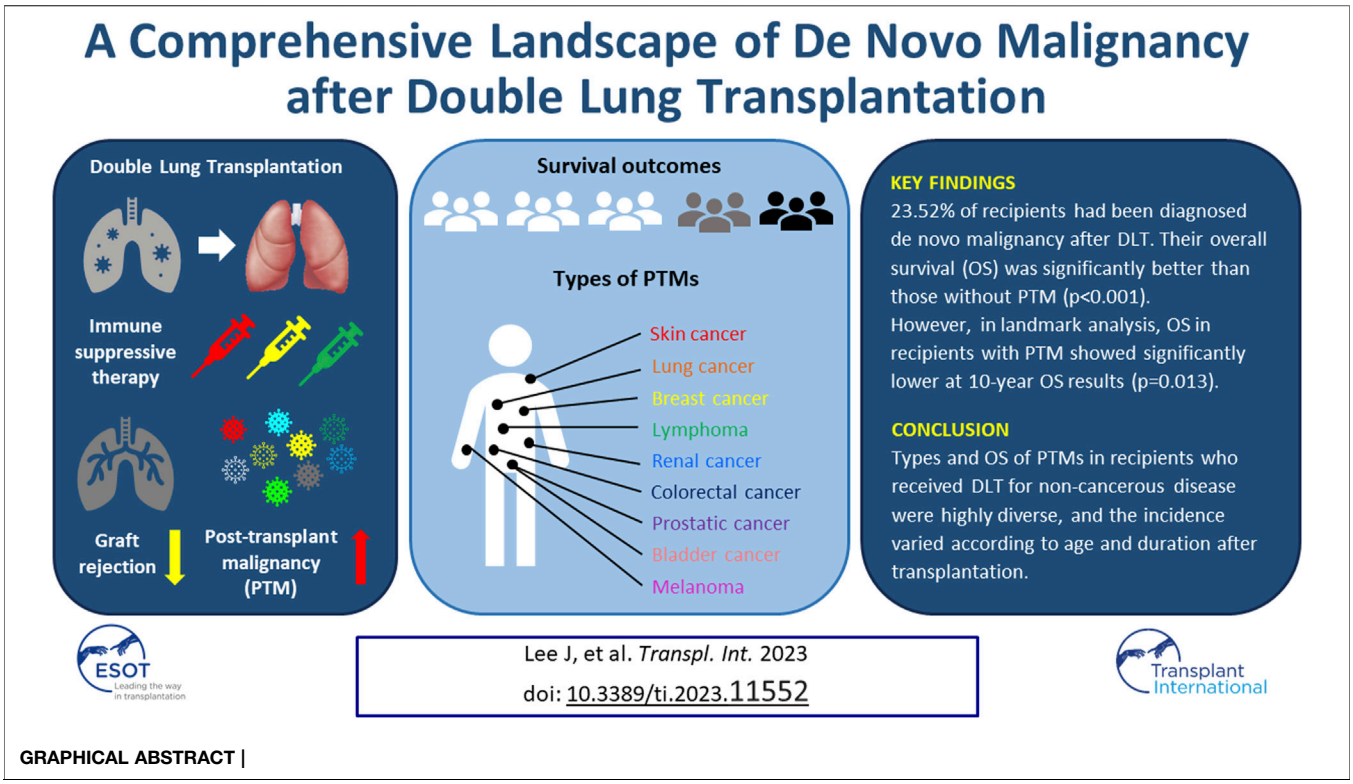
INTRODUCTION

Over the past 20 years, there has been a notable increase in thoracic organ transplantation, with double lung transplantation (DLT) surpassing single lung transplantation nearly two-fold since 2005 [1–4]. Immunosuppressive therapy has substantially improved post-transplant outcomes by mitigating acute and chronic rejection episodes [5–7]. The standard immunosuppressive regimen for lung transplantation consists of calcineurin inhibitors, antimetabolites, and corticosteroids [8, 9]. This regimen has effectively reduced allograft tissue rejection and graft failure, enhancing transplant recipients' survival outcomes [10, 11].

The immunosuppressive regimen attenuates the signaling between antigen-presenting cells and T-cells, inhibits T-cell activation and proliferation, reduces antibody production by B cells, and

Abbreviations: DLT, double lung transplantation; OPTN, Organ Procurement and Transplantation Network; PTM, post-transplant malignancy; UNOS, United Network for Organ Sharing.



GRAPHICAL ABSTRACT |

suppresses antibody-mediated complement system activation [12–14]. However, this immunosuppressive microenvironment may inadvertently promote tumor development and progression, facilitating immune evasion by cancer cells [15, 16]. Consequently, while immunosuppressive therapy has successfully suppressed allograft rejection, malignancies associated with immunosuppression are increasingly acknowledged as a significant post-transplant complication [17, 18].

Although the relationship between post-transplant malignancy (PTM) and immunosuppressive therapy has been suggested, PTM remains a leading cause of mortality in thoracic transplantation patients [19–21]. Transplant recipients face a lifelong risk of PTM, necessitating diligent screening for *de novo* PTM. A thorough examination of PTM, accounting for transplant recipient characteristics and time since transplantation, is crucial for informing PTM management strategies.

In this study, we investigated the annual incidence, cumulative risk, and survival outcomes of PTM in patients who underwent DLT for non-cancerous diseases. We utilized data from the Organ Procurement and Transplantation Network (OPTN) to better understand PTM characteristics following DLT.

MATERIAL AND METHODS

Data: Data pertaining to thoracic transplantation was procured from the United Network for Organ Sharing (UNOS)—a non-profit organization committed to its mission of overseeing the nation's transplant system under the purview of the federal government[1]. The data, which were de-identified, anonymized, and accompanied by coding files in STATA format, were sourced from the thoracic transplant registry of the OPTN as of 7 October 2022. Only DLT recipients were included while single or multi-organ transplants were excluded given the potential for confounding bias. Among the 29,335 documented DLT cases conducted between 1993 and June 2022, a total of 23,935 recipients who had eligible data were ultimately assessed for *de novo* PTMs following DLT, upon reviewing data suitability. Recipients who had undergone DLT for malignancy were excluded from the study, which received approval from Northwestern University's Institutional Review Board Committee in Chicago, IL, United States (IRB#: STU00207117). The collected data encompassed recipient age at the time of transplantation, sex, smoking history, prior indication for DLT, presence, and date of *de novo* PTM, PTM type, date and cause of death. The recipient cohorts were divided into two groups: those without *de novo* PTM (n = 18,306) and those with *de novo* PTM (n = 5,629). The incidence, annual and cumulative risks of each PTM subtype were scrutinized, and survival outcomes were contrasted.

Analysis: Clinical factors and survival outcomes were evaluated at 5 and 10 years post-DLT for all recipients. Incidence, as well as annual and cumulative risks of PTM, were computed according to PTM type. Furthermore, the variation in annual risk proportion was compared as the follow-up period extended. With a follow-up period of at least 18 years, the cumulative risk was ascertained

[1]www.unos.org

TABLE 1 | Characteristics of recipients with or without *de novo* post-transplant malignancy (PTM) who had received double lung transplantation for non-cancerous diseases.

Variables	Total (*n* = 23,935)	Recipients without PTM (*n* = 18,306)	Recipients with PTM (*n* = 5,629)	*p*-value*
Age at transplantation (mean, ±SD)	51.91 ± 4.95	51.13 ± 41.72	54.46 ± 19.09	<0.001
Gender (*n*, %)				<0.001
Male	13,768 (57.52)	9,983 (54.53)	3,785 (67.24)	
Female	10,167 (42.48)	8,323 (45.47)	1,844 (32.76)	
Smoking history (*n*, %)				<0.001
Non-smoker	9,148 (38.22)	7,490 (40.92)	1,658 (29.45)	
Smoker	11,129 (46.50)	8,282 (45.24)	2,847 (50.58)	
Unknown	3,658 (15.28)	2,534 (13.84)	1,124 (19.97)	
Death (*n*, %)				<0.001
No	12,216 (51.04)	9,794 (53.50)	2,421 (43.01)	
Yes	11,719 (48.96)	8,512 (46.50)	3,208 (56.99)	
Onset of PTM from transplantation				N/A
Median (months, range)	—	—	47.97 (0.00–316.10)	
Mean (months, ±SD)	—	—	60.87 ± 49.26	

**Quantitative variables were compared using a* t*-test, and categorical variables were analyzed using the* χ^2 *test.*

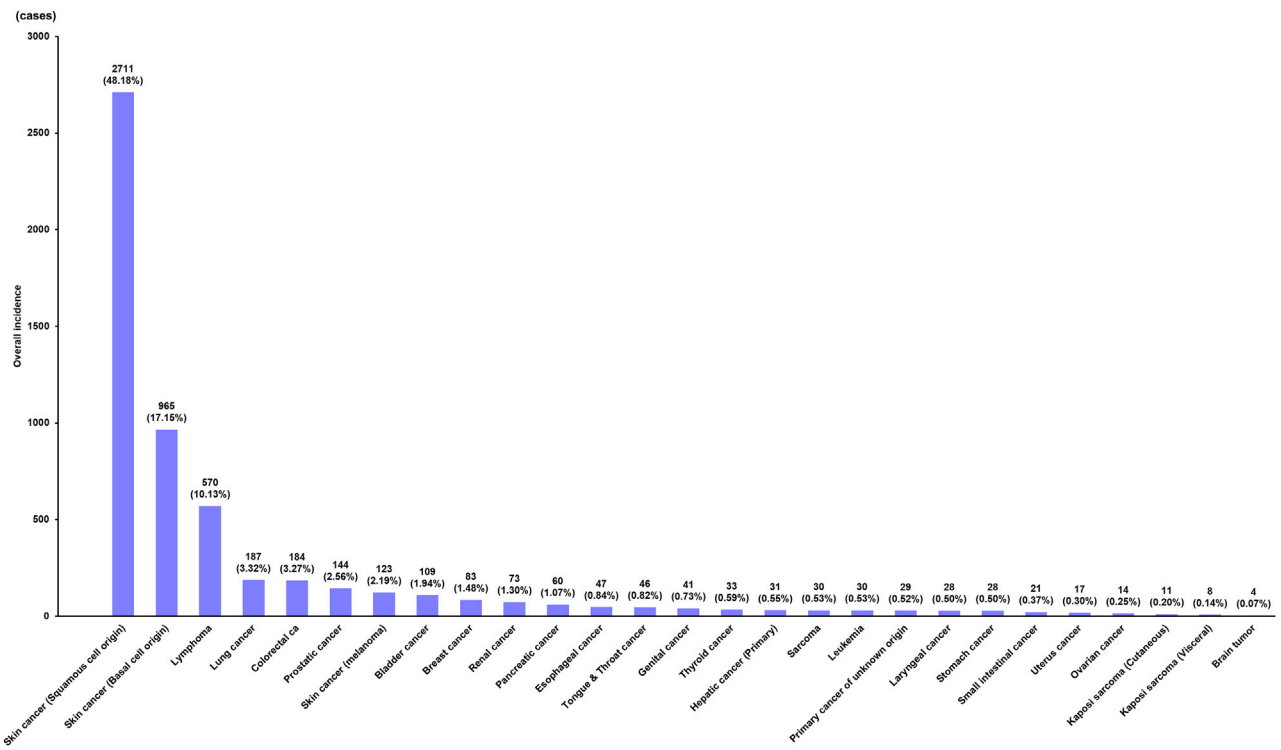


FIGURE 1 | Incidence of *de novo* post-transplant malignancy after double lung transplantation.

for the four most prevalent PTM causes: squamous cell skin cancer (SCC), basal cell skin cancer (BCC), lymphoma, and lung cancer. Recipients with PTM were further categorized based on the number of PTMs they developed, and the overall survival (OS) was analyzed for statistical differences based on the number of PTMs.

Statistics: Quantitative variables were compared using the *t*-test, and categorical variables were analyzed using the χ^2 test. The survival outcomes were analyzed with the Kaplan–Meier survival method. For multivariate analysis, the Cox regression analysis was performed, considering age, sex, and cigarette use at the time of DLT as the variables. For the landmark analysis, we chose 3, 5, 7, 10, 15, and 20 years after transplantation as landmark time points. Only patients alive at this point were included in this analysis and performed an analysis with recipients with or without PTM before time points. All statistical analyses were performed using the SPSS software (version 29.0 SPSS, IBM, Chicago, IL, United States), and a *p*-value of <0.05 was used to determine statistical significance.

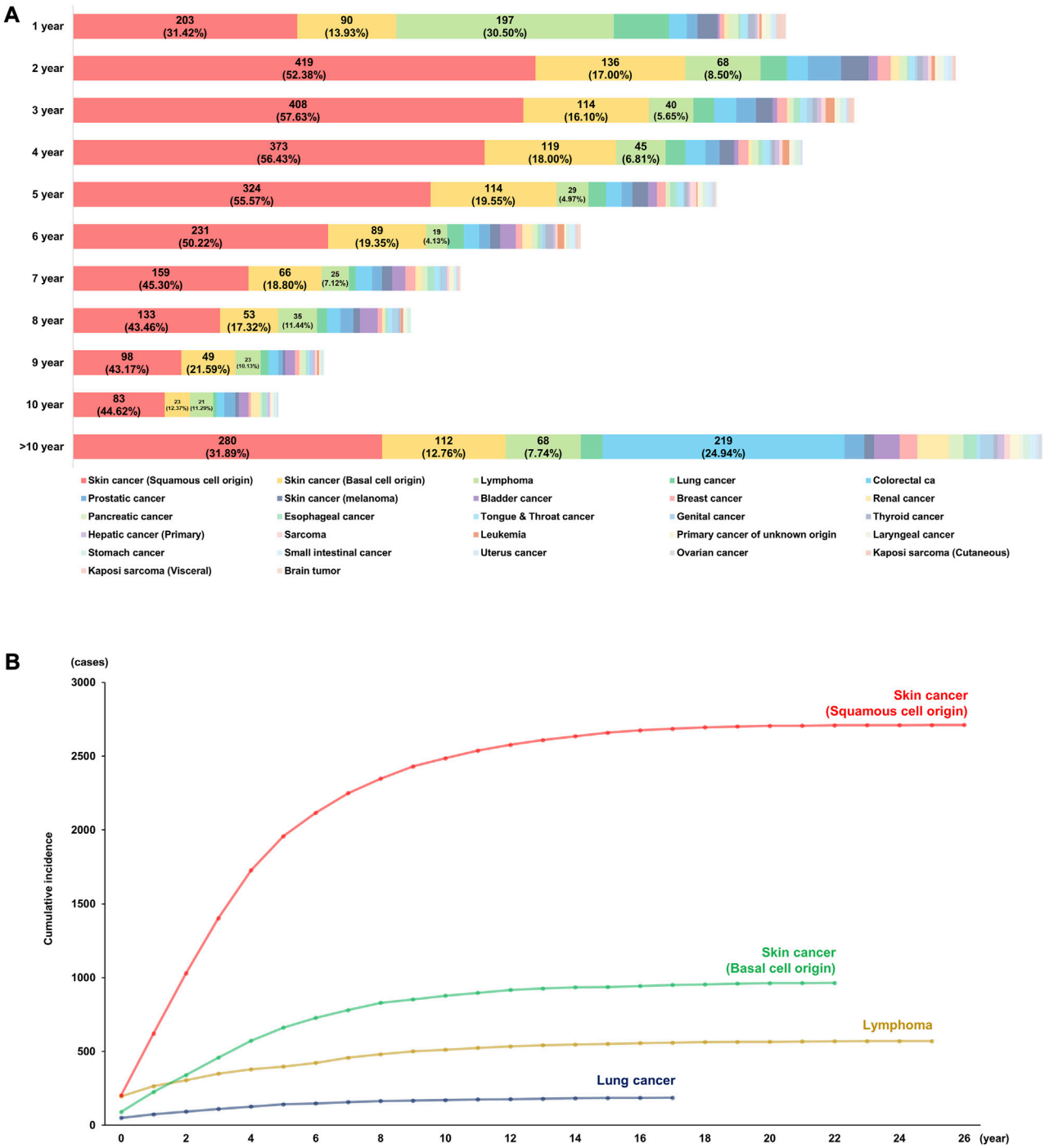


FIGURE 2 | Incidence of *de novo* malignancy after double lung transplantation. **(A)** Annual incidences of post-transplant malignancy. **(B)** Cumulative risks of top four causes of post-transplant malignancy.

RESULTS

Clinical and Demographic Features

Among the 23,935 DLT recipients, 13,768 (57.52%) were males, and 11,129 (46.50%) had a smoking history. The mean age of the recipients was 51.91 years (SD, ±4.95). During the follow-up period, 5,629 cases (30.75%) of PTM occurred, and the mean age of recipients with PTM was significantly greater than that of those without PTM [without PTM: 51.13 years (SD, ±41.72) versus those with PTM: 54.46 years (SD, ±19.09), $p < 0.001$].

TABLE 2 | Sex and age distributions in recipients with post-transplant malignancy (PTM) after double lung transplantation.

Variables (*n*, %)	Sex	Age groups					
	Male: Female	19–29	30–39	40–49	50–59	60–69	70–79
Total recipients	13,768 (57.52): 10,167 (42.48)	2,466	2,457	3,312	7,005	7,842	853
Recipients with PTM	3,785 (67.24): 1,844 (32.76)	304 (12.33)	463 (18.84)	744 (22.46)	1,759 (25.11)	2,140 (27.29)	219 (25.67)
Type of PTMs							
Skin cancer (Squamous cell origin)	1,942 (71.63): 769(28.37)	89 (29.28)	176 (38.01)	340 (45.7)	876 (49.8)	1,110 (51.87)	120 (54.79)
Skin cancer (Basal cell origin)	655 (67.88): 310 (32.12)	44 (14.47)	91 (19.65)	140 (18.82)	289 (16.43)	365 (17.06)	36 (16.44)
Lymphoma	336 (58.95): 234 (41.05)	103 (33.88)	75 (16.20)	72 (9.68)	148 (8.41)	162 (7.57)	10 (4.57)
Lung cancer	127 (67.91): 60 (32.09)	6 (1.97)	15 (3.24)	22 (2.96)	52 (2.96)	75 (3.50)	17 (7.76)
Colorectal ca	94 (51.09): 90 (48.91)	3 (0.99)	32 (6.91)	41 (5.51)	60 (3.41)	44 (2.06)	4 (1.83)
Prostatic cancer	144 (100.00): 0 (0.00)	0 (0.00)	0 (0.00)	15 (2.02)	43 (2.44)	78 (3.64)	8 (3.65)
Skin cancer (melanoma)	82 (66.13): 42 (33.87)	6 (1.97)	7 (1.51)	11 (1.48)	39 (2.22)	56 (2.62)	5 (2.28)
Bladder cancer	81 (73.64): 29 (26.36)	5 (1.64)	0 (0.00)	13 (1.75)	38 (2.16)	51 (2.38)	3 (1.37)
Breast cancer	2 (2.41): 81 (97.59)	4 (1.32)	14 (3.02)	14 (1.88)	32 (1.82)	19 (0.89)	0 (0.00)
Renal cancer	56 (76.71): 17 (23.29)	4 (1.32)	8 (1.73)	14 (1.88)	31 (1.76)	16 (0.75)	0 (0.00)
Others	266 (55.65): 212 (44.35)	40 (13.16)	45 (9.72)	62 (8.33)	151 (8.58)	164 (7.66)	16 (7.31)

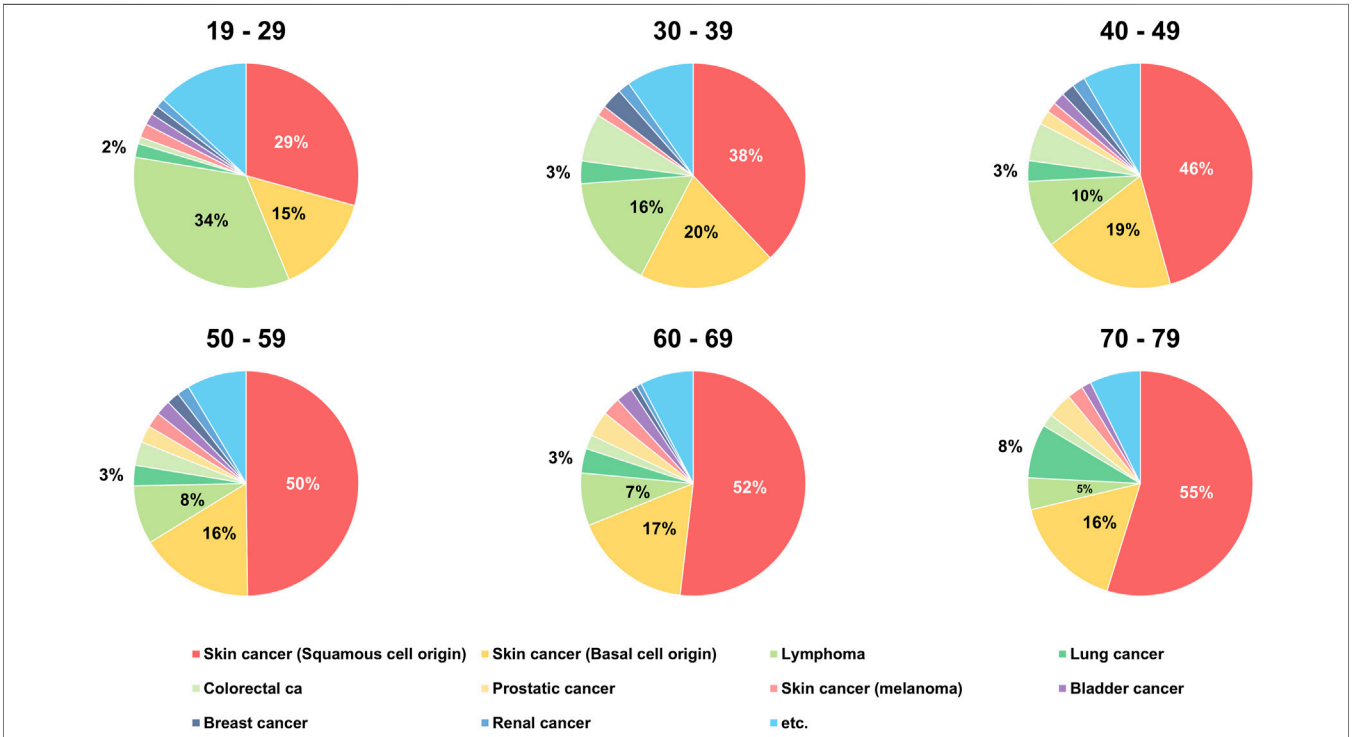


FIGURE 3 | Age distribution of recipients with *de novo* post-transplant malignancy after double lung transplantation.

Male DLT recipients (n = 3,785, 67.24%) were more frequently diagnosed with PTMs ($p < 0.001$), and the mean age at the onset of PTMs was 60.87 years (SD, ±49.26) (**Table 1**).

Indications of DLT for Non-Cancerous Disease

There were 87 different indications for DLT, with the most common being idiopathic pulmonary fibrosis/usual interstitial pneumonitis (n = 6,400; 23.74%). The second and third most common indications for DLT were chronic obstructive pulmonary disease/emphysema (n = 5,276; 22.04%), and cystic fibrosis (n = 4,075; 17.03%). The order of common indications for DLT was identical in recipients with and without PTM (**Supplementary Table S1**).

Types and Incidences of *De Novo* PTM

Twenty-seven types of *de novo* PTM were detected after DLT for non-cancerous disease during the surveillance. The common tumor

TABLE 3 | Incidence of *de novo* malignancy after double lung transplantation by order of occurrence in recipients with single or multiple post-transplant malignancy (PTM).

Types of PTM (*n*, %)	Orders of *de novo* PTM			
	First malignancy (*n* = 4,403)	Second malignancy (*n* = 1,047)	Third malignancy (*n* = 160)	Fourth malignancy (*n* = 19)
Skin cancer (Squamous cell origin)	495 (11.24)	58 (5.54)	15 (9.38)	2 (10.53)
Skin cancer (Basal cell origin)	2,401 (54.53)	287 (27.41)	23 (14.38)	0 (0.00)
Lymphoma	526 (11.95)	403 (38.49)	33 (20.63)	3 (15.79)
Lung cancer	128 (2.91)	44 (4.20)	10 (6.25)	5 (26.32)
Colorectal ca	144 (3.27)	30 (2.87)	5 (3.13)	5 (26.32)
Prostatic cancer	102 (2.32)	28 (2.67)	13 (8.13)	1 (5.26)
Bladder cancer	77 (1.75)	37 (3.53)	10 (6.25)	0 (0.00)
Skin cancer (melanoma)	74 (1.68)	24 (2.29)	11 (6.88)	1 (5.26)
Breast cancer	70 (1.59)	7 (0.67)	6 (3.75)	0 (0.00)
Renal cancer	53 (1.20)	16 (1.53)	4 (2.50)	0 (0.00)
Pancreatic cancer	44 (1.00)	10 (0.96)	5 (3.13)	1 (5.26)
Esophageal cancer	31 (0.70)	12 (1.15)	4 (2.50)	0 (0.00)
Tongue and Throat cancer	29 (0.66)	14 (1.34)	3 (1.88)	0 (0.00)
Genital cancer	28 (0.64)	12 (1.15)	1 (0.63)	0 (0.00)
Thyroid cancer	26 (0.59)	5 (0.48)	2 (1.25)	0 (0.00)
Hepatic cancer (Primary)	21 (0.48)	9 (0.86)	1 (0.63)	0 (0.00)
Primary cancer of unknown origin	23 (0.52)	5 (0.48)	2 (1.25)	0 (0.00)
Sarcoma	16 (0.36)	10 (0.96)	3 (1.88)	1 (5.26)
Leukemia	18 (0.41)	8 (0.76)	3 (1.88)	0 (0.00)
Laryngeal cancer	21 (0.48)	5 (0.48)	2 (1.25)	0 (0.00)
Stomach cancer	22 (0.50)	4 (0.38)	2 (1.25)	0 (0.00)
Small intestinal cancer	11 (0.25)	9 (0.86)	1 (0.63)	0 (0.00)
Uterus carcinoma	15 (0.34)	2 (0.19)	0 (0.00)	0 (0.00)
Ovarian cancer	10 (0.23)	4 (0.38)	0 (0.00)	0 (0.00)
Kaposi sarcoma (Cutaneous)	8 (0.18)	2 (0.19)	1 (0.63)	0 (0.00)
Kaposi sarcoma (Visceral)	6 (0.14)	2 (0.19)	0 (0.00)	0 (0.00)
Brain tumor	4 (0.09)	0 (0.00)	0 (0.00)	0 (0.00)

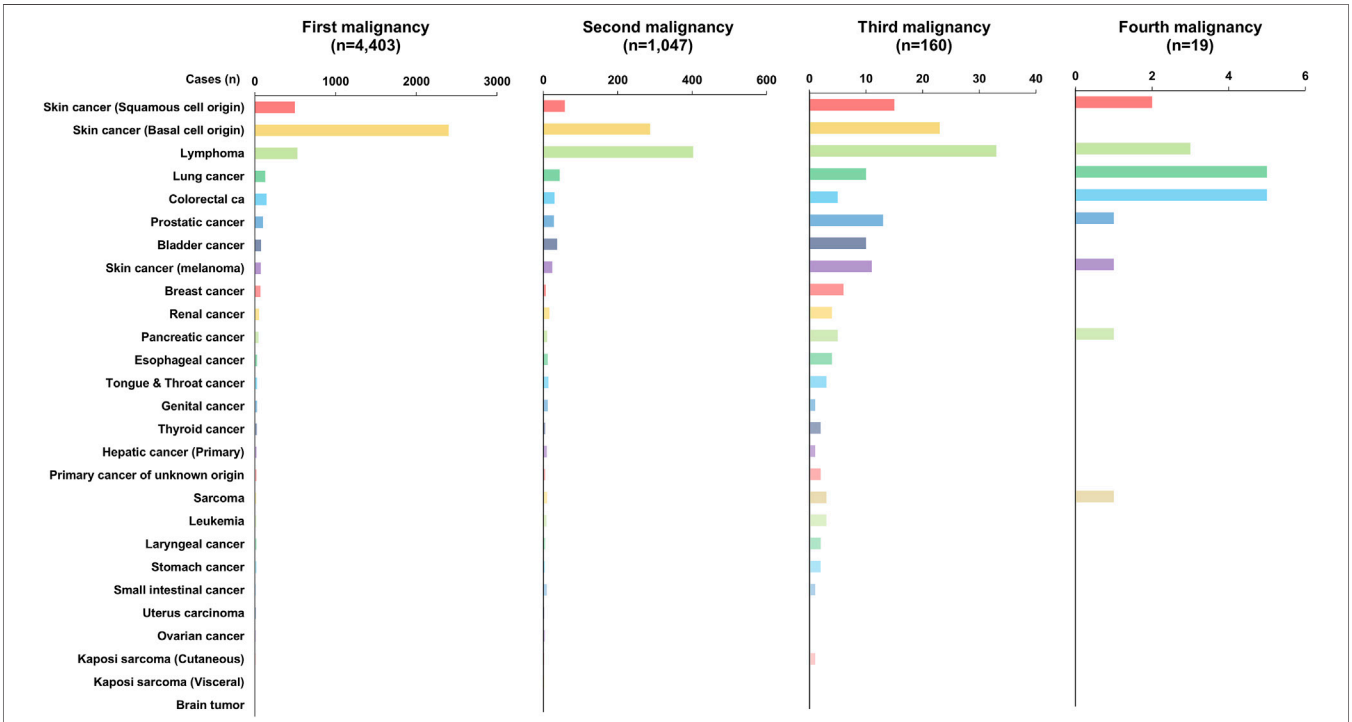


FIGURE 4 | Incidence of *de novo* PTM depending on the order of occurrence in recipients with PTM.

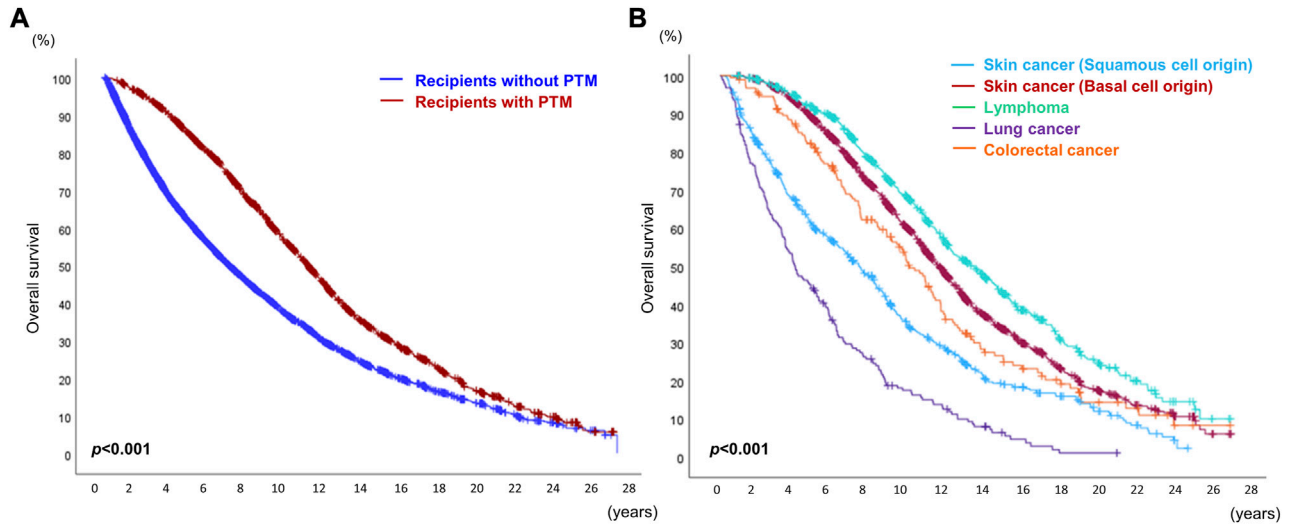


FIGURE 5 | Overall survival of recipients who received double lung transplantation. **(A)** Overall survival of recipients with or without post-transplant malignancy (PTM). **(B)** Overall survival of recipients with the top five causes of *de novo* PTM after double lung transplantation.

types were SCC (n = 2,711; 48.16%), BCC (n = 965; 17.14%), lymphoma (n = 570; 10.13%), lung cancer (n = 187; 3.32%), colorectal cancer (n = 184; 3.27%), prostatic cancer (n = 144; 2.56%), skin cancer (melanoma) (n = 123; 2.19%), bladder cancer (n = 109; 1.94%), breast cancer (n = 83; 1.47%), renal cancer (n = 73; 1.30%), pancreatic cancer (n = 60; 1.07%), esophageal cancer (n = 47; 0.83%), tongue and throat cancer (n = 46; 0.82%), genital cancer including vulva, peritoneum, penis, and scrotum (n = 41; 0.73%), thyroid cancer (n = 33; 0.59%), primary hepatic cancer (n = 31; 0.55%), sarcoma (n = 30; 0.53%), leukemia (n = 30; 0.53%), primary cancer of unknown origin (n = 29; 0.52%), stomach cancer (n = 28; 0.50%), laryngeal cancer (n = 28, 0.50%), small intestinal cancer (n = 21; 0.37%), uterus cancer (n = 17; 0.30%), ovarian cancer (n = 14; 0.25%), Kaposi sarcoma (cutaneous type) (n = 11; 0.20%), Kaposi sarcoma (visceral type) (n = 8; 0.14%), and brain tumor (n = 4; 0.07%) (**Figure 1**).

Annual Risks of Each *De Novo* PTM After DLT

The lifetime incidence of *de novo* PTM following DLT was identified as 23.52% (5,629/23,935), and the annual risks of *de novo* malignancy after DLT are shown in **Figure 2A**. During the first year following DLT, SCC (n = 203; 31.42%) occurred most frequently, followed by lymphoma (n = 197; 30.50%), BCC (n = 90; 13.93%), and lung cancer (n = 50; 7.74%). SCC was diagnosed more than twice as frequently in the first year and most frequently in the second year following DLT (n = 419; 52.38%), and then gradually decreased. Although BCC also occurred more frequently in the second year than in the first year following DLT, the range of change was smaller than that of SCC. Lymphoma and lung cancer most frequently occurred during the first year of DLT; the incidence decreased to less than half in the second year following DLT and gradually decreased thereafter. Although the incidence of colorectal cancer was less than 20 per year during the first 10 years, they continued to occur even 10 years after DLT.

Cumulative Risks of Each *De Novo* PTM After DLT

The incidence of SCC increased until 10 years and rarely occurred 20 years following DLT, and the total cumulative incidence was 2,711 (48.16%). On the other hand, BCC was the second most common PTM after DLT and the rate of increase was slower than that of SCC. Lymphoma was the third most common PTM with a cumulative incidence of 570 (10.13%), and one-third of cases occurred during the first year of transplantation (n = 197/570, 34.56%). Lung cancer was the fourth most common PTM with a cumulative incidence of 119 (3.55%) during the 18 years of follow-up.

The cumulative risks of PTM after DLT are shown in **Figure 2B** (the top four causes: SCC, BCC, lymphoma, and lung cancer) and **Supplementary Figure S1** (the other causes of PTM).

Age Distribution of Recipients With *De Novo* PTM After DLT

When the incidence of PTMs was analyzed by age group, SCC was the most common PTM in all age groups except for recipients aged 19–29 years. In recipients aged 19–29 years, lymphoma (n = 103, 33.88%) was the most common tumor type after DLT. While the incidence of lymphoma gradually decreased with age, BCC showed similar rates of incidence in all age groups (range, 14.47%–19.65%). The incidence of lung cancer after DLT showed similar rates among recipients in the 19–69 age group except for those in the 70–79 age group (n = 17, 7.76%). The incidence of colorectal cancer after DLT was higher in recipients aged 30–39 and 40–49 years than in other age groups (**Table 2**; **Figure 3**).

TABLE 4 | Factors associated with overall survival in recipients who received double lung transplantation for non-cancerous disease.

Variables	Total (*n* = 23,935)	Univariate analysis			Multivariate analysis		
		HR	95%-CI	*p*-value	HR	95%-CI	*p*-value
Age at transplantation (mean, ±SD)	51.91 ± 4.95	1.012	1.010–1.013	<0.001	1.017	1.015–1.019	<0.001
Gender (*n*, %)				0.069			0.006
Male	13,768 (57.52)	1*			1*		
Female	10,167 (42.48)	0.967	0.932–1.003		0.943	0.905–0.983	
Smoking history (*n*, %)				<0.001			0.095
Non-smoker	9,148 (38.22)	1*			1*		
Smoker	11,129 (46.50)	1.151	0.938–0.986		0.961	0.916–1.007	
Unknown	3,658 (15.28)	0.888			0.547	0.498–0.600	
Occurrence of post-transplant malignancy (*n*, %)				<0.001			<0.001
No	18,306 (76.48)	1*			1*		
Yes	5,629 (23.52)	0.586	0.562–0.610		0.604	0.575–0.635	
Occurrence of graft failure (*n*, %)				<0.001			<0.001
No	21,619 (90.32)	1*			1*		
Yes	2,316 (9.68)	2.541	2.420–2.667		3.093	2.936–3.257	

**reference*

Incidence of *De Novo* PTM by Order of Occurrence in Recipients With Multiple PTM

A total of 4,403 recipients (78.22%) were diagnosed with a single *de novo* PTM after DLT, and 1,047 recipients (18.60%) were diagnosed with double *de novo* PTMs simultaneously or subsequently. Furthermore, 160 recipients (2.84%) and 19 recipients (0.34%) were diagnosed with three and four *de novo* PTMs, respectively. While BCC was the most common tumor type (n = 2,401; 54.53%) in the first malignancy group, lymphoma was the most common tumor type in the second (n = 403, 38.49%), and third (n = 33, 20.63%) malignancy groups. Brain tumors (n = 4, 0.09%) occurred only in recipients who had a single PTM (**Table 3**; **Figure 4**).

OS of Recipients With *De Novo* PTM After DLT

According to the OPTN/UNOS data, the OS of all recipients who received DLT for the non-cancerous disease was 51.04% (12,216/23,935) [OS of the recipients without *de novo* PTM: 53.50% (9,794/18,306); OS of the recipients with *de novo* PTM: 43.01% (2,421/5,629). However, OS in recipients with PTM was significantly higher than that in recipients without PTM (**Figure 5A**). And the median and mean survival periods were significantly longer in the recipients with PTM group [median, recipients without PTM: 36.67 months (range, 0.03–330.73) vs. recipients with PTM: 97.20 months (range, 0.90–328.50); mean, recipients without PTM: 66.11 months (SD, ±66.94) vs. recipients with PTM: 106.32 months (SD, ±73.77)].

While the 5-year and 10-year OS rates in recipients with PTM were higher than in those without PTM (5-year, without PTM 67.32% vs. with PTM 83.57%; 10-year, without PTM 57.90% vs. with PTM 62.00%), the 15-year and 20-year OS rates in recipients with PTM were lower than in those without PTM (15-year, without PTM 54.68% vs. with PTM 48.80%; 20-year, without PTM 53.77% vs. with PTM 44.45%).

Among the top five causes of PTM (SCC, BCC lymphoma, lung cancer, and colorectal cancer), the OS rate of recipients with lymphoma was the highest, and that of those with lung cancer was the lowest ($p < 0.001$). The OS of recipients with SCC was worse than that of those with BCC (**Figure 5B**). Kaposi sarcoma (visceral type) showed the worst prognosis among the 27 different types of PTM (**Supplementary Figure S2**).

Age at transplantation, smoking history, occurrence of PTM and GF were associated with OS, in univariate analysis. However, in Cox regression analysis, while the occurrence of PTM was associated with lower risk of overall mortality (HR = 0.604, 95% CI: 0.575–0.635, $p < 0.001$) after adjustment for age (continuous), sex, and smoking history (non-smoker vs. smoker), the occurrence of GF was associated with higher risk of overall mortality (HR = 3.093, 95% CI: 2.936–3.257, $p < 0.001$) (**Table 4**).

Landmark Analysis for OS in Recipients With or Without PTM

To compensate for the immortal time bias of PTM, OS was calculated using landmark analysis (**Table 5**; **Figure 6**). Using 3 and 5 years as the landmark time points, the OS in recipients with PTM was found to be significantly better than those without PTM (3 years, HR = 0.797, 95% CI: 0.759–0.836, $p < 0.001$; 5 years, HR = 0.925, 95% CI: 0.873–0.979, p = 0.007). However, at the 7-year landmark time point, the difference in OS between the two groups disappeared (p = 0.217), and after 10 years of surveillance, the OS in recipients without PTM was better (HR = 1.123, 95% CI: 1.025–1.231, p = 0.013). However, after 15 years, there was no statistical difference in OS between the two groups (15 years, HR = 1.173, 95% CI: 0.986–1.394, p = 0.071; 20 years, HR = 1.055, 95% CI: 0.737–1.509, p = 0.770).

TABLE 5 | Number of recipients and the occurrence of post-transplant malignancy (PTM).

Overall survival (*n*, %)	Total (*n* = 23,935)	Recipients without PTM (*n* = 18,306)	Recipients with PTM (*n* = 5,629)
No landmark			
Number of death events	11,719 (48.96)	8,512 (46.50)	3,208 (56.99)
Median (month, range)	48.67 (0.03–330.73)	36.67 (0.03–330.73)	97.20 (0.90–328.50)
Mean (month, ±SD)	66.11 ± 66.94	53.74 ± 27.62	106.32 ± 73.77
5-year	75.01%	67.32%	83.57%
10-year	58.86%	57.90%	62.00%
15-year	53.29%	54.68%	48.80%
20-year	51.58%	53.77%	44.45%
3-year landmark	18,782 (78.47)	13,754 (75.13)	5,028 (89.32)
Number of death events	6,758 (28.23)	4,050 (22.12)	2,708 (48.10)
5-year landmark	16,742 (69.95)	12,201 (66.65)	4,541 (80.67)
Number of death events	4,718 (19.71)	2,497 (13.64)	2,221 (39.46)
7-year landmark	15,323 (64.02)	12,289 (67.13)	4,034 (71.66)
Number of death events	3,299 (13.78)	1,585 (8.66)	1,714 (30.45)
10-year landmark	13,436 (56.14)	10,303 (56.28)	3,133 (55.66)
Number of death events	1,412 (5.90)	599 (3.27)	813 (14.44)
15-year landmark	12,349 (51.59)	9,834 (53.72)	2,515 (44.68)
Number of death events	325 (1.36)	130 (0.71)	195 (3.46)

Comparison of Survival Outcomes Depending on the Number of PTMs

When the patients were divided into two cohorts, single and multiple PTM groups, the survival outcome in recipients with multiple PTMs was significantly better than that of recipients with single PTMs ($p < 0.001$). However, there was no statistically significant difference in the number of PTMs among the recipients in the multiple PTM group ($p = 0.375$) (**Figure 7**).

Causes of Death in Recipients Who had Received DLT

Among the 23,935 recipients who received DLT, 11,719 recipients (48.96%) died from 74 different causes of death. The main categories of causes of death in recipients who received DLT were as follows: infection (with 13 subcategories), cardiovascular cause (with 11 subcategories), graft failure (with 8 subcategories), pulmonary cause (with 7 subcategories), malignancy (with 6 subcategories), hemorrhagic (with 6 subcategories), cerebrovascular cause (with 5 subcategories).

The mortality rate in recipients without PTM was highest within 1 year after DLT, whereas that in recipients with PTM was highest after 3 years of DLT (**Figure 8**). Although graft failure was the most common cause of death in recipients without PTM, infection (including bacterial, viral, and fungal) was the most common cause of death during the first year after DLT. On the other hand, the most common cause of death in the recipients with PTM was a metastatic malignancy, which occurred most frequently in the 3 years after DLT (**Supplementary Figures S3, S4**).

DISCUSSION

Our study's major findings include: 1) around one-fourth of the recipients who underwent DLT for non-cancerous diseases experienced PTM, with 27 different PTMs occurring during the follow-up period; 2) annual and cumulative risks of each PTM varied based on elapsed time post-DLT, with the highest PTM incidence in the second year after transplantation; 3) PTM incidence differed among age groups, particularly post-transplant lung cancer, which had the highest incidence in the 70–79 age group; 4) one in five recipients with PTM after DLT was diagnosed with multiple PTMs (up to four different types), with the most common tumor types differing based on the order of occurrence; 5) OS after DLT was better in recipients with PTM than those without PTM at the 3-year, and 5-year landmark time points and in recipients diagnosed with multiple PTMs rather than a single PTM.

Organ transplantation has increased, and survival outcomes have improved due to advancements in immunosuppressive therapy [22, 23]. However, *de novo* malignancy development post-transplantation, mainly related to immunosuppressive therapy [17, 24]. In the context of lung transplantation, although the immunosuppressive protocols are similar for both single and bilateral transplantations, our study exclusively focused on DLT. This approach was adopted to mitigate potential confounding factors such as the presence of latent lung cancer in the native lung or underlying conditions like pulmonary fibrosis that could elevate the risk of lung cancer [25, 26].

