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Kidney rejection: Let there be light!

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DOI: 10.3389/ti.2022.10841

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High-density lipoprotein (HDL) are nanoparticles with pleiotropic endothelioprotective properties. We demonstrated that low HDL-cholesterol concentrations in lung transplant candidates are strongly associated with one-year mortality after lung transplantation.

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Letter to the Editor

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DOI: 10.3389/ti.2022.11104

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DOI: 10.3389/ti.2022.10979

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ABSTRACT SUBMISSION

ABSTRACT SUBMISSION

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EDTCO ORGAN DONATION CONGRESS 2023

Towards a new era in donor coordination

16 September 2023
Athens, Greece

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30th ANNIVERSARY

TRANSPLANT TRIAL WATCH
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Transplant Trial Watch

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Keywords: kidney transplantation, immunosuppression, systematic review, liver transplantation, octreotide

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

RANDOMISED CONTROLLED TRIAL 1 |
Revisiting maintenance immunosuppression in patients with renal transplant failure: early weaning of immunosuppression versus prolonged maintenance-systematic review and meta-analysis.
by Elgenidy, A., et al. Journal of Nephrology [Online ahead of print].

Aims
This study aimed to evaluate whether early or late withdrawal of maintenance immunosuppression in patients with kidney transplant failure is linked with better outcomes.

Interventions
Electronic databases including PubMed, WOS, Ovid, and Scopus databases were searched. Titles and abstracts were screened for eligiblity by four independent reviewers. Data extraction was conducted by two independent reviewers. The Newcastle–Ottawa Scale was used to assess the methodological quality of the included studies.

Participants
10 studies were included in the review.

Outcomes
The outcomes of interest were incidence of infection, cancer, mortality (infection-related, malignancy-related, cardiovascular-related), transplant nephrectomy, re-transplantation, panel reactive antibody (PRA) and admission to hospital.

Follow-Up
Not applicable.

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CET Conclusion
This systematic review and meta-analysis investigated the role of continued immunosuppression in the patient with a failed kidney transplant. The authors identified 10 studies including 1,187 patients. There was no difference in overall survival, sensitisation, malignancy, infection or retransplant rates between patients who withdrew immunosuppression early or remained on maintenance immunosuppression. Review methodology appears good, with independent reference screening and searches across multiple databases. The majority of studies were retrospective cohorts with a risk of selection bias. Many meta-analyses included only 2 or 3 studies, with quite wide confidence intervals around estimated effect sizes and some degree of heterogeneity. It is possible, therefore, that a true effect could have been missed due to selection bias or lack of power.

Trial Registration
Not applicable.

Funding Source
Not reported.

RANDOMISED CONTROLLED TRIAL 2 |
Impact of Octreotide on Early Complications After Liver Transplant: A Randomized, Double-Blind Placebo-Controlled Trial.
by Bagheri Lankarani, K., et al. Experimental & Clinical Transplantation 2022; 20(9):835–841.

Aims
This study aimed at investigating the role of octreotide on early outcomes following liver transplantation.

Interventions
Participants were randomised to receive either octreotide or placebo.

Participants
50 patients who underwent deceased donor orthotopic liver transplantation.

Outcomes
The primary endpoint was renal function. The secondary endpoints were length of intensive care unit (ICU) and hospital stays, rate of nosocomial infection, and rate of early allograft dysfunction (EAD).

Follow-Up
16.4 days posttransplant.

CET Conclusion
This is a clear report of a randomised controlled study in liver transplantation. A prior power calculation was conducted, and the trial was therefore adequately powered. The method of randomisation was through allocation software. The trial is described as double-blinded; however, the trial drugs were provided in vials labelled as either "A" or "B." This is unfortunately not as robust for maintaining blinding as having individual codes for every dose administered. The study found a significant reduction in AKI after liver transplantation when octreotide was administered. This was particularly for stage II AKI. As the diagnosis of AKI includes drop in urine output, this is not as robust as relying on serum markers alone. There was also a significant reduction in early allograft dysfunction with octreotide (16% versus 47%). The length of ICU stays, and total hospital stay were also reduced. It is speculated that all these effects are a result of improved renal perfusion and the anti-inflammatory effect of octreotide. A reduction in nosocomial infection was also seen with octreotide, but the mechanism of action for this is not clear.