Major PTM incidences after DLT was highest in the second-year post-transplantation. However, lymphoma was most frequent at the first year than second year. Lymphoma, a post-transplant lymphoproliferative disease, typically occurs within 4–6 months after hematopoietic stem cell transplantation and mainly after the first year of solid organ transplantation [27–29]. Notably, lymphoma post-solid organ transplantation occurs 11.8-fold more frequently than in the non-transplant population ($p < 0.001$), and the age-stratified relative risk is higher in children under 10 years old and adults over 60 years. This PTM is often life-threatening, with a higher risk in heart, lung, intestinal, and multi-organ transplants [30–32]. The occurrence of post-transplant lymphoma is strongly

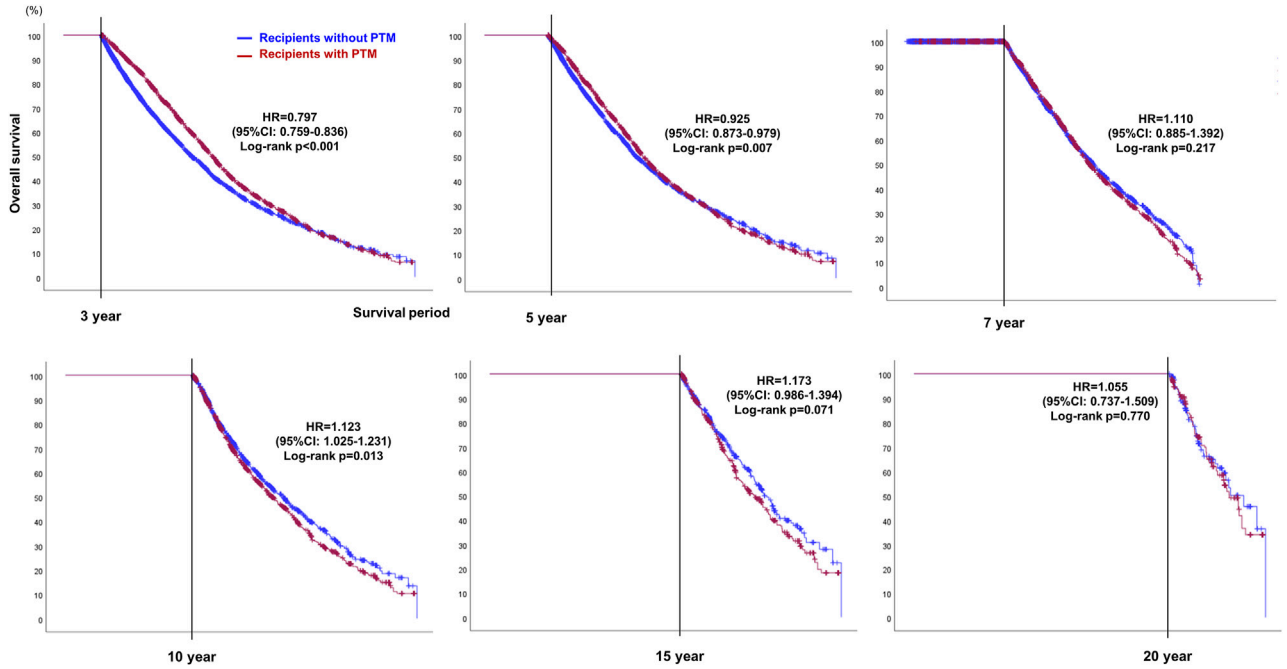


FIGURE 6 | Landmark analysis plots showing OS at 3, 5, 7, 10, 15, and 20-year landmark time points in recipients with or without post-transplant malignancy (PTM) after double lung transplantation.

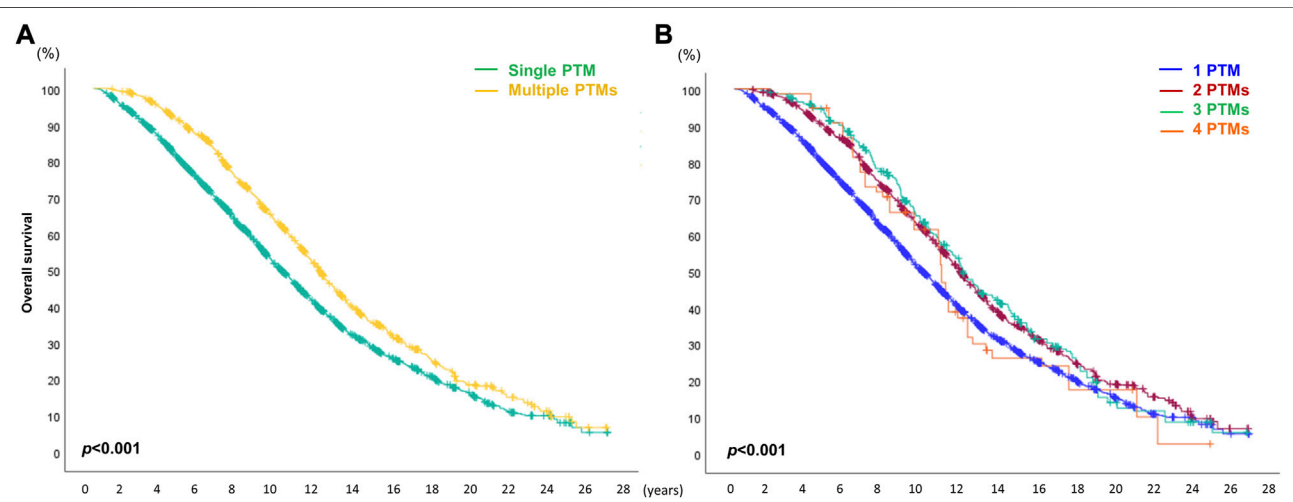


FIGURE 7 | Overall survival (OS) depends on the number of *de novo* post-transplant malignancies (PTMs) after double lung transplantation. **(A)** Comparison of OS between single and multiple PTM. **(B)** Comparison of OS depending on the number of PTMs.

associated with immunosuppressants, such as FK506, OKT3, and ATG [33, 34].

After DLT for non-cancerous diseases, approximately 24% of all recipients were diagnosed with PTMs in their lifetimes, with one-fifth of them being diagnosed multiple times. There were four types of post-transplant skin cancer, including SCC, BCC, melanoma, and cutaneous Kaposi sarcoma. While BCC is more prevalent than SCC in the general population at a 4:1 ratio, where SCC occurs more frequently in transplant patients with an incidence rate 65- to 250-fold higher [35]. Particularly, SCC in organ transplant recipients shows a worse prognosis with nine times higher cancer-specific mortality than in the general population [36–38]. In our study, post-transplant SCC was 3-fold higher than BCC after DLT, and the OS of recipients with BCC was better than that of those with SCC which was similar to the trend observed in the general population [39].

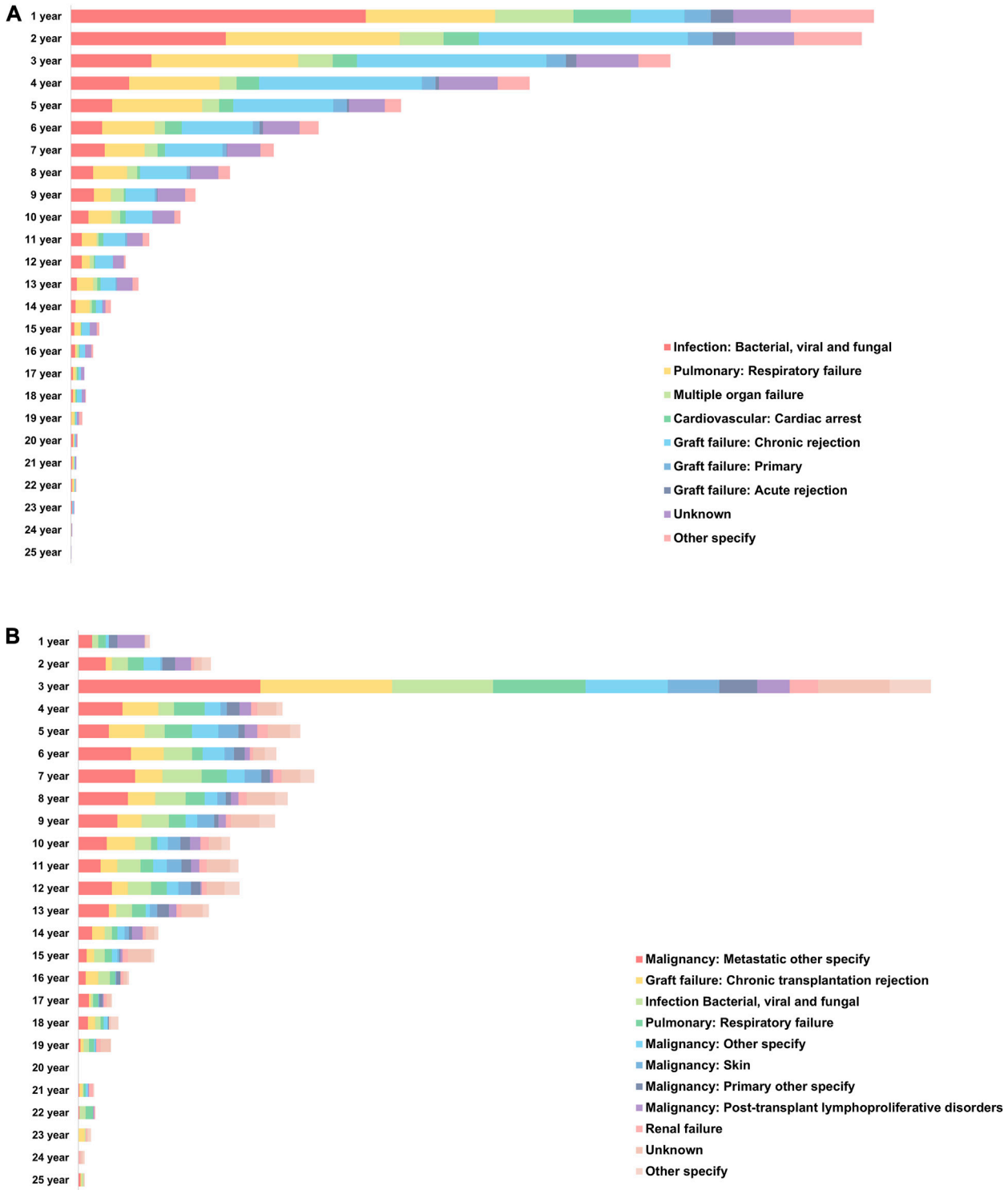


FIGURE 8 | Causes of death in recipients who had received double lung transplantation. **(A)** Annual causes of death in recipients without *de novo* malignancy after double lung transplantation. **(B)** Annual causes of death in recipients with *de novo* malignancy after double lung transplantation.

SCC was the most common type of PTM in most age groups, and lymphoma was the most prevalent only in the 19–29 age group. Colorectal cancer ranked as the 5th most common PTM after DLT and mainly occurred within 1 year after DLT in recipients in their 50s. After DLT, the risk of developing lymphoma and lung cancer was highest within the first year,

while bladder cancer was most likely to occur 8 years after DLT. Other types of PTM occurred mainly in the second year after transplantation, with the incidence gradually decreasing over time. Interestingly, lung cancer was the 4th most common PTM after DLT, despite recipients having received bilateral allogenic lung transplantation. The incidence rates of lung cancer after DLT were only 2%–3% in most age groups, and its incidence was the highest at 8% in the 70–79 age group. While the incidence of lung cancer in the general population gradually increases with age, the recipients who received DLT showed lower occurrence rates until their 60s, which then rapidly increased in their 70s [40, 41].

Although immunosuppressive therapy after solid organ transplantation is necessary to prevent complications after transplantation [6, 42, 43]. However, long-term immunosuppression may promote cancer progression, whether it is a pre-existing or new lesion and the risk of PTM is increased approximately 3- to 4-fold compared with the general population [44–46]. A conventional protocol for maintenance immunosuppressive therapy for lung transplantation is the "triple regimen," which includes a calcineurin inhibitor (cyclosporine or tacrolimus), antiproliferative agents (azathioprine, mycophenolate, sirolimus, and everolimus), and corticosteroids. Tacrolimus has a pro-oncogenic effect by producing transforming growth factor β1 [47], and azathioprine is known to increase the risk of skin cancers after organ transplantation, especially SCC [48, 49]. Cyclosporine use is also associated with lymphoma and skin cancer [50]. And the use of Voriconazole increases the risk for cutaneous SCC among solid organ transplant recipients [51, 52]. However, the association between mycophenolate mofetil and increased cancer incidence after transplantation is unclear. Moreover, sirolimus is known to have both an anticancer effect (by targeting mTOR) and an immunosuppressive effect [53]. To summarize, different PTMs occur depending on the regimen of immunosuppressive agents [21, 28, 54–56]. However, information on PTM remains insufficient, and there are no guidelines for modified immunosuppressive therapy that can minimize the occurrence of PTMs.

In this study, we found that recipients with PTM had significantly better survival outcomes than those without PTM. However, since an earlier study had reported significantly lower 1-year and 3-year survival rates for patients with PTM [57], we conducted a landmark survival analysis to shed more light on this discrepancy. We assumed this was because of the immortal time bias, which means that longer recipients have a higher chance of being diagnosed with PTM. To compensate for this error, which refers to a bias that can occur in observational studies when the time between a defined event (e.g., transplantation) and the start of follow-up (e.g., diagnosis of PTM) is not considered [58], we performed landmark analysis with 3, 5, 7, 10, 15, and 20 years as the landmark time points. Recipients with PTM had better short-term survival (3–5 years) but worse long-term survival (10 years and beyond). Immunosuppressive therapy may contribute to PTM while preventing graft rejection. Graft failure was a major cause of death in recipients without PTM. Factors like age and comorbidities may have a greater impact on long-term survival. Beyond 15–20 years, there was no statistical difference in survival, possibly due to other factors and decreased statistical power.

The major limitation of this study is that not all patients had the same length of follow-up period and actual incidence of PTM could not be calculated for individuals who did not reach the 1-year follow-up after transplantation. And although at least 10 years of follow-up results were investigated for most PTMs, only 9, 6, and 4 years of follow-up results were available for leukemia, Kaposi sarcoma, and brain tumor, respectively. Another limitation of this study is that we did not completely correct for the higher chance of developing cancer over time, even though we performed a landmark analysis. However, this study provided general information on PTMs in recipients who received DLT for non-cancerous diseases, offering a comprehensive landscape in this field.

In conclusion, the types and characteristics of PTMs in recipients who received DLT for non-cancerous diseases were highly diverse, and the incidence varied according to age and duration after transplantation. Additionally, the survival outcomes showed significant differences depending on the existence or types of PTM. Nevertheless, we were able to identify the specific times at which each type of PTM frequently occurred. By gaining a more comprehensive understanding of the characteristics of PTMs in recipients who have undergone DLT, it may become possible to predict with greater accuracy the specific types of PTM that are most likely to occur over time and to facilitate their early detection. Such insights can potentially revolutionize our approach to monitoring and managing PTMs in DLT recipients, ultimately leading to improved clinical outcomes and a better quality of life for those who have undergone this procedure.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: The data that support the findings of this study are available from the corresponding author upon reasonable request. Requests to access these datasets should be directed to ychae@nm.org.

ETHICS STATEMENT

The studies involving humans were approved by Northwestern University's Institutional Review Board Committee in Chicago, IL, United States (IRB#: STU00207117). 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

Conceptualization, JL and YKC; methodology, YKC, JL, AWJY, JY, HJS, and Y-GC; investigation, JL and JY; resources, AWJY, YKC, and AB; data curation, JL, AWJY, YL, and HSK;

writing–original draft preparation, JL, AWJY, and YL; writing, review, and editing, JL, YKC, Y-GC, and LIC, visualization, JL, AWJY, YL, and YKC; supervision, YKC, AB, HJS, and Y-GC; project administration, JL, LIC, AB, and YKC. All authors contributed to the article and approved the submitted version.

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AUTHOR DISCLAIMER

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

CONFLICT OF INTEREST

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

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11552/full#supplementary-material

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Intestinal Donation and Utilization: Single-Center Analysis Within Eurotransplant

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Intestinal donor criteria are classically kept strict, thereby limiting donor supply. Indications for intestinal transplantation (ITx) are rare, but improved outcome and new emerging indications lead to increased demand and relaxing donor criteria should be considered. We sought to compare the donor criteria of intestines transplanted at our center with predefined (per protocol) criteria, and to determine how relaxing donor criteria could impact the potential donor pool. Donor criteria used in 22 consecutive ITx at our center between 2000 and 2020 were compared with predefined criteria. Next, multiorgan donors effectively offered by our Donor Network to Eurotransplant between 2014 and 2020 were retrospectively screened, according to predefined and effectively used intestinal donation criteria. Finally, utilization rate of offered intestines was calculated. In our ITx series, the effectively used donor criteria were less strict than those initially predefined. With these relaxed criteria, a favorable 5-year graft/patient survival of 75% and 95%, respectively was reached. Applying these relaxed criteria would lead to a 127% increase in intestinal offers. Paradoxically, 70% of offered intestines were not used. In conclusion, a significant increase in intestinal donation could be obtained by relaxing donor criteria, while still achieving excellent outcome. Offered intestines are underutilized.

Keywords: awareness, intestinal transplantation, organ donation, organ allocation, utilization

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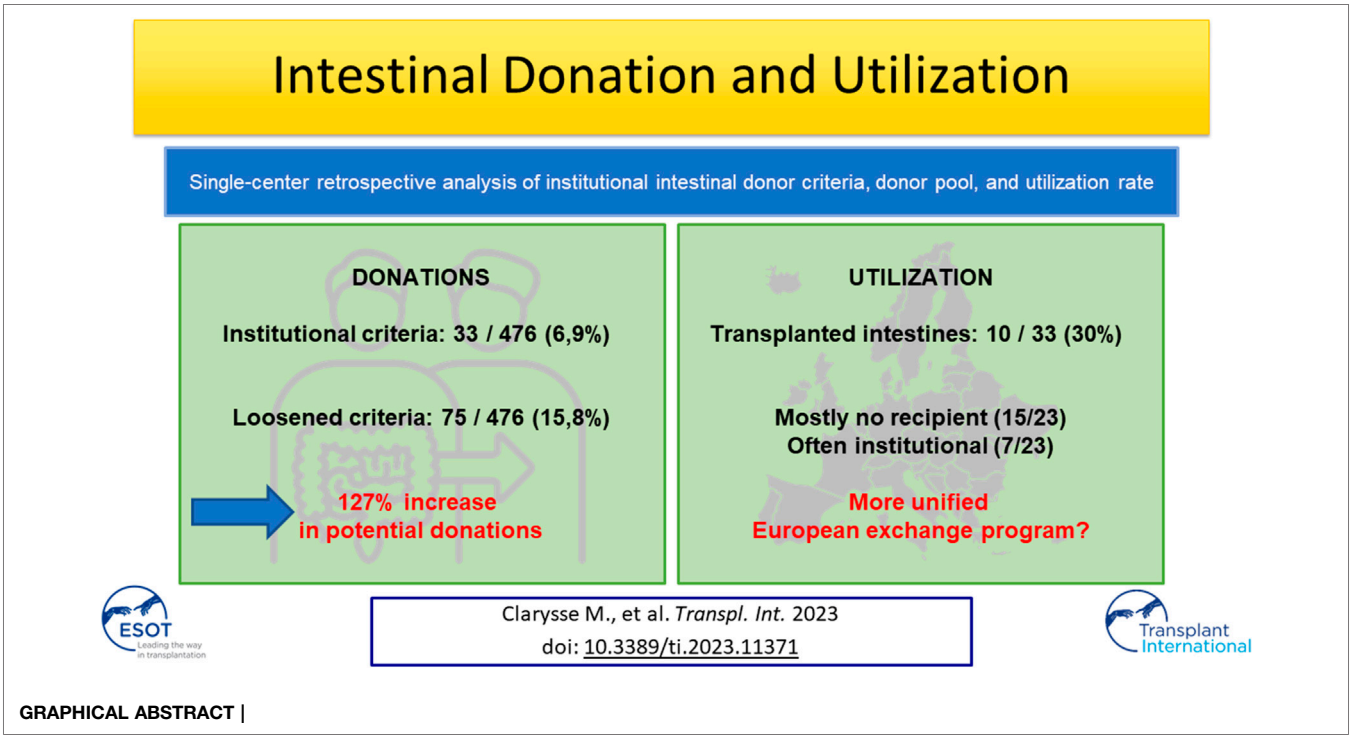
Abbreviations: [Na+], Serum Sodium Concentration; γ, μg/kg/min; ALT, Alanine Transaminase; AST, Aspartate Transaminase; BMI, Body Mass Index; CMV, Cytomegalovirus; CPR, Cardiopulmonary Resuscitation; DBD, Donation after Brain Death; DCD, Donation after Circulatory Death; ELIAC, ET Liver Intestine Advisory Committee; ET, Eurotransplant; ICU, Intensive Care Unit; INR, International Normalized Ratio; ITx, Intestinal Transplantation; LSGO, Leuven Samenwerkende Groep voor Orgaandonatie (Leuven cooperating group for organ donation); NHS, National Health Service; NRP, Normothermic Regional Perfusion; UNOS, United Network for Organ Sharing; OPTN, Organ Procurement and Transplantation Network.



GRAPHICAL ABSTRACT |

INTRODUCTION

Intestinal transplantation (ITx) is indicated in patients with intestinal failure and life-threatening complications of parenteral nutrition [1, 2]. So far, ITx has only been advocated as a "salvage procedure," due to the complexity of the procedure and to outcomes traditionally inferior to other solid organ transplants. However, patient survival reaching 90% and 75% at 1- and 10-year post-transplantation has been reported [2–5]. In addition, new indications like extensive mesenteric thrombosis or other diffuse abdominal diseases, necessitating multivisceral transplantation, are emerging [6]. As a consequence, the demand for suitable donor intestines is increasing.

The intestine is very susceptible to ischemia and preservation injury and for this reason, ITx centers usually only use so called "excellent donors" e.g., donors with very strict predefined criteria. These donors are rare and waiting time can be long. Within United Network for Organ Sharing (UNOS), more than 50% of the listed patients wait more than 1 year prior to transplantation and the risk of deterioration and mortality on the list is high [4]. Since the start of an ITx waiting list in Eurotransplant (ET) on 1st October 2012, a mortality rate of 27% (n = 30/113) has been observed, and 3% of the patients (n = 3/113) were delisted because deemed unfit for ITx (personal communication with ET). "Extended" donor criteria are now accepted for the majority of solid organs [donation after circulatory death (DCD), advanced age, prolonged intensive care unit (ICU) stay, co-morbidity . . .] [7]. Because the intestine is extremely vulnerable to warm ischemia, DCD donors are not routinely used and donation after brain death (DBD) donors represent the largest source of intestinal grafts. In several European countries, there is a shift from DBD towards DCD donors, thereby further reducing the availability of intestinal grafts. For the aforementioned reasons, relaxing the criteria for intestinal donation is becoming necessary.

At the start of our program, we predefined strict donor criteria and we now wanted to determine whether these criteria had actually been respected in our ITx series. Secondly, we determined, in our own donor pool, how slightly relaxing donor criteria would increase the number of intestinal grafts. Lastly, we studied the utilization rate of offered intestines.

PATIENTS AND METHODS

UZ Leuven Donor Network

Belgium has opt-out legislation for organ donation since 1986 and no separate informed consent is needed for intestinal donation. Each of the seven Belgian transplant centers has its own procurement organization, consisting of the respective transplant university hospitals and their own network of cooperating "donor" hospitals. The UZ Leuven Donor Network for organ procurement includes The University Hospitals Leuven and its 37 cooperating hospitals across Flanders, Belgium (LSGO). Belgium is part of ET, and solid organs procured in the LSGO are allocated by ET. Allocation of intestinal grafts to ET waitlisted patients occurs in a patient-driven manner, over three active ITx centers in three countries in 2022. In the time period of the study, 2014–2020, there were seven active ITx centers in four countries.

TABLE 1 | Comparison between predefined and effective intestinal donation criteria in Leuven.

		Demographics and medical history												Lab results									
		Type of donor	Age (years)	Weight (kg)	BMI (kg/m²)	Weight ratio	Blood group (ABO) compatibility	Hypotens time (min)	Hypotens episodes	Arrest time (min)	Cardiac arrest	CPR time (min)	CPR	Highest AST (U/L)	Highest ALT (U/L)	Highest bilirubin (mg/dL)	# of packed cells	Highest INR	ICU stay (days)	Highest amylase (U/L)	Highest lipase (U/L)	Highest creatinine (mg/dL)	Last serum [Na+]
Predefined	Leuven	DBD	<50	≤80	≤25		Compatible																<155
Effective	Leuven	DBD	16 (1–37)	50 (12–75)	19.6 (11–26)	0.9 (0.5–1.5)	Compatible							63 (12–269)	25 (9–198)	0.4 (0.05–3.4)		1.27 (0.9–1.7)		105 (16–603)	16 (6–101)	0.68 (0.2–1.3)	148 (130–157)
N° of patients exceeding predefined criteria		0	0	0	1	0		0						0	0	0		0		0	0	0	1

		Hemodynamics							Inotropics and transfusion and ICU stay									Multi-organ donor				
		CPR	CPR time (min)	Cardiac arrest	Arrest time (min)	Hypotens episodes	Hypotens time (min)	Smoking	NOR	γ of NOR	Alcohol	Drug	DOB	Diabetes	γ of DOB	EPI	γ of EPI	Packed cells	# of packed cells	ICU stay (days)	Heart offered	Lungs offered	Liver offered	Pancreas offered	Kidneys offered
Predefined	Leuven	No	0	No	0	No	0	Yes	<0.1		Yes	<5								<5	Yes	Yes	Yes	Yes	Yes
Effective	Leuven	Yes	0 (0–30)	Yes	0 (0–45)	Yes	0 (0–15)	Yes	Yes *n* = 15	0.1 (0.01–0.68)	Yes	No	Yes *n* = 2	No	2 (1–3)	Yes *n* = 1	0.14	Yes *n* = 8	0 (0–5)	2 (1–9)	Yes *n* = 20	Yes *n* = 20	Yes *n* = 22	Yes *n* = 22	Yes *n* = 22
N° of patients exceeding predefined criteria		3	3	3	3	2	2	0	8		0	0	0	0	0	0	0	0	0	2	2	2	0	0	0

Green = equal; red = predefined violation. ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; DBD, donation after brain death; DOB, dobutamine; EPI, epinephrine; ICU, intensive care unit; INR, international normalized ratio; [Na+], Serum Sodium Concentration; N°, number; NOR, norepinephrine; REA, resuscitation; γ, μg/kg/min.

Predefined Intestinal Organ Donor Criteria at Our Center

At the start of our ITx program in 2000, intestinal organ donor criteria were predefined and are summarized in **Table 1** [8]. All deceased intestinal donors should be DBD, age < 50 years, weight ≤ 80 kg, Body Mass Index (BMI) ≤ 25 kg/m^2. Blood group matching is identical or compatible. Exclusion criteria included smoking, alcohol, drug abuse, and diabetes. Liver and kidney function tests must be normal. Recent cardiac arrest, cardiopulmonary resuscitation (CPR), or hypotensive episodes are excluded. The donor should be hemodynamically stable, with minimal transfusions and inotropic support (<2 drugs at low dosage). ICU stay should be less than 5 days. As a result, intestinal donors are often multi-organ donors (Heart, Lungs, Liver, Pancreas, Kidneys).

Donor Criteria of Intestines Transplanted at Our Center

A retrospective analysis of our prospectively collected ITx database (October 2000—September 2020) was performed. One living donor ITx was excluded and only deceased ITx recipients (n = 22) were analyzed. Data analyzed were: donor type, age, weight, length, BMI, donor/recipient weight ratio, ABO blood group compatibility, smoking/alcohol/drug abuse, diabetes, latest lab results (Aspartate Transaminase (AST), Alanine Transaminase (ALT), total bilirubin, International Normalized Ratio (INR), amylase, lipase, creatinine, [Na+], CPR time, cardiac arrest time, hypotensive episodes, inotropic use and dosage, number of transfused packed cells, ICU stay, and other organs offered (heart, lungs, liver, pancreas, kidneys).

Retrospective Screening of Donor Pool According to Predefined Versus Actually Used Donor Criteria

Data of donors offered by LSGO to ET during a 6-year period (1st January 2014—31st December 2019), and prospectively collected in an *ad hoc* donor database, were analyzed. Data included: donor type, age, weight, length, BMI, ABO and rhesus blood group, smoking/alcohol/drug abuse, diabetes, virology status (human immunodeficiency virus, hepatitis B, hepatitis C), CPR time, cardiac arrest time, hypotensive episodes, inotropic use (number and dosage), transfused packed cells, ICU stay, organs offered and transplanted (heart, lungs, liver, pancreas, kidneys, and intestine), and reasons for not offering or for refusing the intestine.

This donor cohort was screened, first according to the aforementioned predefined intestinal donor criteria, and second according to the criteria effectively applied in our ITx program and defined in the first part of the study.

Statistics

Data were collected using Excel (Microsoft Office 2019). Results are reported as median (range). Subgroup analysis was performed by non-parametric Mann-Whitney Test in GraphPad Prism

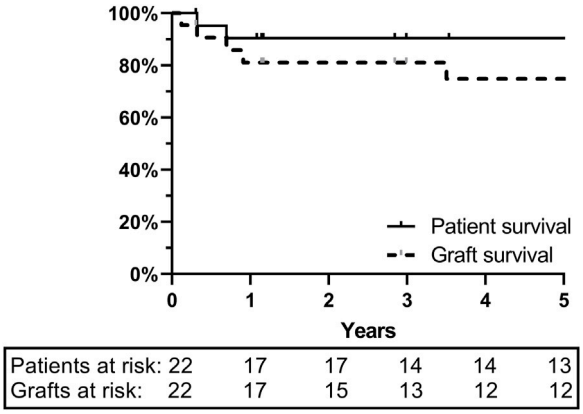


FIGURE 1 | One- and 5-year graft and patient survival at University Hospitals Leuven: ITx cohort of 22 patients.

version 9.0.0 for Windows (GraphPad Software, La Jolla, CA, United States). p-value < 0.05 was considered statistically significant.

Ethics

All ITx patients gave consent for database recording of transplant-related data and their use for research purposes. This study was approved by the ethics committee of University Hospitals Leuven, Belgium (S63306) and was conducted according to the revised version of the Declaration of Helsinki (October 2013, Brazil).

RESULTS

Leuven ITx Series

Between 19 October 2000 and 01 September 2020, 22 deceased ITx were performed. Ten were isolated ITx, five multivisceral transplants, and seven combined liver-ITx. Two were retransplants. Eight donors (36%) were from our local network (LSGO) and the remaining 14 (64%) from ET. Pre-, peri-, and post-transplant surgical and medical management have been described extensively elsewhere [8]. One- and 5-year graft and patient survival were 81%/75% and 95%/95%, respectively (**Figure 1**).

Intestinal Donor Criteria Used in Our ITx Cohort are Less Strict Compared to Predefined Criteria

All donors used for ITx were DBD with a median age of 16 years (1–37). Median weight was 50 kg (12–75). BMI was 19.6 kg/m^2 (11–26). BMI was higher than the predefined maximum of 25 kg/m^2 in one donor. Donor/recipient weight ratio was 0.9 (0.5–1.5). Blood group was compatible in 5 and identical in 17. Smoking and alcohol abuse were present in 2 and 1 donors, respectively. Drug abuse and diabetes were not reported. The latest lab results were acceptable for liver transplantation and kidney transplantation, and last [Na+] was 148 mmol/L (130–157). The predefined maximum [Na+] was overruled in one donor with a [Na+] of 157 mmol/L. In three donors, CPR had been performed for 20 min (10–30) and cardiac arrest time in these donors was 25 min (5–45). Hypotensive episodes of 10 min were noted in two donors (5–15). Norepinephrine was used at a dosage of 0.1 μg/kg/min (γ) (0.0155–0.68) as single inotropic in 13 donors and in combination in 2 others. In eight donors, norepinephrine dosage exceeded the predefined maximum of 0.1γ. Dobutamine was used in two donors, in one as single inotropic, at a dosage of 2γ (1–3). In one donor, epinephrine was used, as second inotropic, at a dosage of 0.14γ. Packed cells transfusion was given in eight donors with a median of 2 units of packed cells (1–5). Median ICU stay was 2 days (1–9) and exceeded the predefined maximum in two donors, with 5 and 9 days, respectively. All abdominal organs were offered in all donors. Heart and lungs were not offered in three donors due to thoracic trauma, atrial fibrillation, and for unknown reason (**Table 1**).

In 55% (n = 12/22) of these effective intestinal donors, the strict predefined criteria had been overruled for BMI, CPR, inotropic use, and ICU stay.

DBD Pool Filtered by Predefined ITx Criteria

From 1st January 2014, to 31st December 2019, the LSGO had referred 664 donors to ET. Of them, 188 (28%) were DCD and 476 (72%) were DBD donors.

Of the 476 DBD donors, 68% (n = 326/476) were excluded for age ≥ 50 years (**Figure 2**). Of the remaining 150 donors, 47 were excluded due to a BMI > 25 kg/m^2. Six additional donors were excluded for weight > 80 kg. Hence, one out of five DBD donors (n = 97/476; 20%) matched the predefined theoretical anthropomorphic criteria for ITx. Thirty-three intestines from these donors (n = 33/97; 34%) were offered to ET. Ten were effectively transplanted within ET, of which 6 in our own center. Out of the 33 offered intestines, only 10 were transplanted and 23 could not be allocated, representing a utilization rate of 30.3% (n = 10/33).

In 64 out of 97 donors (66%), the intestine was not offered, for which the reasons are listed in **Table 2**. Of these 64 donors, predefined criteria were not met in 49 (77%). The three main reasons were > 10 min of CPR (n = 21/49; 43%), ICU stay of > 5 days (n = 8/49; 16%) and high inotropic need (n = 6/49; 12%). In the 15 remaining donors, the most important reasons for not offering the intestine were low cardiac ejection fraction on ultrasound in 3 (5%) and malignancy in 2 (3%) (a grade IV glioblastoma in one and a grade I–II astrocytoma with previous tumor surgery, in another).

In 55 of these non-offered donors (86%), only one exclusion criterium for not offering the intestine (CPR) was present. In 7 (11%), two criteria (among them CPR time, hypotension, ICU stay, and/or inotropic need) were present. In 2 (3%), 3 exclusion criteria were present (CPR time, hypotension, and inotropic need).

In 33 donors, the intestine was offered despite the presence of one exclusion criteria in 20 (61%) and two exclusion criteria in 4 (12%). Overruled criteria were mainly: CPR time, hypotension, ICU stay, and/or inotropic need. Other reasons for overruling

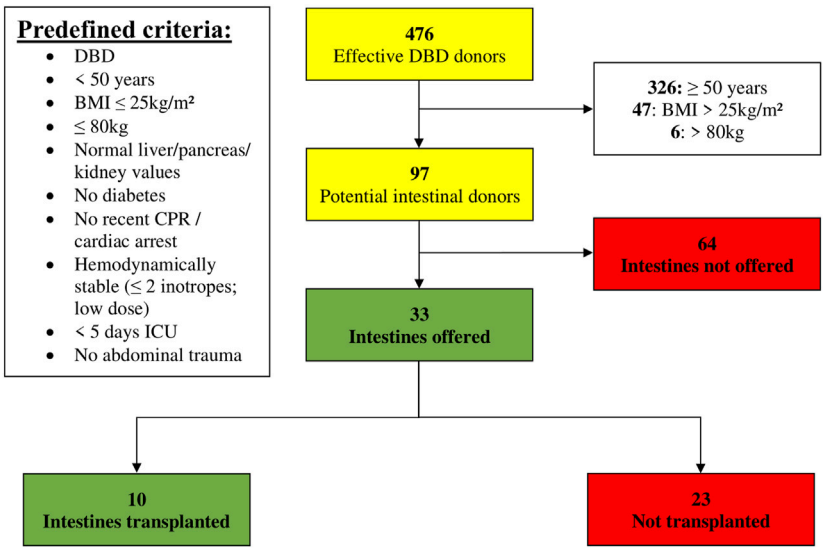


FIGURE 2 | Donor pool (01 January 2014–31 December 2019) filtered by predefined criteria (BMI, Body Mass Index; CPR, Cardiopulmonary Resuscitation; DBD, Donation after Brain Death; ICU, Intensive Care Unit).

TABLE 2 | Reasons for not offering the intestine.

64 intestines not offered
Predefined criteria (*N* = 49)
×21 > 10 min CPR
×8 > 5 days ICU
×6 high inotropic need
×5 > 20 min hypotension
×4 drug abuse
2 × 4 units packed cells
×1 abdominal trauma
×1 diabetes mellitus type 1
×1 diabetes mellitus type 2
Other reasons (*N* = 15)
×3 low cardiac ejection fraction
×2 malignancy
×1 extreme height + weight
×1 gastric bypass surgery
×1 hemochromatosis
×1 infectious
×1 legal issues
×5 unknown

CPR, cardiopulmonary resuscitation; ICU, intensive care unit; N, Number.

criteria were noted in four donors but were not specific to intestinal donation: bacterial meningitis in two, meningioma in one, and a thyroid tumor in another (**Supplementary Table S1**).

In 5 out of the 10 transplanted intestines (50%), one predefined criterium was overruled (CPR time, ICU stay, or inotropic need) and in one donor (10%) two criteria were overruled (prolonged ICU stay and high inotropic need) (**Supplementary Table S2**).

Reasons for Not Using Offered Intestines

Of the 23 refused donor intestines, 7 (30.4%) had directly been offered to our own center but could not be used for organizational reasons. They were then offered to ET but could not be transplanted either. Fifteen intestines directly offered to ET (65%) could not be used, potentially due to absence of suitable recipients within ET or for organizational reasons (**Table 3**). Finally, in one donor, a low cardiac ejection fraction was diagnosed after the intestine was offered and subsequently declined for transplantation.

To determine whether donor factors would account for the non-acceptance of intestinal offers, we compared the donor data of transplanted vs. not transplanted intestines (**Table 4**). No difference was seen in anthropomorphic criteria (age, BMI, weight, height) and in ICU stay. In the non-transplanted cohort, there were two children under 1 year of age, with a very low BMI and weight. Other reasons are reported in four donors, as mentioned above (two bacterial meningitis, one meningioma and one thyroid tumor).

Donor Pool Filtered by Effectively Applied Criteria and Potential Impact on Intestine Donor Pool

The predefined criteria were extended with the following effectively applied criteria: BMI ≤ 26 kg/m^2, hemodynamic parameters (CPR < 45 min; hypotensive episodes, norepinephrine < 0.68γ, transfused packed cells), and ICU < 10 days.

Of the 476 DBD donors, 68% (n = 326/476) were excluded for age ≥ 50 years. Of the remaining 150 donors, 28 were excluded due to a BMI > 26 kg/m^2 and an additional 16 donors were excluded for weight > 80 kg. Hence, 22% of DBD donors (n = 106/

TABLE 3 | Reasons for not transplanting the offered intestine.

23 intestines offered—not transplanted
×15 no recipients
×7 capacity reasons in Leuven
×1 low cardiac ejection fraction

476) met the effective anthropomorphic criteria. Seventy-five donors (71%) met all of the effectively applied criteria and could have been potential intestinal donors (**Figure 3**).

Only 31 non-offered donors would then remain. Twenty-three percent had a CPR ≥ 45 min (n = 7/31) and another 23% had an ICU stay ≥ 10 days (n = 7/31). Drug abuse would still lead to the exclusion of 16% (n = 5/31) and low cardiac ejection fraction to 10% (n = 3/31). Other reasons are mentioned in **Table 5**. In all these 31 non-offered donors, there was only one reason why the intestine would not have been offered.

If the effective criteria would have been applied, this would have resulted in an additional 42 intestinal offers. Main reasons for additional inclusion would have been CPR 10–45 min in 17 donors, 5 donors with hypotensive episodes, and 7 with inotropic need (**Table 6**). Inclusion of these 42 additional intestinal offers, would result in a potential donor increase of 127%. In 98% (41/42) of these additional donors, application of the effective criteria would have resulted in only one violation to the predefined criteria. In the latter one, there would have been two violations: CPR time exceeding 10 min (i.e., 15 min) and usage of two inotropics (0.4γ norepinephrine and 0.3γ epinephrine).

DISCUSSION

The only limiting factor for wider application of solid organ transplantation is the shortage of suitable organs. To meet the higher demand, donor criteria have been progressively relaxed over time. The intestine stands in stark contrast because intestinal donor criteria have usually remained very strict, mostly because the intestine is highly vulnerable to ischemia. Here, we show that outcomes similar to other solid organ transplants can be obtained despite using slightly relaxed criteria. By applying these relaxed criteria, we could substantially increase the pool of intestinal donors. Importantly, we found that a substantial portion of offered intestines are not utilized, suggesting the need for a European-wide intestinal donor organ sharing program.

At first sight, the need for extension of the intestinal donor pool appears less urgent than for other solid organs. Indeed, ITx remains a rare procedure representing less than 0.5% of the overall solid organ transplant activity. That is because the incidence of intestinal failure is much lower compared to failure of other organs and, in case of "uncomplicated" intestinal failure, parenteral nutrition is still the first treatment option [9]. Obstacles to wider application of ITx are the complex surgical and immunobiological challenge that the transplantation of this naturally infected and immunologically active organ represents, and the reported results which were historically inferior -on average-to other solid organ transplants. This has contributed in some reluctance to refer patients for ITx [10]. However, results similar to other solid organ transplants can now be achieved in experienced centers [4]. Excellent outcome, improved quality of life and the proven cost-effectiveness of ITx versus parenteral nutrition (similar to kidney Tx vs. dialysis) is an incentive to propose ITx earlier in the course of intestinal failure [11]. Finally, new indications for intestinal and multivisceral transplantation are emerging such as splanchnic thrombosis and certain tumors [6]. For all these reasons, the demand of suitable intestinal grafts is increasing.

Among solid organs, criteria for intestinal donation are the strictest. This is due to the extreme vulnerability of the bowel to ischemia and reperfusion injury and the wish "not to add additional risks to an already high-risk" procedure. According to the majority of published criteria, age limits are set around 50 years, weight at 80 kg, and BMI at 25–28 kg/m^2. Liver and kidney function are to be normal, and only limited resuscitation time is accepted. An ICU stay of less than a week and cold ischemia time of max 9 h are other standard cut offs (**Supplementary Table S3**) [8, 12–16]. At our center, we defined strict criteria for intestinal donation at the onset of our program (**Table 1**, **Supplementary Table S3**) [8].

By retrospectively analyzing the characteristics of the intestinal grafts we actually procured and transplanted, we observed that we had overruled our own center-predefined strict criteria in 55%. The most frequently overruled criteria were BMI, CPR, inotropic dosage, and ICU stay. Of note, overruling was more frequent in more recent years which suggests a learning and experience effect (data not shown). When comparing the outcome of our "strict" vs. "relaxed"

TABLE 4 | Donor criteria in transplanted versus "no-recipients" cohort.

	Transplanted cohort (N = 10)				No-recipients cohort (N = 22)				p-value
Age (years)	21 (1–41)				26 (0–46)				0.6964
BMI (kg/m^2)	20.7 (16.6–23.1)				21.4 (10.7–24.8)				0.7410
Weight (kg)	67.5 (12–75)				62.5 (3–80)				0.5003
Height (cm)	180 (85–185)				171 (53–185)				0.1497
ICU stay (days)	2 (0–6)				2 (0–9)				0.6546
Blood group	O	A	B	AB	O	A	B	AB	
	4	6	0	0	10	9	2	1	

Numbers represented as median (range). p-*value < 0.05 was considered statistically significant. CPR, cardiopulmonary resuscitation; ICU, intensive care unit; N, Number.*

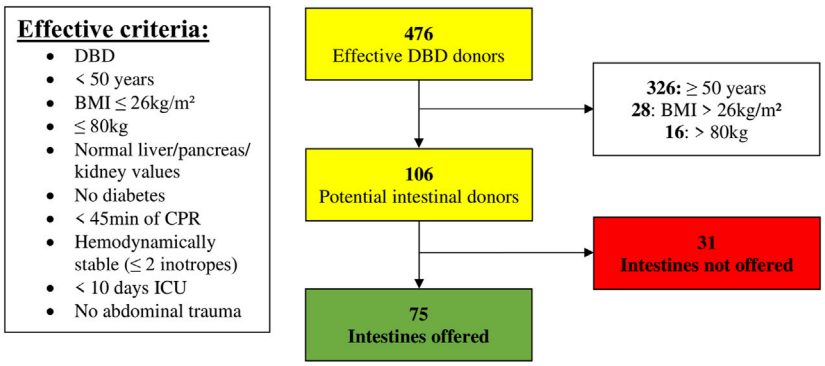


FIGURE 3 | Donor pool (01 January 2014–31 December 2019) filtered according to effective criteria (BMI, Body Mass Index; CPR, Cardiopulmonary Resuscitation; DBD, Donation after Brain Death; ICU, Intensive Care Unit).

TABLE 5 | Reasons for not offering the intestine according to effective criteria analysis.

31 intestines not offered
Effective criteria (*N* = 22)
×7 ≥ 45 min CPR
×7 ≥ 10 days ICU
×5 drug abuse
×1 abdominal trauma
×1 diabetes mellitus type 1
×1 diabetes mellitus type 2
Other reasons (*N* = 9)
×3 low cardiac ejection fraction
×2 gastric bypass surgery
×2 infectious
×1 hemochromatosis
×1 legal issues

CPR, cardiopulmonary resuscitation; ICU, intensive care unit; N, Number.

TABLE 6 | Additional intestinal donor offers when using effective criteria.

42 potential extra offers
Effective criteria (*N* = 33)
17 × 10–45 min CPR
×7 high inotropic need
×5 hypotension
2 × 4 units packed cells
2 × 5–10 days ICU
Other reasons (*N* = 9)
×5 unknown
×3 malignancy
×1 extreme height + weight

CPR, cardiopulmonary resuscitation; ICU, intensive care unit; N, Number.

donors, no difference was observed (data not shown). Our overall 5-year patient survival of 95% compares favorably to the international registry, suggesting that having slightly relaxed the donor criteria had no impact on outcome in our program [17].

Historically, hemodynamically unstable donors were deemed unfit for intestinal donation, as the intestine is extremely sensitive to ischemia [18–20]. However, donors with an episode of cardiac arrest and CPR time of up to 52min have been used successfully in different centers [16, 21, 22]. In our series, we used intestines from donors with a median CPR time of 20 min and maximum up to 45 min. Therefore, pre-procurement cardiac arrest and CPR should not necessarily exclude intestinal donation. In addition to CPR, high inotropic need is another reason for excluding intestinal donation. However, data from UNOS suggest that donor intestines exposed to prolonged periods of hypotension were not necessarily predestined to inferior outcome [22]. And with adequate management, inotropics can be weaned or reduced before procurement. In our program, we accepted donors with short hypotensive episodes, limited amount of packed cells transfusion, and donors on no more than two inotropics in acceptable dosages.

Another discriminatory factor in our predefined criteria is ICU stay < 5 days. In literature, mostly 1 week is used as upper limit [16]. However, longer ICU stays have been reported without a clear impact on outcome [21, 22]. Hence, a prolonged stay on ICU should not *per se* limit intestinal organ donation if other criteria are acceptable.

Another option to increase the intestinal donor pool is to accept older donors. The arbitrary upper age limit has usually been fixed at 50 years [13, 14, 16, 23]. However, several publications report successful ITx with donors older than 50 years [13, 14, 24]. Accordingly, age criteria for intestinal donation have been increased to 60 years in Japan and to 65 years in the United Kingdom [22, 25].

Based on our findings and the data published by others, we recommend a slight extension of the intestinal donation criteria. Donor age up to 60 years, BMI up to 28 kg/m^2 (if donor/recipient size mismatch allows it), ICU stay up to 10 days, limited inotropic usage, previous episodes of hypotension, short period of cardiac arrest and CPR, and limited packed cells transfusions should -separately- not be seen as absolute contraindication for intestinal donation, particularly for

patients already more than 1 year on the waiting list or who need a suitable organ more urgently [22].

We showed that the pool of suitable bowels could be substantially enlarged by using these slightly relaxed donor criteria. Indeed, applying these extended criteria to our whole potential donor pool resulted in a 127% increase in intestinal donors.

Of note, a multivisceral graft has been recently procured in a DCD donor after normothermic regional reperfusion (NRP) and the transplant was successful [26]. This strategy may allow access to an important pool of currently not utilized intestinal grafts. Especially for extreme young (<1 year of age) recipients, waiting time has more impact on the outcome after ITx. In our cohort, there were no such young recipients and only two extreme young donors (<1 year of age) were offered for transplant. When analyzing the LSGO donor pool of 664 for suitable DCD donors, 11 additional potential intestinal donors could be found with the predefined criteria (**Supplementary Figure S1**). By applying the effective criteria, 27 potential intestinal DCD donors could be withheld (**Supplementary Figure S2**). However, more experience on DCD-NRP for ITx is obviously needed.

Strikingly, we noticed that 70% of the intestines offered by our network, were ultimately not used for transplantation. These organs did not differ with regard to anthropometric data and ICU stay, from those grafts that were effectively used. We see two reasons for this underutilization. First, a substantial number of intestinal offers were declined for organizational reasons. Indeed, ITx and procurement require full mobilization of experienced transplant surgeons, transplant anesthesiologists, and gastroenterologists and these are not necessarily permanently standby. On the other, several heart and lung procurement/transplant teams fear inferior outcomes of their graft, if an intestinal graft is concomitantly procured and if special donor preprocurement therapies were initiated. However, the study by Farinelli et al. showed that these intestinal donor preprocurement therapies might even be beneficial for other transplanted organs, without impacting allocation, quality or long-term outcome [27]. ITx may also compete with other organ transplant activities for theater and personnel. In COVID times, travel restrictions for the procurement team and intensive care capacity to take care of these highly demanding patients, further limits the organizational possibilities. Between 1st January 2014, and 31st December 2020, 28 intestinal offers offered by all ET centers were declined explicitly for capacity reasons [28]. In our own center, about 25% of the offered donors had to be declined for organizational reasons as well. The permanent availability of highly specialized ITx services for a small number of patients is challenging and this pleads for more centralization of the procedure.

Second, one intestinal offer at a given time may not necessarily fit all recipients. It is likely that perfectly transplantable intestines were turned down for size-, age-, CMV-mismatch, or other surgical or medical reasons. Size-mismatch is very common in ITx. Most recipients have had previous abdominal surgery and multiple intestinal resections, leaving them with little abdominal domain. Concerning the abdominal domain, there is an important difference whether the transplant graft is liver-containing (combined liver-intestinal or multivisceral) versus isolated small bowel. This limitation can be overcome -to a certain extent-by techniques such as fascia or even full thickness abdominal wall transplantation, graft reduction, etc. [29]. However, these are not commonly used as it further complicates the surgical procedure. It is possible that intestines could not be allocated due to absence of a blood group identical or compatible recipient, and not all centers accept blood group compatible grafts.

Offering these unused intestines outside ET should be considered to increase organ utilization and optimize donor-recipient matching, thereby reducing waiting time and associated mortality. Such cross-program exchange structures already exist for other organs between allocation organizations such as NHSBT (United Kingdom) and Scandiatransplant. Rushton et al. already suggested the possibility to implement a formalized European-wide intestinal donor organ sharing program [25]. A prerequisite for such a European exchange is to keep cold ischemia time short, which would require excellent coordination. Potentially, this could be performed by looking for a second, back up, recipient within or outside ET, at the moment of allocation, in case the intestinal graft gets turned down for own usage by the explant team.

A limitation of our study is that -in all surveyed donors-, a specific reason for not offering the intestine was sought in retrospect. We cannot exclude that in several cases, one simply "forgot" to offer the intestine. That is because ITx is a relatively poorly known activity. We cannot quantify this, but we suspect this has been a relatively frequent reason for not offering the intestine. This could be overcome by mandatory reporting of the intestinal graft if a donor fits the intestinal donation criteria. Another option could be with a UK-like system where all DBD are potential intestinal donors and are allocated through the system to potential recipients. Thereby reducing the subjectivity of the initial offering process [25]. In general, more awareness needs to be given to the importance of intestinal procurement.

In conclusion, this study makes three points. First, the strict intestinal donor criteria that we had predefined are not routinely followed in our actual practice and -despite that-excellent outcomes are obtained. Second, slightly relaxing intestinal donor criteria and in particular accepting donors with prolonged ICU stay, limited CPR time, and mild inotropic usage can substantially increase the nu mber of offered intestines. Third, the pool of offered intestines is paradoxically underutilized, which is multifactorial in origin. A European intestinal donor organ sharing program should be considered to facilitate donor-recipient matching.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the University Hospitals Leuven, Herestraat 49, 3000 Leuven, Belgium (S63306). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

MC, TV, LC, and JP reviewed literature and 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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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11371/full#supplementary-material

Supplementary Figure S1 | Donor pool (01 January 2014–31 December 2019) filtered by predefined criteria for potential DCD intestinal donors (BMI, Body Mass Index; CPR, Cardiopulmonary Resuscitation; DBD, Donation after Brain Death; ICU, Intensive Care Unit).

Supplementary Figure S2 | Donor pool (01 January 2014–31 December 2019) filtered according to effective criteria for potential DCD intestinal donors (BMI, Body Mass Index; CPR, Cardiopulmonary Resuscitation; DBD, Donation after Brain Death; ICU, Intensive Care Unit).

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ORIGINAL RESEARCH
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Thrombotic Microangiopathy in the Renal Allograft: Results of the TMA Banff Working Group Consensus on Pathologic Diagnostic Criteria

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The Banff community summoned the TMA Banff Working Group to develop minimum diagnostic criteria (MDC) and recommendations for renal transplant TMA (Tx-TMA) diagnosis, which currently lacks standardized criteria. Using the Delphi method for consensus generation, 23 nephropathologists (panelists) with >3 years of diagnostic

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Abbreviations: ABMR, antibody-mediated rejection; (a)HUS, (atypical) hemolytic uremic syndrome; BWG, Banff working group; Clin, clinical data; CNI, calcineurin inhibitor; EM, electron microscopy; #D, differential diagnosis; DIC, disseminated intravascular coagulation; EC, endothelial cells; Gen, genetic criterion; GN, glomerulonephritis; HTN, hypertension; ICC, intraclass correlation; IF/IHC, immunofluorescence microscopy/immunohistochemistry; IgAN, IgA nephropathy; Lab, laboratory; LDH, lactate dehydrogenase; LM, light microscopy; MPGN, membranoproliferative GN; MDC, minimum diagnostic criteria; NT-ABMR, non-TMA-associated antibody-mediated rejection; PI GN, post-infectious glomerulonephritis; R, round; SLE, systemic lupus erythematosus; TTP, thrombotic thrombocytopenic purpura; TMA, thrombotic microangiopathy; Tx-TMA, transplant TMA; %A, percentage agreement; %AL, percentage agreement level.

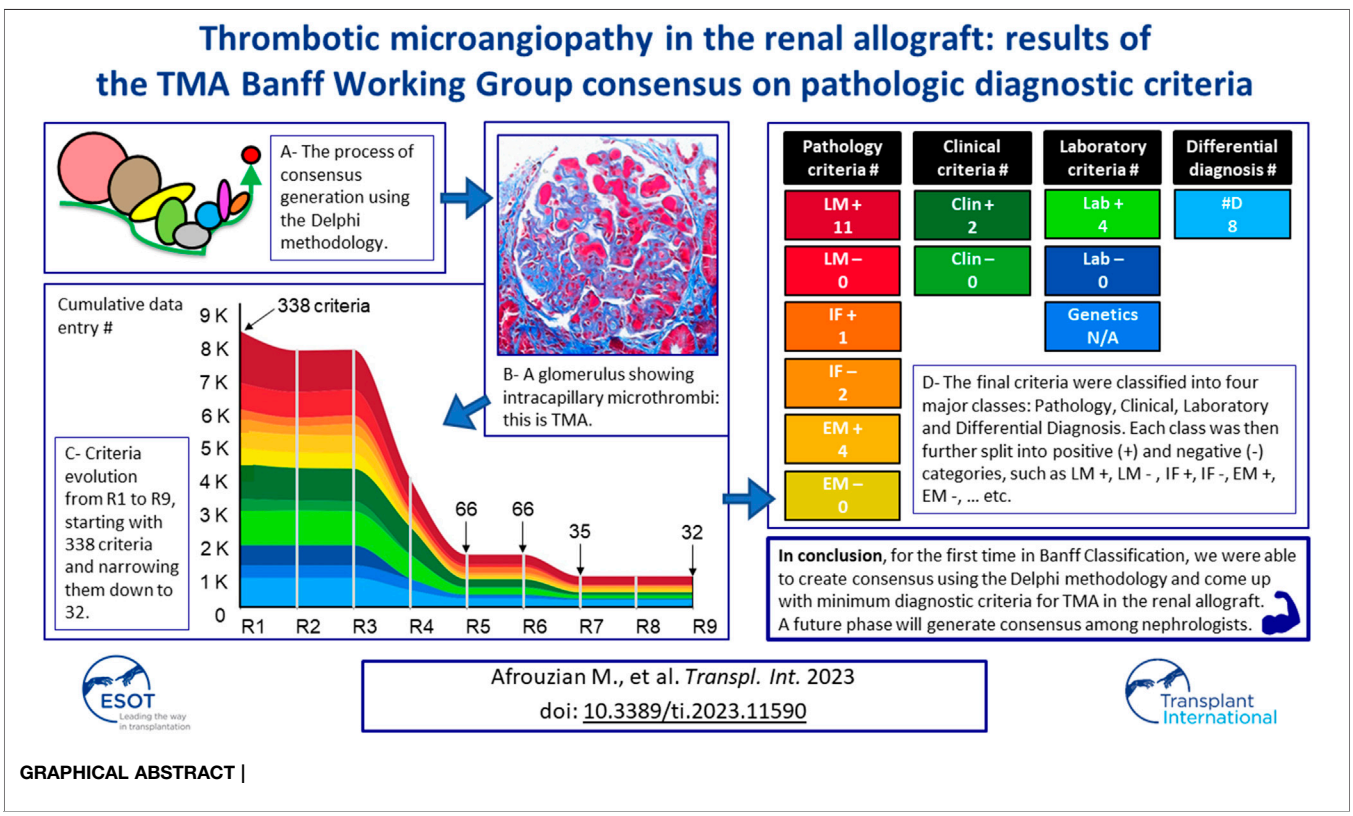
experience with Tx-TMA were asked to list light, immunofluorescence, and electron microscopic, clinical and laboratory criteria and differential diagnoses for Tx-TMA. Delphi was modified to include 2 validations rounds with histological evaluation of whole slide images of 37 transplant biopsies (28 TMA and 9 non-TMA). Starting with 338 criteria in R1, MDC were narrowed down to 24 in R8 generating 18 pathological, 2 clinical, 4 laboratory criteria, and 8 differential diagnoses. The panelists reached a good level of agreement (70%) on 76% of the validated cases. For the first time in Banff classification, Delphi was used to reach consensus on MDC for Tx-TMA. Phase I of the study (pathology phase) will be used as a model for Phase II (nephrology phase) for consensus regarding clinical and laboratory criteria. Eventually in Phase III (consensus of the consensus groups) and the final MDC for Tx-TMA will be reported to the transplantation community.

Keywords: thrombotic microangiopathy, kidney, transplant, pathology criteria, Delphi, Banff

INTRODUCTION

Transplant thrombotic microangiopathy (Tx-TMA) is caused by endothelial injury which is hallmarked by thrombotic occlusion of small vessels resulting in often clinically unexpected allograft failure [1, 2]. Immunologic, genetic, hematologic disorders and drugs may trigger the disorder [1, 3]. A transplant kidney biopsy is performed for definitive diagnosis [4].

The histopathologic diagnosis of Tx-TMA relies on the subjective interpretation of a multitude of histopathologic findings of which thrombi is the major one, but varies in extent and frequency, and depends on its acute or chronic character, and, finally, on the pathologist. There is a long list of morphologies making the diagnosis challenging and often delaying initiation of targeted therapy. The Banff TMA working group (WG) was formed in 2016 under the auspices of the Banff Foundation for Allograft Pathology, with the aim of standardizing TMA diagnostic criteria and coming up with recommendations [5]. A survey circulated in January 2016 among the WG participants, showed considerable



GRAPHICAL ABSTRACT |

heterogeneity among nephropathologists, using a multitude of known TMA features (as mentioned above) with vague or subjective definitions. Therefore, the first aim of the WG was to provide the Banff community with a standardized set of minimum diagnostic criteria for Tx-TMA. A secondary ambition which was identified during the study was to investigate specific lesions that could potentially determine specific etiologies of Tx-TMA. Diagnosis of TMA in the renal allograft, is not merely a morphologic exercise; clinical and laboratory information is crucial for diagnosis. The Delphi approach was considered by the co-chairs as a suitable method to generate consensus, among an expert panel [6–9].

MATERIALS AND METHODS

A detailed description of the materials and methods used in this project including establishing a steering committee, literature review, definition of a panelist, the role of the facilitator, and the process and sequences of events during Delphi rounds is presented in paper 1 [10]. Herewith in paper 2, the authors describe those specific aspects of the materials and methods that are related to pathology.

In the preliminary round, R0, the facilitator asked several questions related to the diagnosis of Tx-TMA and requested the panelists to send their areas of difficulty with Tx-TMA diagnosis in free text. The questions are listed in **Supplementary Table S1**.

Cut-Offs

At the end of each R and after receipt of panelists' responses and data analysis, the cut-off for that R was chosen by the facilitator. It is important to emphasize that the Delphi methodology allows the facilitator to arbitrarily set cut-offs for Rs. This is to allow the facilitator to set the cut-off at a level where redundancies can be eliminated, but the most important information could be retained for the next R. In our study, a cut-off of 80% was set for all Rs, except for R4 and R5. To make sure that no important criterion is dropped for the next R, the cut-off for these two Rs was set at 60%, as a cut-off of 80% would have eliminated well-known TMA lesions, such as presence of double contours.

Pathological Validation of the Criteria

The original Delphi method used in other disciplines or in earlier pathology manuscripts did not contain a histology-based validation round. In this study, we designed a modified version of Delphi to adapt the methodology to the needs of our study, which was a pathology project, where the results of the rounds needed to be validated using real-life cases. Therefore, at the beginning of the study, the facilitator asked the panelists to submit transplant kidney biopsy (TxBx) cases from their institutional collection. A total of 37 cases of TxBx was collected and shipped to the facilitator (MA) at the Department of Pathology. For each case, 2–3 micron-thick paraffin-embedded sections, stained with hematoxylin & eosin (H&E), periodic-acid-Schiff (PAS), Masson's trichrome (TCR) and Jones silver or periodic-acid-methenamine-silver (PAMS) stains were submitted. IF and immunohistochemistry (IHC) including C4d staining, as well as EM findings were provided in free text. Only some cases were supplemented by EM images. Slides were de-identified and scanned at ×400 using an Aperio scanner at the University of Toronto. Central review of the cases was performed by the steering committee before circulating the cases among the panelists.

The Cases

Histological evaluation was included in the Delphi process during rounds R6 and R7, where 66 criteria (56 pathological, clinical and laboratory criteria and 10 differential diagnoses) were validated against 37 real-life cases. The panelists were asked to list the criteria they used to make their diagnosis on each case. The cases validated in this study were composed of TMA cases ($n = 28$) and non-TMA cases or look-alikes ($n = 9$), displayed in **Supplementary Table S2**. The original diagnosis of the 37 validated anonymized cases along with the patients' demographics reflected a random selection of real-life situations encountered by our panelists in their practice. Each case was accompanied by a short clinical history, relevant laboratory information available at the time of biopsy. The co-chairs also received the original pathology report and diagnosis, and information regarding treatment and outcome, which were not shared with the panelists.

Percentage Agreement (%A) and Percentage Agreement Levels (%AL)

%A shows agreement amongst the panelists concerning a diagnosis or criterion. Moreover, we computed the level of agreement as the number of cases falling into a %AL. For example, a 97–100%AL was the level on which 97%–100% of the panelists agreed on the same diagnosis on X number of cases. Further, a %AL was considered: 0–40 = poor; 41–60 = fair; 61–80 = good; 81–96 = excellent and 97–100 = total.

Statistics

All statistical modeling were performed using SAS, version 9.4 (SAS, Inc., Cary, NC). Details on the statistics are published in paper 1 [10]. Some figures were drawn using the open source data visualization tool RAWGraphs [11].

Of note, this study used a retrospective collection of cases to validate criteria resulting from the consensus and was not designed to measure outcome, therapy, or intervention.

RESULTS

Pathological Criteria

Table 1 lists the six pathological categories and their related criteria. A total of 18 pathological criteria (16 positive or 2 negative) were obtained at the end of R7.

The following lists the pathological criteria:

- *11 LM+ criteria* including presence of bloodless, dilated, congested glomerular capillaries; fibrin thrombi in arterioles/small arteries ± fibrinoid change; fibrin thrombi

TABLE 1 | Pathological criteria classified in 6 categories and panelists' percentage of agreement (%A) for each criterion.

Category 1	LM + criteria	%A
1	1A. bloodless, dilated, congested glomerular capillaries	54
2	1B. fibrin thrombi in arterioles/small arteries ± fibrinoid change	100
3	1C. fibrin thrombi in glomerular capillaries/hilum	100
4	1D. arterial or arteriolar intimal edema/mucoid changes	95
5	1E. glomerular endothelial swelling (acute lesion)	73
6	1F. mesangiolysis (acute lesion)	82
7	1G. double contours (chronic lesion)	59
8	1H. platelet thrombi in glomerular capillaries (CD61)	50
9	1I. fragmented/extravasated RBCs	50
10	1J. onion skin changes (chronic lesion)	41
11	1K. collapsed capillaries	18
Category 2	**LM – criteria**	
0	There is no LM finding that can help ruling out TMA	73
Category 3	**IF + criteria**	
1	3A. glomerular intraluminal staining with fibrin-related antigens	91
Category 4	**IF – criteria**	
1	4A. C4d positivity in peritubular capillaries (favoring AMR vs. TMA)	82
2	4B. presence of immune complexes	77
Category 5	**EM + criteria**	
1	5A. sub-endothelial widening/rarefaction + accumulation of "fluff"	91
2	5B. fibrin tactoids in the lumen/widened sub-endothelial space (glomerular or vascular)	91
3	5C. glomerular endothelial swelling, loss of/decreased fenestration (acute lesion)	86
4	5D. GBM duplication/lamination/multilayering with mesangial (or mesangial cell) interposition (chronic lesion)	86
Category 6	**EM – criteria**	
0	There is no EM lesion that can help you rule out TMA	82

in glomerular capillaries/hilum; arterial or arteriolar intimal edema/mucoid changes; glomerular endothelial swelling (acute lesion); mesangiolysis (acute lesion); double contours (chronic lesion); platelet thrombi in glomerular capillaries; fragmented/extravasated red blood cells (RBCs); onion skin changes (chronic lesion); collapsed capillaries.

- *1 IF+ criterion* including presence of glomerular intraluminal staining with fibrin-related antigens.
- *2 IF- criteria* including C4d-positivity in peritubular capillaries (favoring AMR vs. TMA), and presence of immune complexes.
- *4 EM+ criteria* including sub-endothelial widening/ rarefaction + accumulation of "fluff"; fibrin tactoids in the lumen/widened sub-endothelial space (glomerular or vascular); glomerular endothelial swelling, loss of/decreased fenestration (acute lesion); GBM duplication/lamination/ multilayering with mesangial (or mesangial cell) interposition (chronic lesion).

During this process, the panelists put an emphasis on the temporal character of the lesions, for instance, intracapillary thrombi reflecting acute and/or sub-acute Tx-TMA, while double contours, representing chronic Tx-TMA. Of note, acute, sub-acute and chronic TMA were considered as phenomena that can be present simultaneously.

Clinical Criteria

The 2 Clin+ criteria shown in **Table 2** included pregnancy/post-partum/history of pre-eclampsia/eclampsia HELLP syndrome and past history of TMA/HUS/aHUS/TTP.

Laboratory Criteria

Table 2 also shows the results on the laboratory criteria.

The 4 Lab+ criteria included elevated LDH, low haptoglobin levels (in the absence of history of recent transfusion), dropping hematocrit/anemia/hemolytic anemia and thrombocytopenia. Two Lab-criteria were dropped because of insufficient votes (<20%): absence of donor ABO-incompatibility and absence of proteinuria.

Differential Diagnoses

Table 3 presents the eight differential diagnoses most used during the validation of the 37 cases. They were entertained during the two validation Rs and included thrombotic thrombocytopenic purpura (TTP)/acquired HUS/atypical HUS (aHUS); donor-related TMA: observed in the donor in the first week/first month post Tx; chronic Tx glomerulopathy; disseminated intravascular coagulation (DIC); acute or chronic non-TMA-related ABMR (NT-ABMR); anti-phospholipid syndrome; immune complex-mediated glomerulonephritis (GN) including *de novo* or recurrent membranoproliferative GN, IgA nephropathy (IgAN), lupus nephritis (LN), post-infectious GN and accelerated hypertension.

Definitions

At the end R8, the need to generate consensus regarding morphological definition of key lesions was recognized. In R9, eight criteria were defined. **Table 4** lists the definition of 4 LM and 4 EM criteria on which consensus was obtained among the panelists.

Criteria Evolution During Nine Rounds

Figure 1 shows criteria evolution from R1 to R9. A detailed explanation of the evolution of the criteria is reported in the result and discussion sections of paper 1 [10].

Basically, starting with 338 criteria obtained at the end of R1, the facilitator was able to narrow them down to a final number of 24 criteria and 8 differential diagnoses at the end of the study.

Quality of the Panelists' Agreement

The panelists' diagnostic performance on the 37 cases computed at 61–80%AL, 81–96%AL and 97–100%AL is shown in **Table 5**: The 61–80%AL column shows that up to 80% of the panelists agreed on 83.78% of cases (31/37) which represents a "good" level of agreement. The 81–96%AL column shows that up to 96% of panelists agreed on 54.05% of the cases (20/37) which is

TABLE 2 | Clinical and laboratory criteria and panelists' percentage of agreement (%A) for each criterion.

Category 7	Clin + criteria	%A
1	7A. pregnancy/post-partum/history of pre-eclampsia/ eclampsia/HELLP syndrome	91
2	7B. past history of TMA/HUS/aHUS/TTP	91
Category 8	**Clin - criteria**	
0	There is no clinical info that can help you ruling out TMA	77
Category 9	**Lab + criteria**	
1	9A. elevated LDH	91
2	9B. low haptoglobin levels (in the absence of history of recent transfusion)	91
3	9C. dropping hematocrit/anemia/hemolytic anemia	91
4	9D. thrombocytopenia	91
Category 10	**Lab - criteria**	
0	No criterion was retained	00
Category 11	**Gen criteria were not assessed**	
0	none	00

TABLE 3 | Differential diagnoses.

Category 12	#D	%A
1	12A. TTP/Acquired HUS/aHUS	82
2	12B. donor-related TMA: observed in the donor in the first week/first month post Tx	86
3	12C. chronic TX glomerulopathy	82
4	12D. DIC	73
5	12E. acute or chronic NT-ABMR	77
6	12F. anti-phospholipid syndrome	59
7	12G. immune complex-mediated GN (*de novo* or recurrent, MPGN, IgAN, LN, post-infectious GN)	45
8	12H. accelerated hypertension	41

considered an "excellent" level of agreement on more than the half of the cases. Total agreement or 97–100%AL between the panelists was obtained on 10.81% of cases (4/37). In each column, those cases marked with (-) did not reach the %AL indicated for that column. It is worth noting that regarding choosing between a diagnosis of Tx-TMA vs. no TMA, on six cases (16.21%), the panelists' opinions were split (12 vs. 11). Agreement on these six cases was therefore judged as "equivocal". A more detailed information about the cases and their respective %AL is provided in **Table 5**.

R8 was originally planned to produce major and minor criteria according to the panelists' ranking; however, after examination of the results, the facilitator decided that future validation studies are needed to develop the concept of major/minor criteria.

Literature Review

An exhaustive literature search and review (12–27) regarding the incidence of the selected lesions of Tx-TMA obtained at the end of R8 revealed that there is a lack of systematic reporting on the incidence of 12 pathological lesions/criteria obtained in the current study. **Supplementary Table S3** summarizes the result of the literature review [12–26].

DISCUSSION

TMA in the Native and the Transplanted Kidney: Similarities and Differences

TMA in the native kidney shares many morphological features with TMA in the transplanted kidney. They both are caused by endothelial cell injury, and presence of intravascular thrombi, and especially when the lesions are diffuse, they are strong diagnostic tools for the pathologist. However, similarities between the two conditions stop at the morphological level as a transplanted organ is involved with and targeted by many factors that a native organ is not. TMA in the native kidney: 1. is typically part of a larger

TABLE 4 | Definitions for selected light and electron microscopy lesions.

Light microscopy	
1A. bloodless, dilated, congested glomerulus	Ischemic wrinkling (="deflation", = "ghost glomerulus", = "implosion") of capillary loops mostly devoid of RBCs, ± enlarged endothelial cells, ± luminal occlusion, ± thickened GBM appearing less dense on Jones silver stain (=sub-endothelial accumulation by EM)
1D. arterial or arteriolar intimal edema/mucoid change	Arterial or arteriolar intimal expansion or widening with edema and accumulation of basophilic material (mucoid/mucinous/myxoid change) ± luminal narrowing
1F. mesangiolysis (acute lesion)	Poorly stained (="dissolution") widened mesangium, ± dilated capillary loops or microaneurysms, ± loss or degenerative changes of mesangial cells
1I. Fragmented, extravasated RBC	Arterial or arteriolar intramural fragmented RBCs
Electron microscopy	
5A. sub-endothelial widening/rarefaction + accumulation of "fluff"	Sub-endothelial loose and finely granular, flocculent electron lucent material ("fluff") ± fibrin* tactoids in glomeruli, arteries or arterioles. (* = fibrin in the lumen can be caused by other diseases)
5B. fibrin tactoids in the lumen/widened sub-endothelial space (glomerular or vascular)	Spiculated, needle- or spindle-shaped tactoid material, at times striated
5C. glomerular endothelial swelling, loss of/decreased fenestration (acute lesion)	EC loss of fenestration with cytoplasmic vacuolization
5D. GBM duplication/lamination/multilayering with mesangial (or mesangial cell) interposition (chronic lesion)	Mesangial cell or matrix interposition indicating chronic lesion*. (*: presence of fluff indicates acute lesion)

TABLE 5 | Original diagnoses on the 37 cases, panelists' responses, percentage agreement (%A) and percentage agreement levels (%AL).

Case #	Original diagnoses	Panelist responses		(%A)		(%AL)		
		TMA	No TMA	TMA	No TMA	61–80 %AL in 31/37 cases (83.8%)	81–96 %AL in 20/37 cases (54.1%)	97–100 %AL in 4/37 cases (10.8%)
1	TMA (diffuse)	19	4	83	17	X	X	—
2	TMA (focal) + AMR	22	1	96	4	X	X	—
3	TMA (acute & chronic)	23	0	100	0	X	X	X
4	TMA (classical case)	22	1	96	4	X	X	—
5	TMA (classical case)	22	1	96	4	X	X	—
6	TMA (early)	11	12	48	52	—	—	—
7	TMA found on EM only	8	15	35	65	X	—	—
8	TMA found on EM only	4	19	17	83	X	X	—
9	TMA (classical case)	22	1	96	4	X	X	—
10	AMR + TMA	12	11	52	48	—	—	—
11	TMA (classical case)	19	4	83	17	X	X	—
12	No TMA (suspicious for AMR)	7	16	30	70	X	—	—
13	No TMA (TCMR +C4d-neg AMR)	5	18	22	78	X	—	—
14	Subtle TMA + CNI tox	14	9	61	39	—	—	—
15	TMA (classical case)	20	3	87	13	X	X	—
16	TMA (classical case)	17	6	74	26	X	—	—
17	TMA with rare thrombi	19	4	83	17	X	X	—
18	TMA with small thrombi	5	18	22	78	X	—	—
19	No TMA (GN with deposits)	4	19	17	83	X	X	—
20	TMA (acute and chronic)	22	1	96	4	X	X	—
21	TMA (acute and chronic)	21	2	91	9	X	X	—
22	TMA + Nephrosclerosis	18	5	78	22	X	—	—
23	No TMA (chronic AMR + TG + weak C4d+)	10	13	43	57	—	—	—
24	No TMA (chronic AMR + TG + weak C4d+)	6	17	26	74	X	—	—
25	TMA (classical case)	22	1	96	4	X	X	—
26	TMA (classical case)	21	2	91	9	X	—	—
27	TMA + Hypertensive arteriopathy	21	2	91	9	X	—	—
28	TMA (classical case)	23	0	100	0	X	X	X
29	TCMR	5	18	22	79	X	—	—
30	TMA (focal) + AMR	12	11	52	48	—	—	—
31	TMA (classical case)	21	2	91	9	X	X	—
32	No TMA	12	11	52	48	—	—	—
33	TMA (classical case)	23	0	100	0	X	X	X
34	No TMA (rec. MPGN)	14	19	42	58	X	—	—
35	No TMA (rec. IgA nephropathy)	2	21	9	91	X	X	—
36	TMA (classical case)	23	0	100	0	X	X	X
37	TMA + AMR	21	2	91	9	X	X	—

picture and one of the manifestations of a systemic disease such as Hemolytic Uremic Syndrome (HUS); 2. is associated with laboratory indicators of microvascular thrombosis, such as thrombocytopenia, elevated LDH and decreased haptoglobin; 3. is usually the only main finding in the biopsy; 4. is often the manifestation of a single disease, for example, systemic sclerosis or systemic lupus erythematosus. On the other hand, Tx-TMA often: 1. presents as localized TMA (L-TMA or renal TMA), and not as part of a systemic disease. While recurrent disease is the cause of a small proportion of Tx-TMAs, most transplant L-TMAs are *de novo* [27]; 2. lacks the laboratory indicators of microvascular thrombosis such as thrombocytopenia, presence of schistocytes, elevated LDH; 3. is difficult to diagnose as there are many confounding factors, such as antibody-mediated rejection (C4d-positive or C4d-negative), T cell-mediated rejection, drug toxicity, and recurrence of the pre-existing disease that blurs the picture for both clinical and pathological diagnosis. Therefore, while endothelial injury is central to the pathogenesis in both renal native and allograft TMA leading to similar lesions in the glomerulus and renal vasculature, diagnosis of Tx-TMA involves a different mindset, algorithm, and differential diagnosis, and sometimes, different criteria.

Literature Review

Up-to-date and to the authors' knowledge, there is no study dealing with the standardization of diagnostic criteria for Tx-TMA (**Supplementary Table S3**). The paper published by Haas et al [28], addresses the diagnostic criteria for TMA, however, only touches TMA in the native kidney and TMA in the renal

allograft is not approached. Most scientific literature does not provide a detailed description of Tx-TMA-associated lesions, including the pathological criteria for which our study reached a consensus. Thus, our study fills this gap and provides, for the first time, diagnostic criteria as prerequisite for further comparative studies.

The TMA BWG Mandates: The Why and the What

As the results of the 2016 Banff TMA WG clearly showed, nephropathologists use many different criteria/lesions to diagnose Tx-TMA. The TMA BWG was formed with specific objectives and goals to standardize the existing biopsy lesions, retrospectively [29]. The goals of the TMA BWG, according to the Banff 2017 meeting report were to: "1- establish uniform diagnostic criteria for Tx-TMA; 2- determine the frequency with which TMA occurs in renal allograft biopsy; and 3- determine if there are specific features of TMA in renal allografts that help resolve the differential diagnosis of Tx-TMA when the cause is not readily apparent from clinical history, DSA/C4d, etc. . ."

The authors achieved the first goal in 5 years and generated consensus among Banff participants regarding establishing a list of diagnostic criteria. The second goal was accomplished by reviewing the current literature: the authors unveiled the lack of data on the incidence of the Tx-TMA lesions Tx-TMA lesions identified through this Delphi study. The third goal could not be achieved entirely as further input from nephrologists will be needed to finalize the clinical and laboratory criteria. The Phase II of the study with nephrologists is currently in progress and will address the third goal.

Novelty of the Study: Introducing Delphi to the Banff Classification

Since 1991 and for the past 30 years, the Banff Classification on Allograft Pathology group used the NIH model of consensus generation as a tool to define transplant-related pathological lesions. This required resources for travelling and live meetings amongst expert pathologists, nephrologists, and transplant surgeons. The debates resulted in recommendations known as Banff criteria, which were proposed to the transplantation community, and applied for patient management, following rigorous validation studies. Although Delphi by itself is not a new methodology, it solves many of the inconveniences of the use of the NIH consensus format within the Banff community: anonymous yet democratic approach of consensus generation; first-time introduction of digital pathology to Delphi for case validation; and dramatic reduction of the costs of a Banff-related process. The total cost of the study was below US$20,000.00. As no travelling was required, in the era of global warming and the COVID pandemic, this methodology suggests a new approach for consensus generation to the Banff community. In the joint paper of our working group describing the Delphi process, readers will find why they should choose one method over the other [10].

It took 5 years to complete this study and come up with 24 criteria and 8 differential diagnoses. The time may seem long, however, if compared to allograft rejection introduced in Banff in 1991 which took 20 years for the Banff community, to reach consensus on final diagnostic criteria, this appears a speedy process. An example is the glomerulitis lesion (g lesion) which was introduced in Banff in 1993 [30]. Although the criteria were introduced at that time, their definition and application evolved continuously throughout the years, discussions continued for years regarding threshold for number of glomerular leukocytes, the degree of endothelial cell enlargement/capillary luminal occlusion or even the exact application of the g score [31, 32]. The consensus for these lesions took 18 years, 9 Banff conferences held in multiple locations including Banff/Canada, Aberdeen/Scotland, La Coruna/Spain, Edmonton/Canada, and Paris/France to come up with final diagnostic criteria on glomerulitis. In comparison, our Delphi study started with 338 suggestions, involved 23 panelists (all nephropathologists) and 4 nephropathologists who conducted the study. The study was completed in 5 years (despite the pandemic turmoil), with significantly smaller budget. The low cost of the Delphi method is not specific to this study and is a known advantage of Delphi.

Panelists' Performance

Panelists' performance from a statistical point of view, is briefly discussed in paper 1 [10]. In the current paper, the authors would like to put an emphasis on the impact that the complexity of TX-TMA cases have on the pathologists' performance.

Light, immunofluorescence and electron microscopy criteria listed in **Table 1** are the results of nine rounds of survey. The listed criteria do not represent any new lesions and every pathologist dealing with Tx-TMA uses some of them during his/her practice. This list is basically a guideline on the most important lesions that need to be considered when dealing with Tx-TMA. Some aspects of Tx-TMA also will need to be tested by additional studies with prediction analysis. For example, the distinction between chronic and acute lesions of Tx-TMA seems to be important, as they are manifested by different microscopic lesions. The presence of acute TMA lesions generally means the patient has an on-going treatable condition, while chronic TMA lesions generally mean the patient has potentially irreversible damages in the renal allograft. The usefulness of distinguishing chronic from acute TMA therefore could be the subject of such prediction analysis.

At this point the authors draw the reader's attention to an important point: The "subjects" in this Delphi study are neither the criteria nor the real-life cases that were validated. The "subjects" are "the panelists." Therefore, statistics usually expected from an NIH-type study such as adequacy of the sample size or number of validated cases, and reporting of p-values and ICCs related to criteria, should not be expected from this Delphi study. Only %A and %AL which reflect subjects' or panelists' performance can be reported. This is one of the main differences between Delphi and NIH-type consensus methods. Delphi evaluates performance at different agreement levels, not the criteria nor the cases. Therefore,

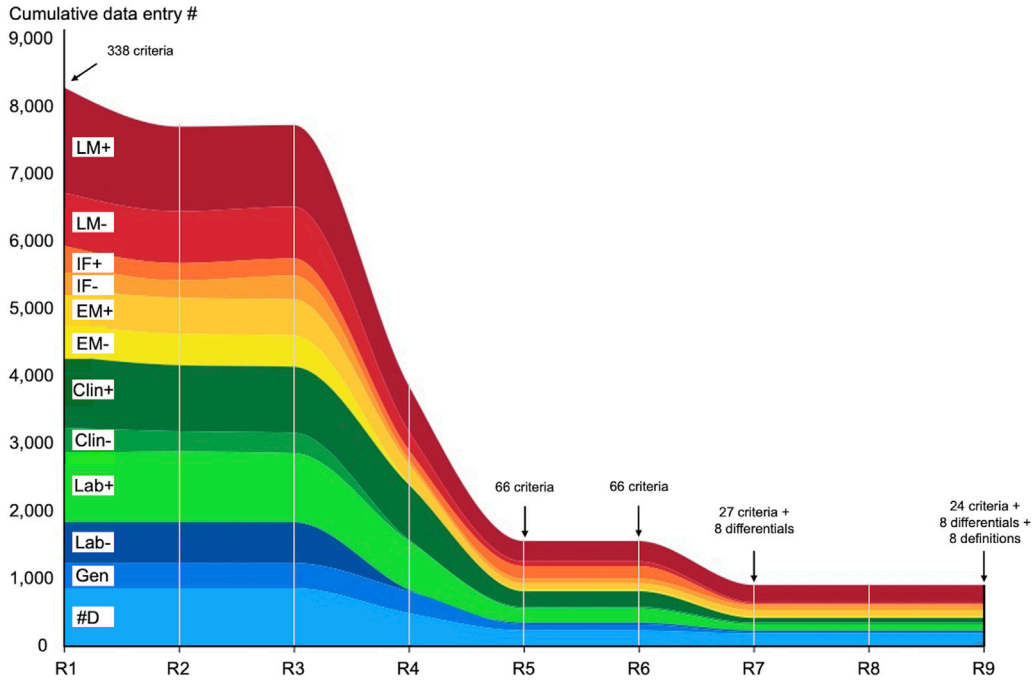


FIGURE 1 | Criteria evolution during eight rounds of Delphi. The X-axis represents each R and the Y-axis the cumulative number of data entries. R1 started with 338 criteria (arrow) which were narrowed down to 66 in R5 and remained 66 in R6. The criteria were further narrowed down to 35 (comprising eight differential diagnosis) in R7. R6 and R7 were two validation rounds and R9 was the control round also called the definition round. Abbreviations: Clin-, clinical data negative; Clin+, clinical data positive; #D, differential diagnosis; EM-, electron microscopy negative; EM+, electron microscopy positive; IF-, immunofluorescence microscopy negative; IF+, immunofluorescence microscopy positive; Lab-, laboratory negative; Lab+, laboratory positive; LM-, light microscopy negative; LM+, light microscopy positive.

the final results will not be presented with *p*-values or ICC but as total, excellent, good, fair or poor agreement levels.

Supporting Clinical and Laboratory Criteria

For the pathological diagnosis of Tx-TMA, the clinical situations such as arterial hypertension, acute renal or multi-system organ failure were deemed unnecessary, as well as laboratory items such as donor specific antibodies (DSA), positive crossmatch, low complement levels or high serum levels of CNIs, since the panelists believed none of these criteria can stand alone.

Despite the fact that clinical and laboratory information are essential for renal biopsy interpretation, consensus was reached on only a few criteria. Early on during the Delphi process, our renal transplant pathology expert panelists suggested and listed both therapeutic agents (for example, Tacrolimus or mTOR inhibitors) and complement-related disorders as items that could be considered in the final list of diagnostic criteria. However, as the list was narrowed down to reflect minimum diagnostic criteria, these items were eliminated by consensus. Additionally, the majority of the 37 cases shared by the panelists and validated, did not have any initial information about complement factors, as it happens in real-life situation and early in the course of diagnosing a case of Tx-TMA. Therefore, these items are not listed in this phase of the study. Importantly, this information is not lost, and being entertained in Phase II (as mentioned above) by the nephrologists.

This is consistent with the difficulty that nephrologists and nephropathologists have in diagnosing Tx-TMA. Even though in the pathology phase (Phase I) these criteria were agreed on, they will need to be approved by the nephrologists in Phase II. They are, therefore, not final.

Emergence of Areas of Controversy

After reviewing the panelists' responses on the 37 cases, the most common confounding factor for pathology diagnosis of Tx-TMA emerged: ABMR. It became a source of considerable intellectual conflict every time a case that had a clinical, laboratory (C4d or DSA results) or morphological hint of ABMR was encountered by the panelists. To explain the magnitude of the problem: one of the most challenging questions for our panelists was whether ABMR is in the differential diagnosis list of Tx-TMA or is causing Tx-TMA? Therefore, ABMR and its attributes were mentioned both as negative criteria when the panelists were trying to rule out Tx-TMA, and at the same time as criteria for diagnosis of Tx-TMA. The authors believe this area of conflict needs to be addressed by the Banff community, requiring further research and debate, and is out of the scope of this paper.

Strengths and Weaknesses of the Study

Comparisons between the Delphi method and other consensus generation tools, including the NIH-type method, have been discussed in detail in the literature [8]. For our study, the reasons why we chose the Delphi methodology, which we consider a strength, were multiple: its anonymous aspect, its capacity to generate consensus among many participants, on numerous items, and in a short period of time, as well as its huge advantage on cost-effectiveness. The Delphi

methodology has recently been used in surgical pathology [33, 34], however, this is the first time that the method is being used in the Banff classification group. Leading to rapid and inexpensive consensus, this process could represent a precedent in consensus generation within the Banff community. One of the advantages of Delphi is the flexibility that the facilitator has in designing the rounds. However, our study went beyond a general survey on opinions related to Tx-TMA and included histological evaluation of real-life cases within consensus generation to define diagnostic lesions. Online surveys allowed to respect our initial wish for anonymous responses.

The lack of accepted criteria that would play the role of gold standard in the diagnosis of the 37 cases not only was one of the main hurdles of this study, but also the main motivation behind initiating this work. During the two validation Rs, to circumvent this obstacle, it was decided to adhere to the original diagnosis provided by the panelist/expert who had submitted the cases.

Perhaps a further caveat of the study is the lack of correlation with treatment and outcome.

Despite the above-mentioned weaknesses, this study represents a significant step forward to tackle the pathology issues associated with Tx-TMA. A second Delphi study, with the collaboration of over 30 nephrologists, is currently ongoing.