Jadad Score
5.

Data Analysis
Strict intention-to-treat analysis.

Allocation Concealment
Yes.

Trial Registration
IRCT20190619043942N1.

Funding Source
Non-industry funded.

CLINICAL IMPACT SUMMARY

This is an interesting RCT in liver transplantation investigating the potential for octreotide infusion to reduce the risk of post-operative Acute Kidney Injury (AKI). The study was relatively small, with only 50 transplants included across both arms. However, the scale of the reduction in AKI was so great that a significant reduction was demonstrated with octreotide; The study group had a 20% risk of AKI compared to 44% in the control group. The difference was particularly evident in stage 2 AKI. There was also a significant reduction in early allograft dysfunction of a similar magnitude (16% versus 47%). A significant reduction in post-operative infections was also seen with octreotide, but the mechanism for this is not clear.

There are some concerns about the methodology that do weaken the strength of the conclusions though. For example, the use of vials marked as A or B can lead to the loss of allocation concealment, despite an otherwise acceptable method of randomisation.

Octreotide has some evidence base in ischaemia reperfusion injury for both liver and kidney, that may support the results seen in this study. However, the scale of the apparent benefit was really quite extreme in this study. As the study authors acknowledge, further studies are needed to really understand if this is a true effect of the intervention alone.

AUTHOR CONTRIBUTIONS

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

CONFLICT OF INTEREST

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

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ORIGINAL RESEARCH
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doi: 10.3389/ti.2023.10840

Extracorporeal Photopheresis Improves Graft Survival in a Full-Mismatch Rat Model of Kidney Transplantation

Gaston J. Piñeiro[1,2], Marta Lazo-Rodriguez[2], Pedro Ventura-Aguiar[1,2], Maria J. Ramirez-Bajo[2,3], Elisenda Banon-Maneus[2,3], Miquel Lozano[4], Joan Cid[4], Natalia Hierro-Garcia[2,3], David Cucchiari[1,2], Ignacio Revuelta[1,2,3], Enrique Montagud-Marrahi[1,2], Eduard Palou[5], Beatriu Bayés-Genís[1,2], Josep M. Campistol[1,2], Fritz Diekmann[1,2,3] and Jordi Rovira[2]**

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Extracorporeal photopheresis (ECP) is an immunomodulatory therapy based on the infusion of autologous cellular products exposed to ultraviolet light (UV) in the presence of a photosensitizer. The study evaluates the ECP efficacy as induction therapy in a full-mismatch kidney transplant rat model. Dark Agouti to Lewis (DA-L) kidney transplant model has been established. ECP product was obtained from Lewis rat recipients after DA kidney graft transplantation (Lew^{DA}). Leukocytes of those Lew^{DA} rats were exposed to 8-methoxy psoralen, and illuminated with UV-A. The ECP doses assessed were 10×10^6 and 100×10^6 cells/time point. Lewis recipients received seven ECP infusions. DA-L model was characterized by the appearance of donor-specific antibodies (DSA) and kidney function deterioration from day three after kidney transplant. The dysfunction progressed rapidly until graft loss (6.1 ± 0.5 days). Tacrolimus at 0.25 mg/kg prolonged rat survival until 11.4 ± 0.7 days ($p = 0.0004$). In this context, the application of leukocytes from LewDA sensitized rats accelerated the rejection (8.7 ± 0.45, $p = 0.0012$), whereas ECP product at high dose extended kidney graft survival until 26.3 ± 7.3 days, reducing class I and II DSA in surviving rats. ECP treatment increases kidney graft survival in full-mismatch rat model of acute rejection and is a suitable immunomodulatory therapy to be explored in kidney transplantation.