CONCLUSION

The current work is a starting point in the process of diagnosing renal Tx-TMA. The TMA BWG looked at Tx-TMA from many different perspectives including its patterns of appearance (systemic versus localized), temporal occurrence (acute versus chronic), the difficulties pathologists face in identifying some of its lesions by LM, relationship between Tx-TMA and ABMR, and other potentially confounding conditions, and finally, the multitude of its mimickers (differential diagnoses). The authors generated consensus on 24 criteria, providing a list of differential diagnoses and identifying areas of diagnostic difficulty. While this realization undoubtedly conveys valuable recommendations for nephropathologists involved in the management of patients with Tx-TMA, its satisfactory implementation will require attentive validation and refinement, starting with consensus generation among nephrologists, who will fortify the clinical and laboratory criteria. Once Phase II and Phase III are completed, this study may serve as a baseline for diagnosing Tx-TMA, and Delphi be considered a useful methodology facilitating the process of consensus generation within the transplantation community.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

MA designed the study and acted as facilitator for Delphi. MA and HL conceived the study and selected cases for validation. HL supervised the study. NK, VB, LT, CA-C, HR, JA, AF, DB, PC, MC-vG, SdA, DE, MF, DG, EH, NM, YO, MR, VR, HS, and DT participated as panelists, suggested the initial 338 criteria, participated in Delphi consensus rounds and performed two validation rounds on 37 cases. EH, VB, LT, CA-C, JA, NK, MC-vG, SdA, and SS provided crucial contribution by submitting cases. LC performed data collection and participated in data analysis. SS contributed to the development of the initial framework. MA, NK, and HL reviewed the results of R9 and wrote the manuscript. VB, LT, CA-C, HR, JA, and AF critically reviewed, commented on, and edited the manuscript. MA and NK acquired funding for the research. 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.2023.11590/full#supplementary-material

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Delphi: A Democratic and Cost-Effective Method of Consensus Generation in Transplantation

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The Thrombotic Microangiopathy Banff Working Group (TMA-BWG) was formed in 2015 to survey current practices and develop minimum diagnostic criteria (MDC) for renal transplant TMA (Tx-TMA). To generate consensus among pathologists and nephrologists, the TMA BWG designed a 3-Phase study. Phase I of the study is presented here. Using the Delphi methodology, 23 panelists with >3 years of

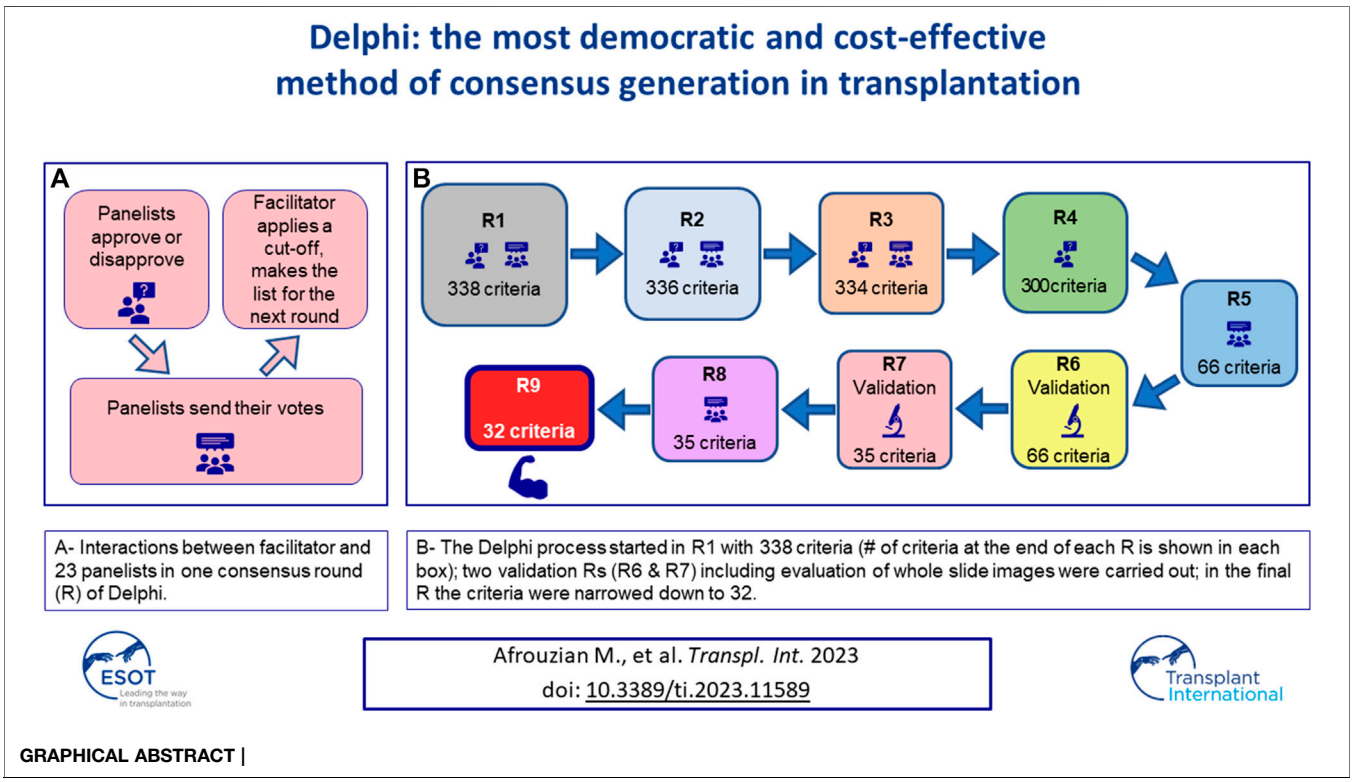
Abbreviations: ABMR, antibody-mediated rejection; (a)HUS, (atypical) hemolytic uremic syndrome; BWG, Banff Working Group; Clin, clinical data; CNI, calcineurin inhibitor; EM, electron microscopy; #D, differential diagnosis; DIC, disseminated intravascular coagulation; EC, endothelial cells; Gen, genetic criterion; GN, glomerulonephritis; HTN, hypertension; ICC, intraclass correlation; IF/IHC, immunofluorescence microscopy/immunohistochemistry; IgAN, IgA nephropathy; Lab, laboratory; LDH, lactate dehydrogenase; LM, light microscopy; MPGN, membranoproliferative GN; MDC, minimum diagnostic criteria; NT-ABMR, non-TMA-associated antibody-mediated rejection; PI GN, post-infectious glomerulonephritis; R, round; SLE, systemic lupus erythematosus; TTP, thrombotic thrombocytopenic purpura; TMA, thrombotic microangiopathy; Tx-TMA, transplant TMA; %A, percentage agreement; %AL, percentage agreement level.

diagnostic experience with Tx-TMA pathology listed their MDC suggesting light, immunofluorescence, and electron microscopy lesions, clinical and laboratory information, and differential diagnoses. Nine rounds (R) of consensus resulted in MDC validated during two Rs using online evaluation of whole slide digital images of 37 biopsies (28 TMA, 9 non-TMA). Starting with 338 criteria the process resulted in 24 criteria and 8 differential diagnoses including 18 pathologic, 2 clinical, and 4 laboratory criteria. Results show that 3/4 of the panelists agreed on the diagnosis of 3/4 of cases. The process also allowed definition refinement for 4 light and 4 electron microscopy lesions. For the first time in Banff classification, the Delphi methodology was used to generate consensus. The study shows that Delphi is a democratic and cost-effective method allowing rapid consensus generation among numerous physicians dealing with large number of criteria in transplantation.

Keywords: Delphi, Banff, thrombotic microangiopathy, kidney, transplantation

INTRODUCTION

Transplantation is a relatively young and undoubtedly challenging science. In 1991, to address the main questions of organ transplantation a group of 20 experts composed of transplant clinicians/surgeons/pathologists gathered in Banff/Canada to build the Banff classification on allograft pathology [1]. Since then and for the past 30 years, experts have met every 2 years at Banff meetings, and generated many guidelines thankfully used by the Transplantation community. The Banff Working Group (BWG) for Thrombotic Microangiopathy (TMA) was formed in 2015 under the auspices of the Banff Foundation for Allograft Pathology to standardize criteria for diagnosing and classifying renal transplant TMA (Tx-TMA) [2]. In January 2016, a survey was circulated among the BWG participants regarding Tx-TMA. The results presented at the 2017 Banff conference, revealed considerable heterogeneity among nephropathologists regarding the criteria used for Tx-TMA diagnosis [3]. Therefore, standardization of diagnostic criteria deemed necessary. To achieve this goal, three phases were designed: Phase I (consensus among nephropathologists), Phase II (consensus among nephrologists), and Phase III



GRAPHICAL ABSTRACT |

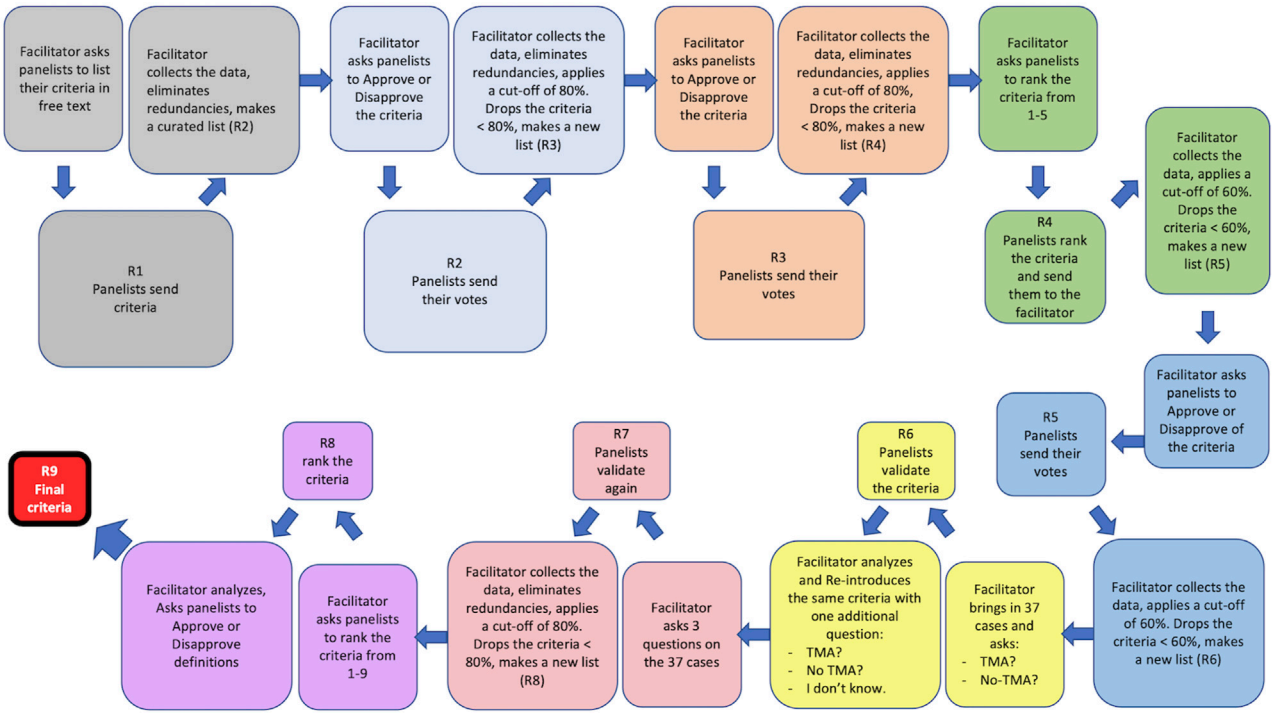


FIGURE 1 | The Delphi process applied to this study. Nine rounds of survey (R1–R9) were designed. At the beginning of each round or R, the facilitator presented the panelists with the results (criteria) obtained from the previous R and asked them to either approve/disapprove of the listed criteria or to rank them. The panelists individually responded to this call and sent their votes to the facilitator who would collect the responses, eliminate redundancies, and apply a cut-off (80% or 60%) to that R. The results of the cut-off application were then shared with the panelists. A new list composed of all criteria that were above the cut-off was made by the facilitator and presented in the next R to the panelists. R6 and R7 were two rounds during which the criteria obtained from R5 were validated against 37 real-life cases by the panelists. R9 was a control round during which the integrity of the entire Delphi process was assessed. R9 was used to fine tune the definitions of the lesions that the panelists had difficulty with, during the validation R and was therefore called the Definition R.

(consensus of the consensus groups). The Delphi method of consensus generation was chosen to be used for the first time in Banff classification. Delphi is a structured process in which a panel of experts (the panelists) reaches consensus through iterative surveys with controlled feedback from the facilitator [4–7]. The panelists remain anonymous during surveys to ensure that their interactions remain devoid of biases that are usually introduced by group dynamics [4, 8, 9]. In addition, in contrast to other techniques like the nominal group technique or the NIH's consensus conference, as the Delphi method does not require the physical presence of the participants in an actual meeting [10, 11], all interactions are designed to be online. The current work represents Phase I or the pathology phase of the study. Phase II, representing consensus among nephrologists, has already started and its results will be reported in the future. Phase I generated two interconnected papers that are being presented here. To omit redundancy, the results obtained from applying the Delphi method to transplantation, specifically to the diagnosis of Tx-TMA are reported in the current paper; in paper 2, published in the same issue [12], the pathology criteria themselves are being discussed in terms of their importance in the diagnosis of Tx-TMA in the practice of transplantation pathology.

MATERIALS AND METHODS

Figure 1 illustrates the process of Delphi applied to this study. The pathological aspects of the material and methods are presented in paper 2 [12].

Steering Committee and Panelists

A steering committee composed of two nephropathologists (MA, HL) performed literature review, identified areas of difficulty in Tx-TMA diagnosis and defined the terms "experts or panelists" by introducing inclusion and exclusion criteria, as required by Delphi [8]. Panelist was defined as a nephropathologist who had reported or published on Tx-TMA biopsies in the past 3 years (2012–2015). The steering committee members as well as the facilitator (MA) were excluded from the expert panel to avoid bias. Twenty-three nephropathologists from five continents met the above criterion and qualified as panelists.

Design of the Delphi Rounds

To develop a core set of histopathological lesions (hereafter called "criteria") a total of 10 rounds R) of survey (R0, R1 . . . R9) were launched at different points of the study, which spanned over a

total of 5 years. Detailed information about each R and statistical analysis are provided below.

R0: The panelists were asked to send in free text their questions and areas of difficulty or ambiguity in the diagnosis of Tx-TMA. The panelists' responses were shared with them at the end of R0. This survey was inserted based on the critique of the Delphi method by Keeney et al. and Diamond et al. [9, 10].

R1: The facilitator created a curated list of the criteria/opinions of the panelists and categorized them into positive (+) and negative (−) criteria. A positive criterion was defined as a criterion that, when present, would help the panelist make the diagnosis of TMA. A negative criterion was, by definition, a criterion which, when present, would help the panelist in ruling out the diagnosis of TMA. Based on the list obtained, 4 classes and 12 categories were formed: As shown in **Supplementary Figure S1**, the Pathology Class included six categories: Light microscopy positive (LM+); Light microscopy negative (LM−); Immunofluorescence microscopy positive (IF+); Immunofluorescence microscopy negative (IF-); Electron microscopy positive (EM+); and Electron microscopy negative (−). The Clinical Class comprised two categories: Clinical positive (Clin+); and Clinical negative (Clin−). The Laboratory Class included two categories: Laboratory positive (Lab+); and Laboratory negative (Lab−). Genetic criteria (Gen) were composed of tests that would help confirm the diagnosis of TMA. As some panelists had suggested a number of differential diagnoses, the facilitator also created a separate class for Differential Diagnosis Class (#D). Of note, TMA is a lesion with many mimickers. At the same time, different conditions may cause TMA. Therefore, the category of differential diagnosis included both mimickers and conditions that could cause TMA. After data collection and elimination of redundancies by the facilitator, the results were communicated to the panelists.

R2: Panelists were asked to either approve or disapprove of the results obtained from R1. Responses were collected, a cut-off of 80% called 80% agreement level (80%AL) was established by the facilitator: those criteria approved by 80% or more panelists were retained and the remaining criteria were held as potential candidates in the list that would be circulated in the next R. Results of R2 were shared with the panelists. In other words, an 80%AL would be, by definition, the level at which 80% of the participants would reach an agreement on a criterion. It is worth noting that according to the Delphi literature, the decision regarding the cut-off for each R, is totally arbitrary and can be changed from one R to another [6, 10, 13].

R3: Panelists were asked to approve or disapprove of the criteria including the differential diagnoses. A reasonable deadline was set, after which, the panelists' R3 responses were collected. At this point, the facilitator eliminated redundancies, unified those criteria/opinions that were close in terms of meaning, and included in the same line terminologies that described the same phenomenon. These actions were taken to near opinions that were similar or at least not contradictory. The cut-off for this R was chosen to be 80% therefore, criteria approved by 80% or more panelists were retained and shared with the panelists. The remaining criteria approved by less than 80% of the panelists, were still shared with the panelists for the sake of transparency, however, were not included in the list circulated in the next R.

R4: A curated list of criteria was presented to the panelists who were asked to rank the criteria. The ranking was performed on a Likert-scale from 1 to 5 with anchors on 1 (highly suggestive of TMA), 2 (moderately suggestive of TMA), 3 (mildly suggestive of TMA), 4 (rather less favorable for diagnosis of TMA) and 5 (non-specific for diagnosis of TMA). After receiving all panelists' responses, the mean rank for each criterion was calculated at one-decimal numbers. To make sure that no important criteria are dropped for the next R, the cut-off for this R was set at 60%. Criteria with mean ranks between 1 and 2.9 were considered being above the 60% cut-off and therefore were retained for the next R, while those with mean ranks between 3 and 5 were considered below the 60% cut-off and eliminated. Any criterion below the cut-off was also presented to the panelists at the end of R4 but dropped from the next R's list. Based on the application by Jones et al [14], the facilitator provided feedback to panelists regarding all positive and negative criteria and the differential diagnoses.

R5: A curated list of criteria was presented to the panelists. To further narrow down the criteria, the panelists were asked to repeat the ranking of the criteria obtained from R4, using the scale of 1–5, with 1 being the most diagnostic criteria and 5 being the least favorable criteria. Responses were collected by the facilitator. To make sure that no important criteria are dropped for the next R, the cut-off for this R was set at 60% (as in R4); Mean ranks were calculated, and results shared with the panelists.

R6: This R was the first validation R. At this point, 37 cases collected and scanned by the facilitator were shared with the panelists who were asked to label the cases as either "TMA" or "No TMA." Additionally, the panelists were asked to indicate which criterion on the list was used to make their diagnosis. For each biopsy the panelists ought to provide a mandatory comment about the case in free text, providing suggestions and criticizing the adequacy of the case or, the process. After receipt of all responses, facilitator and statistician analyzed R6 responses. **Supplementary Table S1** reflects a snapshot of R6's process. Comments not fitting in the "yes" or "no" responses were counted in a separate line called "N/A". Based on the commentaries, it became clear that the R6 clearly needed to be re-designed, as some panelists were undecided regarding the diagnosis of some cases and could not decide if those cases were TMA or not. Therefore, the facilitator did not establish any cut-off for R6 and did not share the results of R6 with the panelists. To re-design the validation R, a third choice of "equivocal" (meaning I do not know) was added by the facilitator to the other two choices of "TMA" and "No TMA" and a new validation R called R7 was launched.

R7: In R7 panelists validated the criteria against the same 37 cases. During this R the panelists were asked to label each case as either "TMA," "No TMA," or "Equivocal." Like R6, the panelists were asked to indicate which criteria on the list were used to make the final diagnosis on each case and enter their opinion in free text. After receipt of all responses, the facilitator and the statistician analyzed the responses. The cut-off for R7 was

set at 80% i.e., a new analysis calculated the 80%AL for each of the 37 cases, and on each criterion. Criteria with <80% agreement were dropped for the next R. For clarification, the authors provide an example on criterion 1A here: in R7, the number of panelists who used criterion 1A for ANY of the 37 cases was counted. If out of 23 panelists, 19 or more (≥82.65%) used criterion 1A in at least 1 case, it was considered that criterion 1A was "used by more than 80% of the panelists" and therefore should be kept in the list for the next R. Results of R7 were shared with the panelists.

R8: The panelists were challenged in this R with the criteria obtained from R7 and asked to rank the criteria from 1 to n (1 being the most favorable criterion and n being the least favorable criterion), depending on the number of criteria in each category. Mean ranks of the criteria obtained from this R were calculated and shared with the panelists. This list contained the final criteria for diagnosis of Tx-TMA. It should be emphasized that R8 was originally planned to produce major and minor criteria by taking in to account panelists' ranking. However, after examination of the results, the facilitator decided that future validation studies are needed to develop the concept of major/minor criteria.

R9: This R is usually used as a "control R" to assess the internal integrity of the process. The facilitator decided to use R9 to generate consensus on the definition of some lesions, that appeared to be morphologically problematic for some of the panelists during the previous Rs. Therefore, a consensus was needed regarding their definition. For example, the lesion "mesangiolysis", an important diagnostic tool, did not receive sufficient vote in one of the rounds and was eliminated. The facilitator had to modify the cut-off for that round to keep this lesion as a criterion on the list. Therefore, R9 was called the definition R during which panelists were asked to define some terms used for a few light and electron microscopy criteria. All panelists had to provide in text format their own definition on these selected lesions. These definitions were then curated with elimination of redundancies, assembled in sentences by the steering committee, and shared with the panelists.

Percentage Agreement (%A) and Percentage Agreement Levels (%AL)

Two terms were used to reflect the agreement between the panelists. The first term, %A, showed the agreement amongst the panelists concerning a diagnosis or criterion. The second term, %AL, reflected %A falling into a cut-off of agreement. For example, a 100%AL was the level on which 97%–100% of the panelists agreed on the same diagnosis on X number of cases. A 100%AL was therefore interpreted as 'total agreement". By the same token, a %AL was considered: poor if in the range of 0–40; fair if between 41 and 60; good if between 61 and 80; excellent if between 81 and 96 and total if between 97 and 100.

Statistical Analysis

A detailed explanation of the statistical analysis is rendered below.

In R0 and R1 no statistical analysis was performed.

In R2 and R3, we calculated the approval percentage for each criterion based on the following formula:

$$\%approval\ for\ criterion\ k = \frac{\#of\ participants\ approved\ criterion\ k}{\#of\ participants\ in\ the\ round} \times 100\%$$

In R4 and R5, we calculated the percentage of ranking based on the following formula:

$$\%ranking\ for\ criterion\ k = \frac{average\ ranking\ (1\ to\ 5)\ for\ criterion\ k}{5} \times 100\%$$

In R6 and R7, we calculated the %A for each criterion based on the following formula:

$$\%agreement\ for\ criterion\ k = \frac{\#of\ participants\ used\ criterion\ k\ in\ some\ cases}{\#of\ participants\ in\ the\ round} \times 100\%$$

To assess the relative importance of the criteria, in R8, we calculated the percentage of favorable ranking based on the following formula:

$$\%of\ favorable\ ranking\ for\ criterion\ k = \frac{\#of\ participants\ ranked\ criterion\ k\ (1\ to\ 6)}{\#of\ participants\ in\ the\ round} \times 100\%$$

All statistical modeling were performed using SAS, version 9.4 (SAS, Inc., Cary, NC). Some figures were drawn using the open source data visualization tool RAWGraphs [15].

RESULTS

Table 1 lists the original diagnoses of the 37 cases that were chosen to be validated (for panelists' response, see below). The project started with 338 items/criteria obtained at the end of R1. **Table 2** summarizes the evolution of the criteria from R1 to R9. By the end of R5, the facilitator was able to narrow down the items to 66 which included 56 criteria and 8 differential diagnoses. A list of the items entering R6 is provided in **Supplementary Table S1**. At the end of R7 the items were narrowed down to 35 including 27 criteria and 8 differential diagnoses. In R8, the number of items remained at 35. After R9, the facilitator eliminated three negative criteria that were expressed as "there is no criterion to help ruling out TMA." These were eliminated because they could not be counted as criteria. Therefore, at the end of R9 the study ended up with 32 items including 24 criteria and 8 differential diagnoses. A detailed list of criteria and discussion about each criterion is outside the scope of this manuscript and will be published in the future.

Supplementary Table S2 lists the number of the final criteria classified in each of the 12 categories which included 18 Pathological criteria (16 positive or 2 negative including 11 LM+, 1 IF+, 2 IF−, 4 EM + criteria); 2 Clinical criteria (2 Clin + criteria); 4 Laboratory criteria (including 4 Lab+ criteria). The 2 Lab- criteria were dropped because of insufficient votes (<20%). The process generated eight

TABLE 1 | Diagnosis on the original 37 cases and percentage of agreement.

Cases	Original diagnoses	Panelists' responses		% of agreement	
		TMA	No TMA	TMA	No TMA
1	TMA (diffuse)	19	4	83	17
2	TMA (focal) + ABMR	22	1	96	4
3	TMA (acute and chronic)	23	0	100	0
4	TMA (classical case)	22	1	96	4
5	TMA (classical case)	22	1	96	4
6	TMA (Early)	11	12	48	52
7	TMA found on EM only	8	15	35	65
8	TMA found on EM only	4	19	17	83
9	TMA (classical case)	22	1	96	4
10	ABMR + TMA	12	11	52	48
11	TMA (classical case)	19	4	83	17
12	No TMA (suspicious for ABMR)	7	16	30	70
13	No TMA (TCMR + C4d-neg ABMR)	5	18	22	78
14	Subtle TMA + CNI tox	14	9	61	39
15	TMA (classical case)	20	3	87	13
16	TMA (classical case)	17	6	74	26
17	TMA with rare thrombi	19	4	83	17
18	TMA with small thrombi	5	18	22	78
19	No TMA (GN with deposits)	4	19	17	83
20	TMA (acute and chronic)	22	1	96	4
21	TMA (acute and chronic)	21	2	91	9
22	TMA + Nephrosclerosis	18	5	78	22
23	No TMA (Chronic ABMR + TG + weak C4d+)	10	13	43	57
24	No TMA (Chronic ABMR + TG + weak C4d+)	6	17	26	74
25	TMA (classical case)	22	1	96	4
26	TMA (classical case)	21	2	91	9
27	TMA + Hypertensive arteriopathy	21	2	91	9
28	TMA (classical case)	23	0	100	0
29	TCMR	5	18	22	78
30	TMA (focal) + ABMR	12	11	52	48
31	TMA (classical case)	21	2	91	9
32	No TMA	12	11	52	48
33	TMA (classical case)	23	0	100	0
34	No TMA (recurrent MPGN)	14	19	42	58
35	No TMA (recurrent IgA glomerulopathy)	2	21	9	91
36	TMA (classical case)	23	0	100	0
37	TMA + ABMR	21	2	91	9

The original diagnoses of the 37 cases chosen to be validated for panelists' response is shown along with the percentage agreement.

TABLE 2 | Evolution of criteria from R1–R9.

Classes	R1	R2	R3	R4	R5	R6	R7	R8	R9
1. LM (LM+ & LM–)	90	89	87	85	16	16	12	12	11
2. IF (IF+ & IF–)	27	26	26	26	10	10	3	3	3
3. EM (EM+ & EM–)	43	43	43	32	5	5	5	5	4
4. Clin (Clin+ & Clin–)	55	55	55	52	12	12	3	3	2
5. Lab (Lab+ & Lab–)	70	70	70	70	9	9	4	4	4
6. Gen	16	16	16	14	4	4	N/A	N/A	N/A
Differential diagnosis	37	37	37	21	10	10	8	8	8
Total criteria	338	336	334	300	66	66	35	35	32

The number of criteria was narrowed down significantly during the Delphi process, starting from R1 and ending in R9. The table summarizes this evolution.

differential diagnoses entertained during the two validation Rs. Defining of eight criteria including 4 LM+ and 4 EM+ criteria emerged as a necessity at the end of R8. The panelists achieved this task during R9 which also served as a control R for the entire Delphi process.

Agreement Among Panelists

The facilitator observed the panelists' performance looking at multiple agreement levels and at different points of the study. At the end of R6, the first validation R, %AL was assessed at 50%, 60%, 70%, 80%, 90%, and 100% levels (shown in **Figure 2**). The

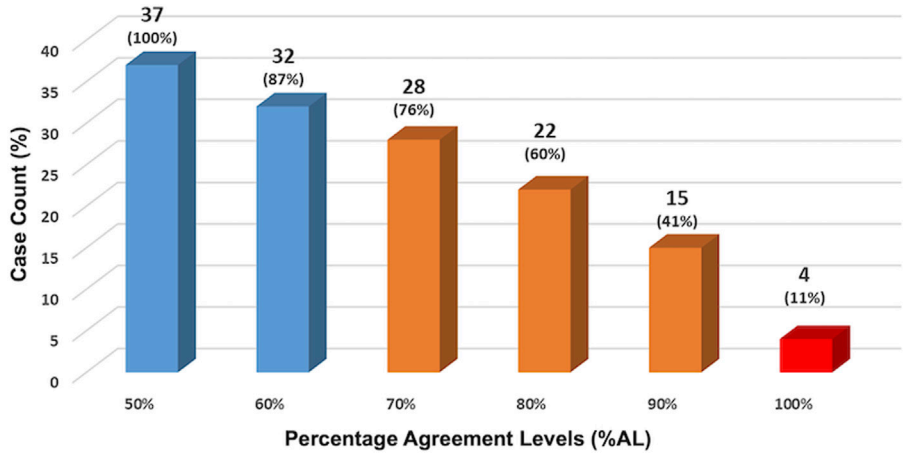


FIGURE 2 | Panelists' performance assessed at the end of R6. The facilitator observed the panelists' performance looking at multiple agreement levels. At the end of R6, the first validation R, %AL was assessed at 50%, 60%, 70%, 80%, 90% and 100% levels. The results show that at 70%AL (middle bar), consensus was reached on 28/37 (76%) of cases. This means that almost three-quarters of the panelists agreed on three-quarters of the cases.

TABLE 3 | Cumulative agreement levels among panelists.

Fair agreement 41–100%AL	Good agreement 61–100%AL	Excellent agreement 81–100%AL	Total agreement 97–100%AL
Obtained in 37/37 cases (100%)	Obtained in 31/37 cases (83.78%)	Obtained in 20/37 cases (54.05%)	Obtained in 4/37 cases (10.81%)
Case #: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37	Case #: 1, 2, 3, 4, 5, 7, 8, 9, 11, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, 24, 25, 26, 27, 28, 29, 31, 33, 34, 35, 36, 37	Case #: 1, 2, 3, 4, 5, 8, 9, 11, 15, 17, 19, 20, 21, 25, 28, 31, 33, 35, 36, 37	Case #: 3, 28, 33, 36

Different %ALs between the panelists regarding the diagnosis of the 37 validated cases: 41–100%AL, 61–100%AL, 81–100%AL and 97–100%AL.

results show that at 70%AL (middle bar), consensus was reached on 28/37 (76%) of cases. This means that almost three-quarters of the panelists agreed on three-quarters of the cases.

A deeper look at the %AL at the end of the study is shown in **Table 3** which shows the cumulative agreement levels among panelists and reveals that: 1- Total agreement (97–100%AL) was achieved in 4 cases (10.81% of cases; 2- Excellent agreement (81–100%AL) in 20/37 cases (54.05%); Good agreement (61–100%AL) in 31/37 cases (83.78%) and Fair agreement (41–100%AL) in all 37 cases (100%).

DISCUSSION

Delphi and Consensus

The term consensus is clarified in Delphi and defined as "general agreement, but not necessarily unanimity," and includes the process to resolve objections by interested parties. A process would be considered a consensus, if all comments have been fairly considered, each objector has been advised of the disposition of his or her objection(s) and the reasons why, and the consensus body members have been given an opportunity to change their votes after reviewing the comments [16]. Delphi is a structured process of consensus generation in an iterative fashion through repeated anonymous surveys with controlled feedbacks given by the facilitator [6]. In Delphi a panel of experts (the panelists) can reach consensus through multiple online interactions, that would prevent introduction of bias from group dynamics. In contrast to other techniques like the nominal group technique or the NIH's consensus conference, the Delphi method does not require the physical presence of the participants in an actual meeting [11].

Comparing the NIH Type of Consensus Generation With Delphi

To compare the NIH type of consensus with the Delphi method, and why the Delphi method is preferred in some situations, a point-by-point description of both methods is presented below.

The NIH Type of Consensus Generation

The reader of the current paper is most probably familiar with the rules of the usual NIH type consensus generation. In this type of consensus: 1. Opinions/questions/criteria are usually pre-designed by a steering committee composed of the most experienced members of the group at the beginning of the process; 2. The literature has already covered some information about the incidence and/or definitions of the criteria/lesions and all panelists are on the same page; 3. Communications are in person or through online video-conferencing, therefore, the identity and opinions of the panelists, the most experienced, the less famous, the loudest and the silent, the most and least popular members of the group are known by all participants, introducing "human interaction bias" into the process. Therefore, "discussions" in this consensus model are performed by directly addressing one or multiple panelists and accepting or not an argument, *in situ* and within the group; 4. The criteria or the pathology cases brought to the consensus are the "subjects" of the study. This means that in the NIH model, the study is expected to validate the criteria with a significant number of cases, report *p*-values and inter-correlation coefficients (ICC), which evaluate criteria performance when put to test.

The Delphi Method of Consensus Generation

Delphi, however, has a fundamentally different approach to the panelists and the criteria. In Delphi: 1. Questions or criteria are not set in advance by the steering committee and the entire group of panelists set the tone by expressing their own opinions/questions/criteria at the beginning of Delphi; 2. Definitions of the criteria/lesions are not known at the start of the process as no one knows which lesions are going to reach the finish line. For example, this study started with 338 criteria and lesions suggested by the panelists. It is obvious that the steering committee could not possibly define all the 338 criteria at the beginning of the study, as this would introduce an external bias. Hence, such interventions from the steering committee or the facilitator are strictly prohibited during Delphi, allowing a democratic process devoid of any peer pressure, interference, and bullying. All 338 criteria had to enter R1, and those reaching the finish line by R8 were the result of a vigorous election process; 3. In Delphi, the "subjects" are the panelists, not the criteria nor the validated cases, therefore, *p*-values and ICCs are not expected to be generated; 4. All opinions are expressed anonymously, not only to eliminate peer pressure but also to allow a different type of "discussion." To expand on this notion, it suffices to mention that in Delphi, the cognitive exercise starts with the first Rs when each panelist faces the list of criteria voted by other panelists, permitting self-reflection on personal knowledge, opinion, and experience. Later, in-mid process, after multiple Rs of voting and elimination of the criteria that have not received enough vote, a cognitive connection is automatically established between this panelist and the rest of the group creating a collective mind ready to validate the final list.

Results show that 3/4 of the panelists agreed on the diagnosis of 3/4 of cases. Comparing these results to the results of similar studies that used the NIH-type of consensus, one can draw the following conclusion regarding the quality of consensus: our results are comparable to other studies even though different methodologies and statistical analyses were used. For example, Liapis et al. reported that consensus was reached among about 75% of the pathologists who agreed on 75% of cases when scoring the number of glomeruli present in implantation (donor) biopsies. The consensus was below 75% when scoring was performed on glomerulosclerosis and other parameters such as number of arteries, and tubular atrophy (Data obtained from table 2, ICC results, Liapis H et al, AJT 2017) [17]. Although the quality of consensus appears to be similar in both studies, it is worth mentioning that the present study generated consensus on criteria and on diagnoses, not just on a single factor such as number of glomeruli. Therefore, a much complex consensus process was applied to our Delphi-based study.

Performance of the Panelists

When analyzing panelists' performance, the results are encouraging: a "good" level of agreement, was obtained on 31 cases, and consensus was reached among 70% of panelists on 28/37 (76%) of cases, basically implying that about three-quarters of the panelists agreed on three-quarters of the cases. This result shows that the Delphi process was able to generate an acceptable level of consensus among our panelists.

Novelty

The use of Delphi as a consensus building method started in the last decade of the 20th century and, therefore, has been used by some disciplines for years. However, its introduction to the world of pathology is recent [18, 19], moreover, it has never been used in Banff classification. Furthermore, the novelty of our study is in the integration of a classical histopathology workup into the Delphi process, including interpretation of digital whole slide images accompanied by clinical history and laboratory data. This approach which is a modification of the usual Delphi method can be used in medicine, especially in transplantation pathology, where criteria generated during multiple consensus rounds could be validated against real-life cases. This modified Delphi method is, therefore, adapted to the needs of the pathology consensus process.

As in other methods, Delphi is partly an exercise to educate a group of participants to think and re-think about their definitions/cutoffs, adapting alternative terminologies in the process (in this case histopathologic criteria) and running the risk of less than 100% agreement. The latter, however, is not unexpected in an observational discipline, like histopathology, thus agreement cutoffs have to be introduced and are generally valid. Finally, the world has changed since the initiation of consensus building on allograft pathology and the creation of the Banff classification. Pandemic-related travel and contact restrictions, financial constraints, and global warming concerns—also related to academic air travel—all advocate for a revision of old practices. In this perspective, the Delphi methodology represents a great solution for consensus building in general and the Banff Classification operating through consensus in particular.

In conclusion, the Delphi methodology is a method of consensus generation that has not been used in Transplantation. For the first time in Banff classification, and in the Phase I of the study, Delphi was used by the TMA-BWG to generate consensus on MDC for TMA in renal allograft biopsies.

We adapted the Delphi methodology to the needs of consensus building in pathology by using digital imaging during validation Rs. Delphi proved to be a highly efficient method of consensus generation among pathologists. The novelty of the study is in its anonymous yet democratic approach, online implementation, low cost, and ability to reach many participants from around the globe.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

MA designed the study and acted as facilitator for Delphi. MA and HL conceived the study and selected cases for validation. HL supervised the study. NK, VB, LT, CA-C, HR, JA, AF, DB, PC, MC-vG, SdA, DE, MF, DG, EH, NM, YO, MR, VR, HS, and DT participated as panelists, suggested the initial 338 criteria, participated in Delphi consensus rounds and performed two validation rounds on 37 cases. EH, VB, LT, CA-C, JA, NK, MC-vG, SdA, and SS provided crucial contribution by submitting cases. LC performed data collection and participated in data analysis. SS contributed to the development of the initial framework. MA, NK, and HL reviewed the results of R9 and wrote the manuscript. VB, LT, CA-C, HR, JA, and AF critically reviewed, commented on, and edited the manuscript. MA and NK acquired funding for the research. 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.2023.11589/full#supplementary-material

Supplementary Figure S1 | Classification of the initial suggested criteria. The panellists were initially asked to suggest their criteria for diagnosis of Tx-TMA. After 338 opinions/criteria were collected, the facilitator classified them into four classes including Pathologic, Laboratory and clinical criteria and differential diagnoses. Each of the classes were further divided into positive and negative categories, for example the pathologic criteria were divided into LM+, LM–, IF+, IF–, EM+ and EM– categories.

Supplementary Table S1 | A snapshot of R6's process. After receipt of all responses, facilitator and statistician analyzed R6 responses. This is a snapshot from the excel sheet that showed the result of the analysis on a few cases (labelled as KB1, KB2, . . .etc.). In the left column, the validation questions are shown including question 1 (Is this a case of TMA or No TMA?), and question 2 (Please mark the criteria—listed below—which helped you in your final diagnosis).

Supplementary Table S2 | Number of final criteria obtained at the end of the study. The final criteria were classified in to 12 categories which included 18 Pathological criteria (16 positive or 2 negative including 11 LM+, 1 IF+, 2 IF–, 4 EM+ criteria); 2 Clinical criteria (2 Clin+ criteria); 4 Laboratory criteria (including 4 Lab+ criteria). The 2 Lab– criteria were dropped because of insufficient votes (<20%). The genetic category remained without any criteria due to lack of sufficient information. The process generated eight differential and the definition of eight criteria including 4 LM+ and 4 EM+ criteria was refined through consensus in R9.

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BRIEF RESEARCH REPORT
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Immunoadsorption-Based HLA Desensitization in Patients Awaiting Deceased Donor Kidney Transplantation: An Interventional, Non-Randomised, Single Cohort Study

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Whether immunoadsorption (IADS) as part of desensitization protocols could facilitate deceased donor kidney transplantation (KT) in highly sensitized (HS) patients remains to be proven. We retrospectively analyzed our IADS based desensitization protocol for deceased donor KTs between 2013 and 2018. Fifteen HS patients (age 52 years [40–56]) were included. Waiting time before IADS was 6 years [5–10] and the interval between IADS initiation and KT was 5 months [1–12] for the 14 transplanted patients. Nine patients had prior KT. Calculated panel reactive antibody decreased significantly during the protocol (99.3% [92.5–99.9] vs. 79.4% [56.7–81.9]; $p = 0.004$). Death-censored graft survival was 85.7% at 1 and 2 years post-transplantation. One-year median plasma creatinine level was 135 µmol/L [111–202]. Six developed active antibody mediated rejection (ABMR) at 1 year, with a median delay of 13 days [11–26]. Eight patients developed severe infections, including two fatal outcomes. Finally, compared to 93% of patients who received desensitization receiving a KT, only 43% of a control with similar characteristics underwent transplantation. However, no difference was found in overall probability of being alive with a functioning graft at the end of follow-up. The results indicate that our IADS-based desensitization strategy was not effective due to a high rate of ABMR and severe infectious complications which pose a challenge to its universalization.

Keywords: kidney transplant, graft survival, HLA desensitization, apheresis, immunoadsorption

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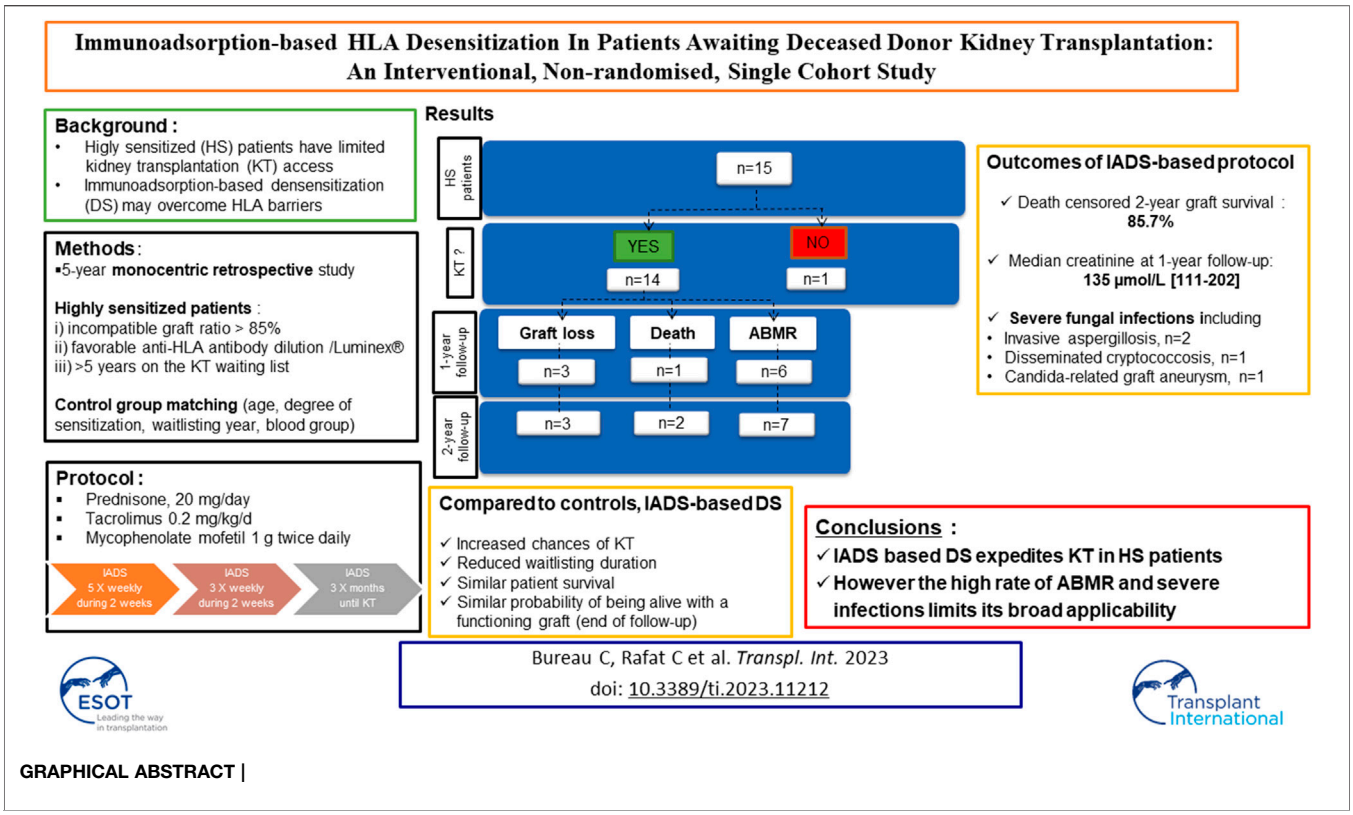
INTRODUCTION

Kidney transplantation (KT) is universally acknowledged to be the treatment of choice for patients with end stage kidney disease (ESKD) in terms of survival and quality of life. In France, as elsewhere, the population affected by chronic kidney disease has grown steadily over the past decades totaling 51,000 patients with ESKD on dialysis and 9,675 on KT waiting lists in December 2021, whilst deceased donor organ procurement has plateaued, resulting in a shortage of organs [1].



GRAPHICAL ABSTRACT |

Sensitization against HLA epitopes through blood transfusion, pregnancy and prior organ transplantation also hampers KT access resulting in protracted waiting time and increased mortality. Within the Eurogroup Transplantation Consortium it is thus estimated that 5% of patients are deemed highly sensitized (HS), as determined by an HLA antibody profile that reacts to ≥85%–100% of donors in the donor population [2]. The advent of novel assays providing enhanced HLA antibody detection—first and foremost being highly sensitive bead-based Luminex® single antigen assays—has further increased the proportion of patients categorized as HS. In addition, the most recent studies have stressed the preeminence of donor specific antibodies (DSAs) as a predictor of post-KT active antibody mediated rejection (ABMR) and graft survival. They have prompted new risk stratification strategies and, in turn, novel therapeutic procedures designed to circumvent the negative outcomes occasioned by alloimmunization [3].