Keywords: kidney transplantation, acute rejection, extracorporeal photopheresis, induction therapy, animal model

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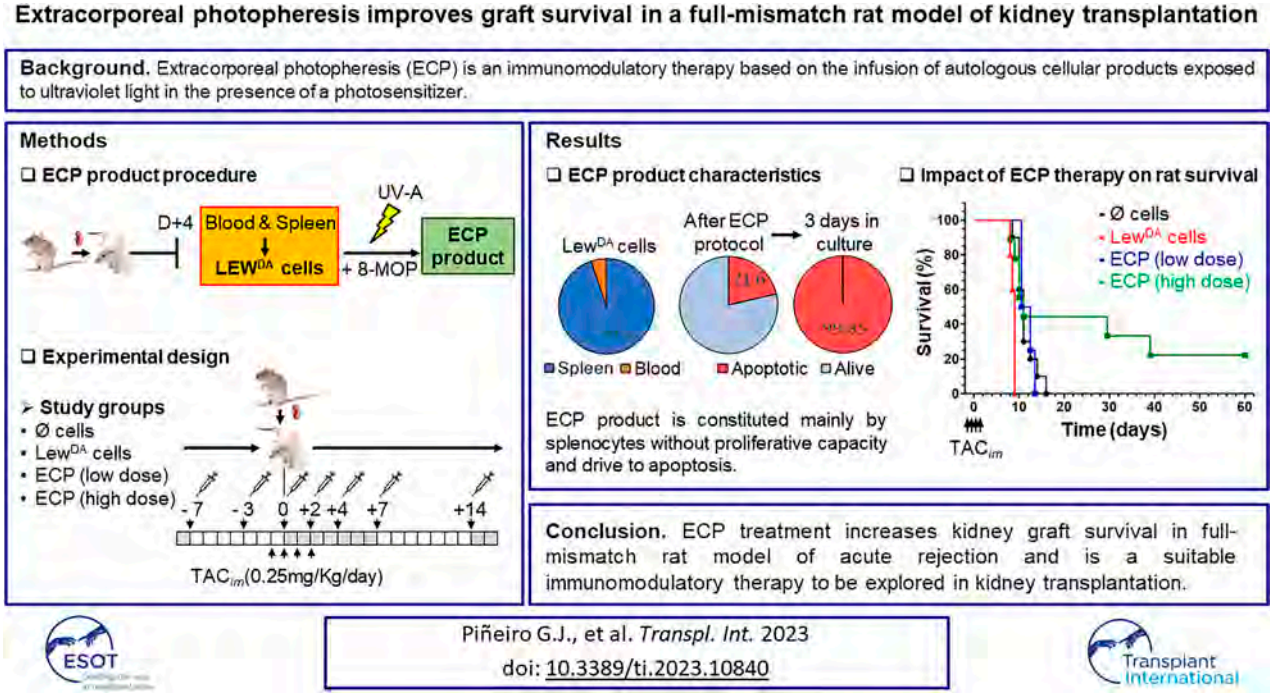
Piñeiro GJ, Lazo-Rodriguez M, Ventura-Aguiar P, Ramirez-Bajo MJ, Banon-Maneus E, Lozano M, Cid J, Hierro-Garcia N, Cucchiari D, Revuelta I, Montagud-Marrahi E, Palou E, Bayés-Genís B, Campistol JM, Diekmann F and Rovira J (2023) Extracorporeal Photopheresis Improves Graft Survival in a Full-Mismatch Rat Model of Kidney Transplantation. Transpl Int 36:10840. doi: 10.3389/ti.2023.10840

INTRODUCTION

Kidney transplantation is the best therapeutic option for patients with end-stage renal (1–4), however, optimal control of the alloimmune response is still challenging. Antibody-mediated rejection (ABMR), and complications related to immunosuppression, are critical aspects that need to be addressed (5–7). To improve kidney graft survival, novel treatments should demonstrate a good security profile while attaining the desired control of the alloimmune



GRAPHICAL ABSTRACT |

response. In this sense, cell therapies, such as regulatory T cells (Tregs), regulatory macrophages (Mregs), Tolerogenic dendritic cells (TolDC), or Mesenchymal stromal cells (MSC), may be a suitable option providing control of the alloimmunity without increasing immunosuppression (8–10). Cell therapies are usually used as induction therapy to minimize the immune response against the graft as soon as possible or to minimize the immunosuppressive load reducing side effects.

Extracorporeal photopheresis (ECP) is an immunomodulatory therapy based on the infusion of autologous cellular products, obtained through leukopheresis, and exposed to ultraviolet light A (UV-A) in the presence of a photosensitizer, 8-methoxypsoralen (8-MOP). ECP has demonstrated to suppress various autoimmune and alloreactions without increasing overall immunosuppression and, therefore, without increasing infection rates (11–13).

The two main indications for ECP therapy are cutaneous T-cell lymphoma (CTCL) (14) and graft-versus-host disease (GvHD) (15, 16). Moreover, ECP has been previously applied in solid organ transplantation and demonstrated efficacy in (lung, heart and liver) improving response in steroid-resistant rejection episodes through no randomized clinical trials. In all of these transplants, it has also been used as an add-on therapy to standard immunosuppression to reduce the incidence of acute graft rejection during the first months following transplantation (17–26).

In kidney transplantation, most of the information is derived from case reports, small retrospective series, and a prospective study with short follow-up (18, 27–32). But the lack of evidence in kidney transplantation makes the use of ECP controversial.

The present study aims to evaluate the use of ECP as induction therapy in a full-mismatch kidney transplant model in rats. We hypothesized that ECP treatment could effectively prevent acute rejection, improving kidney allograft function and survival.

MATERIAL AND METHODS

Animal Model

Inbred male Dark Agouti rats (DA, RT1-A^{av1}) were used as donors for allogenic renal transplantation for Lewis recipient rats (L, RT1-A^{1}). The surgical technique was performed as previously described (33). Briefly, donor kidney procurement and kidney transplantation were performed under anesthesia with isoflurane. Donor kidneys were flushed with Celsior solution at 4°C and were stored in Celsior solution at 4°C until the implantation. Renal transplants were performed with an end-to-side anastomosis of the aortic stump of the donor kidney and recipient's aorta, and between the recipient inferior vena cava and donor renal vein, respectively. Uretero-ureterostomy was performed with an end-to-end interrupted sutures technique. Recipient rats were bi-nephrectomized at transplantation.

The animals were kept at constant temperature, humidity, and at a 12-h light/dark cycle with free access to water and rat chow.