Desensitization protocols have emerged as one approach to overcome the HLA barrier and allow for KT in HS ESKD patients [4, 5]. Various strategies have been utilized but most protocols are built around pharmacological immunosuppression combined with apheresis. They also share a common goal, which is to deplete B cell populations and to reduce DSA to levels amenable to KT with a negative complement-dependent cytotoxic (CDC) crossmatch. Immunoadsorption (IADS), using Immunosorba® columns (Globaffin®, Fresenius), has established itself as one of the preferred techniques among different apheresis options [6]. Compared to plasmapheresis, it provides semi-specific plasma treatment, superior immunoglobulin clearance, and obviates the need for albumin or plasma substitution [7]. IADS-based desensitization has shown good results for living donor KT [8]. There are few data in the setting of deceased donor KT where such an approach implies strict compliance with repeated sessions of apheresis and sustained immunosuppression pending allocation of an acceptable KT [9–11]. This study comprises a single center (Tenon Hospital, Paris, France) report of the outcomes associated with 15 consecutive HS patients who were on a kidney transplant waiting list and included in an IADS-based desensitization protocol.

METHODS

Patient Population Selection and Definitions

We retrospectively analyzed all patients between January 2013 and September 2018, who underwent IADS-based HLA desensitization protocol for deceased donor KT. Patients deemed eligible for the procedure had to fulfill the following criteria: 1) an incompatible graft ratio >85% calculated for a given individual on the basis of his anti-HLA antibodies profile and the HLA pattern stemming from nationwide kidney procurement performed over the last 5 years, 2) a favorable anti-HLA antibody dilution test performed using the Luminex® technique to mitigate a prozone effect and to predict adequate depletion through IADS, 3) more than 5 years on the KT waiting list, 4) protocol acceptance. Concerning antibody dilution test, before single antigen flow bead testing, a 0.1-M solution of disodium EDTA (Sigma-Aldrich®, St Louis, United States) at pH = 7.4 was

diluted 1:10 in the sera and incubated for 10 min to avoid prozone effect. The French kidney allocation system offers organs at a national level. A national priority is given to highly sensitized patients based on their immunological profile ("incompatible graft ratio" >85%). During IADS protocol as the sensitization decrease that national priority can be removed. The French allocation system also allows for a locally retrieved kidney to be offered locally depending on match ability (ABO blood group and HLA compatibility measured by CDC crossmatch).

For each patient receiving the desensitization protocol, two control patients were selected from the same KT center. Controls were matched for age, degree of sensitization, had been waitlisted for a KT in the same year as the study patient, were of the same ABO blood group, and needed to have not died or be transplanted before the study patient had started the IADS protocol. Follow-up data for controls included KT status and date of KT and/or date of death.

Kidney biopsies were scored according to the 2017 Banff classification.

Desensitization Protocol

The description of the desensitization protocol is presented in the **Supplementary Methods S1**.

Immunoadsorption Therapy and Immunosuppressive Therapy for KT

The descriptions of the immunoadsorption therapy and the induction and maintenance immunosuppressive therapy for KT are presented respectively in the **Supplementary Methods S2, S3**.

Anti HLA Antibodies and CDC Crossmatch Assessment

The description of the anti HLA antibodies and CDC crossmatch assessment is presented in the **Supplementary Methods S4**.

Clinical Data

We obtained clinical data from medical records in our center and the CRISTAL database from the Agence de la Biomédecine. Each recipient from the present study gave written informed consent to be included in the CRISTAL database networks. The follow-up was terminated in August 2021.

Statistical Analysis

Continuous variables were expressed as median (interquartile range) and categorical variables as numbers (percentages). Continuous variables were compared using the nonparametric two-tailed Mann-Whitney test. Qualitative variables were compared using the Chi squared test.

Ethics Statement

The study was conducted in accordance with the ethical guidelines of the Assistance Publique—Hôpitaux de Paris. No institutional review board approval was necessary at the time of the study as it was a retrospective study involving no intervention. The study was conducted according to the ethical standards of the 2000 Declaration of Helsinki as well as the Declaration of Istanbul 2008.

RESULTS

Demographics

From 2013 to 2018, a total of 15 HS ESKD patients were included in the IADS-based desensitization protocol for deceased donor transplantation in our center. During the same period, 497 kTs were performed in the center (66 from a living donor and 431 from a deceased donor). A total of (**Table 1** and **Supplementary Table S1**) 4 men and 11 women with a median age of 52 years [40–56] were cleared for the protocol. Their median body mass index was 26 [21–29] and 12 out of 15 had African-Caribbean origins. Nine patients presented with hypertension and one with diabetes mellitus. All patients had a history of more than 3 blood transfusions. Women (n = 11) presented with a median 3 [2–5] previous pregnancies. The median duration of renal-replacement therapy was 11 years [8–14] and the median time on the waiting list was 6 years [5–10]. Nine patients had received either 1 (n = 6) or 2 (n = 3) previous KTs. Upon starting the desensitization program, patients exhibited a calculated panel reactive antibody (cPRA) of 99.3% [92.5–99.9] and an anti-class I PRA-CDC of 30% [18–41]. Kidney diseases are detailed in **Supplementary Table S1**. All were seronegative for HIV and HCV but six patients had a past HBV infection (positivity for anti-HBc and anti-Hbs antibodies) and two had a chronic HBV infection. In total, 12 out of 15 patients had received previous immunosuppressive therapy (for initial kidney disease or for previous KT). For all but one patient the dilution test performed on their serum showed a significant decrease in anti-HLA antibody titers.

Impact of Desensitization Protocol

After initiation of the IADS-based desensitization protocol, KT was performed after a median of 5 months [1–12] in 14 out of 15 patients (**Figure 1**). The patients received a median of 23 IADS sessions [14–32] over a median of 110 days [35–141] before KT. All the patients received IADS using an arteriovenous fistula. cPRA fell significantly from 99.3% [92.5–99.9] before IADS to 79.4% [56.7–81.9] following completion of the final IADS session (p = 0.004). Side effects observed during desensitization were mycophenolate-induced diarrhea (n = 4), hypocalcemia (n = 3) and cytopenias (n = 1). In one case the IADS-based protocol was terminated after 19 sessions (2 months) due to diarrhea and cytopenia but the response in terms of anti-HLA antibody titer was favorable and the patient was transplanted 8 months later. The protocol was discontinued in one case after 16 sessions (1 month) due to a lack of efficacy—the cPRA remained at 100%. For all desensitized patients who were transplanted, the day 0 CDC crossmatch was negative. However, 7 out of 14 KT

TABLE 1 | Demographic and nephrological features before transplantation.

Number of patients	*n* = 15
Demographic features	
Sex, male n (%)	4/15 (27)
Age, years	52 [40–56]
Ethnicity	
Sub Saharan African, *n* (%)	9/15 (60)
North African, *n* (%)	1/15 (7)
Caucasian, *n* (%)	3/15 (20)
Caribbean, *n* (%)	2/15 (13)
Sensitization-associated characteristics	
Previous kidney transplantation, *n* (%)	9 (60)
Number of pregnancies for women, *n* (%)	3 [2–5]
Transfusions > 3, *n* (%)	15/15 (100)
cPRA, %	98 [88–99]
PRA-CDC (anti-HLA class I), %	30 [18–41]
Historical positive CDC Crossmatch	8/15 (53)
Nephrological features pre-Tx	
Initial kidney disease, n (%)	
Undetermined	3 (20)
Nephrosclerosis	3 (20)
Membranous nephropathy	1 (6.7)
Anti-GBM disease	1 (6.7)
ADPKD	1 (6.7)
FSGS	1 (6.7)
Chronic hemodialysis duration, years	11 [8–14]
Time span between waiting list registration and IA initiation, years	6 [5–10]
Donor's characteristic	
Age, years	65 [37–70]
Hypertension, *n* (%)	3/14 (21)
Diabetic, *n* (%)	2/14 (14)
Serum creatinine, μmol/L	69 [56–84]
Proteinuria, g/24 h	0 [0–0.16]

Abbreviations: ADKP, autosomal dominant polycystic disease; CDC, complement-dependent cytotoxic; cPRA, calculated panel reactive antibodies; FSGS, focal segmental glomerulosclerosis; GBM, glomerular basal membrane; IA, immunoadsorption; Tx, transplantation.

ID: immunoadsorption-based desensitization waiting time: time elapsed between transplantation list registration and transplantation; waiting time after IA initiation: time elapsed between immunoadsorption-based desensitization and transplantation.

recipients had displayed a historic CDC positive crossmatch (3/14 IgG against T and B lymphocytes, 4/14 IgM only). Flow cytometry crossmatch is not routinely performed in France for deceased donor transplantation. The median cumulated historical DSA MFI value before transplantation was 21,222 [12,067–42,095] in class I and 6,157 [1,730–20,455] in class II antibodies. At the day of transplantation, median DSA number and sum total MFI of DSA were 3 [1.8–4.3] and 7,625 [2,771–10,201], respectively.

Donor Characteristics

The median donor age was 66 years [40–71], and a history of hypertension and diabetes mellitus was disclosed in 3/14 (21%) and 2/14 (14%) patients, respectively. Donor serum creatinine was 69 μmol/L [56–84]. A cerebrovascular event was the recorded cause of death in 10 of 14 (71%) donors. The median number of HLA mismatches was 5 [5–6]. All KT recipients displayed at least one DSA on the day of transplantation: cumulative day 0 DSA-MFI was 4,505 [2,133–7,125] for class I and 1,150 [0–4,320] for class II. Median cold ischemia time was 15.5 h [12.5–18.5].

Transplant Follow-Up

The median post-transplant follow-up was 3.1 years [1.7–4.9]. At 3 months post-transplant, cumulative DSA-MFI was 5,962 [4,229–11,200] for class I and 5,209 [2,599–8,593] for class II antibodies. Individual post-transplant DSA kinetics is shown in **Supplementary Figure S1**. Active ABMR was diagnosed in 7/14 (50%) patients (six within the first-year post-transplant). For two patients, active ABMR was subclinical and diagnosed based on protocol biopsies. All 7 patients were treated with steroids, plasma exchange or IADS, combined with eculizumab (n = 3), and/or IVIg (n = 6). Histopathological Banff scores are shown in **Table 2**. In 3/14 cases chronic ABMR was diagnosed during follow-up. Banff score on the available 3rd month protocol biopsies is shown in **Supplementary Table S2**.

At 1-year post-KT, 2 KT recipients had died from severe infections but with functioning grafts and there were two graft losses (one due to recurrent focal and segmental glomerulosclerosis [FSGS] and one due to active ABMR). For the 10 functional grafts at 1-year, median serum creatinine and estimated glomerular filtration rate (eGFR) were 135 μmol/L [111–202] and 47 mL/min/1.73 m [3] [29–51], respectively. Uncensored graft survival was 71.4% at both 1 and 2 years. Death-censored graft survival was 85.7% at the same time points. By the end of study follow-up, 8/14 patients had lost their graft due to chronic allograft dysfunction (n = 3), death (n = 2), acute rejection (n = 1), renal arterial mycotic aneurism (n = 1), and FSGS recurrence (n = 1). There were a number of infectious complications that are listed in **Supplementary Table S3**.

Comparing Outcomes of KT-Waitlisted Highly-Sensitized ESKD Patients With or Without IADS Desensitization

Finally, we compared outcomes between our 15 HS ESKD patients receiving IADS desensitization and a group of patients matched for age, degree of HLA sensitization and time of KT waitlisting (n = 30: 2:1) (**Table 3**). Compared to 93% of patients who received desensitization receiving a KT, only 43% of our control group underwent transplantation. Time from waiting list enrollment to KT was 6.5 [5.7–10.2] years in desensitized patients and 10.5 [8.3–11.7] years in controls. However, we did not find a significant difference in overall patient survival (87% vs. 96%, p = ns) and in the percentage of patients alive with a functioning graft (40% in both groups) at the end of follow-up.

The results are summarized in **Figure 1**.

DISCUSSION

Management of highly sensitized ESKD patients represent a conundrum for KT teams. It is well recognized that patients who are denied a KT have an increased mortality compared to recipients of an HLA incompatible KT, and this holds true in the presence of a historic positive cytotoxic crossmatch [12]. In addition, in the current kidney allocation system, anti-HLA sensitization decreases

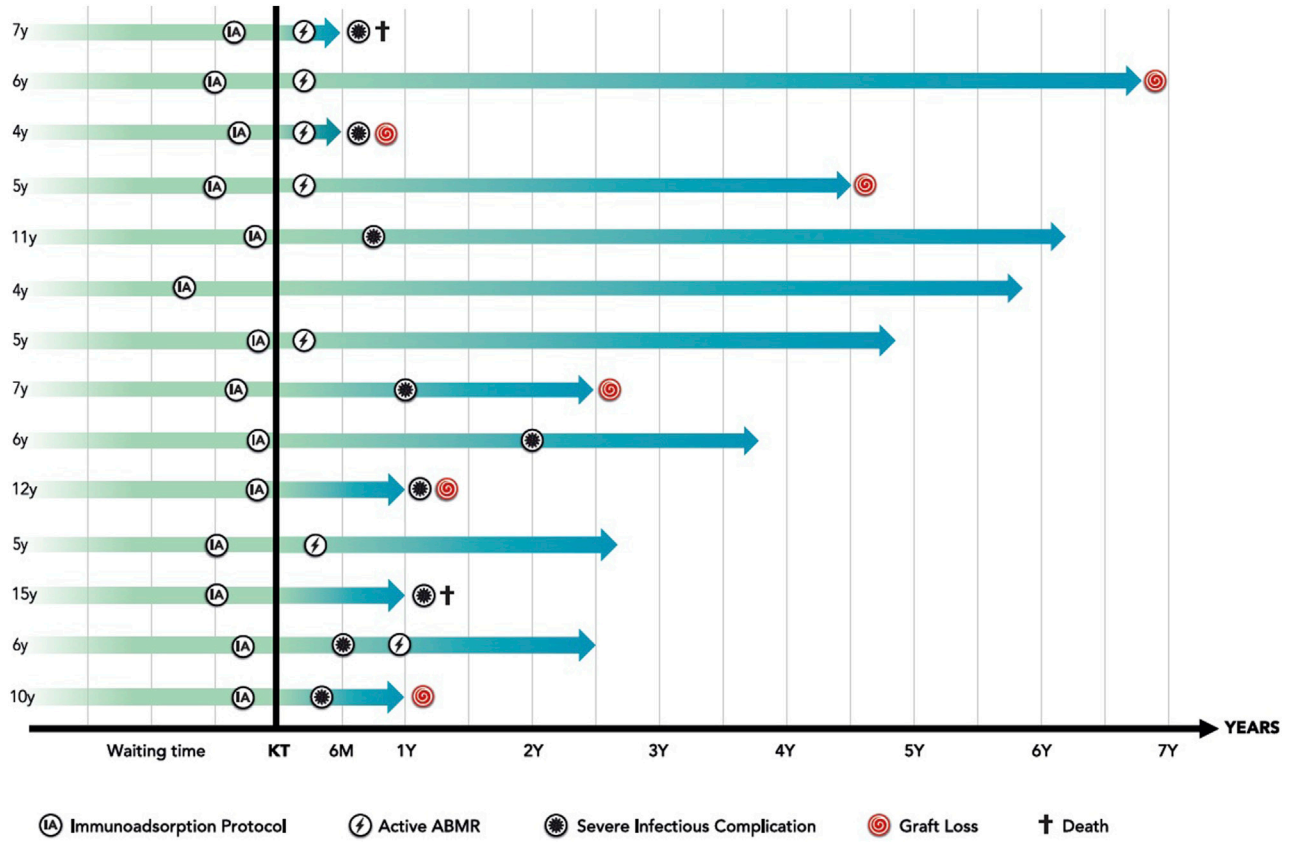


FIGURE 1 | Immunoadsorption based desensitization protocol: overall results.

TABLE 2 | Post-kidney transplantation active ABMR episodes.

Patient	Delay between KT and active ABMR diagnosis (days)	Banff classification	Plasma creatinine at biopsy (μmol/L)	Urine protein to creatinine ratio (g/mmol)	Treatment	Outcome	Biopsy indication
1	8	g3 i0 t0 v0 ptc2 cg0 mm1 ci0 ct0 cv1 ah0 C4d3	282	NA	IADS, PE, steroids, IVIg	Sepsis and bleeding Death < M3	AKI
2	10	g2 i1 t0 v0 ptc1 cg0 mm0 ci0 ct0 cv1 ah0 C4d3	376	NA	IADS, eculizumab, steroids, IVIg	Chronic ABMR	AKI
4	12	g2 i2 t1 v0 ptc2 cg0 mm0 ci0 ct0 cv0 ah0 C4d0	697	0.17	PE, steroids, eculizumab, IVIg	Graft loss < M3	AKI
5	30	g2 i0 t0 v0 ptc1 cg0 mm0 ci0 ct0 cv2 ah0 C4d3	183	0.02	Steroids, PE, IVIg	Chronic ABMR Graft loss Y4	AKI
7	13	g2 i1 t0 v1 ptc2 cg0 mm0 ci0 ct0 cv0 ah0 C4d3	170	0.05	PE, eculizumab, steroids, IVIg		AKI
11	95	g1 i0 t0 v0 ptc0 cg0 mm0 ci0 ct0 cv1 ah0 C4d3	200	0.01	PE, steroids, IVIg		Protocol month 3
13	398	g2 i0 t0 v0 ptc0 cg0 mm1 ci0 ct0 cv0 ah0 C4d0	116	0.01	PE, steroids		Protocol month 12

Abbreviations: ABMR, antibody mediated rejection; AKI, acute kidney injury; IADS, immunoadsorption; IVIg, intravenous immunoglobulins; KT, kidney transplantation; NA, not available; PE, plasma exchange; g, glomerulitis; i, interstitial inflammation; t, tubulitis; v, intimal arteritis; cpt, peritubular capillaritis; cg, transplant glomerulopathy; mm, mesangial matrix increase; ci, interstitial fibrosis; ct, tubular atrophy; cv, arterial fibrous intimal thickening; ah, hyaline arteriolar thickening.

TABLE 3 | Comparing outcomes of KT-waitlisted highly-sensitized ESKD patients with or without IADS desensitization.

	IADS group	Controls	*p*
	n = 15	*n* = 30	
Demographics			
Age, years	52 [40–56]	52 [43–58]	0.68
ABO group, *n* (%)	AB 3/15 (20%)	AB 4/30 (13%)	0.67
	O 7/15 (47%)	O 19/30 (63%)	0.35
	A 3/15 (20%)	A 4/30 (13%)	0.67
	B 2/15 (13%)	B 3/30 (10%)	0.98
Degree of sensitization (TGI, %)	99 [92–100]	98 [72–99]	0.11
Outcomes at the end of follow-up			
Transplantation, *n* (%)	14/15 (93%)	13/30 (43%)	0.001
Time from waitlisting to KT, years	6.5 [5.7–10.2]	10.5 [8.3–11.7]	0.07
Death-censored graft loss, *n* (%)	6/15 (40%)	1/30 (3%)	0.003
Death, *n* (%)	2/15 (13%)	2/30 (6%)	0.85
Alive and functioning graft, n (%)	6/15 (40%)	12/30 (40%)	ns

Abbreviations: IADS, immunoadsorption; TGI, "taux de greffons incompatibles," French sensitization score « percentage of incompatible kidney transplants »; KT, kidney transplantation.

the chances of patients being allocated a kidney graft and may even preclude KT as in the case of some highly sensitized patients [13]. Conversely preformed DSA are acknowledged to expose patients to an increased risk of graft failure and ABMR [14].

Several strategies aimed at reducing anti-HLA antibody levels and enhancing the chances of HS patients being offered a KT have been elaborated. Early protocols utilizing exclusively IVIg [4, 15] have been replaced by those combining apheresis—either plasma exchanges [16–18] or IADS [6, 9, 10, 19, 20]—with immunosuppressive drugs (steroids, calcineurin inhibitors, mycophenolate mofetil, eculizumab, rituximab), and IVIg [21]. Recently, IdeS an IgG degrading endopeptidase has been shown to allow for greater anti-HLA antibody depletion after a single dose thus representing another potential option for HS transplant candidates in the near future [22, 23]. Imlifidase dispenses with the repeated and cumbersome IADS sessions and allows for a greater reduction in DSA, at least on the day of KT. Besides, it is effective in even the most highly sensitized patients and 3-year follow-up graft survival was encouraging (90%) [24].

With regards to apheresis techniques, IADS has been shown to be more efficient than plasma exchange for lowering anti-HLA antibody titers [22, 25], and obviates the need for plasma replacement with its attendant side effects. To date, the implementation of IADS-based protocols has been chiefly restricted to living donor HLA and ABO incompatible KT. Our data is a further contribution to the few prior experiences in the setting of deceased donors [8–10]. Our protocol led to a decrease in cPRA so that 93% of the patients were ultimately transplanted. These patients had been on the waiting list for several years with a low likelihood of ever receiving a KT. Our comparison with a relevant control group suggests that the desensitization protocol used here increases the probability of HS patients being transplanted and also expedites KT.

Only few experiences with IADS-based desensitization have been reported so far. Using a protocol akin to ours, Noble et al reported on 36 patients including 8 living donors. In six cases (16.7%) the IADS protocol was aborted due to failure to clear DSA or complications. With a different approach [9, 10] patients displaying a positive complement-dependent cytotoxicity crossmatch received a single session of IADS immediately prior to KT and were cleared for transplantation provided the crossmatch was rendered negative. Compared to our cohort, the patients exhibited lower HLA sensitization, and a significant proportion of the patients were deemed unsuitable for KT having failed to yield a negative crossmatch (around 20%). However, the study disclosed favorable graft survival rates.

However, the shortcomings of desensitization protocols should be recognized. While these have been instrumental in offering a therapeutic opportunity for HS patients, post-KT DSA rebound significantly increases the risk of ABMR as shown in the **Supplementary Figure S1**. Fifty percent of patients suffered active ABMR, and there were three cases of chronic ABMR. One team [26] (Schwaiger et al.) opted for systematic post KT IADS, yet the group of highly sensitized patients CDCXM+/DSA+ patients which most closely resembles our cohort nonetheless exhibited increased rates of ABMR (44%). In fact, irrespective of the desensitization approach, the rate of ABMR was a cause of concern ranging from 38% [24] to 41% [11]. From an immunological perspective, half of our patients were free of adverse immunological events post-KT despite high DSA levels. Taken together, these results suggest that 1) for any given patient, DSA alone should not preclude KT; 2) within the group of HS patients, current immunological risk stratifiers may incorrectly classify these patients as untransplantable. 3) the same stratifiers are ineffective at delineating HS patients who may enjoy a satisfactory post-KT course from those at risk of early ABMR.

We observed significant infectious complications in our cohort. This is unsurprising for a number of reasons. HS ESKD patients may have impaired immunity due to previous immunosuppression for native kidney disease, previous KT and on top of the burden of dialysis and ESKD itself. The multi-targeted immune desensitization protocol used here would have further enhanced the infectious risk. The high rate of invasive fungal infections (n = 4) including two fatal cases of aspergillosis and one case of disseminated cryptococcosis is a likely reflection of the patients' defective adaptive cellular immunity [27–29]. In line with this, infection has been highlighted as the principal contributor to death in other cohorts of IADS-desensitized patients [26].

We recognize the limitations of the current study. Our data are observational and from a single center with a modest number of patients. However, these types of patients, that is those who are HS and who have been waitlisted for a significant amount of time, are uncommon. Nevertheless, as the fraction of HS patients is expected to grow over the coming years there is a dire need to devise strategies to raise the prospects of KT. Currently, there is no consensus on how to manage this very high-risk group resulting in divergent strategies around the world. Unfortunately, outcomes for these patients are dismal and so transplantation remains their only hope, albeit with the risks described here.

When contemplating IADS-based desensitization KT the risks entailed by remaining on the waiting list should be carefully weighed against the hazards of a potentially short-lived graft function, the high likelihood of ABMR and severe infections. Importantly, immunoadsorption strategy is not associated with an improved probability of being alive with a functioning graft at the end of

the follow-up compared to those receiving no immunoadsorption. Indeed, those who received immunoadsorption had poorer graft outcomes following transplantation.

In our experience, the exceedingly high risk of ABMR and lethal infections outweighed the potential benefits of KT, precluding the universalization of our IADS-based desensitization strategy in its current scheme. However, there may be select groups of patients that might benefit from immunoadsorption and these should be defined in future studies. Single IADS, or better yet imlifidase, may represent less cumbersome options. Regardless of the adopted strategy, clinicians should be wary of the high rate of ABMR and candidates should be selected and informed accordingly.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

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

AUTHOR CONTRIBUTIONS

Conception and design: CB, CR, ER, PG, and YL. Data acquisition: CB, CR, PG, and YL. Analysis and interpretation: CB, CR, JT, SM, DB, ER, PG, and YL. Drafting the manuscript: CB, CR, ER, PG, and YL. Final approval: CB, CR, JT, SM, LM, HF, CP-H, NO, AH, MJ, DB, ER, PG, and YL. 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.2023.11212/full#supplementary-material

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Tacrolimus Exposure Before and After a Switch From Twice-Daily Immediate-Release to Once-Daily Prolonged Release Tacrolimus: The ENVARSWITCH Study

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LCP-tacrolimus displays enhanced oral bioavailability compared to immediate-release (IR-) tacrolimus. The ENVARSWITCH study aimed to compare tacrolimus $AUC_{0-24\ h}$ in stable kidney (KTR) and liver transplant recipients (LTR) on IR-tacrolimus converted to LCP-tacrolimus, in order to re-evaluate the 1:0.7 dose ratio recommended in the context of a

Abbreviations: AE, adverse event; AUC, area under the curve; BID, twice a day; C_0, trough concentration; CI, confidence interval; CKD-EPI, Chronic Kidney Disease EPIdemiology collaboration; C_{max}, maximal concentration after drug intake; DBS, dried blood spot; eGFR, estimated glomerular filtration rate; FAS, full analysis set; ICH, International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; IR-tacrolimus, immediate-release tacrolimus; ISBA expert system, ImmunoSuppressive Bayesian dose Adjustment expert system; KTR, Kidney transplant recipient; LCP-tacrolimus, extended-release tacrolimus; LTR, Liver transplant recipient; MedDRA, Medical Dictionary for Regulatory Activities; PPS, per-protocol set; QD, once a day; RMSE, root mean square error; SCr, serum creatinine; SD, standard deviation; TDM, therapeutic drug monitoring; V, visit.

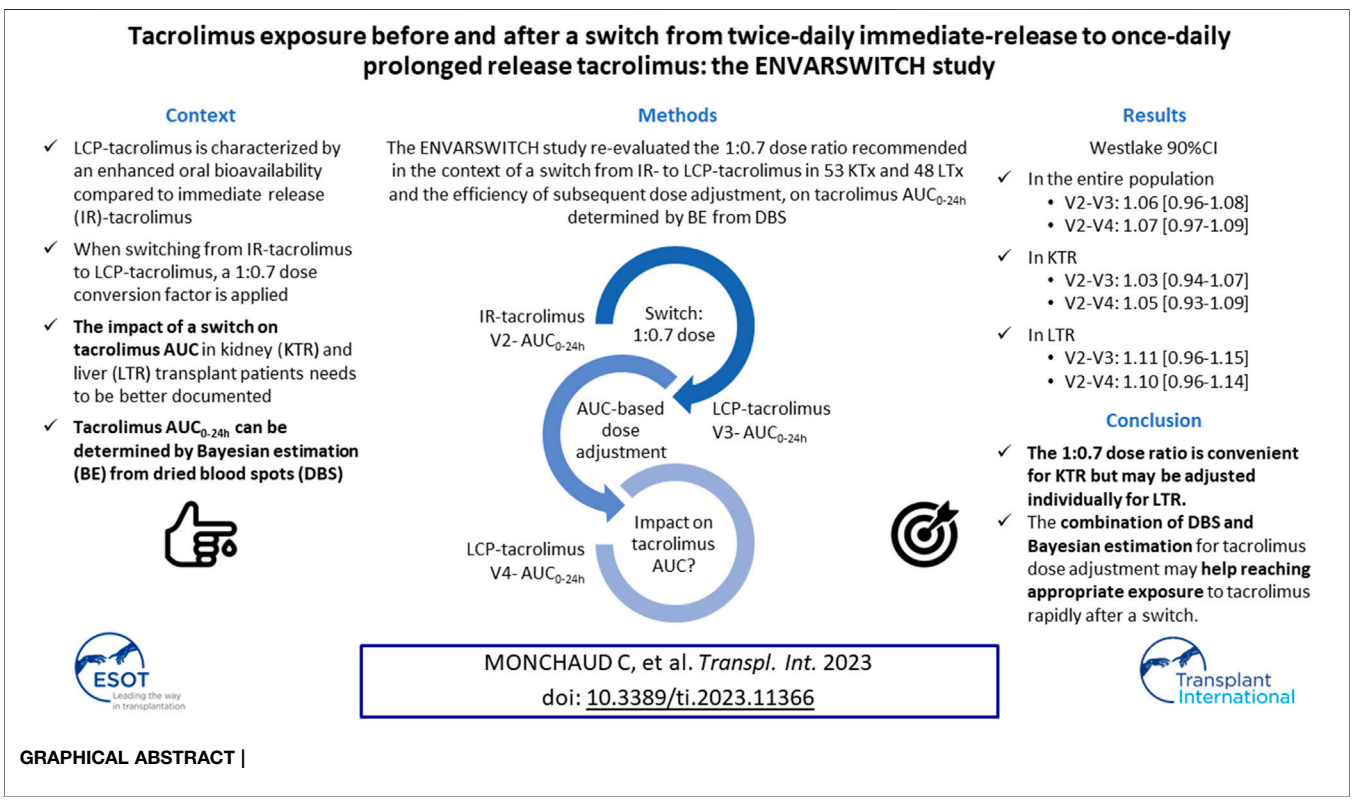
switch and the efficiency of the subsequent dose adjustment. Tacrolimus $AUC_{0–24\ h}$ was obtained by Bayesian estimation based on three concentrations measured in dried blood spots before (V2), after the switch (V3), and after LCP-tacrolimus dose adjustment intended to reach the pre-switch $AUC_{0–24\ h}$ (V4). $AUC_{0–24\ h}$ estimates and distributions were compared using the bioequivalence rule for narrow therapeutic range drugs (Westlake 90% CI within 0.90–1.11). Fifty-three KTR and 48 LTR completed the study with no major deviation. $AUC_{0–24\ h}$ bioequivalence was met in the entire population and in KTR between V2 and V4 and between V2 and V3. In LTR, the Westlake 90% CI was close to the acceptance limits between V2 and V4 (90% CI = [0.96–1.14]) and between V2 and V3 (90% CI = [0.96–1.15]). The 1:0.7 dose ratio is convenient for KTR but may be adjusted individually for LTR. The combination of DBS and Bayesian estimation for tacrolimus dose adjustment may help with reaching appropriate exposure to tacrolimus rapidly after a switch.

Keywords: kidney transplantation, liver transplantation, LCP-tacrolimus, AUC monitoring, dried blood spots, conversion, therapeutic drug monitoring, dose individualization

INTRODUCTION

The pharmacokinetics of LCP-tacrolimus (Envarsus®) has been sparsely investigated [1], and clinical trials [2–5] have left some uncertainty on the exact starting dose, dose ratio with regards to other prolonged-release formulations, and blood levels to be expected in kidney (KTR) and liver transplant recipients (LTR). Previous experience with Advagraf® showed that absorption could be almost nil in the first days post-transplantation, and that in stable patients, the 1:1 dose ratio resulted in lower C_0 but comparable $AUC_{0–24\ h}$ [6].

The relationship between tacrolimus exposure and effects renders individual dose adjustment essential to avoid under- or overexposure [7]. The exposure index best associated with clinical effects is the area under the concentration–time curve (AUC) [7]. To overcome the inconveniences of collecting 10–12 blood samples over the dose interval,



GRAPHICAL ABSTRACT |

Bayesian estimators based on sparse sampling strategies have been developed for the AUC estimation of all tacrolimus formulations [1, 8–12] and are routinely used through the ISBA expert system[1] [13]. However, the collection of several blood samples by venipuncture in a medical environment induces costs and logistical constraints. Therefore, dried blood spot (DBS) sampling, which can easily be performed at home, has been proposed for the therapeutic drug monitoring (TDM) of tacrolimus [14–20]. After a fingerprick, blood is applied onto a special filter paper, which is subsequently mailed to the laboratory. Good acceptability by the patients [21] and reliability of measured drug levels [16–20] are arguments in favor of DBS for the TDM of tacrolimus in transplantation. Furthermore, DBS are particularly suited to LCP-tacrolimus for which the optimal sampling times for $AUC_{0-24\,h}$ estimation are 0, 8, and 12 h post-dose [1].

In this context, we hypothesized that implementing DBS home sampling for the Bayesian estimation of tacrolimus $AUC_{0-24\,h}$ before and after a conversion, and considering the pre-switch $AUC_{0-24\,h}$ as a reference for LCP-tacrolimus dose adjustment after the switch, would allow maintaining of tacrolimus $AUC_{0-24\,h}$. Therefore, the aims of the ENVARSWITCH study were to verify, in KTR and LTR, the equivalence of the $AUC_{0-24\,h}$ values before and after a switch from IR-tacrolimus to LCP-tacrolimus at a 1:0.7 dose, followed by individual dose adjustment targeting the pre-switch $AUC_{0-24\,h}$. The study also aimed to compare tacrolimus exposure indices ($AUC_{0-24\,h}$, C_{max} and C_0) before vs. after the switch, before and after dose adjustment.

PATIENTS AND METHODS

Study Design, Patients and Procedures

The ENVARSWITCH study (EudraCT number: 2016-001014-22) was a multicenter prospective open clinical study conducted in 16 French transplantation centres, in accordance with the Declaration of Helsinki, Good Clinical Practice and the International Conference on Harmonization (ICH) guidelines. The protocol received approval from the Independent Ethics Committee (ref. CPP16-022/2016-001014-22) and authorization from the French National Agency for Medicines and Health Products Safety (ref. 160372A-11). All enrolled patients gave their written informed consent.

The primary objective was to verify the absence of difference between pre- and post-switch tacrolimus $AUC_{0-24\,h}$ calculated by Bayesian estimation, in KTR and LTR switched from IR-tacrolimus (Prograf®) to LCP-tacrolimus (Envarsus®) at a 1:0.7 dose, possibly followed by individual dose adjustment targeting the pre-switch $AUC_{0-24\,h}$.

We enrolled adult (≥18 year-old) kidney and liver transplant recipients, transplanted for between 2 weeks and 1 year, in whom a switch from IR-tacrolimus to LCP-tacrolimus had been decided, and in whom the IR-tacrolimus dose had been unchanged for at least 1 week or since the last two C_0 measurements. At the first protocol visit (V1), tacrolimus C_0 had to be between 4 and 12 μg/L and hematocrit >0.27.

After inclusion, real-time Bayesian estimation of $AUC_{0-24\,h}$ was performed (**Figure 1**): on the day before the switch (V2), after the IR-tacrolimus morning and evening doses (two $AUC_{0-12\,h}$ estimations); 2–4 days after the switch (V3); 7–14 days after V3 (V4). Conversion from IR-tacrolimus to LCP-tacrolimus was done on a 1:0.7 (mg:mg) total daily dose basis. Further dose adjustment could be performed between days 7 and 9, according to the AUC_{0-24h} estimated at V3, to target the pre-switch $AUC_{0-24\,h}$ calculated by summing the morning and the evening tacrolimus $AUC_{0-12\,h}$. $AUC_{0-24\,h}$ at V4 was compared to the individual target $AUC_{0-24\,h}$ (V2). No standardized $AUC_{0-24\,h}$ target was considered for the study.

Tacrolimus AUC Determination

$AUC_{0-24\,h}$ was obtained by Bayesian estimation and a limited sampling strategy (pre-dose then 1 h and 3 h post-dose for IR-tacrolimus; pre-dose and 8 h then 12 h post-dose for LCP-tacrolimus) [1, 8, 10]. DBS were collected on Whatman™ 903 protein saver cards. At V2, the study nurses collected the DBS necessary for the determination of IR-tacrolimus morning $AUC_{0-12\,h}$ and trained the patients to collect DBS autonomously. Afterwards, DBS collection was performed at home by the patients. DBS were post-mailed within 24 h after sampling to Limoges University Hospital for centralized analysis. Tacrolimus concentrations were determined using a high performance liquid chromatography–tandem mass spectrometry method on a 4500 AB-Sciex system (Forster City, CA, United States) validated in accordance with the IATDMCT recommendations [22], covering a concentration range of 1–100 μg/L. AUC estimation and the recommended dose were transmitted to the clinicians *via* a dedicated website within 24 h following DBS reception (maximum 5 days).

Endpoints

The primary endpoint was the Westlake 90%-confidence interval (CI) of the ratio of the dose-adjusted LCP-tacrolimus steady-state $AUC_{0-24\,h}$ (V4) over the pre-switch IR-tacrolimus steady-state $AUC_{0-24\,h}$ (V2) after log-transformation, in the entire population.

Secondary endpoints were the Westlake 90%-CI of the ratio of $AUC_{0-24\,h}$ at V4 over $AUC_{0-24\,h}$ at V2 in KTR and in LTR patients and the differences in and ratios of $AUC_{0-24\,h}$, C_{max} and C_0 between V2 and V3 in each subgroup.

Renal function was assessed as serum creatinine (SCr) and glomerular filtration rate estimated using the CKD-EPI equation [23]. For regulatory reasons, whenever missing, the eGFR was estimated from SCr by applying the CKD-EPI equation and considering the individuals as "not Black," since there was a very high probability for patients to be of Caucasian or North-African ancestry.

Post hoc analyses were performed to examine, in the entire population and in each subgroup: 1) the correlation between the theoretical LCP-tacrolimus dose (calculated by applying the 1:0.7 ratio) and the actual dose at V3; 2) the correlation between the

[1]https://abis.chu-limoges.fr/

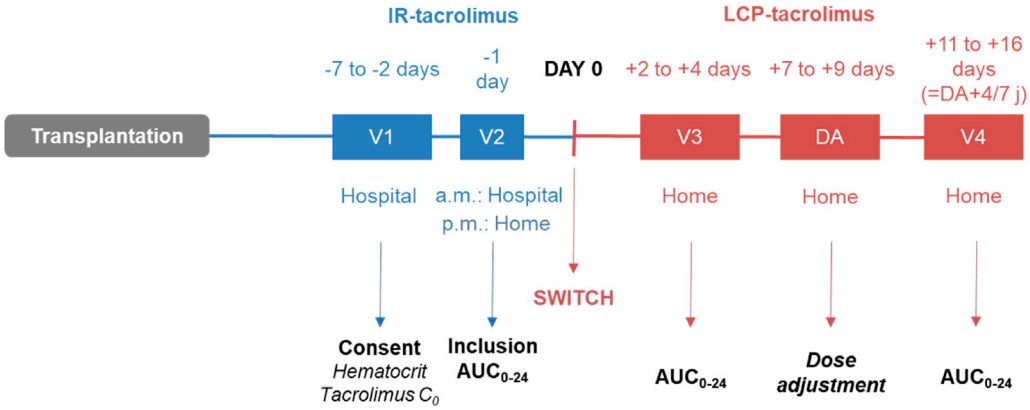


FIGURE 1 | ENVARSWITCH study design.

LCP-tacrolimus dose proposed after V3 and the actual dose at V4. Doses and exposure indices were also compared between subgroups and periods.

Adverse Events (AEs)

All AEs occurring between enrollment and the end of the trial were recorded on an ongoing basis, regardless of whether they were related or not to IR-tacrolimus or LCP-tacrolimus. Seriousness was assessed according to ICH E2A [24] and severity (mild, moderate, severe) according to its impact on activities of daily life. The causality to the investigational drug was independently assessed by the investigator and the sponsor (worst causality) at the time of the event. All AEs were coded using the MedDRA dictionary (version 23.0).

Statistical Analyses

Statistical analyses were performed using R version 4.0 (R Project for Statistical Computing: [2]). Categorical data are reported as frequencies and percentages, continuous data as means ± standard deviations (SD). Continuous variables were compared between periods using Student paired-t test.

Data were analyzed for the intent-to-treat population (Full Analysis Set, FAS; KTR and LTR, referred to as "the entire population") and for the per-protocol set (PPS). The FAS comprised included patients who complied with all study visits, while the PPS was restricted to patients of the FAS with no critical protocol deviation. Unless stated, all results are based on the FAS. Safety analyses were based on all included patients.

The comparison of $AUC_{0-24\,h}$ between V2 and subsequent visits was based on the mean ratios between log-transformed $AUC_{0-24\,h}$ and their Westlake 90%-CI. $AUC_{0-24\,h}$ between visits were deemed bioequivalent if the Westlake 90%-CI fell within the 0.90–1.11 range defined by the European Medicine Agency for the bioequivalence of drugs with a narrow therapeutic index [25–28].

[2]http://www.r-project.org

The comparison of the exposure indices between the three periods was done by computing Pearson's coefficient tests, and calculating the mean relative difference and root mean square error (RMSE) of exposure indices at V3 and V4 with respect to those measured at V2.

Sample Size

It was estimated that 96 patients would demonstrate a mean ratio of 1 [90%CI within 0.90–1.11] between V4 and V2 log-transformed $AUC_{0-24\,h}$, with an expected coefficient of variation = 25% for tacrolimus $AUC_{0-24\,h}$ and 80% power. Anticipating that 10% patients may not meet the requirements of tacrolimus C_0 between 4 and 12 μg/L and hematocrit >0.27, and that 20% may drop out (including missing or poor DBS collection or analysis), the total number of patients to enroll was set to 134.

RESULTS

Patients

Overall, 134 patients (70 KTR and 64 LTR) were enrolled. Three patients did not meet the inclusion criteria at V1 and 30 either discontinued study participation or displayed unexploitable $AUC_{0-24\,h}$ at V2 (**Figure 2**). Thus, the FAS comprised 101 patients, of whom 75 constituted the PPS. The KTR and LTR subgroups (**Table 1**) were comparable in terms of sex ratio, weight, body mass index and haematocrit, but LTR were characterized by a significantly older age (p = 0.001), later post-transplantation period (p = 0.002), and better kidney function (p = 0.022 for SCr and <0.001 for eGFR).

Tacrolimus Dose and Exposure Indices

AUC_{0-24} were distributed normally (Shapiro-Wilk test p between 0.067 and 0.2195). At V2, the mean IR-tacrolimus daily dose was significantly higher in KTR than in LTR (p < 0.001; **Table 2**), and so were C_0 and $AUC_{0-24\,h}$ (p < 0.001) (**Table 1**). The difference on daily dose and $AUC_{0-24\,h}$

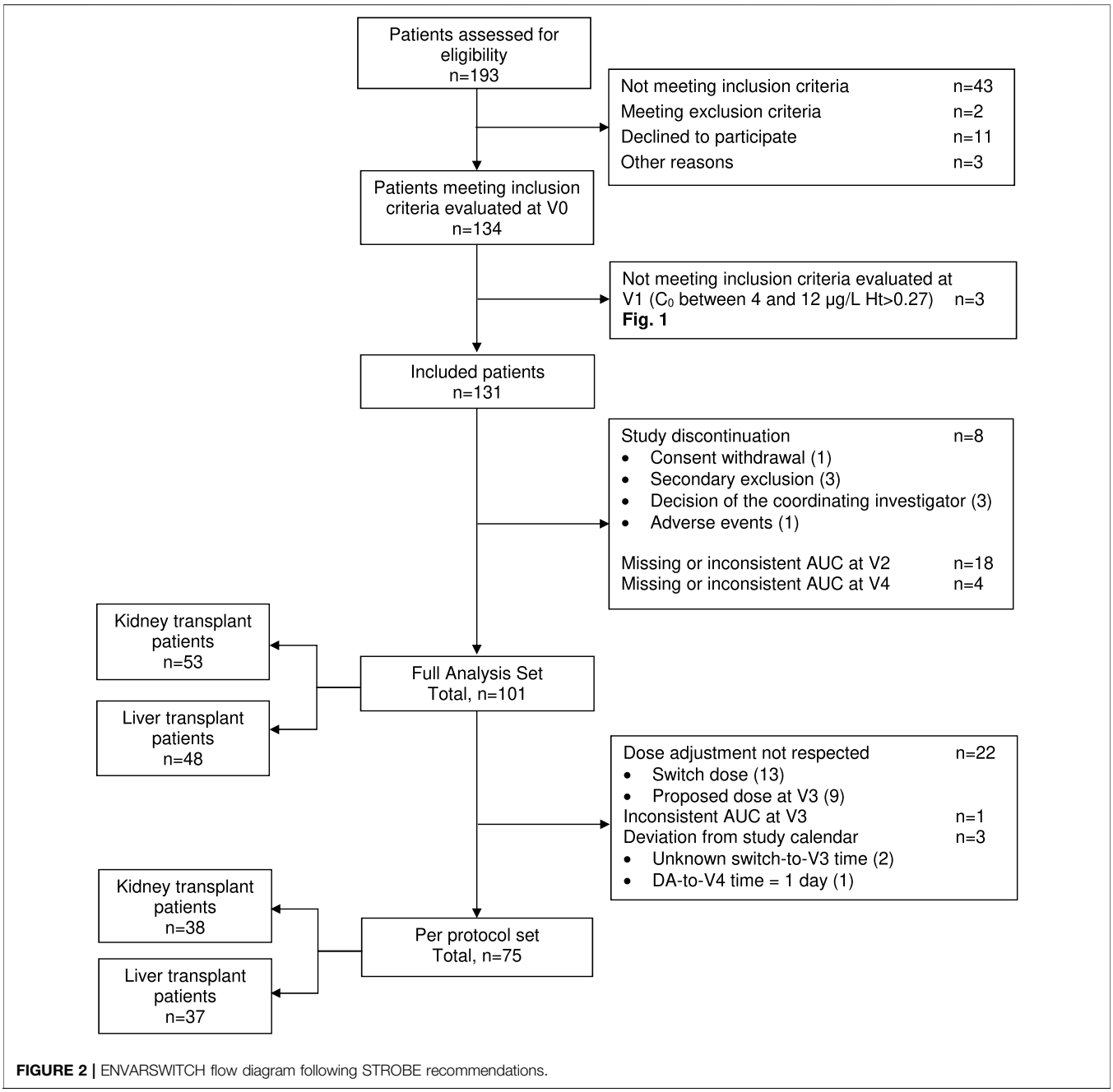


FIGURE 2 | ENVARSWITCH flow diagram following STROBE recommendations.

of LCP-tacrolimus between KTR and LTR persisted at V4 (p = 0.001 and $p < 0.001$, respectively).