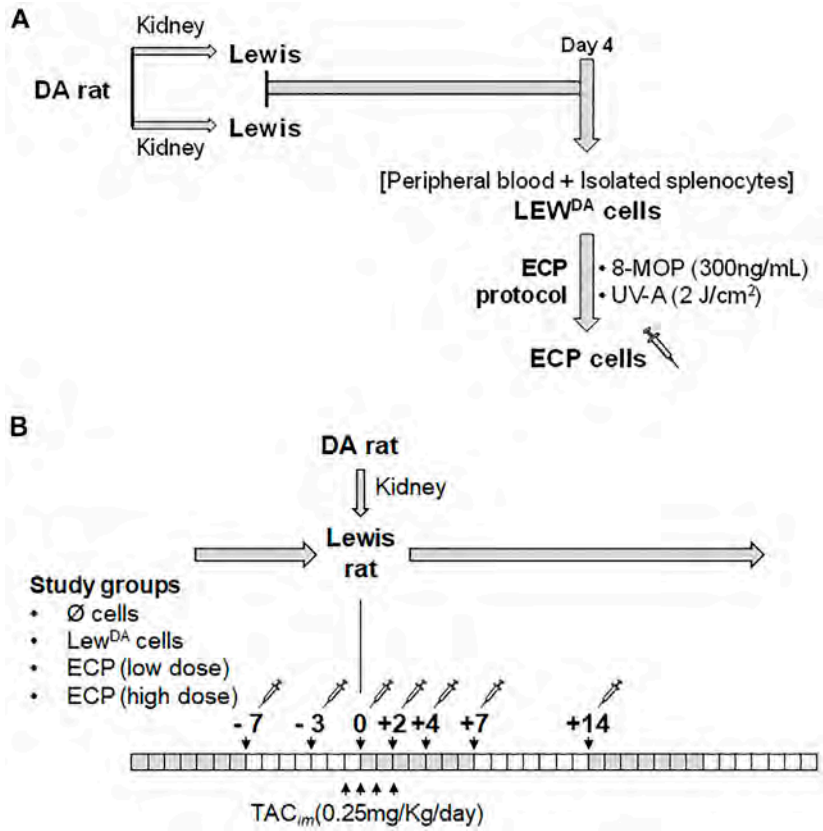


FIGURE 1 | Generation of ECP cells and ECP induction therapy in kidney transplant rat model. **(A)** Generation of ECP cells. Each cell product batch contains LEW^{DA} leukocytes from 2 Lewis recipients' rats. Four days after kidney transplantation, leukocyte cell suspention (LEW^{DA}) were obtained from peripheral blood and spleen. LEW^{DA} cells were incubated with 8-MOP for 30 min, later cell suspension was illuminated with UV-A ($2J/cm^2$) into MacoGenic (MACOPharma). **(B)** Extracorporeal photopheresis (ECP) treatment scheme in DA to Lewis kidney transplant rat model. Four groups were established according to the cell product used: without cells, Lew^{DA} cells, ECP at low dose (10^7 cells/infusion) and ECP at high dose (10^8 cells/infusion). DA, Dark Agouti rats; LEW, Lewis rats; Lew^{DA}, leukocytes from sensitized Lewis rats; TAC_{im}, intramuscular TAC injection; 8-MOP, psoralen; ECP, Extracorporeal photopheresis.

Immunosuppressive Therapy

A short dose of tacrolimus (TAC) was established to favor initial graft function, avoiding a rapid loss due to acute rejection. TAC was administered during four consecutive days (−1, 0, +1 and +2 with respect to transplantation). To set the TAC dose to be used, the Lewis rat recipients were divided into three TAC dose groups, 0.1, 0.25, or 0.5 mg/kg.

Detection of Donor-Specific Antibodies

DSA detection was performed in serum samples collected throughout the experiment. Dark Agouti donor rat splenocytes (5×10^5 cells/sample) were suspended in MACS buffer for 10 min at room temperature and then incubated with 25 µL of recipient Lewis rat serum samples for 30 min. Cells were washed three times and then incubated with a panel of markers that include FITC-conjugated mouse anti-rat IgG (1:100 dilution), MHC class I (RT1A, OX-18 antibody) and MHC class II (RAT1B, HIS19 antibody) markers for a further 20 min. After washing (3 times), cells were suspended in 150 µL of MACS buffer and analyzed on FACS Canto II cytometer. As negative controls, cells were incubated with serum from non-immunized Lewis rats. The class I and II DSAs were quantify by mean fluorescence intensity (MFI) of FITC-IgG staining in cells that expressed MHC class I or II.

Conventional Histology and Immunofluorescence

At 6 days of transplantation, a morphological and immunohistochemical study was performed in five Lewis rat recipients to identify the type of graft rejection. Formalin-fixed tissue was embedded in paraffin. Sections (3-µm thick) mounted on xylene glass slides (Dako, Carpinteria, CA) were used for immunohistochemistry. After antigen retrieval had been carried out, endogenous peroxidase blocking for 10 min in 3% hydrogen peroxide (Merck, Darmstadt, Germany) was performed before primary antibody incubation. The primary antibody, rat anti-C4d (Hycult Biotech, PA), was incubated overnight at 4°C. Envision system-specific anti-rabbit secondary antibody labeled with horseradish peroxidase polymer (Dako, Glostrup, Denmark) was applied for 1 h. All sections were counterstained with Mayer hematoxylin. Immunohistochemical procedure was