Evaluation of the Overall Dose-Conversion and Individual Dose-Adjustment Strategy

The bioequivalence criterion between V2 and V4 was met in the FAS (mean ratio [90% CI] = 1.07 [0.97–1.09]) and the PPS (1.08 [0.97–1.11]). The violin plots of AUC_{0-24h} by subgroup at V2 and V4 in the FAS are presented in **Figure 3**. No significant difference was observed on the mean $AUC_{0-24\ h}$ between V2 and V4 (p = 0.297), but correlation was poor (r = 0.608), with a mean relative difference between V4 and V2 of 0.074 ± 0.330 h.µg/L and RMSE = 34%.

The bioequivalence criterion between V2 and V4 was met in KTR (1.05 [0.93–1.09]) and almost met in LTR (1.10 [0.96–1.14]). The correlation between V2 and V4 AUC_{0-24h} was poor in both subgroups (r = 0.462 and 0.571, respectively), and even poorer for C_0 (r = 0.100 and 0.429) (**Figure 4**). No statistically significant C_0 difference was observed between V2 and V4 for either subgroup

TABLE 1 | Patient characteristics at V2.

Variables	Full analysis set			Per protocol set		
	Total	Kidney transplant patients	Liver transplant patients	Total	Kidney transplant patients	Liver transplant patients
	***N* = 101**	***N* = 53**	***N* = 48**	***N* = 75**	***N* = 38**	***N* = 37**
Age, years	**53.2 (11.9)**	49.6 (13.2)	57.4 (8.80)	**53.8 (12.0)**	49.0 (13.4)	58.7 (7.93)
Gender (M/F)	**70/31**	32/21	38/10	**52/23**	23/15	29/8
Post-transplantation time, days	**138 (91.8)**	112 (87.7)	168 (87.9)	**137 (90.0)**	107 (83.8)	167 (86.9)
Weight, kg	**74.7 (15.3)**	75.2 (14.3)	74.1 (16.4)	**75.3 (15.8)**	75.9 (14.8)	74.7 (16.9)
Body mass index, kg/m^2	**25.4 (4.41)**	25.2 (4.01)	25.5 (4.84)	**25.3 (4.64)**	25.1 (4.16)	25.6 (5.11)
Serum creatinine, µmol/L	**118 (51.2)**	129 (32.0)	106 (64.5)	**121 (56.4)**	130 (34.4)	112 (71.8)
eGFR (CKD-EPI), mL/min/ 1.73 m^{2a}	**62.3 (21.1)**	53.4 (16.1)	72.1 (21.7)	**60.8 (20.4)**	53.6 (17.0)	68.2 (21.2)
Tacrolimus total daily dose, mg	**6.36 (4.12)**	7.75 (4.49)	4.81 (3.03)	**6.03 (3.62)**	7.63 (3.75)	4.39 (2.64)
Tacrolimus C_0, µg/L	**7.97 (2.01)**	8.58 (1.60)	7.31 (2.21)	**7.90 (1.93)**	8.55 (1.60)	7.26 (2.04)
Hematocrit, %	**37.1 (5.03)**	36.8 (4.85)	37.5 (5.25)	**37.0 (4.97)**	36.8 (5.00)	37.2 (5.00)

Data are presented as mean (SD).
[a]*eGFR considering patients as Caucasians: in FAS,* n = *35 (22 KTx, 13 LTx); in PPS,* n = *28 (17 KTx, 11 LTx).*
Bold characters are for totals.

TABLE 2 | Tacrolimus daily dose (mg/day) and $AUC_{0-24\ h}$ (h.µg/L) at each study visit in the full analysis set.

		Total	Kidney transplant patients	Liver transplant patients
		***N* = 101**	***N* = 53**	***N* = 48**
V2	Tacrolimus daily dose	**6.36 (4.12)**	7.75 (4.49)	4.81 (3.03)
Before conversion	$AUC_{0-24\ h}$	**229 (77.2)**	266 (70.5)	187 (61.9)
V3	Tacrolimus daily dose	**4.43 (2.87)**	5.51 (3.25)	3.22 (1.73)
After conversion	$AUC_{0-24\ h}$	**237 (88.6)**	273 (89.1)	198 (70.3)
V4	Tacrolimus daily dose	**4.48 (3.32)**	5.63 (3.81)	3.22 (2.06)
After dose adjustment	$AUC_{0-24\ h}$	**236 (84.0)**	269 (72.2)	200 (82.1)

Data are presented as mean (SD).
Bold characters are for totals.

(C_0 = 7.87 ± 2.60 vs. 8.14 ± 2.41, p = 0.671 and 5.71 ± 2.12 µg/L vs. 6.33 ± 3.14 µg/L, p = 0.150, respectively).

Evaluation of the Recommended Dose-Conversion Ratio

The bioequivalence criterion between V2 and V3 was met in the entire population (1.06 [0.96–1.08]) and in KTR (1.03 [0.94–1.07]), but not in LTR (1.11 [0.96–1.15]). The correlation between V2 and V3 $AUC_{0-24\ h}$ was poor in both subgroups (r = 0.724 and 0.531, respectively). Additionally, despite the absence of significant C_0 differences between V2 and V3 in either subgroup (7.87 ± 2.60 vs. 7.72 ± 2.53 µg/L, p = 0.680 and 5.71 ± 2.12 vs. 6.30 ± 2.51 µg/L, p = 0.120, respectively), the correlation between V2 and V3 C_0 was poorer than that of the $AUC_{0-24\ h}$ (r = 0.516 and 0.391, respectively) (**Figure 4**). As expected, the mean C_{max} was significantly lower at V3 than at V2 in both subgroups (15.6 ± 5.60 vs. 22.1 ± 9.42 µg/L, p < 0.001 and 11.4 ± 3.99 vs. 16.1 ± 6.69 µg/L, p < 0.001, respectively).

Compliance With the Recommended Dose

Correlations between the IR-tacrolimus dose at V2 × 0.7 and the LCP-tacrolimus dose at V3, and between the LCP-tacrolimus dose proposed at V3 and the administered dose at V4 were strong in both subgroups (r > 0.9, **Figure 5**), showing overall good compliance of the clinicians with the doses recommended at all steps.

Impact of Dose Adjustment on $AUC_{0-24\ h}$

The impact of dose adjustment on AUC_{0-24h} was evaluated by comparing $AUC_{0-24\ h}$ V4 vs. V2 depending on whether the patients needed dose adjustment after V3 (88 patients) or not (13 patients) and whether dose adjustment was done (53 patients) or not (35 patients) (**Figure 6**). No patient benefited from a dose adjustment if no dose adjustment had been proposed. The $AUC_{0-24\ h}$ at V4 and V2 were well correlated in KTR who did not require and did not have a dose adjustment (r = 0.982), but not in LTR in the same situation (r = 0.225). In contrast, among patients for whom we proposed dose adjustment, the correlation was poor in KTR, whether dose adjustment had been applied or not (r = 0.458 and 0.356, respectively) and fair in LTR recipients (r = 0.581 and 0.794, respectively).

Renal Function

No difference in renal function was found between V2 and V4 in the entire population (Scr at V4 = 119 ± 50.2 µmol/L, p = 0.826; eGFR = 62.5 ± 22.3 mL/min, p = 0.974), nor in subgroups separately. The average SCr and eGFR at V4 were, respectively

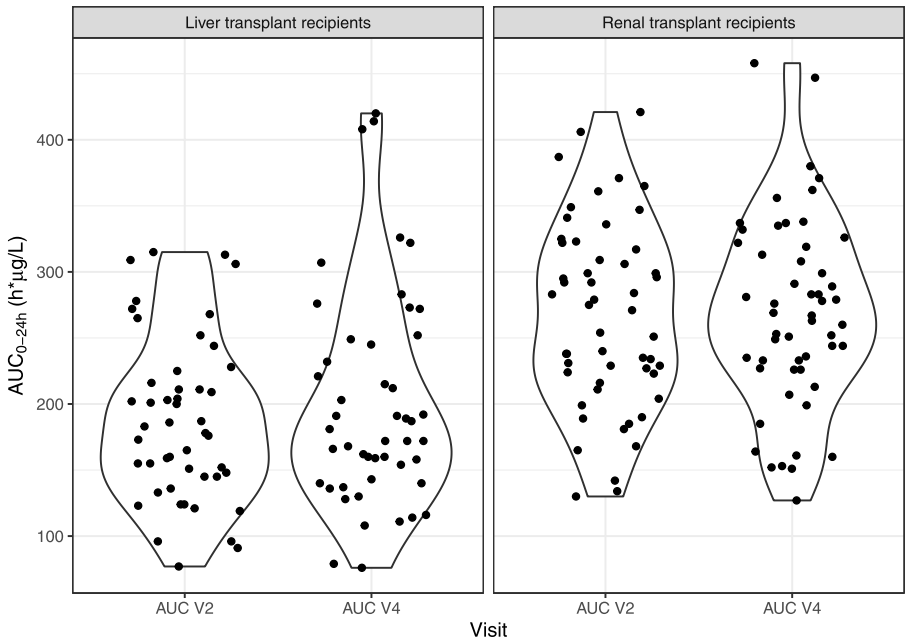


FIGURE 3 | Violin plots of IR-tacrolimus $AUC_{0-24\ h}$ (V2) and LCP-tacrolimus $AUC_{0-24\ h}$ after dose adjustment (V4) in the full analysis set, split in two subgroups (liver and kidney transplant patients).

130 ± 30.0 µmol/L ($p = 0.953$) and 53.4 ± 16.0 mL/min ($p = 0.708$) in KTR and 107 ± 63.0 µmol/L ($p = 0.780$) and 72.4 ± 24.0 mL/min ($p = 0.818$) in LTR.

Safety

The safety analysis set comprised the 134 patients enrolled (**Table 3**). Patients were on IR-tacrolimus for a maximum of 7 days and on LCP-tacrolimus for a maximum of 3 weeks during their participation in the study. Nineteen and fifty AEs occurred respectively while on IR-tacrolimus and LCP-tacrolimus. Eleven patients (8.2%) experienced at least one AE while on IR-tacrolimus and 33 (25.8%) while on LCP-tacrolimus. One patient (0.7%) while on IR-tacrolimus and twelve (9.4%) while on LCP-tacrolimus experienced at least one AE considered as possibly related to tacrolimus. The majority of AEs were of mild-to-moderate severity (100% on IR-tacrolimus and 94% on LCP-tacrolimus). The incidence of tremor, diarrhea, and hyperglycemia on LCP-tacrolimus was respectively 3.1%, 1.6%, and 0.8%.

Three serious AEs occurred in three patients, among which two were related to tacrolimus: one pneumopathy and one sub-therapeutic dosage. This latter AE occurred in a patient who had subtotal colectomy, resulting in a decreased $AUC_{0\text{-}24}$ at V3 confirmed by a low C_0 value, explained by the lower absorption of tacrolimus in its extended-release formulation. The patient was switched back to IR-tacrolimus and excluded from the study.

DISCUSSION

ENVARSWITCH confirms bioequivalent exposure to tacrolimus in terms of $AUC_{0-24\ h}$ in 101 stable KTR or LTR converted from IR-tacrolimus to LCP-tacrolimus using a 1:0.7 dose ratio. It is the first clinical study proposing the combination of DBS and Bayesian estimation for tacrolimus $AUC_{0-24\ h}$ determination and dose adjustment. The Westlake interval in the entire population fell within the bioequivalence criteria for narrow therapeutic index drugs [26–28], and no significant difference was found between the mean $AUC_{0-24\ h}$ before the switch and after the switch followed by individual dose adjustment. Despite the removal of 30/131 (23%) patients from the FAS, the study remained sufficiently powered to validate its primary objective (N > 96 patients). The higher than expected proportion of drop-outs was compensated by the lower than expected proportion of patients not meeting the inclusion criteria at V2 (hence not eligible for formulation switching). Also, although the analysis was less powered, the Westlake interval calculated from data of the PPS still fulfilled the bioequivalence criteria. These results suggest that the 1:0.7 dose conversion ratio combined with individual dose adjustment is overall adapted. Importantly, as patients' ethnicity was not collected for regulatory reasons, and because the 1:0.7 conversion factor is not recommended for patients of African origin, we hypothesize that the patients to whom this study was proposed by their treating physician were of other origins, and mostly white Europeans.

The Westlake interval between the $AUC_{0-24\ h}$ measured before and right after the switch also fell within the bioequivalence criteria in the entire population. This suggests that before any individual dose adjustment, the 1:0.7 dose conversion ratio is adapted, as proposed from the conversion studies [2–4]. Nevertheless, while subgroup analyses found no difference between $AUC_{0-24\ h}$ at V2 and at V3 and V4 in KTR recipients, the Westlake interval was close to, but did not fall

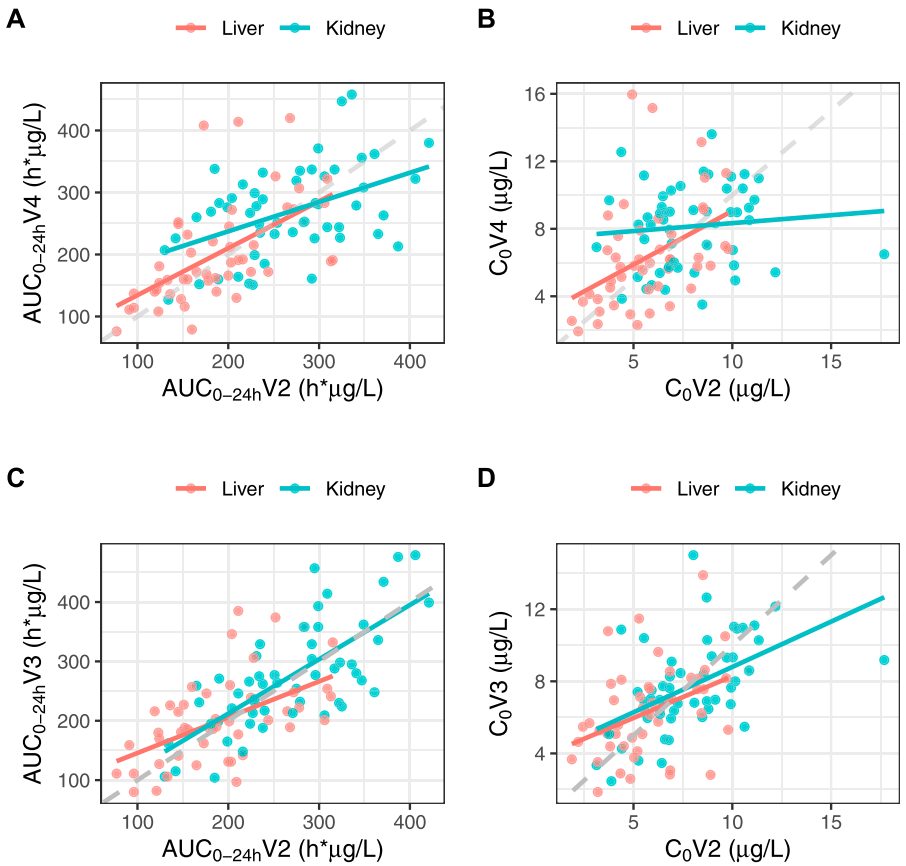


FIGURE 4 | Correlations between IR-tacrolimus (V2) and dose-adjusted LCP-tacrolimus (V4) $AUC_{0-24\ h}$ **(A)** and C_0 **(B)** and between IR-tacrolimus (V2) and dose-converted LCP-tacrolimus (V3) $AUC_{0-24\ h}$ **(C)** and C_0 **(D)** in the full analysis set split in the two transplant subgroups.

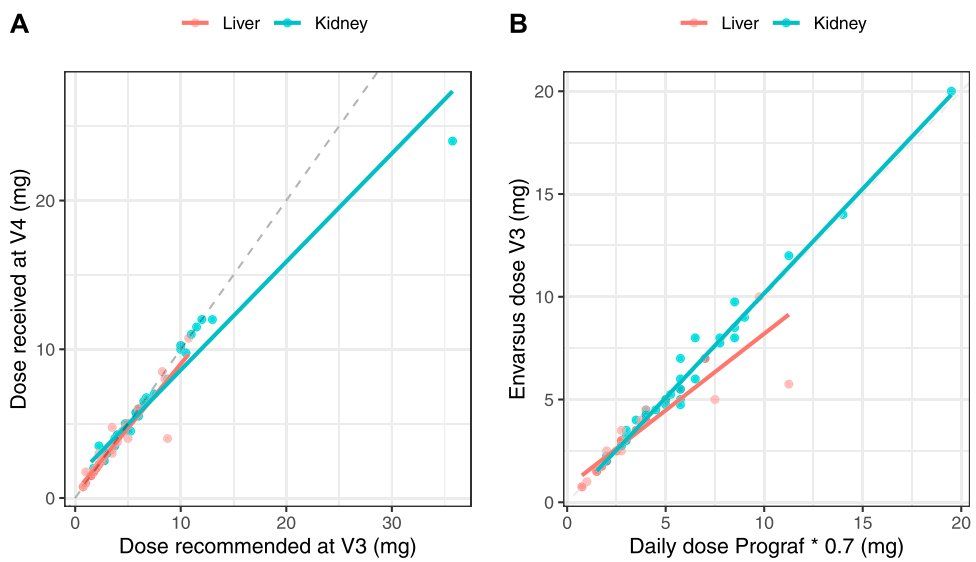


FIGURE 5 | Correlations between the theoretical converted dose (IR-tacrolimus daily dose × 0.7) and the actual dose received by the patient at V3 **(A)** and between the LCP-tacrolimus dose proposed based on the $AUC_{0-24\ h}$ at V3 and the dose actually received at V4 **(B)** in the full analysis set split in the two transplant subgroups.

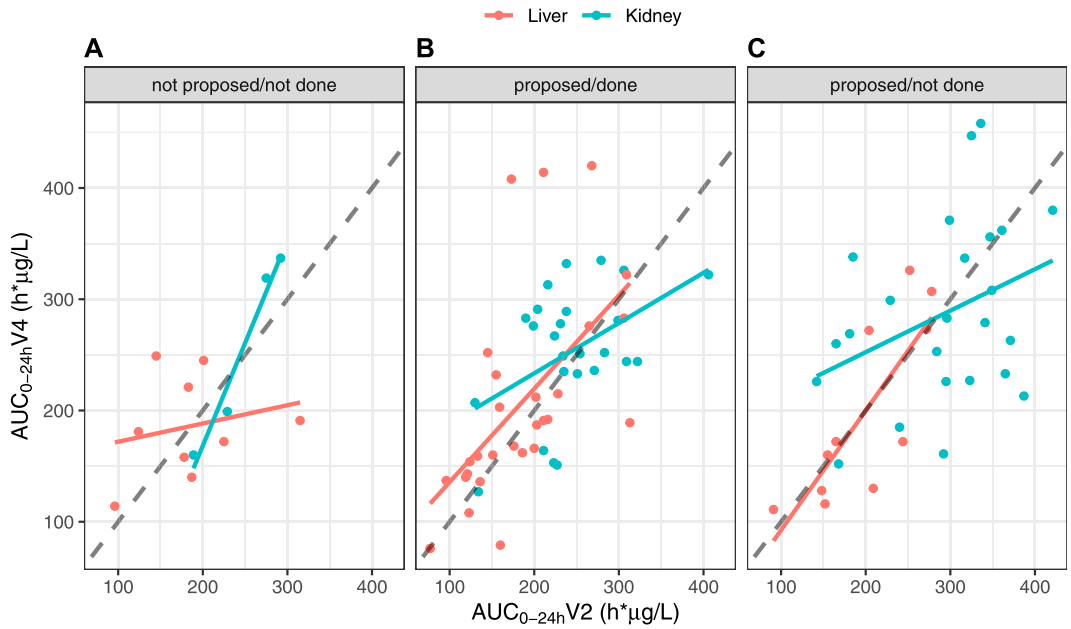


FIGURE 6 | Correlations between $AUC_{0-24\ h}$ values at V4 and V2, split by organ and depending on the scenario, in the FAS: **(A)** Dose adjustment after V3 not proposed and not done; **(B)** Dose adjustment after V3 proposed and done; **(C)** Dose adjustment after V3 proposed but not done.

within, the bioequivalence criteria in LTR. This might partly be due by a lack of power in the subgroup analysis. Additionally, the correlation between the theoretical and actual doses in both contexts of the conversion and dose adjustment proposal was better in KTR than in LTR (**Figure 5**). More precisely, the LTR group tended to receive lower doses than those they were supposed to receive, especially for theoretical doses above 5 mg/day, while KTR overall received the theoretical doses. This lack of compliance may partly explain why the Westlake interval did not meet the bioequivalence criteria for narrow therapeutic index drugs in LTR. Still, the correlation between $AUC_{0-24\ h}$ at V4 and at V2 in LTR who did not need and did not have dose adjustment after the conversion was poorer than that observed in KTR in the same situation (**Figure 6**). This observation clearly suggests that the 1: 0.7 dose conversion ratio is adequate for KTR patients overall but may need to be slightly decreased and followed by dose adjustment in LTR patients. Still, the Westlake interval fell within the larger acceptance interval [0.8–1.25] recommended by the FDA for bioequivalence studies [29].

The poor correlation between $AUC_{0-24\ h}$ at V2 and at the subsequent visits confirms the wide intra-individual variability in tacrolimus exposure [7], which was unfortunately not compensated for by individual dose adjustment. Given the short time of participation in the study, this variability cannot be attributed to long-term tacrolimus clearance variation observed mainly in patients in the late vs early period after transplantation (after M12 vs. before M1). Correlations between C_0 values at V2 and the subsequent visits were even poorer. Although a decrease in the intra-individual variability of tacrolimus exposure on LCP-tacrolimus vs. IR-tacrolimus could be expected, studies in solid organ transplantation have reported comparable intra-patient variability on C_0 [30, 31]. Only one study has reported a significantly lower intra-patient variability of the AUC on LCP-tacrolimus (10.9%) vs. IR-tacrolimus (14.1%) [32]. In any case, the poorer correlation between C_0 values is in favour of considering the $AUC_{0-24\ h}$ rather than C_0, at least when patients are converted from IR-to LCP-tacrolimus, then at regular time points during follow-up.

The poor correlation between $AUC_{0-24\ h}$ values before the switch and afterwards may also be due to the relatively short time period between the switch and the subsequent $AUC_{0-24\ h}$ measurement. It was ≤3 days in 85/101 patients, so that V3 $AUC_{0-24\ h}$ may not reflect steady-state. This may have led to imprecise or even wrong dose recommendations. Furthermore, steady state may not even have been reached at V4 in all patients, as suggested by the poor correlation between $AUC_{0-24\ h}$ at V2 and V4 in LTR who did not need and did not have tacrolimus dose adjustment (**Figure 6**).

Variability may have also come from the use of DBS collected using non-volumetric devices and from the study design, where nurses collected the morning AUC at V2 while the other AUCs were collected by the patients. A comparison of AUC at V3 vs. V4, all sampled by the patients themselves, confirmed the high intra-individual variability (data not shown), dwarfing inter-operator differences as a source of variability. At the time the study was launched, analytical validation data for the measurement of tacrolimus concentrations were available only for the Whatman™ 903 protein saver cards [14, 17, 33]. In the meantime, experience has shown that the insufficient standardization of the volume of blood drops contributes to a relative imprecision of concentration

TABLE 3 | Incidence of adverse events (AE) by system organ class and for each treatment in the safety analysis set.

	IR-tacrolimus		LCP-tacrolimus	
	AEs *n* (%)	Patients *n*[a] (%)	AEs *n* (%)	Patients *n*[a] (%)
	N = 19	*N* = 134	*N* = 50	*N* = 128
Number of patients with at least one AE		11 (8.2)		33 (23.8)
Number of patients with at least one serious AEs	0	0	3 (6.0)	3 (2.3)
Severity				
Mild	12 (63.2)	8 (6.0)	24 (48.0)	18 (14.1)
Moderate	7 (36.8)	4 (3.0)	23 (46.0)	16 (12.5)
Severe	0	0	3 (6.0)	3 (2.3)
MedDRA classification (System Organ Class and Preferred Terms)[b]				
Blood and Lymphatic System Disorders				
Anemia	1 (5.3)	1 (0.7)	1 (2.0)	1 (0.8)
Bicytopenia	—	—	1 (2.0)	1 (0.8)
Leukopenia	2 (10.5)	2 (1.5)	—	—
Lymphopenia	—	—	1 (2.0)	1 (0.8)
Neutropenia	1 (5.3)	1 (0.7)	—	—
Eye Disorders				
Retinal detachment	—	—	1 (2.0)	1 (0.8)
Gastrointestinal Disorders				
Abdominal pain	—	—	2 (4.0)	2 (1.6)
Constipation	1 (5.3)	1 (0.7)	—	—
Diarrhea	—	—	2 (4.0)	2 (1.6)
Dyspepsia	1 (5.3)	1 (0.7)	1 (2.0)	1 (0.8)
Gastrointestinal motility disorder	—		1 (2.0)	1 (0.8)
Gastroesophageal reflux disease	1 (5.3)	1 (0.7)	—	—
Hemorrhoids	1 (5.3)	1 (0.7)	—	—
Mucous stools	—	—	1 (2.0)	1 (0.8)
General Disorders and Administration Site Conditions				
Fatigue	2 (10.5)	2 (1.5)	1 (2.0)	1 (0.8)
Edema peripheral	1 (5.3)	1 (0.7)	—	—
Hepatobiliary Disorders				
Jaundice	—	—	1 (2.0) (severe)	1 (0.8)
Infections and Infestations				
Cytomegalovirus gastrointestinal infection	—	—	1 (2.0)	1 (0.8)
Influenza	—	—	1 (2.0)	1 (0.8)
Sinusitis	—	—	1 (2.0)	1 (0.8)
Investigations				
Alanine aminotransferase increased	—	—	1 (2.0)	1 (0.8)
BK polyomavirus test positive	—	—	1 (2.0)	1 (0.8)
Blood creatinine increased	1 (5.3)	1 (0.7)	3 (6.0)	3 (2.3)
Blood phosphorus decreased	2 (10.5)	2 (1.5)	—	—
Immunosuppressant drug level decreased	—	—	1 (2.0) (severe)	1 (0.8)
Immunosuppressant drug level increased	—	—	2 (4.0)	2 (1.6)
Metabolism and Nutrition Disorders				
Hypercalcemia	1 (5.3)	1 (0.7)	—	—
Hyperglycemia	—	—	1 (2.0)	1 (0.8)
Iron deficiency	—	—	1 (2.0)	1 (0.8)
Vitamin D deficiency	1 (5.3)	1 (0.7)	—	—
Musculoskeletal and Connective Tissue Disorders				
Back pain	1 (5.3)	1 (0.7)	—	—
Tendonitis	—	—	1 (2.0)	1 (0.8)
Nervous System Disorders				
Headache	—	—	1 (2.0)	1 (0.8)
Neuropathy peripheral	—	—	1 (2.0)	1 (0.8)
Sciatica	—	—	1 (2.0)	1 (0.8)
Tremor	1 (5.3)	1 (0.7)	4 (8.0) (1 severe)	4 (3.1)
Psychiatric Disorders				
Anxiety	—	—	1 (2.0)	1 (0.8)
Irritability	—	—	1 (2.0)	1 (0.8)
Nightmare	—	—	1 (2.0)	1 (0.8)

(Continued on following page)

TABLE 3 | (*Continued*) Incidence of adverse events (AE) by system organ class and for each treatment in the safety analysis set.

	IR-tacrolimus		LCP-tacrolimus	
	AEs *n* (%)	Patients *n*[a] (%)	AEs *n* (%)	Patients *n*[a] (%)
	***N* = 19**	***N* = 134**	***N* = 50**	***N* = 128**
Renal and Urinary Disorders				
Hematuria	—	—	1 (2.0)	1 (0.8)
Pollakiuria	—	—	1 (2.0)	1 (0.8)
Renal failure	—	—	1 (2.0)	1 (0.8)
Urine abnormality	—	—	1 (2.0)	1 (0.8)
Reproductive System and Breast Disorders				
Testicular swelling	—	—	1 (2.0)	1 (0.8)
Respiratory, Thoracic and Mediastinal Disorders				
Cough	—	—	1 (2.0)	1 (0.8)
Lung disorder	—	—	1 (2.0)	1 (0.8)
Wheezing	—	—	1 (2.0)	1 (0.8)
Skin and Subcutaneous Tissue Disorders				
Pruritus	—	—	2 (4.0)	2 (1.6)
Surgical and Medical Procedures				
Eventration repair	—	—	1 (2.0)	1 (0.8)
Vascular Disorders				
Blood pressure inadequately controlled	—	—	1 (2.0)	1 (0.8)
Hot flush	—	—	1 (2.0)	1 (0.8)
Hypotension	1 (5.3)	1 (0.7)	—	—
Total	**19**		**50**	

[a]*Patients with ≥2 AEs in the same preferred term are counted only once for that preferred term.*
[b]*MedDRA version 23.0.*
Bold characters are for totals.

measurements [22]. Another potential source of imprecision could have been the hematocrit (varying between 26.2% and 47.0% among patients at V2), as no correction of the analytical results was performed based on the hematocrit. Various patient-centered volumetric micro-sampling devices are now favored for the TDM of immunosuppressants [15, 16, 22, 34].

Twenty-two patients (17%) were withdrawn from the FAS because of unexploitable AUCs, mostly due to non-compliance with sampling times or poor quality of the DBS samples. Yet, training and a user manual had been provided to the healthcare teams and patients. This suggests that using home-based collection of microsamples requires may require even more training for certain patients, in order for them to understand the importance of respecting the sampling schedule, rigorously collect sampling information, and proceed to proper sample collection.

Interestingly, significantly lower exposure was observed in LTR compared to KTR. This may be related to the large C_0 target window at inclusion, allowing liver transplant physicians to target lower C_0 than kidney transplant doctors. This hypothesis is confirmed by the lower daily doses received by LTR compared to KTR (**Table 2**; $p < 0.001$). As the individual $AUC_{0-24\,h}$ at V2 was used as a target for LCP-tacrolimus dose adjustment after V3, the lower exposure in LTR compared to KTR was carried forward throughout the study. Of note, no AUC target has been validated so far for either kidney or liver transplant patients in late periods after transplantation; the only proposed $AUC_{0-12\,h}$ target of 150 h.µg/L [7], was derived from a study performed in 100 kidney transplant patients in the early post-transplantation period [35].

The ENVARSWITCH study used an original approach, where the Westlake interval served to compare the mean exposure obtained with the twice daily IR-tacrolimus vs the once daily LCP-tacrolimus formulation at a 0.7 dose ratio and after dose adjustment. The Westlake interval is generally used in bioavailability studies comparing generic to reference formulations, or newer to reference formulations of brand name drugs for instance. The results obtained here allow for the conclusion that LCP-tacrolimus and IR-tacrolimus had bioequivalent AUCs (since C_{max} and T_{max} were not studied), despite the above-mentioned sources of intra-individual variability. This is consistent with previous studies showing that respecting the 1:0.7 dose ratio obviated the need for dose adjustment in the majority of patients [30]. This means that the IR-tacrolimus to LCP-tacrolimus 1:0.7 conversion dose ratio is appropriate on average, in particular in KTR, but may deserve to be refined in LTR. We calculated that the dose ratio that would zero-in the Westlake interval within the acceptance limits for LTR should be 5% lower, i.e., 1:0.665. However, given the small difference and the impossibility of giving each patient a very precise dose, this option was not considered. The best recommendation would therefore be that tacrolimus exposure should be closely monitored in LTR, preferably based on the $AUC_{0-24\,h}$, in order to adjust their dose individually, i.e., to compensate for the largest individual exposure differences.

Finally, a relatively low incidence of adverse events was reported in the ENVARSWITCH study. This is related to the short duration of patient participation in the study, especially while on IR-tacrolimus (mean of 6 days between inclusion at V1 and the switch to LCP-tacrolimus).

In conclusion, while the design of the ENVARSWITCH study does not allow comparing therapeutic drug monitoring strategies

(AUC or C_0 monitoring from venous blood or DBS samples), its results suggest that the combination of DBS and Bayesian estimation for tacrolimus dose adjustment elicits reaching rapidly appropriate exposure to tacrolimus after the switch from IR-tacrolimus to LCP-tacrolimus. The use of volumetric microsampling devices should further improve the reliability of $AUC_{0-24\,h}$ estimation and individual dose adjustment.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comité de Protection des Personnes (ref. CPP16-022/2016-001014-22) and French National Agency for Medicines and Health Products (ref. 160372A-11). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CMo, J-BW, SC, and PMa participated in research design, the writing of the paper, the performance of the research, and data analysis. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

CMo reports receiving lecture fees and support for participation in International Congress from Astellas. SD reports receiving lecture fees from Chiesi, Novartis, Sandoz, Intercept, Astellas, Roche, Ipsen and Abbvie and serving as a board member of Novartis and Biotest. AT reports a research grant from Astellas. MB reports financial support from Chiesi for participation in International Congress. CG has received consulting fees from Chiesi and speaker's honoraria from Astellas. DA has received travel grant and honoraria for lectures from Chiesi. FS has received speaker's honoraria and/or research grants from Chiesi, Novartis, Astellas, Gilead, Neovii, Merck Sharp & Dohme, Pfizer and Baxter. CMa reports research grants, financial support for participation in congresses and expertise fees from Chiesi and Astellas. PMa has received speaker and consultant honoraria and/or research grants from Chiesi, Sandoz, Astellas and BMS.

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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Survival Benefit of Kidney Transplantation in Patients With End-Stage Kidney Disease and Prior Acute Myocardial Infarction

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Patients with end stage kidney disease (ESKD) and a previous acute myocardial infarction (AMI) have less access to KT. Data on ESKD patients with an AMI history who underwent first KT or dialysis between January 2007 and December 2018 were extracted from the Korean National Health Insurance Service. Patients who underwent KT (n = 423) were chronologically matched in a 1:3 ratio with those maintained on dialysis (n = 1,269) at the corresponding dates, based on time-conditional propensity scores. The 1, 5, and 10 years cumulative incidences for all-cause mortality were 12.6%, 39.1%, and 60.1% in the dialysis group and 3.1%, 7.2%, and 14.5% in the KT group. Adjusted hazard ratios (HRs) of KT versus dialysis were 0.17 (95% confidence interval [CI], 0.12–0.24; $p < 0.001$) for mortality and 0.38 (95% CI, 0.23–0.51; $p < 0.001$) for major adverse cardiovascular events (MACE). Of the MACE components, KT was most protective against cardiovascular death (HR, 0.23; 95% CI, 0.12–0.42; $p < 0.001$). Protective effects of KT for all-cause mortality and MACE were consistent across various subgroups, including patients at higher risk (e.g., age >65 years, recent AMI [<6 months], congestive heart failure). KT is associated with lower all-cause mortality and MACE than maintenance dialysis patients with a prior AMI.

Keywords: mortality, acute myocardial infarction, end stage kidney disease (ESKD), kidney transplantation (KT), major adverse cardiovascular events (MACE)

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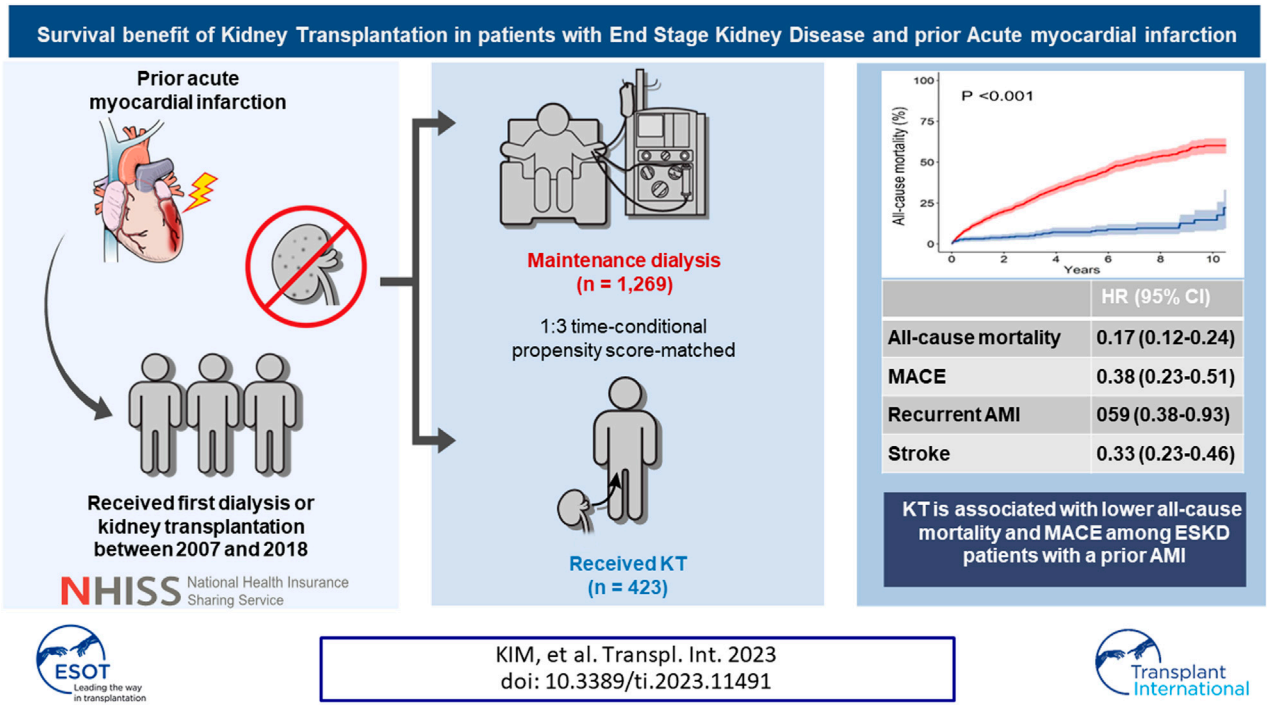
Abbreviations: AMI, acute myocardial infarction; CABG, coronary artery bypass graft; CHF, congestive heart failure; CVD, cardiovascular disease; ESKD, end stage kidney disease; KT, kidney transplantation; MACE, major adverse cardiovascular events; PCI, percutaneous coronary intervention.



GRAPHICAL ABSTRACT |

INTRODUCTION

Cardiovascular disease (CVD) is the most common cause of death in patients with end-stage kidney disease (ESKD) [1]. For patients with ESKD requiring renal replacement therapy, kidney transplantation (KT) is the best treatment option to reduce the risk of CVD [2]. However, approximately 50% of patients with ESKD already have CVD before initiating renal replacement therapy [3]. Furthermore, the number of KT candidates with a history of CVD is gradually increasing because of the increasing number of KT candidates who are older or who have waited for an extended period of time for a deceased donor kidney [4]. Prior CVD history is the strongest risk factor for posttransplant coronary artery disease [5, 6] and affects physicians' decisions regarding whether to proceed with KT.

The Kidney Disease Improving Global Outcome guidelines suggest that patients with ESKD who have CVD can be candidates for KT after appropriate cardiologic evaluation [7]. However, in the real world, patients with CVD have low access to KT, as reported in a French registry study [8]. A United States (US) registry study demonstrated that underlying CVD was more frequent in patients who were not informed about KT than in those who were informed [9]. Furthermore, in a recent Australian study, patients with CVD were half as likely to be waitlisted for deceased donor KT or to undergo living-donor KT, compared with individuals without CVD [10]. This low access to KT may be attributed to both patients and physicians assuming that the comorbid CVD can lead to poorer outcomes from KT than remaining on dialysis; however, the validity of this assumption has not been well investigated.

Among CVD events, acute myocardial infarction (AMI) is one of the strongest risk factors for mortality in patients with ESKD [11]. According to the US Renal Data System report, mortality after AMI in patients with chronic kidney disease stages 4 or 5 was more than 50% after 2 years [12]. To our knowledge, only one study including patients with a prior AMI has compared survival between patients treated with KT and those treated with dialysis. Using an Argentina registry [13], this study showed a survival benefit with KT in patients older than 60 years and with multiple comorbidities. However, less than 20% of patients included in the analyses had a previous AMI, emphasizing the need for more studies to inform physician decisions about KT in patients with prior AMI. Moreover, AMI is distinct from other comorbidities when considering KT because of the possibility for postoperative acute CVD events, as well as the risk of bleeding associated with potent antiplatelet agents [14, 15].

To determine an optimal treatment strategy for patients with ESKD who have a history of AMI, it is necessary to compare major adverse cardiovascular events (MACE) and mortality after KT with the same outcomes in patients remaining on dialysis. Therefore, we used a nationwide database to compare the survival benefit of KT with that of maintenance dialysis in patients with ESKD and a prior AMI.

PATIENTS AND METHODS

Data Sources

The Korean government uses the National Health Insurance Service (NHIS) database, which covers 97% of all citizens (almost 50 million people) in the Republic of Korea. All hospitals in Korea send information about inpatient and outpatient visitations, procedures, prescriptions, and national health examination data to the NHIS. The NHIS then assigns diagnosis codes based on the International Classification of Disease (ICD), 10th edition. These data resources are widely validated and used for epidemiologic studies [16]. The NHIS provides information from claims data for research purposes and includes mortality records with the cause and date of death, which are retrieved from the Statistics Korea database[1]. Data are available with the approval and oversight of the NHIS (NHIS-2019-1-448) through the Korean National Health Insurance Sharing Service[2]. The specific codes used to define every diagnosis, procedure, and drug in this study are shown in **Supplementary Tables S1, S2**.

Study Population

This study used NHIS data of patients newly diagnosed with ESKD (defined as requiring hemodialysis, peritoneal dialysis, and/or KT) between January 2007 and December 2018. As KT was usually performed after a period of dialysis treatment (except for cases of pre-emptive KT), comparing KT and dialysis based on the date of ESKD diagnosis would inevitably lead to immortal time bias of patients receiving KT, thereby resulting in an overestimation of the survival of these patients [17]. To minimize this bias, we applied a "prevalent new user design," which has been used in pharmacoepidemiology. Treating ESKD as a "disease," dialysis as a "former drug," and KT as a "new drug," in accordance with the components of a prevalent new user design, we established separate time-based exposure cohorts for dialysis and KT [18, 19]. The time interval of ±3 months surrounding the date of KT was used to select the dialysis control patients (**Supplementary Figure S1**). The cohort entry date was defined as the KT date for the KT cohort and the corresponding date of dialysis prescription for the dialysis cohort. When patients included in the dialysis cohort at certain cohort entry dates subsequently underwent KT, they were censored and reused as KT subjects based on the date of KT. This provides an intention-to-treat approach for comparing the effects of proceeding with KT versus continuing on dialysis alone or waiting for further KT at the given entry date. Baseline characteristics, including prior AMI and exclusion criteria, were based on the cohort entry date of each subject. Prior AMI was defined as the first diagnosis of AMI with a hospital admission duration of >2 days.

In this study, we included only patients with a prior AMI within 5 years before each cohort entry date. We excluded patients who were <19 or >75 years of age at the time of cohort entry. Patients diagnosed with cancer (because of its effects on KT eligibility) and those diagnosed with stroke, valvular heart disease, and/or cardiac conduction abnormality (because of the effects of these non-AMI CVDs on KT accessibility and outcomes) within 5 years before cohort entry were also excluded. In addition, patients receiving dialysis for >10 years before KT were excluded to eliminate individuals in excellent medical condition while on dialysis, who then received KT.

Matching

The KT and dialysis cohorts were matched according to these steps: 1) the dialysis date corresponding to the KT date was set as the cohort entry date in the dialysis cohort, 2) exclusion criteria were applied based on the cohort entry date, 3) only patients with an AMI within 5 years before cohort entry were selected, and 4) dialysis patients were matched to KT patients based on time-conditional propensity scores calculated using conditional logistic regression stratified by dialysis cohort or KT cohort [20]. The covariates used for generating the time-conditional propensity scores were age, sex, diabetes mellitus, calendar year of ESKD diagnosis, calendar year of cohort entry date, interval from ESKD to cohort entry date, interval from AMI to cohort entry date, type of AMI treatment (percutaneous coronary intervention [PCI], coronary artery bypass graft [CABG], or medication only), and secondary prevention drugs after AMI (angiotensin converting enzyme inhibitors or angiotensin receptor blockers, beta blockers, statins, antiplatelet agents (aspirin, clopidogrel, cilostazole, ticlopidine, prasugrel, ticagrelor, or triflusal) or calcium channel blockers). Use of a drug was defined as being prescribed the drug >2 times during outpatient visits within 1 year before cohort entry.

Patients with underlying conditions were matched according to the Charlson Comorbidity Index (CCI) calculated using data from the 5 years period before cohort entry [13, 14]. Diabetes mellitus and congestive heart failure (CHF) were matched separately from CCI because of their prominent effects on CVD and survival in patients with ESKD. Matching was performed with a 1:3 ratio, without replacement, and in chronological order. If a matched dialysis subject underwent KT during follow-up, the patient was censored at the time of KT, then included in the KT cohort and matched with other patients in the dialysis cohort based on the newly designated entry date (KT date).

Outcomes

The primary outcome of this study was all-cause mortality and MACE, which was a composite of cardiovascular mortality, recurrent AMI, and stroke. The secondary outcomes were each component of MACE and a coronary revascularization procedure (PCI or CABG). Cardiovascular mortality was defined as any death with an ICD-10 code of I00–I99, as confirmed in the Statistics Korea database. Recurrent AMI was defined as hospitalization for the AMI diagnosis code and/or coronary revascularization. The study population was followed from each cohort entry date until the date of death, 31 December

[1]http://mdis.kostat.go.kr
[2]http://nhiss.nhis.or.kr

2018, or the date of subsequent KT (in the dialysis cohort), whichever came first.

Statistical Analysis

Matching on time-conditional propensity scores was performed with greedy (nearest neighbor) matching techniques [21]. Covariate balances were considered adequate when standardized mean differences after matching were <0.1 [22]. Baseline characteristics were compared between the KT and matched dialysis groups using the t-test or chi-squared test, as appropriate. Continuous variables were expressed as mean ± standard deviation, and categorical variables were expressed as number (percentage). Kaplan–Meier survival curves with the log-rank test were used to compare cumulative outcome incidences. Hazard ratios for each outcome were obtained before and after being adjusted for baseline characteristics using Cox proportional-hazard regression analysis. Death from causes other than CVD and loss to follow-up were considered as competing risks when comparing MACE and each component. Moreover, regression analyses were performed by Fine and Gray's model for those outcomes. Sensitivity analyses were performed in various subgroups for all-cause mortality and MACE: age (<65 vs. ≥65 years), sex, AMI treatment method (PCI/CABG vs. medication alone), interval from AMI to cohort entry date (<6 vs. 6–12 vs. ≥12 months), year of cohort entry date (2007–2012 vs. 2013–2018), interval from ESKD to cohort entry date (<1 vs. 2–5 vs. 5–10 years), CCI (<9 vs. ≥9), and CHF (presence or absence). The sensitivity of the effect of KT was analyzed by creating an interaction of the *p*-value between KT versus non-KT and each subgroup. Furthermore, to confirm whether KT adversely affected outcomes during the early post-KT period, we performed several independent analyses (<3, <6, and <12 months after cohort entry), where administrative censoring was applied to the maximum time point (or earlier if the patient was lost to follow-up).

All p values were two-sided, and p values <0.05 were considered significant. Analyses were performed using the statistical package SAS 9.4 (SAS Institute, Cary, NC, United States) and R version 4.2.0 for Windows[3].

The Institutional Review Board (IRB) of the Yonsei University Wonju College of Medicine (Wonju, Korea) approved this study (IRB number: CR319308). Informed consent was waived because anonymous and de-identified information was used for the analyses. This trial was registered with the Clinical Research Information Service, Republic of Korea (KCT0005759).

RESULTS

Patient Characteristics

Of the 331,994 first diagnosed with ESKD during the study period, 325,785 were in the dialysis cohort and 13,428 were in the KT cohort (**Figure 1**). From these, a 1:3 matched ESKD population with prior AMI were included in the comparative analyses: 1,269 dialysis patients were matched to 423 KT patients based on time-conditional propensity scores with appropriate balance (**Supplementary Figure S2**). Baseline characteristics are shown in **Table 1**. The mean age was 52.3 ± 10.8 years for the dialysis group and 53.3 ± 11.1 years for the KT group ($p = 0.979$). Men were more frequent in both groups (73.8% in the dialysis group vs. 73.5% in the KT group; $p = 0.924$). Year of first ESKD diagnosis and cohort entry date were similar between the two groups. As a result of chronologic matching, the interval from ESKD diagnosis to cohort entry date was similar between the two groups, not only when stratified by <1 year, 1–5 years and 5–10 years, but also when mean values were compared (30.6 ± 29.1 months vs. 33.1 ± 29.7 months; $p = 0.482$). Mean interval from AMI to cohort entry date was 25.0 ± 18.1 months in the dialysis group and 23.9 ± 18.8 months in the KT group. Additionally, the two groups had similar treatment modalities for their prior AMI (CABG [7.0% vs. 8.0%], PCI [44.7% vs. 40.0%], and medical treatment alone [48.3% vs. 52.0%]; $p = 0.226$), which were consistent with AMI treatment distributions previously reported in patients with chronic kidney disease [23, 24]. Secondary prevention drugs after AMI were used in similar percentages of patients at cohort entry in both groups. The mean CCI value was more than 8 and similar in both groups (8.7 ± 2.6 vs. 8.6 ± 2.4; $p = 0.600$). The frequencies of each CCI component were similar between groups, except peripheral vascular disease, dementia, and hemi- or paraplegia.

Details of Kidney Transplantation Recipients

Of the 423 patients who underwent KT, 185 (43.7%) received <1 year of pre-transplant dialysis before KT, including 66 (15.6%) who underwent pre-emptive KT. The median pre-transplant dialysis duration was 29.6 (interquartile range, 9.7–57.4) months. There were 9 (2.1%) in-hospital deaths after KT: 6 were due to recurrent AMI and 3 were from an unknown cause. There were 13 (3.1%) in-hospital MACE, including 6 cardiovascular deaths and 7 cases of coronary artery disease treated with PCI. The cumulative incidences of graft failure (restart of dialysis or re-transplantation) were 2.4%, 5.2%, and 8.9% at 1, 5, and 10 years after KT, respectively (**Supplementary Figure S3**).

Primary Outcomes

During the mean follow-up period of 48.3 ± 38.6 months (dialysis group, 45.7 ± 37.7 months; KT group, 61.3 ± 40.7 months), 542 patients in the dialysis group and 41 patients in the KT group died, representing incidence rates of 112.2 and 19.0 per 1,000 person-years, respectively. Except for unknown cause, the most common cause of mortality was CVD, followed by cancer and infection in both groups (**Supplementary Figure S4**). All-cause mortality was significantly lower in the KT group than in the dialysis group ($p < 0.001$) based on Kaplan–Meier curve analysis (**Figure 2**). The 1, 5, and 10 years cumulative incidences of all-cause mortality were 12.6%, 39.1%, and 60.1% in the dialysis group and 3.1%, 7.2%, and 14.5% in the KT group (**Table 2**). The adjusted hazard ratio (HR) of KT for all-cause mortality was 0.17, with a 95% confidence interval (CI) of 0.12–0.24 ($p < 0.001$).

[3]http://cran.r-progect.org/

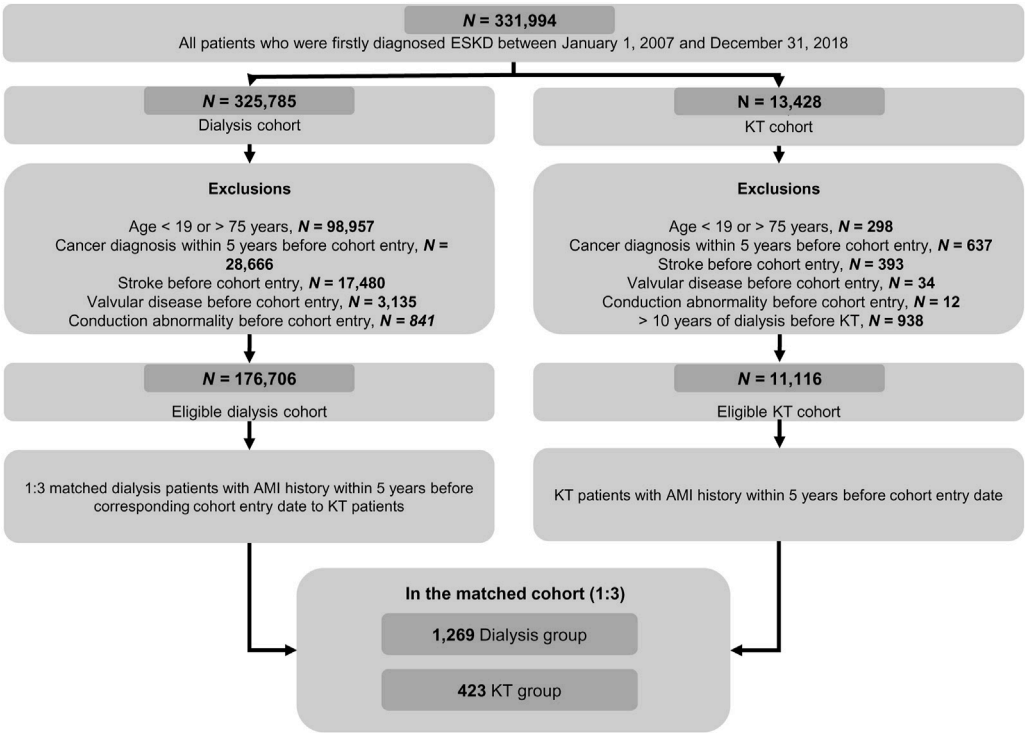


FIGURE 1 | Flow diagram showing selection of the study population from the Korean National Health Insurance Service Database. AMI, acute myocardial infarction; ESKD, end-stage kidney disease; KT, kidney transplantation.

The incidence of MACE was also significantly lower in the KT group than in the dialysis group ($p < 0.001$; **Figure 2**). The 1, 5, and 10 years cumulative incidences of MACE were 15.6%, 37.6%, and 52.1% in the dialysis group and 6.6%, 13.8%, and 29.1% in the KT group. The adjusted HR of KT for MACE was 0.38, with a 95% CI of 0.23–0.51 ($p < 0.001$; **Table 2**).

Secondary Outcomes

The incidences of all MACE components were significantly lower in the KT group than in the matched dialysis controls (**Figure 2** and **Table 2**). KT provided the most protection against cardiovascular death, as indicated by the lowest subdistribution HR (HR, 0.23 [95% CI, 0.12–0.42]; $p < 0.001$). For cardiovascular mortality, the 1, 5, and 10 years cumulative incidences were 3.5%, 11.3%, and 20.5% in the dialysis group and 0.7%, 1.2%, and 9.5% in the KT group. KT was also protective against recurrent AMI (HR, 0.59 [95% CI, 0.38–0.93]; $p = 0.023$) and stroke (HR, 0.33 [95% CI, 0.23–0.46]; $p < 0.001$), compared with maintaining on dialysis. Additionally, the incidence of coronary revascularization (PCI or CABG), regardless of the specific diagnosis, was significantly lower in the KT group than in the dialysis group (HR, 0.38 [95% CI, 0.27–0.52]; $p < 0.001$).

Sensitivity Analyses

The protective effects of KT for all-cause mortality and MACE were seen in all subgroups, especially in higher-risk patients, such as those >65 years of age, patients with an interval from AMI to cohort entry date of <6 months, and those with CHF (**Figure 3**). However, when compared within the stratified time intervals during the early period after cohort entry, the KT group had a higher risk of recurrent AMI in the first 3 months post-KT, compared with the dialysis group (HR, 3.30 [95% CI, 1.46–7.47]; $p = 0.004$) (**Supplementary Tables S3, S4**).

DISCUSSION

To our knowledge, this study is the first population-based cohort study that used nationally representative data to compare all-cause mortality and MACE in patients with ESKD and a prior AMI between patients treated with KT and those maintained on dialysis. KT was associated with a survival benefit in patients with ESKD and an AMI history at certain time points, compared with chronologically matched patients who remained on dialysis at the corresponding time points during the course of their ESKD. Additionally, our results suggested that KT reduced the risk of MACE (overall and all components) in patients with ESKD and a prior AMI, compared with maintenance dialysis. Of the individual MACE components, cardiovascular mortality decreased the most in patients who underwent KT. The beneficial effects of KT for all-cause mortality and MACE were consistent across various subgroups, including patients >65 years, those with a recent (<6 months) AMI, and

TABLE 1 | Baseline characteristics between matched ESKD patients with AMI history.

Variables	Dialysis (*n* = 1,269)	KT (*n* = 423)	*P*
Age	52.3 ± 10.8	53.3 ± 11.1	0.979
Sex, male	936 (73.8)	311 (73.5)	0.924
Year of first ESKD diagnosis			0.188
2007–2012	906 (71.39)	316 (74.7)	
2013–2018	363 (28.61)	107 (25.3)	
Year of cohort entry date			0.998
2007–2012	471 (37.12)	157 (37.12)	
2013–2018	798 (62.88)	266 (62.88)	
Interval from ESKD to cohort entry date			0.999
<1 year	555 (43.7)	185 (43.7)	
1–5 years	525 (41.4)	175 (41.4)	
5–10 years	189 (14.9)	63 (14.9)	
Mean, month	30.6 ± 29.1	33.1 ± 29.7	0.482
Interval from AMI to cohort entry date, months	25.0 ± 18.1	23.9 ± 18.8	0.257
AMI treatment			0.226
CABG	89 (7.0)	34 (8.0)	
PCI	567 (44.7)	169 (40.0)	
Medical treatment	613 (48.3)	220 (52.0)	
Secondary preventive drugs after AMI			
ACEi or ARB	941 (74.2)	313 (74.0)	0.949
Beta blocker	958 (75.5)	319 (75.4)	0.974
Statin	846 (66.7)	265 (62.7)	0.132
Antiplatelet agent	896 (70.6)	287 (67.9)	0.819
Calcium channel blocker	1,124 (88.6)	385 (91.0)	0.284
Charlson Comorbidity Index	8.7 ± 2.6	8.6 ± 2.4	0.600
Diabetes	1,117 (88.0)	375 (88.7)	0.728
Congestive heart failure	761 (60.0)	238 (56.3)	0.180
Peripheral vascular disease	645 (50.8)	184 (43.5)	0.009
Dementia	64 (5.0)	9 (2.1)	0.011
Chronic pulmonary disease	763 (60.1)	249 (58.9)	0.647
Rheumatologic disease	114 (9.0)	46 (10.9)	0.250
Peptic ulcer disease	758 (59.7)	268 (63.4)	0.186
Mild liver disease	713 (56.2)	250 (59.1)	0.294
Moderate or severe liver disease	40 (3.2)	17 (4.0)	0.392
Hemiplegia or paraplegia	54 (4.3)	9 (2.1)	0.045
AIDS	0 (0.0)	2 (0.5)	0.062

Abbreviations: ACEi, angiotensin converting enzyme inhibitors; AIDS, acquired immune deficiency syndrome; AMI, acute myocardial infarction; ARBs, angiotensin receptor blockers; CABG, coronary artery bypass graft; ESKD, end stage kidney disease; KT, kidney transplantation; PCI, percutaneous coronary intervention.

patients with CHF, all of whom are considered at much higher risk for adverse events following KT. Our results, therefore, suggest that clinicians should actively consider KT for patients with ESKD who have survived a prior AMI.

In previous national cohort studies, the presence of multiple comorbidities was associated with reduced access to KT in patients with ESKD [8–10, 25]. This low access likely reflects clinicians assuming that KT in patients with multiple comorbidities can result in poorer survival than remaining on dialysis. In this regard, studies in Denmark and Argentina demonstrated the clinical relevance of recommending KT, even in patients with multiple comorbidities [13, 26]. However, the survival benefits of KT in patients with ESKD who survived a prior AMI have not been fully investigated. To help fill this knowledge gap, the current study provides evidence in support of the use of KT in patients with a previous AMI.

A major strength of this study was that we compared KT patients with chronologically matched dialysis controls who had similar underlying conditions, including a prior AMI, at similar time points during the course of ESKD. Because most KT patients underwent varying durations of dialysis before transplantation, a standard retrospective study design would inevitably lead to immortal time bias between the initial diagnosis of ESKD and the KT procedure. We minimized potential bias by using a prevalent new user design and matching on time-conditional propensity scores, as has been excellently described by Suissa et al [17–19]. Time-dependent Cox analysis adjusted the hazard ratio by considering time-dependent covariates before and after the reference point at the time of KT. On the other hand, in the case of prevalent new user design, chronological matching was performed to reflect the patient's status at each time point during each period. This method more accurately aligned the

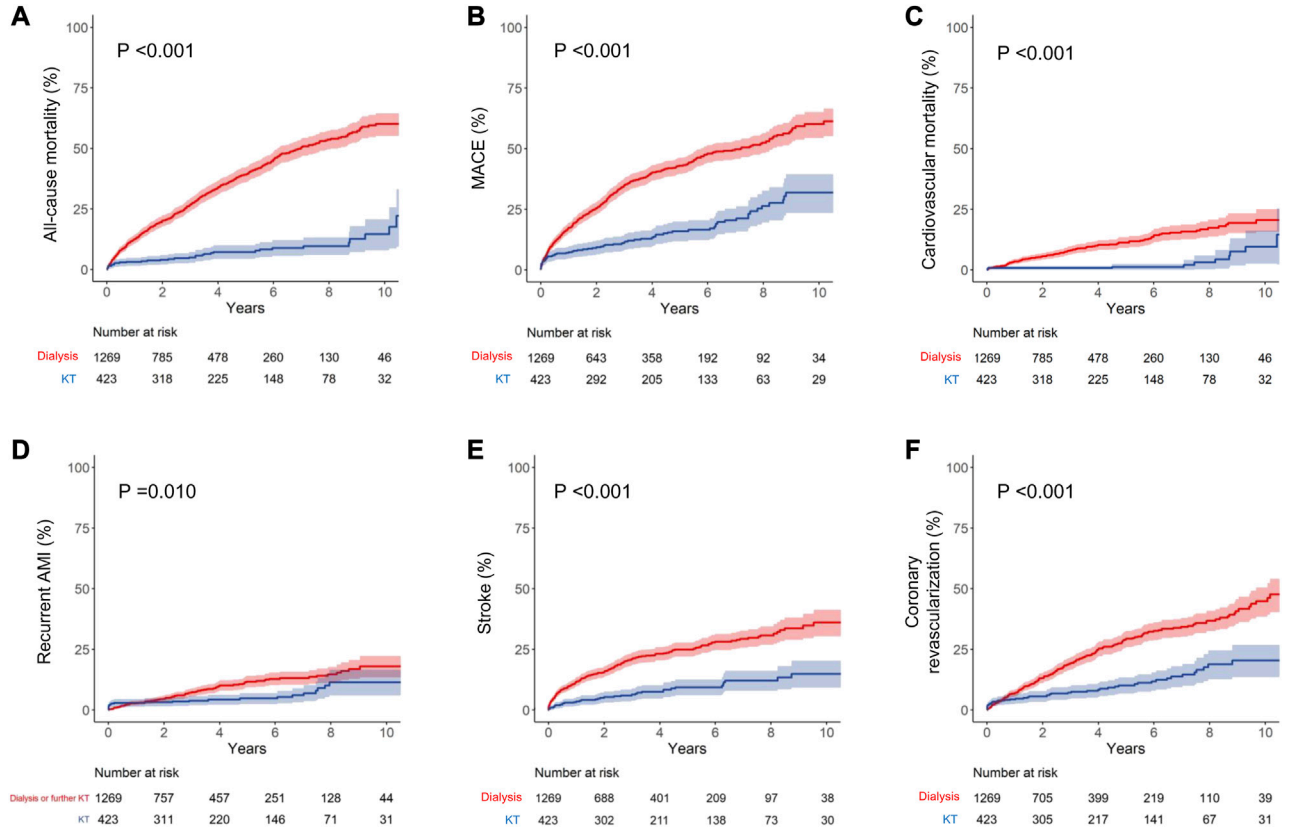


FIGURE 2 | Kaplan–Meier curve analyses for cumulative incidence of each outcome. **(A)** All-cause mortality, **(B)** MACE, **(C)** cardiovascular mortality, **(D)** recurrent AMI, **(E)** stroke, and **(F)** coronary revascularization. Dialysis group data are shown in red and KT group data are shown in blue. MACE is the composite outcome of cardiovascular mortality, non-fatal AMI, and stroke. AMI, acute myocardial infarction; KT, kidney transplantation; MACE, major adverse cardiovascular events.

TABLE 2 | Adjusted hazard ratios of KT for outcomes versus two dialysis control groups.

Outcomes		Cumulative incidence			Fine & gray model			
		1 year	5 years	10 years	Unadjusted sHR (95% CI)	*P*	Adjusted sHR[a] (95% CI)	*P*
All-cause mortality	Dialysis	12.6	39.1	60.1	Reference		Reference	
	KT	3.1	7.2	14.5	0.17 (0.12–0.24)	<0.001	0.17 (0.12–0.24)	<0.001
MACE[b]	Dialysis	15.6	37.6	52.1	Reference		Reference	
	KT	6.6	13.8	29.1	0.37 (0.28–0.48)	<0.001	0.38 (0.23–0.51)	<0.001
Cardiovascular mortality	Dialysis	3.5	11.3	20.5	Reference		Reference	
	KT	0.7	1.2	9.5	0.22 (0.12–0.41)	<0.001	0.23 (0.12–0.42)	<0.001
Recurrent AMI	Dialysis	2.8	11.7	18.0	Reference		Reference	
	KT	2.9	4.7	11.3	0.56 (0.36–0.87)	0.011	0.59 (0.38–0.93)	0.023
Stroke	Dialysis	11.3	24.5	35.7	Reference		Reference	
	KT	3.5	9.3	14.9	0.34 (0.24–0.48)	<0.001	0.33 (0.23–0.46)	<0.001
Coronary revascularization	Dialysis	6.9	29.3	44.8	Reference		Reference	
	KT	4.4	10.1	20.4	0.38 (0.28–0.52)	<0.001	0.38 (0.27–0.52)	<0.001

For MACE, cardiovascular death, recurrent AMI, stroke, coronary revascularization, other causes of mortality except for CVD, and follow-up loss were considered competing risks. Moreover, regression analyses were performed by Fine and Gray's model for those outcomes.

Abbreviations: AMI, acute myocardial infarction; CI, confidence intervals; CVD, cardiovascular disease; MACE, major cardiovascular events; KT, kidney transplantation; sHR, subdistribution hazard ratio.

[a]*Adjusted by age, gender, Charlson comorbidity index, interval from AMI to KT or dialysis, and type of AMI treatment. Year of index date.*

[b]*MACE means the composite outcome of cardiovascular death, non-fatal AMI, and stroke.*

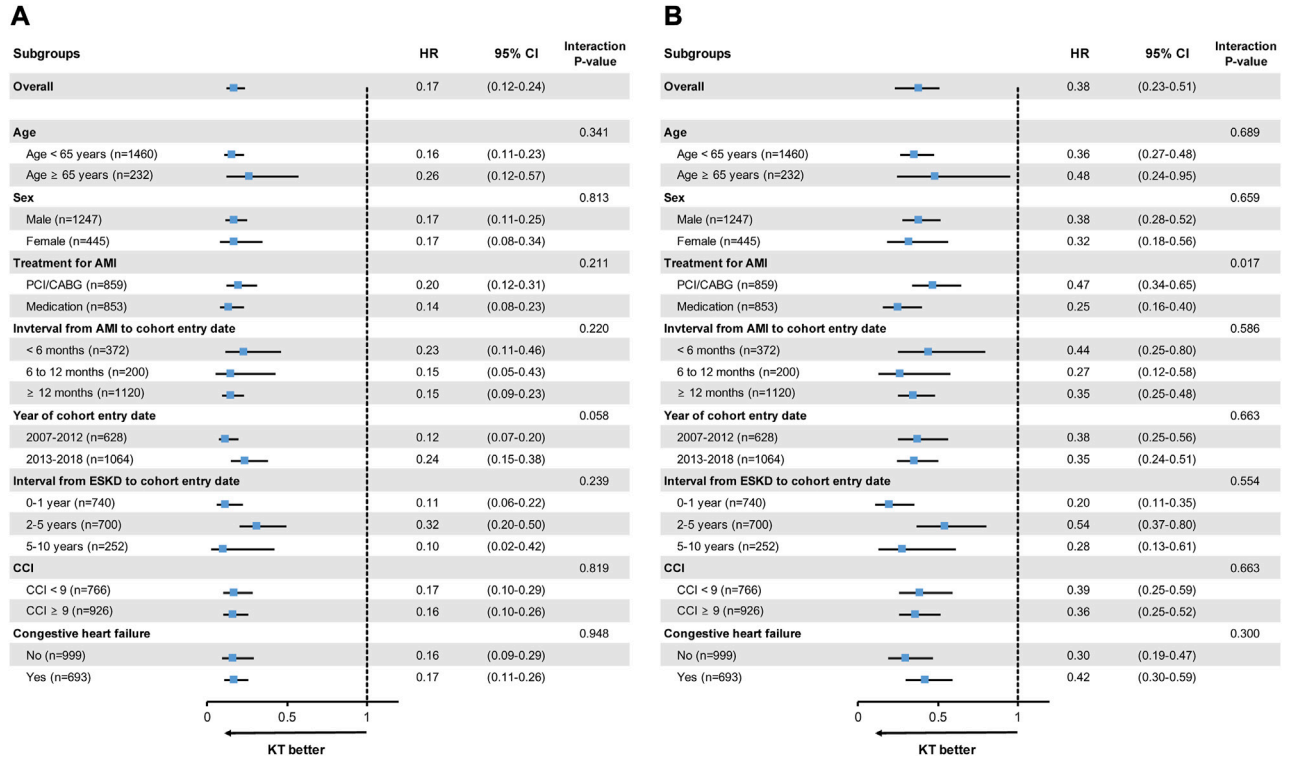


FIGURE 3 | Subgroup analyses for **(A)** all-cause mortality and **(B)** MACE. MACE is the composite outcome of cardiovascular death, non-fatal AMI, and stroke. AMI, acute myocardial infarction; CABG, coronary artery bypass graft; CCI, Charlson Comorbidity Index; CI, confidence interval; ESKD, end-stage kidney disease; HR, hazard ratio; KT, kidney transplantation; MACE, major adverse cardiovascular events; PCI, percutaneous coronary intervention.

time-dependent coefficients and better reflected the characteristics ESKD patients at a specific time point.

Due to limitations in the data characteristics, we were unable to extract the KT waitlist from NHIS data. While it would be more valid to compare outcomes with wait-listed dialysis patients, it is justified to use all-propensity matched dialysis patients as a control group. Therefore, we established matched controls from the entire pool of dialysis patients in one nation, instead of a waiting-list group of patients. From the perspective of nephrologists and transplant surgeons, waiting-list patients are a specially selected population who are planning to proceed with KT, regardless of donor type. Waiting-list analysis is suitable for investigating the benefits of a specific type of KT, such as lymphocyte cross match [27] or ABO-incompatible living-donor KT [28]; however, it cannot help decide whether to proceed with KT (i.e., begin waiting for a deceased donor or undergo living-donor KT) in patients with ESKD and multiple comorbidities, whose access to transplantation would be low. Thus, our study was designed to compare patients who underwent KT with those maintained on dialysis at the corresponding date, regardless of whether they were waitlisted or received a transplant at a later date. To clarify the impact on outcome, disease entity was restricted to AMI, maintaining disease homogeneity. Our findings showed the superiority of KT over dialysis, even for patients with an AMI history, at specific time points after ESKD diagnosis.

A prior study using the US Renal Data System showed that the cumulative incidence of AMI in patients who underwent KT, regardless of whether they received a deceased donor and living-donor kidney, was higher than that of patients on dialysis maintenance until approximately 1 year after KT [29]. Over time, the incidence of AMI in patients who underwent KT eventually became lower than that in patients on maintenance dialysis. Indeed, KT patients have several risk factors for MACE, especially during the early post-transplantation period, such as the stress of surgery, the high dose of immunosuppressive medications, and the possibility of early graft dysfunction [30]. In our study, recurrent AMI in KT patients with an AMI history was also significantly higher than that of dialysis patients in the first 3 months after cohort entry. Given that the overall incidence of recurrent AMI, as well as other MACE components, can be reduced by KT, the risk of recurrent AMI during the early post-transplantation period should not be the reason for automatically avoiding KT in this higher-risk ESKD population. However, clinicians should be cautious about the possibility of early recurrent AMI and monitor patients closely to allow prompt detection of this event.

Given that intravenous contrast and CABG surgery negatively affect residual renal function, patients with ESKD (including those receiving or not receiving dialysis) are less likely to undergo diagnostic coronary angiography or coronary revascularization after AMI [24]. In our study, half of the

patients with ESKD and an AMI history did not undergo CABG or PCI and received only medical treatment. For patients with chronic kidney disease, guidelines recommend standard treatment, regardless of renal function, in the setting of ST-elevation MI; however, in the setting of non-ST-elevation MI, there is insufficient evidence to recommend standard therapy, especially for patients with ESKD [31, 32]. It is difficult to say which treatment is superior for patients with ESKD and an AMI history because prognosis varies depending on the individual circumstances, such as the presence of left anterior descending coronary artery disease [33, 34]. However, regardless of the type of prior AMI treatment (PCI, CABG, or medications alone), subsequent KT showed a survival benefit in our study population.

Dual antiplatelet therapy is usually required for 6 months to 1 year after coronary revascularization by either PCI or CABG [35]. Therefore, in the early post-revascularization period, especially <6 months post-AMI, clinicians are likely reluctant to suggest KT because of the possibility of MACE recurrence or bleeding secondary to antiplatelet therapy. However, our results indicated that KT could be beneficial, even before 6 months after an AMI. Furthermore, the duration and number of antiplatelet agents could be minimized through appropriate stent selection or CABG, thereby reducing the interval from coronary revascularization to KT [36]. In a study from the United Kingdom, of patients who underwent pre-KT assessment with coronary angiography, most revascularization procedures before KT were successful, and the 3 years survival of patients after cardiac revascularization was 88.4% [37]. Considering this report and our results, we suggest that planned KT after a minimized interval with antiplatelet treatment is feasible when patients with ESKD develop AMI.

CHF is closely associated with the general health status of patients with ESKD [38]. When considering KT, CHF is an important factor for determining how well patients tolerate the operation and negatively impacts the likelihood of a clinician considering KT. However, our study showed that among AMI survivors, subgroup with CHF also had a survival benefit from KT. This result provides evidence for more actively planning KT, even in patients with a prior AMI and CHF. However, because information about ejection fraction and New York Heart Failure Association (NYHA) classification was not available for this study, this result should be interpreted with caution.

This study has several limitations. Despite successful matching, we could not completely eliminate selection bias between the KT and dialysis groups because of limited information in the claims database, such as laboratory results, severity of prior AMI, and NYHA functional classes. Another limitation was the lack of information about time-varying CVD risk factors, such as diet, physical activity, and medications during follow-up. We also could not distinguish donor characteristics, such as living or deceased, age, renal function at donation, and underlying disease, all of which are important factors affecting post-transplantation outcomes. Lastly, we could not estimate the likelihood of undergoing KT (especially deceased donor KT after being waitlisted) because the NHIS database does not contain information about the blood group or degree of pre-transplantation sensitization of KT patients.

Despite these limitations, the results of this nationwide population-based cohort study showed that KT was associated with lower all-cause mortality and MACE in patients with ESKD and an AMI history, even in various high-risk subgroups. Thus, KT seems safe among AMI survivors who are planning to receive dialysis or are currently on dialysis, unless another definite contraindication is present.

DATA AVAILABILITY STATEMENT

The database used for this study was provided by the National Health Insurance Service (NHIS) in the Republic of Korea (NHIS-2019-1-448). Only authorized researchers were granted access to the database at the Big Data Research Center of the Big Data Steering Department at the NHIS.

ETHICS STATEMENT

The Institutional Review Board (IRB) of the Yonsei University Wonju College of Medicine (Wonju, Korea) approved this study (IRB number: CR319308). Informed consent was waived because anonymous and de-identified information was used for the analyses. This trial was registered with the Clinical Research Information Service, Republic of Korea (KCT0005759).

AUTHOR CONTRIBUTIONS

Study design: JYL and D-GK; Statistical analysis: SK and D-GK; Supervision/mentoring: D-HC, KK, JL, KH, MK, JY, and BH; Each author contributed important intellectual content during manuscript drafting or revision and agrees to be personally accountable for the individual's own contributions and to ensure that questions pertaining to the accuracy or integrity of any portion of the work.

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.2023.11491/full#supplementary-material

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ORIGINAL RESEARCH
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Influence of Early Postoperative Basal Insulin Treatment and Post-Transplant Diabetes Mellitus Risk on Health-Related Quality of Life in Kidney Transplant Recipients—An Analysis of Data From a Randomized Controlled Trial

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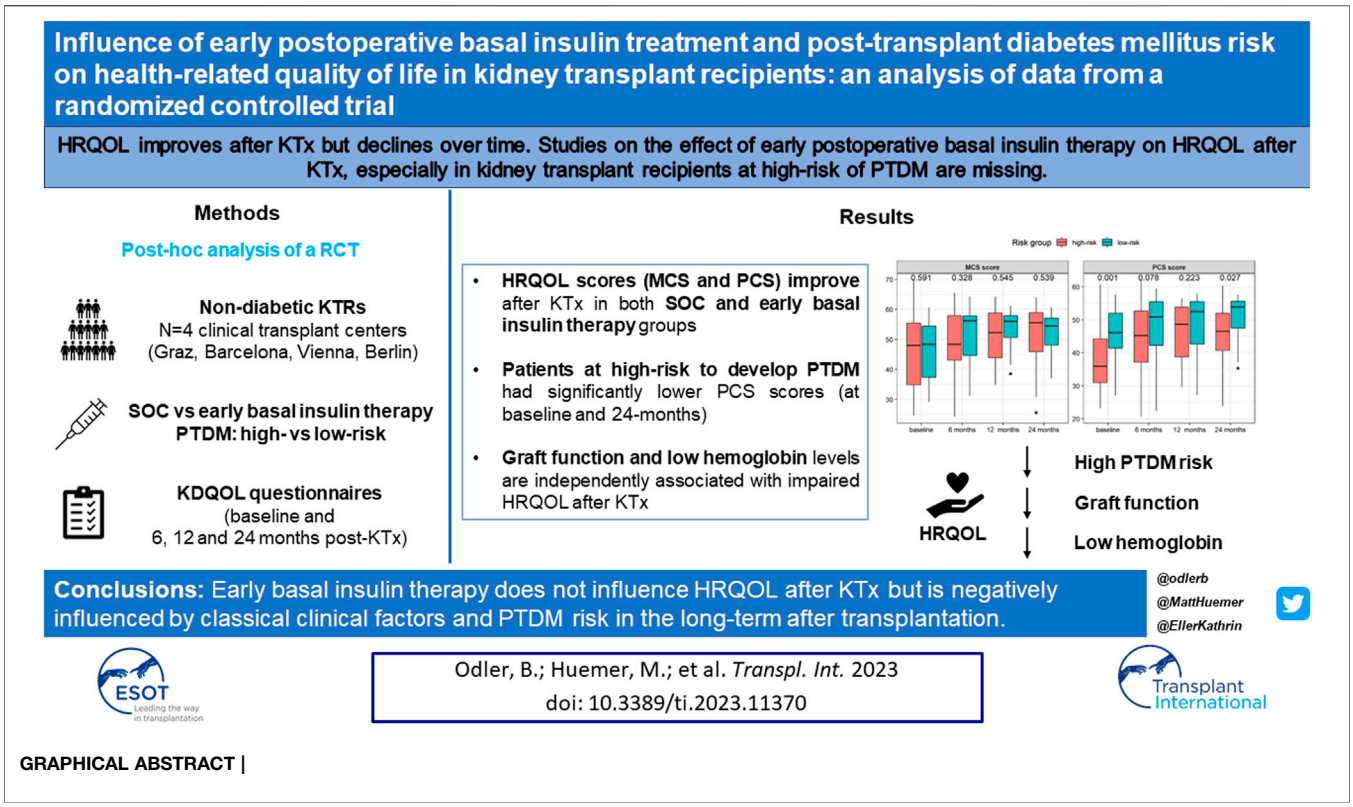
Health-related quality of life (HRQOL) improves after kidney transplantation (KT) but declines over time. Studies on the effect of early postoperative basal insulin therapy on HRQOL after KT, especially KTRs at high risk of developing post-transplant diabetes mellitus (PTDM) are missing. Data from a randomized controlled trial on 148 non-diabetic KTRs were analyzed. HRQOL using the KDQOL-SF™ was compared in KTRs who either received early postoperative basal insulin therapy or standard-of-care and in KTRs at risk of developing PTDM. Determinants of HRQOL outcomes were investigated using multivariable linear regression analysis. In total, 148 patients completed the KDQOL-SF at baseline. Standard-of-care or early basal insulin therapy after KT did not influence HRQOL. Overall, KT improved the mental (MCS) and physical component summary (PCS) scores at 6-month after KT, which remained stable during further follow-up visits. However, patients at high-risk for PTDM had significantly greater impairment in the PCS score (baseline, 24 months) without differences in MCS scores. In the multivariable regression analysis, allograft function and hemoglobin levels were associated with decreased MCS and PCS scores, respectively. A limitation of the study is the fact that only around 50% of the ITP-NODAT study patients participated in the HRQOL evaluation. Still, our data clearly show that early basal insulin therapy does not affect HRQOL after KT but is negatively influenced by classical clinical factors and PTDM-risk at 24 months after KT. The latter might be influenced by older age.

Keywords: kidney transplantation, HRQOL, insulin, PTDM, clinical study



GRAPHICAL ABSTRACT |

INTRODUCTION

Chronic kidney disease (CKD) has a major impact on both physical and mental health, especially in patients with advanced CKD including dialysis dependency [1–4]. Both reduced self-reported and objective physical function as well as mental health are associated with increased mortality rates in this patient population [5]. Kidney transplantation (KT) is considered the optimal and most cost-effective treatment in patients with advanced CKD with improved survival rates and clear benefits on quality of life (QOL) measures [6, 7].

Although clinically relevant improvements are substantive, a proportion of KTRs experience poor health-related quality of life (HRQOL) despite ongoing satisfactory allograft function [8, 9]. In addition, recent evidence suggests a decline in patients' HRQOL in the long-term after KT [10]. Some clinical and psychological factors occurring in a substantial proportion of KTRs such as airflow limitation [11], gastrointestinal symptoms [12], side effects of immunosuppressive drugs [13], or anxiety [9] are well-documented to negatively affect HRQOL.

Post-transplant diabetes mellitus (PTDM) is a frequent complication associated with mortality in individuals after KT [14–16]. While the impact of PTDM on graft loss is debated [17], clear associations on cardiovascular (CV) outcomes, especially in patients with modifiable CV risk factors such as obesity are well known [18–21]. The appropriate management of PTDM remains challenging and has previously been reviewed [22, 23]. Neutral Protamin Hagedorn (NPH) insulin and insulin analogs to treat hyperglycemia are commonly used in the early post-transplant period, however, these require intensive blood glucose monitoring and patients' adherence to avoid therapy-associated adverse events [24, 25].

According to the American Diabetes Association (ADA), HRQOL is a key measure, which should be integrated into the care of patients with diabetes mellitus (DM) in order to improve management and clinical outcomes [26]. Although the occurrence and challenges on treatment of PTDM in KTRs are well recognized, little is known about the impact of this complication on HRQOL of patients after KT. In line, many uncertainties on interventions improving HRQOL of KTRs remain unresolved. Thus, assessing modifiable risk factors and interventions to improve general health status and prevent decline in HRQOL are highly desired.

In this study, we aimed to investigate whether the application of early postoperative basal insulin therapy for the prevention of PTDM might affect HRQOL compared with standard care in KTR using long-term, protocoled HRQOL data obtained in the Insulin Therapy for the Prevention of New Onset Diabetes after Transplantation (ITP-NODAT) study [27]. Moreover, we evaluated the contributors of HRQOL in individuals at high-risk for developing PTDM after KT. Since certain KTRs might benefit from tailored basal insulin therapy, such data are important to allow for more individualized recommendations regarding PTDM prophylaxis strategies after KT.

METHODS

Study Design

The detailed description of the original study protocol (ClinicalTrials.gov registration: NCT03507829) and the primary results have been published previously [27]. Briefly, the ITP-NODAT study was an investigator initiated, open label, prospective, randomized, multi-center clinical trial with an unblinded end-point evaluation to test the efficacy of early postoperative basal insulin therapy for the prevention of PTDM in KTRs. Four clinical transplant centers (Medical University of Vienna, Austria; Medical University of Graz, Austria; Hospital del Mar Barcelona, Spain; Charité Universitätsmedizin Berlin, Germany) participated in the study. Patients were randomized in a 1:1 ratio in each participating center prior to transplantation and stratified by first versus repeated kidney transplant. The 24-month follow-up was finalized in May 2020 and the primary results were published in 2021 [27].

Participants and Interventions

Detailed patient eligibility and the study interventions have been described previously [27]. In brief, n = 263 non-diabetic KTRs receiving standard immunosuppressive therapy (tacrolimus, mycophenolate, and steroids) were included in the study. After randomization, patients were divided into standard of care control and treatment groups. In the standard of care control group, once daily fasting plasma glucose monitoring was performed, and antihyperglycemic treatment was initiated according to the physician`s decision. In contrast, KTRs in the treatment group underwent regular capillary blood glucose monitoring (4-times daily) and received basal insulin therapy with intermediate acting (NPH) insulin (human insulin isophane, Humulin N [Eli Lilly]) combined with short-acting insulin (insulin lispro, Humalog [Eli Lilly]), if the afternoon (pre-supper) glucose values exceeded 140 mg/dL (7.8 mmol/L). Pre-specified dose adjustment schemes for insulin titration and application of antihyperglycemic medication for both study groups, as well as predefined schemes for immunosuppression, were applied in each participating center as described previously [27]. Predefined trial visits were performed at 3, 6, 12, and 24 months after KT.

Study Definitions

PTDM was defined as 2 h post oral glucose tolerance test ≥200 mg/dL (11.1 mmol/L) or hemoglobin A1c (HbA1c) ≥6.5% (48 mmol/mol) according to the ADA guideline criteria [26]. Patients at high risk of developing PTDM after KT were defined using age, serum lipid levels, body mass index (BMI), family history of DM, and the history of polycystic kidney disease (PCKD) based on previously published literature data [27]. Accordingly, patients fulfilling at least one of the following criteria at the time of transplantation were defined as part of the high-risk population:

1. Age ≥ 60 years
2. Age 45–59 plus one of the following criteria: triglycerides ≥200 mg/dL *or* triglycerides 150–200 mg/dL and BMI>27 *or* triglycerides 150–200 mg/dL and high-density lipoprotein (HDL) < 40 mg/dL (men) *or* triglycerides 150–200 mg/dL and HDL<50 mg/dL (women)
3. Family History of DM
4. Polycystic kidney disease (PCKD)

Assessment

HRQOL was evaluated using the kidney disease quality of life short form (KDQOL-SF™) [28]. The KDQOL-SF is a multidimensional patient reported outcome measurement. It is available in different languages including Spanish and German. The questionnaire was developed to assess the health-related disease burden of individuals with CKD and on dialysis with excellent psychometric properties (Cronbach's alpha = 0.61–0.90). The multiple scales of the questionnaire include 43 disease-targeted items focusing on symptoms, effects of kidney disease, burden of kidney disease, work status, cognitive function, quality of social interaction, sexual function, sleep, social support, dialysis staff encouragement, and patient satisfaction. Additionally, the questionnaire includes the short form 36-health survey (SF-36™). The scoring of each scale ranges from zero to 100 and can be calculated if at least 50% of each scales' items were completed by the participant. Higher scores reflect better self-reported QOL.

The KDQOL-SF measurements were self-reported and assessed at baseline, and during the trial visits at 6, 12, and 24-month follow-up. All assessments were carried out in parallel to collection of trial data in the parent trial at the respective study site.

Outcomes

In the original study, pre-specified primary and secondary endpoints were defined at month 12 and 24 post-transplant, respectively and were published previously [27]. The HRQOL was defined as secondary endpoint in the original study as the SF-36 mental component summary (MCS) and SF-36 physical component summary (PCS) scores derived from the KDQOL-SF at 6, 12, and 24 months after KT [27]. Exploratory outcomes include change in the KDQOL-SF subscales in the predefined study groups in the parent trial and in the low- and high-risk groups for PTDM at the same follow-up time points.

Statistical Analysis

Patient characteristics were reported as absolute and relative frequencies for categorical data and for numerical data as means and standard deviation (SD) if normally distributed or medians (range) otherwise. Comparison between groups were done using t-tests, Mann-Whitney U tests, Chi-square, Wilcoxon signed-rank tests or Fisher's exact tests as appropriate. Univariable and multivariable linear regression analyses were performed for physical and mental component scores at 6, 12, and 24 months after kidney transplantation. Treatment group, baseline PCS and MCS scores, risk group (for PTDM), renal function (eGFR), hemoglobin, inflammation (CRP), and glycemic control (HbA1c, OGTT) were included in the univariable analysis as covariates. All variables with a p-value <0.2 in the univariable analysis were included in the multivariable analysis. Beta coefficients were

presented along with their 95% confidence interval (CI). A *p*-value of 0.05 or less was considered statistically significant. All statistical analyses were conducted using R version 4.2.

RESULTS

Patient Characteristics in the Study Groups Stratified by Standard-of-Care and Treatment

The total study sample involved 73 and 75 participants (N = 148 in total, 56.3% overall response rate) in the standard of care and treatment groups, respectively. The low response rate resulted from patient incompliance, if they were unable to fill out the questionnaires or incomplete questionnaires. Baseline characteristics of the participants were balanced between the groups randomized to standard of care control or treatment groups. Participants in the treatment group had a higher proportion of polycystic kidney disease (PCKD) as primary kidney disease and tended to have a higher rate of DM in the family history, and had lower body weight as well as BMI. Patients' characteristics at baseline in the whole cohort and study groups are summarized in **Table 1**.

HRQOL Measures in the Study Groups Stratified by Standard of Care and Treatment

PCS and MCS scores were calculated from available and valid responses for 85 (57%) participants at baseline, 91 (61%) at 6-month, 67 (45%) at 12-month and 61 (41%) at 24-month follow-up. Missing data resulted mainly from patients lost to follow-up or incomplete questionnaires. In both, the standard of care control and the treatment groups, a significant increase in MCS [control: 54.9 (26.0, 63.2) vs. 49.6 (25.6, 67.7), p = 0.046 and treatment: 50.9 (24.3, 65.5) vs. 46.9 (24.6, 69.7), p = 0.004, respectively] and PCS [control: 51.6 (20.7, 62.0) vs. 42.5 (25.1, 60.7), p = 0.001 and treatment: 45.0 (23.0, 59.9) vs. 41.7 (23.2, 57.6) p = 0.015, respectively] scores were observed at 6-month as compared to baseline, which remained stable during the further follow-up visits (**Figure 1**).

Baseline Patient Characteristics in the Low-, and High-Risk Groups

Within the study sample, 65% of the patients were defined as high-risk for developing PTDM. Detailed characteristics of the resampled study sample concerning the risk factors are provided in **Table 2**. The high-risk group included predominantly males (60%, n = 50), had a mean age of 56.4 years (SD: 12.5 years), 41% had glomerular disease as a primary kidney disease and had higher baseline HbA1c. Baseline patient characteristics were similar between high- and low-risk groups (**Table 2**).

HRQOL Measures in the Groups of High- and Low-Risk for PTDM

Patients in the high-risk group had a significantly greater impairment in the PCS scores at baseline [35.9 (23.2, 60.7) vs. 46.1 (27.1, 57.6), $p < 0.001$] and 24 months after transplantation [46.5 (23.9, 60.4) vs. 53.9 (35.3, 57.7), p = 0.027] as shown in **Figure 2**. No significant differences in the MCS scores [baseline: 48.0 (24.6, 69.7) vs. 48.3 (29.1, 60.7), p = 0.591 and 24 months: 55.5 (25.5, 64.0) vs. 54.5 (37.0, 60.7), p = 0.539] were found.

The PCS and MCS scores were comparable between high- and low-risk groups at baseline (**Figure 3A**), only showing a significant difference in the score of physical-role-functioning [0.0 (0.0, 100.0) vs. 50.0 (0.0, 100.0), p = 0.001] at this time-point. At 24 months after transplantation, significant differences in pain [77.5 (20.0, 100.0) vs. 100.0 (32.5, 100.0), p = 0.045], physical-role-functioning [50.0 (0.0, 100.0) vs. 100.0 (0.0, 100.0), p = 0.003], and emotional-role-functioning [100.0 (0.0, 100.0) vs. 100.0 (0.0, 100.0), p = 0.030] between the high-risk and the low-risk groups were found (**Figure 3B**).

Within the disease specific scores of the KDQOL-SF™, there were no significant differences between the scores of the symptom problem list [84.1 (43.2, 100.0) vs. 95.5 (56.8, 100.0), p = 0.064], effects of kidney disease [87.5 (53.1, 100.0) vs. 87.5 (37.5, 100.0), p = 0.453], burden of kidney disease [81.2 (6.2, 100.0) vs. 84.4 (25.0, 100.0), p = 0.946], work status [50.0 (0.0, 100.0) vs. 100.0 (0.0, 100.0), p = 0.076], cognitive function [86.7 (26.7, 100.0) vs. 93.3 (46.7, 100.0), p = 0.671], quality of social interaction [86.7 (46.7, 100.0) vs. 86.7 (53.3, 100.0), p = 0.586], sexual function [62.5 (0.0, 100.0) vs. 100.0 (12.5, 100.0), p = 0.236], sleep [63.8 (32.5, 100.0) vs. 74.2 (25.0, 92.5), p = 0.110], and overall health [80.0 (30.0, 100.0) vs. 80.0 (50.0, 100.0), p = 0.196] at 24 months.

Confounders of HRQOL Measures

In the univariable regression analysis, early postoperative insulin treatment was not significantly associated with the PCS or MCS scores at any timepoint but being in the low-risk group for developing PTDM was significantly related to a better PCS score at 24 months after transplantation (Beta: 5.1, 95% CI: 0.54–9.6, p = 0.029). Hemoglobin, renal function, CRP, baseline PCS and MCS scores, and OGTT were significantly associated with PCS and MCS scores at various timepoints (**Table 3**).

Neither the PCS nor the MCS scores were associated with early postoperative insulin treatment or risk-profile in our multivariable analysis (**Table 4**). However, our model showed a significant association of baseline PCS score at 6 months (Beta: 0.40, 95% CI: 0.04–0.76, p = 0.029) renal function at 12 months (Beta: 0.14, 95% CI: 0.02–0.27, p = 0.025), and hemoglobin at 24 months (Beta: 3.3, 95% CI: 1.50–5.10, $p < 0.001$) after transplantation with PCS score, while baseline MCS score (Beta: 0.32, 95% CI: 0.06–0.59, p = 0.017) and hemoglobin at 6 months (Beta: 2.6, 95% CI: 0.71–4.60, p = 0.009) as well as renal function at 24 months (Beta: 0.19, 95% CI: 0.05–0.32, p = 0.008) after KT were significantly associated with MCS score (**Table 4**).

TABLE 1 | Baseline patient characteristics in the standard of care and treatment groups.

Characteristic	Overall *N* = 148	Control *N* = 73	Treatment *N* = 75	*p*-value
Female	55 (37)	25 (34)	30 (40)	0.5
Age (years)	49.9 (13.9)	50.4 (14.5)	49.4 (13.3)	0.6
Height (cm)	169 (10)	169 (10)	170 (10)	0.6
Weight (kg)	71.2 (63.5, 82.0)	76.4 (66.0, 83.5)	68.4 (63.0, 77.0)	**0.017**
BMI (kg/m^2)	25.5 (4.6)	26.5 (5.2)	24.6 (3.7)	**0.019**
Primary kidney disease				0.054
Glomerular	59 (56)	33 (66)	26 (47)	
Vascular	11 (10)	5 (10)	6 (11)	
Tubulointerstitial	9 (9)	5 (10)	4 (7)	
PCKD	23 (22)	5 (10)	18 (33)	
Other	3 (3)	2 (4)	1 (2)	
Number of previous kidney allografts				0.3
1	126 (85)	60 (82)	66 (88)	
2	20 (14)	12 (16)	8 (11)	
3	1 (1)	0 (0)	1 (1)	
4	1 (1)	1 (1)	0 (0)	
Dialysis prior to KT	134 (91)	63 (86)	71 (95)	0.082
Comorbidities				
Cardiovascular	60 (41)	31 (42)	29 (39)	0.6
Respiratory	11 (7)	7 (10)	4 (5)	0.3
Urinary	14 (10)	8 (11)	6 (8)	0.5
Endocrinological	18 (12)	7 (10)	11 (15)	0.3
Neurological	3 (2)	1 (1)	2 (3)	>0.9
Psychiatrical	8 (5)	1 (1)	7 (9)	0.063
Other	6 (4)	4 (6)	2 (3)	0.4
Laboratory results				
Hemoglobin (g/dL)	11.9 (1.5)	11.6 (1.5)	12.1 (1.5)	0.12
Creatinine (mg/dL)	7.4 (5.8, 9.5)	7.1 (5.4, 9.2)	7.7 (6.1, 9.5)	0.2
eGFR (ml/min/1.73m^2)	7.2 (5.3, 9.3)	7.6 (5.6, 10.1)	6.6 (4.8, 8.8)	0.3
CRP (mg/dL)	2.0 (0.6, 6.7)	1.6 (0.6, 5.8)	2.0 (0.8, 6.8)	0.5
HbA1c (%)	5.2 (4.8, 5.4)	5.3 (4.8, 5.5)	5.1 (4.9, 5.3)	0.4

Statistically significant p*-values in the analysis appear in bold (*p *< 0.05). Continuous variables are expressed as mean (SD) or median (minimum and maximum). Categorical variables are n (%).*

Abbreviations: BMI, body mass index; CRP, C-Reactive Protein; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; KT, kidney transplantation; PCKD, polycystic kidney disease.

DISCUSSION

In this analysis of data from a randomized controlled trial [27], the overall HRQOL of the study population remained stable during the follow-up period but increased significantly in the PCS after KT. In addition, KTRs at high risk of developing PTDM had a significantly greater impairment in physical functioning at baseline and 24 months after KT. Most importantly, early postoperative insulin therapy was not associated with worse HRQOL measures, but kidney allograft function and associated anemia were independent predictors of reduced PCS and MCS scores at certain time points during the 2 years follow-up after KT.

The risk of hypoglycemic complications in PTDM patients was found to be comparable to other types of DM [29], and basal insulin treatment can negatively affect HRQOL by inducing symptomatic albeit mild hypoglycemia [30]. In the ITP-NODAT trial [27], the postoperative basal insulin therapy was initiated early with a preventive intention requiring a stringent risk-benefit assessment. Early basal insulin treatment did show significantly higher hypoglycemic events in the treatment group, but mainly within the first 3 months of post-operative treatment. Some patients escalated their insulin dosage without clear indication resulting in seriously low blood sugar levels and suggesting an insecurity with the handling of insulin at the beginning of the treatment. However, with ongoing treatment no further hypoglycemic events were registered [27]. In accordance, the results of our analysis indicate no negative effect of early basal insulin therapy on the HRQOL of the patients. These data are of critical significance since we need a personalized glucose-lowering therapy for different patient cohorts according to their risk profile without negatively affecting HRQOL.

A possible explanation for why early basal insulin therapy does not affect HRQOL in KT recipients despite having similar diabetes and hypoglycemic associated complications may be a stronger positive effect of KT itself [29, 30]. Basal insulin therapy

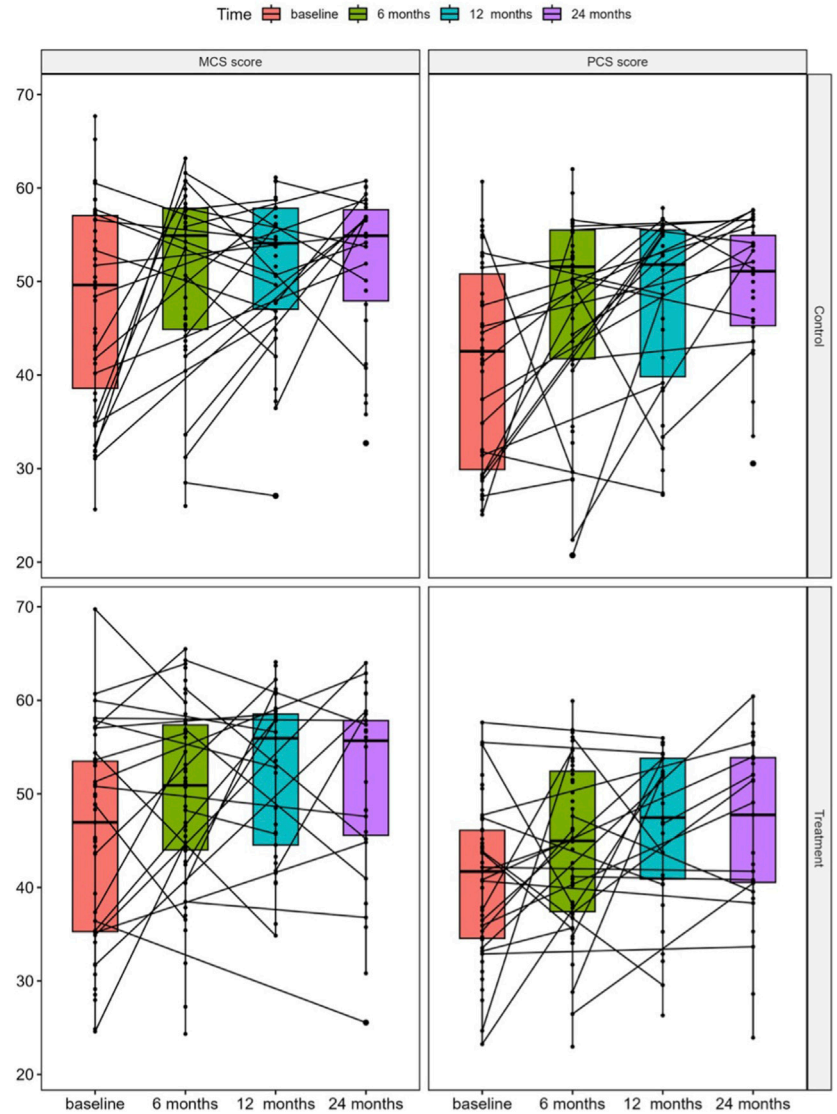


FIGURE 1 | Median changes in KDQOL-SF SF-36 physical component summary (PCS) and mental component summary (MCS) scores in the study groups stratified by standard of care control and treatment at baseline and at 6, 12, and 24 months after KT.

and the accompanied fear of hypoglycemic events is known to negatively affect the HRQOL of non-transplant diabetes patients. While these fears may also appear in KT recipients, the overall positive effects of transplantation including cessation of hemodialysis, increased physical functioning, and decreased effects and burden of kidney disease may outweigh the negative effects of early basal insulin therapy in post-operative KT patients [9].

To further explore the effects of early postoperative insulin therapy on HRQOL, we resampled the study participants according to their risk of developing PTDM using the factors age and clinical predisposition for metabolic dysfunction in addition to laboratory markers. Metabolic dysfunction may be the result of an unfavorable lifestyle or a genetic predisposition including a positive family history of DM. In line, PCKD as a systemic genetic disorder resulting in a progressive growth of cysts not only in the kidneys, but also in the liver, seminal ducts, and/or pancreas are at high-risk to develop PTDM [31, 32]. Nevertheless, metabolic dysfunction and overweight are considered as a distinct pathological entity, the metabolic syndrome, which itself affects the HRQOL of patients [33, 34]. The resulting two groups of high versus low-risk for the development of PTDM showed significant differences in their physical health (SF-36 PCS) 24 months after KT, while there were no differences in mental health (MCS) at any timepoint. Interestingly, at 6 and 12 months after transplantation, both groups showed increased and comparable HRQOL scores, suggesting a valuable benefit of KT during the first year for all patients. Positive changes in HRQOL observed early after KT are particularly driven by increased physical activity, reduced

TABLE 2 | Baseline patient characteristics in the groups of low- and high-risk for post-transplant diabetes mellitus.

Characteristic	High-risk *N* = 84	Low-risk *N* = 45	*p*-value
Female	34 (40)	16 (36)	0.6
Age (years)	56.4 (12.5)	39.7 (10.4)	**<0.001**
Height (cm)	168.4 (10.3)	170.9 (10.4)	0.2
Weight (kg)	75.0 (65.3, 84.7)	70.0 (62.0, 79.0)	0.062
BMI (kg/m^2)	26.6 (4.8)	23.8 (3.8)	**0.002**
Primary kidney disease			**<0.001**
Glomerular	26 (41)	22 (71)	
Vascular	7 (11)	4 (13)	
Tubulointerstitial	5 (8)	4 (13)	
PCKD	23 (37)	0 (0)	
Other	2 (3)	1 (3)	
Number of previous kidney allografts			0.9
1	71 (85)	40 (89)	
2	12 (14)	5 (11)	
3	0 (0)	0 (0)	
4	1 (1)	0 (0)	
Dialysis prior to KT	75 (89)	40 (89)	>0.9
Comorbidities			
Cardiovascular	38 (45)	15 (33)	0.2
Respiratory	9 (11)	2 (4)	0.3
Urinary	9 (11)	4 (9)	>0.9
Endocrinological	13 (15)	3 (7)	0.15
Neurological	2 (2)	1 (2)	>0.9
Psychiatrical	7 (8)	0 (0)	0.10
Other	2 (2)	3 (7)	0.3
Laboratory results			
Hemoglobin (g/dL)	11.7 (1.5)	12.0 (1.6)	0.2
Creatinine (mg/dL)	7.1 (5.6, 9.3)	7.8 (6.2, 9.7)	0.3
eGFR (ml/min/1.73m^2)	7.1 (5.1, 9.0)	7.2 (5.5, 9.3)	0.6
CRP (mg/dL)	2.0 (0.6, 10.0)	1.7 (0.6, 3.4)	0.3
HbA1c (%)	5.3 (5.0, 5.5)	4.8 (4.6, 5.2)	**<0.001**

Statistically significant p*-values in the analysis appear in bold* (p < *0.05). Continuous variables are expressed as mean (SD) or median (minimum and maximum). Categorical variables are n (%).*

Abbreviations: BMI, body mass index; CRP, C-Reactive Protein; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; KT, kidney transplantation; PCKD, polycystic kidney disease.

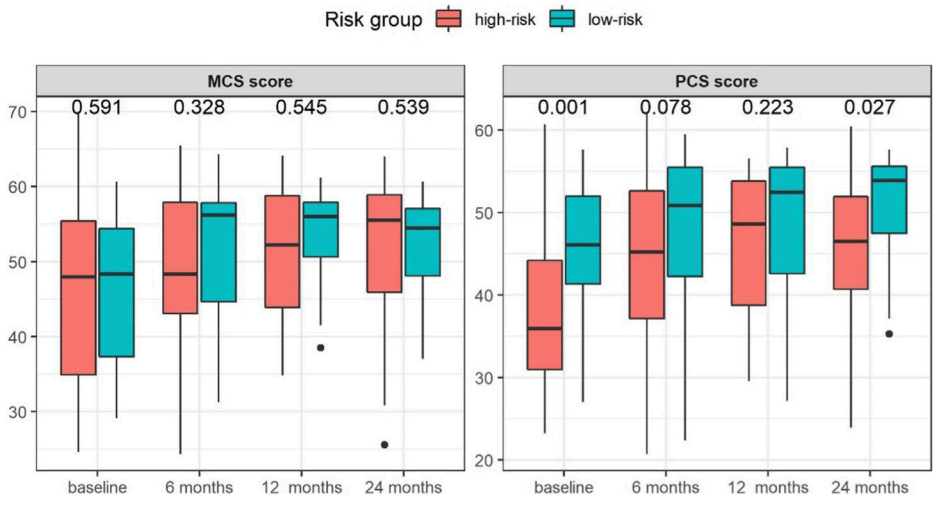


FIGURE 2 | Median changes in KDQOL-SF SF-36 physical component summary (PCS) and mental component summary (MCS) scores in the study groups stratified by risk group for post-transplant diabetes mellitus at baseline and at 6, 12, and 24 months after KT.

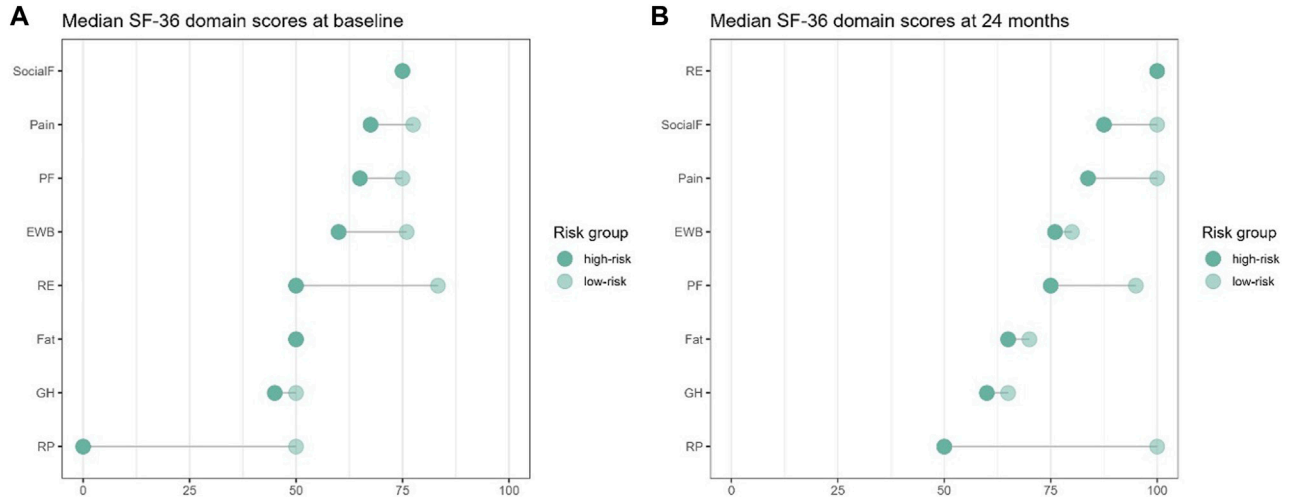


FIGURE 3 | Changes of median KDQOL-SF SF-36 domain scores at **(A)** baseline (BL) and **(B)** 24-month for low-risk compared to high-risk for post-transplant diabetes mellitus.

TABLE 3 | Univariable regression analyses of confounders for changes in KDQOL-SF™ physical and mental component scores at 6, 12, and 24 months after kidney transplantation.

	Physical component summary score								
	Univariable								
	N	**m6 Beta, (95% CI)**	***p*-value**	***N***	**m12 Beta, (95% CI)**	***p*-value**	***N***	**m24 Beta, (95% CI)**	***p*-value**
Group	91		0.120	67		0.541	61		0.136
Control		—			—			—	
Treatment		−3.2 (−7.2, 0.84)			−1.4 (−5.8, 3.1)			−3.2 (−7.4, 1.0)	
Risk group	81		0.128	61		0.320	52		**0.029**
High-risk		—			—			—	
Low-risk		3.6 (−1.1, 8.2)			2.3 (−2.3, 7.0)			5.1 (0.54, 9.6)	
Baseline PCS	48	0.42 (0.13, 0.71)	**0.006**	43	0.24 (−0.04, 0.52)	0.095	40	0.35 (0.05, 0.66)	**0.025**
HbA1c	87	0.61 (−2.3, 3.5)	0.677	62	−1.5 (−5.1, 2.0)	0.384	58	−2.6 (−6.5, 1.4)	0.194
eGFR	89	0.02 (−0.05, 0.09)	0.599	67	0.18 (0.09, 0.28)	**<0.001**	61	0.18 (0.09, 0.28)	**<0.001**
Hemoglobin	86	1.6 (−0.54, 2.7)	**0.004**	64	−1.2 (−0.02, 2.4)	**0.046**	59	2.0 (0.72, 3.2)	**0.003**
CRP	85	−0.1 (−0.18, −0.01)	**0.024**	67	−0.29 (−0.82, 0.25)	0.291	61	−0.26 (−0.54, 0.02)	0.067
oGTT	91	0.02 (−0.01, 0.05)	0.232	67	−0.02 (−0.05, 0.02)	0.314	61	0.00 (−0.08, 0.08)	0.973
	Mental Component Summary Score								
	Univariable								
	N	**m6 Beta, (95% CI)**	***p*-value**	***N***	**m12 Beta, (95% CI)**	***p*-value**	***N***	**m24 Beta, (95% CI)**	***p*-value**
Group	91		0.507	67		0.775	61		0.797
Control		—			—			—	
Treatment		−1.4 (−5.4, 2.7)			0.59 (−3.5, 4.7)			−0.60 (−5.3, 4.1)	
Risk group	81		0.170	61		0.249	52		0.856
High-risk		—			—			—	
Low-risk		3.2 (−1.4, 7.9)			2.4 (−1.7, 6.6)			0.45 (−4.5, 5.4)	
Baseline MCS	48	0.41 (0.16, 0.67)	**0.002**	43	0.38 (0.16, 0.59)	**0.001**	40	0.18 (−0.08, 0.44)	0.178
HbA1c	87	−0.29 (−3.2, 2.6)	0.845	62	2.2 (−0.86, 5.2)	0.158	58	−0.85 (−4.9, 3.2)	0.677
eGFR	89	−0.03 (−0.04, 0.09)	0.445	67	0.08 (−0.02, 0.17)	0.107	61	0.12 (0.01, 0.23)	**0.034**
Hemoglobin	86	1.2 (0.11, 2.3)	**0.032**	64	0.91 (−0.22, 2.0)	0.111	59	0.59 (−0.88, 2.1)	0.427
CRP	85	−0.05 (−0.13, 0.04)	0.259	67	−0.01 (−0.51, 0.49)	0.968	61	−0.10 (−0.42, 0.21)	0.508
oGTT	91	−0.03 (−0.07, 0.00)	**0.042**	67	0.00 (−0.03, 0.04)	0.884	61	−0.01 (−0.10, 0.07)	0.781

Statistically significant p*-values in the analysis appear in bold (*p *< 0.05). *Only variables with a* p*-value <0.2 in the univariable analysis were carried on for multivariable analysis. Numbers represent the beta coefficients and 95% confidence intervals in each group and variable, while* N *represents the number of patients with available data.*
Abbreviations: BMI, body mass index; CRP, C-Reactive Protein; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; MCS, mental component score; PCKD, polycystic kidney disease; PCS, physical component score.

TABLE 4 | Multivariable regression analyses of confounders for changes in KDQOL-SF™ physical and mental component scores at 6, 12, and 24 months after kidney transplantation.

	Physical component summary score								
		Multivariable							
	N	m6 Beta, (95% CI)	*p*-value	*N*	m12 Beta, (95% CI)	*p*-value	*N*	m24 Beta, (95% CI)	*p*-value
Group	91		0.162	67			61		0.272
Control		—			—			—	
Treatment		−4.5 (−11.0, 1.9)						−2.6 (−7.4, 2.2)	
Risk group	81		0.856	61			52		0.604
High-risk		—			—			—	
Low-risk		−0.61 (−7.4, 6.2)			—	—		1.9 (−5.4, 9.1)	
Baseline PCS	48	0.40 (0.04, 0.76)	**0.029**	43	0.16 (−0.12, 0.44)	0.257	40	0.18 (−0.18, 0.54)	0.318
HbA1c	87	—		62	—	—	58	−4.0 (−9.0, 0.90)	0.105
eGFR	89	—		67	0.14 (0.02, 0.27)	**0.025**	61	0.06 (−0.07, 0.20)	0.360
Hemoglobin	86	1.6 (−0.55, 3.7)	0.139	64	0.31 (−1.2, 1.9)	0.688	59	3.3 (1.5, 5.1)	**<0.001**
CRP	85	0.07 (−0.15–0.29)	0.521	67	—	—	61	0.49 (−0.02, 1.0)	0.060
oGTT	91	—		67	—	—	61	—	—
	Mental Component Summary Score								
		Multivariable							
	N	m6 Beta, (95% CI)	*p*-value	*N*	m12 Beta, (95% CI)	*p*-value	*N*	m24 Beta, (95% CI)	*p*-value
Group	91		—	67		—	61		—
Control		—			—			—	
Treatment		—							
Risk group	81		0.160	61		—	52		—
High-risk		—			—			—	
Low-risk		4.2 (−1.7, 10)			—			—	
Baseline MCS	48	0.32 (0.06, 0.59)	**0.017**	43	0.21 (−0.07, 0.49)	0.142	40	0.07 (−0.19, 0.32)	0.592
HbA1c	87	—	—	62	0.41 (−3.6, 4.5)	0.836	58	—	—
eGFR	89	—	—	67	0.06 (−0.08, 0.20)	0.401	61	0.19 (0.05, 0.32)	**0.008**
Hemoglobin	86	2.6 (0.71, 4.6)	**0.009**	64	0.60 (−1.3, 2.5)	0.518	59	—	—
CRP	85	—	—	67	—	—	61	—	—
oGTT	91	−0.03 (−0.07, 0.01)	0.138	67	—	—	61	—	—

Statistically significant p*-values in the analysis appear in bold (*p *< 0.05). Only variables with a* p*-value <0.2 in the univariable analysis were carried on for multivariable analysis. Numbers represent the beta coefficients and 95% confidence intervals in each group and variable, while N represents the number of patients with available data.*
Abbreviations: BMI, body mass index; CRP, C-Reactive Protein; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; MCS, mental component score; PCKD, polycystic kidney disease; PCS, physical component score.

symptom burden or improvements in social functioning, among others [10]. In the long-term course after KT, a significant impairment of physical health, which is associated with a pronounced muscular weakness resulting in a lower physical activity has been—comparable to our results—proven before [9].

The cause of muscular weakness in KTR is multifactorial. Older patients experience geriatric syndromes like sarcopenia, which itself, has a complex pathophysiology including reduced physical activity, systemic inflammation, and neuropathic changes leading to a denervation of muscles [35]. However, in younger patients, the metabolic syndrome and obesity are known to induce a loss of muscular strength relative to their body mass, which is again linked to systemic inflammation and reduced physical activity [35, 36]. Pro-inflammatory cytokines such as tumor-necrosis factor alpha and interleukin 6, are known to stimulate muscle protein degradation and reduce muscle protein synthesis. Pro-inflammatory states have all been described in sarcopenia [37], metabolic syndrome, obesity [38], and PCKD [32], which define the high-risk PTDM group. Accordingly, CRP levels tended to increase in the high-risk group in both the uni- and multivariable model 24 months after KT. The resulting sarcopenia together with the pre-existing metabolic syndrome might potentially explain our findings of reduced physical health 24 months after KT in the high-risk PTDM group. A potential strategy to prevent muscle weakness, sarcopenia, and metabolic risk factors in KT recipients could be controlled physical exercise. Recent randomized controlled trials investigating the effect of a 10–12 weeks training program of either resistance or combined resistance and aerobic exercise compared to no training in post KT showed notable improvements in functional performance, body composition, muscular strength, renal function, fatigue, and HRQOL [39–41]. Promoting physical activity in KT recipients may therefore prevent the observed vanishing effect of KT in high-risk PTDM patients.

The high-risk group also had a greater disease specific symptom burden, pain, and impaired emotional-role-functioning 24 months after KT compared to the low-risk PTDM group, with comparable values between groups at 6 and 12 months, suggesting again a slowly vanishing effect of KT on HRQOL in the high-risk PTDM

group. We also found a statistical association of renal function (eGFR) and hemoglobin levels to the HRQOL 24 months after KT independent from early insulin treatment and PTDM-risk in the multivariable analysis. Hemoglobin levels were associated with the physical health domain (PCS score), while renal function was related to the mental health domain (MCS score) of the SF-36. The symptom burden of KTRs influencing HRQOL is multifactorial including decreased renal function, anemia, depressive symptoms, anxiety, and elevated BMI, as well as treatment-specific encompassing side effects and complications of immunosuppressive therapy [42, 43]. Both severely impaired renal function and anemia lead to uremic symptoms, fatigue, and breathlessness [43, 44], while immunosuppressive agents and metabolic syndrome can cause peripheral neuropathy, thereby enhancing pain and reducing mobility [45]. Particularly calcineurin-inhibitors may induce a disabling pain-syndrome in 5%–15% of KTRs within the first year after KT [46]. Psychological symptoms constitute a burden to patients *per se* but also enhance the perception of physical symptoms in a multidimensional way [47], together potentially creating a vicious cycle amplifying the total symptom burden after KT. The high-risk PTDM group of our study cohort were older and displayed a higher BMI, both associated with more depressive symptoms [33]. Additionally, metabolic dysregulation decreases renal function of the graft in the long-term [48], causing fears of graft rejection and anxiety further enhancing depressive symptoms [49, 50].

Our study has certain limitations including a considerable proportion of missing data within the HRQOL questionnaires, especially towards the end of the study period which might lead to underestimation to detect lower effect sizes. This is mainly explained by patients lost to follow-up or incompliance with completing the questionnaires. Furthermore, this analysis lacks socioeconomic data as well as comparison of different ethnicities since Caucasians were in the majority included in the ITP-NODAT study [27]. Sociodemographic differences may especially affect the definition of metabolic risk profiles limiting our results mainly to Caucasians living in Europe. In addition, the measurement instrument is generic and might miss to capture important diabetes-specific aspects. Nevertheless, the strengths of this analysis are its multicenter randomized design comprising detailed clinical data and the long-term follow-up of the study participants providing unique data on determinants of HRQOL on the long-term after KT.

Taken together, early postoperative insulin therapy, which might be reasonable in selected patient groups, is not compromising the HRQOL. Although KT substantially improves the HRQOL of CKD patients 1 year after transplantation, patients in the high-risk PTDM group experience a significant impairment in HRQOL in the long course after KT. The HRQOL of KTR is significantly dependent on graft function and anemia. Given the complex relationship between manageable risk factors, physical and psychological symptom burden, and nephrological treatment, a multidisciplinary post-transplant care should be considered to meet the multidimensional needs of KTR, especially within high-risk PTDM populations.

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 study was undertaken with independent external monitoring in accordance with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use-Good Clinical Practice principles, and the Declaration of Helsinki. Written informed consent was obtained from all patients after approval from the institutional review board at each participating center.

AUTHOR CONTRIBUTIONS

BO, MHu, AB, and KE contributed to the design of the analysis of the original study, interpretation of data, and wrote the first draft of the manuscript. AB performed the statistical analysis. MHe, ES, AK, MK, HH-G, GE, FB, AF, and MJP-S contributed to the data collection and reviewing of the manuscript. MHe, JP, KB, and AR contributed to the design and writing of the protocol of the parent study and reviewed the manuscript. KE is the principal investigator of the ITP-NODAT study, gave final approval of the published version of the manuscript and is the guarantor of this work and as such had full access to all the data in the study. 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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LETTER TO THE EDITOR
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A Novel Daratumumab-Based Regimen for Desensitization in Highly HLA-Presensitized Patients Awaiting Kidney Transplantation

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Keywords: desensitization, presensitized kidney transplantation, daratumumab, anti-CD38 monoclonal antibody, plasma cells

Dear Editors,

Pre-transplant desensitization is a common strategy to increase the success of renal transplantation in human leukocyte antigen (HLA)-presensitized patients with uremia. Currently, the most efficacious desensitization strategy is to start with rounds of plasmapheresis (PP)/immunoadsorption (IA) together with intravenous immunoglobulin (IVIG) or rituximab [1, 2]. The representative regimens include a combination of rituximab and high-dose IVIG (2 g/kg over 2–4 days) [3], and a combination of 3–5 sessions of PP followed after each session by an infusion of low-dose IVIG (0.1 g/kg) [4]. However, they have limited effectiveness in highly presensitized patients [5]. Daratumumab, an anti-CD38 monoclonal antibody, may be effective in reducing preformed antibodies and desensitizing these patients, given its effect on plasma cell elimination [6]. Two clinical trials are currently investigating daratumumab for desensitization before presensitized kidney transplantation. The first one is the COMBAT trial, in which patients with calculated panel-reactive antibody (cPRA) > 99% will be treated with belatacept in step I, and if ineffective, with four sessions of apheresis, followed by daratumumab monotherapy in step II (ClinicalTrials.gov ID NCT05145296). The second one is the DARDAR trial, in which they investigate daratumumab dose escalation (4–8–16 mg/kg) in step 1 and full dose (16 mg/kg) in step 2 (ClinicalTrials.gov ID NCT04204980). In the literature, cases concerning to daratumumab for pre-transplant desensitization are limited to sensitized heart [7–9] and lung [10] transplant candidates. This letter reports on a case of high HLA sensitization in which a novel daratumumab-based therapy effectively reduced the degree of sensitization and resulted in successful kidney transplantation.

A 32-year-old man (50 kg) was waitlisted for a third kidney transplant. The single-antigen bead assay revealed 22 positive antibodies against HLA-I loci and 14 against HLA-II loci in his serum. Among them, four HLA-I and seven HLA-II antibodies had a high mean fluorescence intensity (MFI) value (>10,000). His cPRA was >99%. With the informed consent, the patient received a new regimen for desensitization to increase the transplant chance.

The treatment was initiated with 200 mg of rituximab. Five days later, PP/IVIG (15–20 g) plus daratumumab (400 mg, intravenously 1 day after PP/IVIG) was given weekly for 19 weeks as an intensive therapy (phase 1, **Figure 1A**). After phase 1, nine HLA-I and seven HLA-II antibodies were reduced (MFI 5,000–10,000 to 1,401–4,827 and >10,000 to <3,000, respectively) and the

Abbreviations: cPRA, calculated panel-reactive antibody; DSA, donor-specific antibody; HLA, human leukocyte antigen; IVIG, intravenous immunoglobulin; MFI, mean fluorescence intensity; PP, plasmapheresis.

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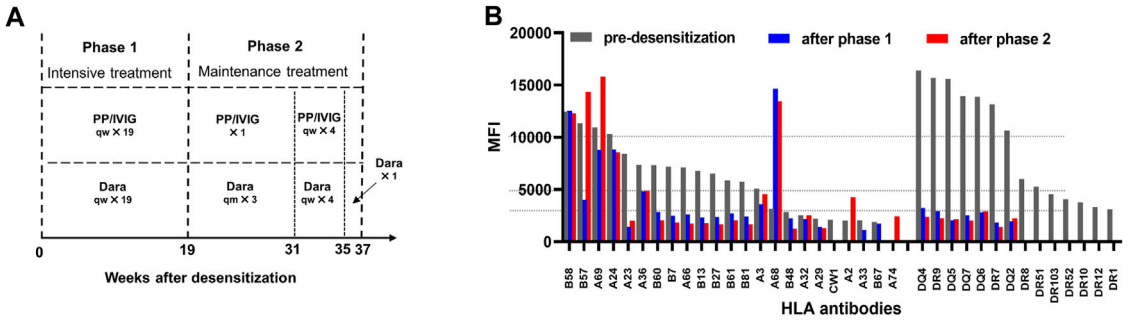


C

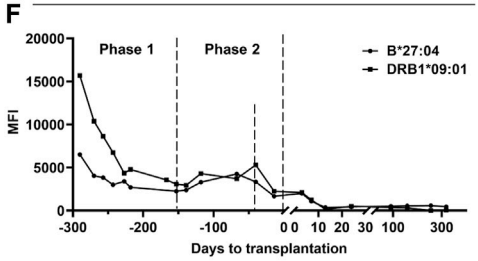
Total cPRA	MFI cut-off		
	1000	3000	5000
Before desensitization	99.9%	99.9%	99.6%
After phase 1 treatment	99.9%	93.6%	65.3%
After phase 2 treatment	99.9%	81.7%	35.7%

D

cPRA (class Ⅰ/Ⅱ)	MFI cut-off					
	1000		3000		5000	
	Ⅰ	Ⅱ	Ⅰ	Ⅱ	Ⅰ	Ⅱ
Before desensitization	94.3%	98.7%	89.8%	98.7%	69.9%	98.7%
After phase 1 treatment	94.0%	98.6%	78.2%	74.4%	65.3%	0
After phase 2 treatment	93.3%	98.6%	81.7%	0	35.7%	0

E

HLA	A	B	DRB1	DQB1
Donor	11:01, 11:02	27:04, 46:01	04:06, 09:01	03:02, 03:03
Recipient	11:01, 11:02	15:01, 15:18	04:04, 04:06	03:02, 03:02



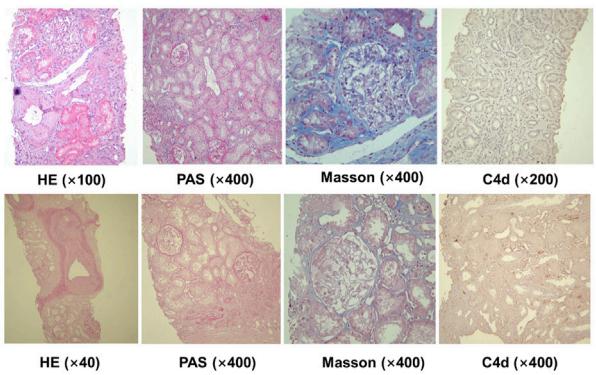


FIGURE 1 | Daratumumab-based HLA desensitization regimen and therapeutic outcomes. **(A)** The desensitization protocol was initiated with 200 mg rituximab. Five days later, plasmapheresis (PP), intravenous immunoglobulin (IVIG, 15–20 g each time) plus daratumumab (Dara, 400 mg, intravenously 1 day after PP/IVIG) were started, with frequencies as indicated. **(B)** Serum HLA antibody levels (1:4 dilution) at various stages. MFI: mean fluorescence intensity. **(C)** Total calculated panel-reactive antibody (cPRA) levels after indicated MFI cutoffs. **(D)** HLA class I/II cPRA levels at the indicated MFI cutoffs. **(E)** Donor-recipient HLA phenotyping indicated two of the preformed antibodies against HLA were donor-specific antibodies (DSA), anti-DRB1 09:01 and anti-B27:04. MM: mismatch. **(F)** DSA levels were significantly decreased after desensitization treatment and did not rebound after transplantation. **(G)** Protocol biopsy results at 3 months (top panel, Banff 2019: i0, t0, g0, v0, ptc0, MVI = 0, ci2, ct1, cg0, cv2, ah2, mm0, Ti0, i-IFTA0, t-IFTA0, C4d0) and 12 months (bottom panel, Banff 2019: i0, t0, g0, v0, ptc0, MVI = 0, ci0, ct0, cg0, cv2, ah2, mm0, Ti0, i-IFTA0, t-IFTA0, C4d1) post-transplantation.

other seven HLA-II antibodies became negative (MFI < 1,000) (**Figure 1B**). The total cPRA decreased from 99.6% to 65.3% and class II cPRA decreased from 98.7% to 0 (MFI cutoff: 5,000, **Figure 1C, D**).

A phase 2 therapy was initiated to maintain the antibody reduction achieved in phase 1 while the patient waited for an HLA-suitable donor. The patient received 400 mg daratumumab monthly and one session of PP/IVIG during the first 12 weeks. Intensive therapy with PP/IVIG plus daratumumab weekly was given to reverse the mild antibody rebound for the following 4 weeks. Daratumumab (400 mg) was administered once during the last 2 weeks (**Figure 1A**). Desensitization was thus maintained and improved during the 18 weeks after phase 1 (**Figure 1B**). The total cPRA further decreased from 65.3% to 35.7% and class II cPRA remained at 0 (MFI cutoff: 5,000, **Figure 1C, D**).

At the end of phase 2, an ABO-compatible kidney obtained from a 52-year-old man after brain death was allocated to the patient via the Chinese organ transplant responding system. The donor-recipient HLA mismatch grade was 4 (**Figure 1E**). There were low levels of anti- DRB1 0901 (MFI: 2,253) and anti- B2704 (MFI: 1,673) donor-specific antibodies (DSAs) in the patient's pre-transplant serum, whereas their MFI values before desensitization had been 15,684 and 6,513, respectively (**Figure 1F**). The complement-dependent cytotoxicity test result was negative.

The allocated kidney was transplanted. The patient received induction therapy of 200 mg rituximab on day −1, 500 mg

methylprednisolone daily on days 0–2 and 25 mg thymoglobulin daily on days 0–5 plus maintenance therapy with oral tacrolimus (trough level 7–10 ng/mL), mycophenolate mofetil (750 mg/12 h) and prednisone (10 mg/d). In addition, 20 g/d IVIG on days 1–5 and 15 g/d on days 6–13 were given to prevent DSA rebound. The patient showed good post-operative recovery without any episodes of rejection. DSAs disappeared 2 weeks post-transplantation (**Figure 1F**). The patient was discharged 30 days after transplantation with a serum creatinine level of 162 μmol/L. Oral compound sulfamethoxazole and valganciclovir were given every 3 days for 3 months to prevent opportunistic infection.

The patient was followed up for 1 year during which graft function remained stable. A 3-month protocol biopsy showed no signs of antibody or T cell-mediated rejections. One-year protocol biopsy results were similar but showed mild peritubular capillary C4d deposition (**Figure 1G**). DSA levels kept negative, while almost all non-DSAs with high levels before transplantation also declined during the 1-year follow-up period and none of those with low levels rebounded after transplantation.

The patient developed some flu-like symptoms, including nasal discharge, dry cough, and fatigue, after the initial daratumumab administration and was given intravenous infusion of 5 mg dexamethasone in advance of subsequent treatments which suppressed the symptoms. The patient developed myelosuppression during the later stages of intensive desensitization therapy. Myelosuppression was relieved during maintenance treatment, possibly due to the reduced frequency of daratumumab treatment. In addition, no significant toxicity to vision, peripheral nerves or liver was observed and no incidents of infection occurred.

High levels of preformed circulatory antibodies generally take a long time to gradually decay without assistance and the expectation was that the antibody-lowering effect of daratumumab monotherapy might be difficult to show in the short term. However, PP directly removed some of the circulating antibodies and IVIG reduced endogenous antibody rebound after PP-mediated depletion. Therefore, the regime was designed to elicit a synergistic action of daratumumab and PP/IVIG in reducing antibodies and improving desensitization. When a significant response to the intensive desensitization therapy had been achieved, phase 2 treatment with daratumumab monotherapy was initiated to maintain the antibody reduction while waiting for an HLA-matched donor.

In conclusion, the present case provides a novel desensitization strategy for kidney transplantation in highly presensitized patients. Further clinical studies are required for validation of this approach.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology. 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. 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

GC designed the study. DZ, ZG, LZ, and GC performed the surgery, analyzed the data, and wrote 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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