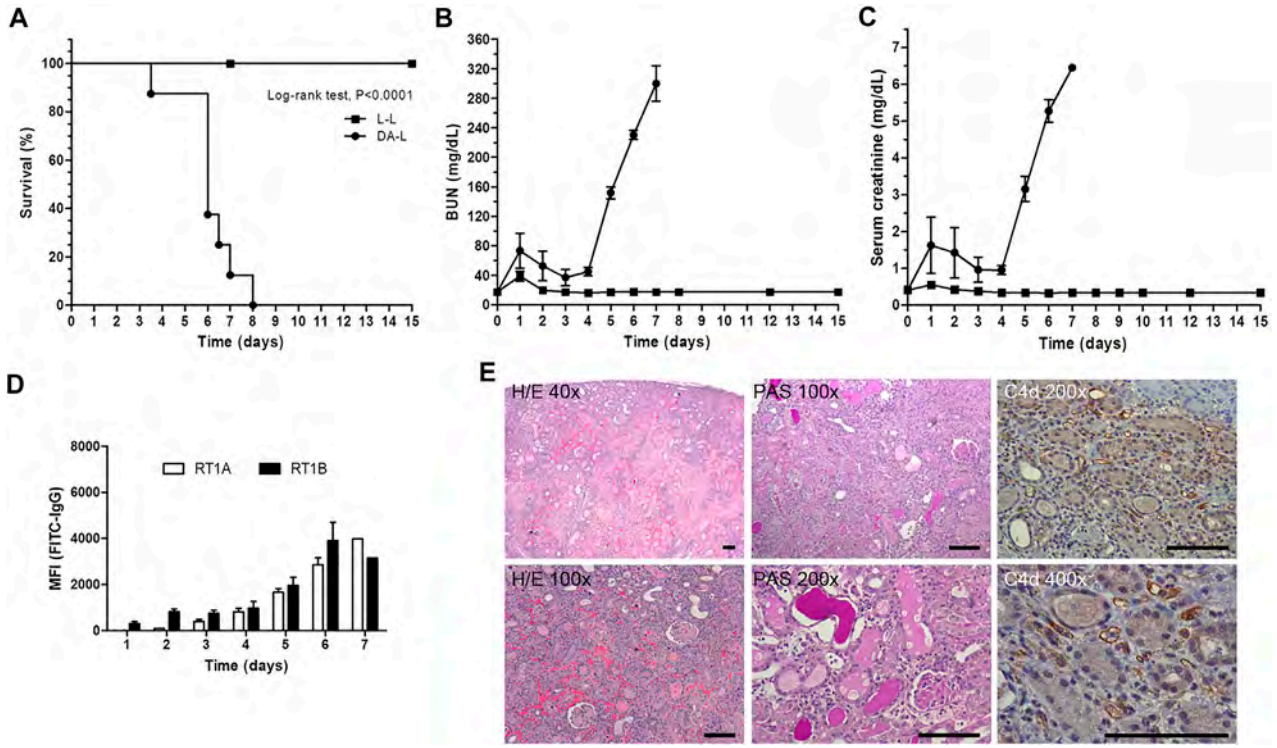


FIGURE 2 | Characterization of DA-L rat kideny transplant model. **(A)** Rat survival analysis, isogenic (L-L, *n* = 11, square) versus allogenic (DA-L, *n* = 8, circle) kidney transplantation. **(B,C)** Renal function analysis including blood urea nitrogen (BUN) and serum creatinine, respectively. **(D)** Donor-specific antibodies analysis including rat MHC class I (RT1A) and MHC class II (RT1B). **(E)** Representatives' images to illustrate kidney graft lesions observed in DA-L model at 6 days after transplantation. Hematoxylin/Eosin (H/E) and periodic acid Schiff (PAS) stains, and C4d immunohistochemistry were performed to determine that the rejection process observed in DA-LEW model is defined as an antibody-mediated rejection process. Scale bars are 100 μm in all pictures.

performed at the same time to avoid possible day-to-day variations in staining performance. All images were acquired using an Olympus BX51 clinical microscope and DP70 digital camera and software (Olympus, Tokyo, Japan).

A renal pathologist evaluated hematoxylin/eosin, periodic acid Schiff and C4d stains to evaluate renal damage.

Extracorporeal Photopheresis on Alloreactive Cells

A total of 27 batches of Lewis rat leukocytes (Lew^{DA}) were obtained from 54 transplanted rats (2 Lewis recipients per batch). Four days after DA-L kidney transplantation (**Figure 1A**), peripheral blood was collected and the spleen from 2 Lewis rat recipients were harvested, mashed, and passed through a cell strainer. Red blood cells were removed from both cell suspensions using RBC lysis buffer (Multispecies 10x, eBioscience), then were washed and counted. Both cell suspensions were characterized and subsequently pooled in a Lew^{DA} suspension.

The characterization of Lew^{DA} cells was performed by flow cytometry. Cell surface markers were stained with antibodies indicated in **Supplementary Table S1**, according to the instructions of the manufacturer. In all samples, Aqua Live/Dead fixable dead cell kit (Thermo Fisher Scientific, Waltham, MA, United States) was used unambiguously to remove dead cells. Flow cytometry analysis was performed on a FACS Canto II (BD Biosciences, Heidelberg, Germany). Data were analyzed using FlowJo software (Tree Star, Ashland, OR, United States). Overview of the gating strategy for T, NK and B cells has been shown in **Supplementary Figure S1**.

To perform the extracorporeal photopheresis, psoralen (8-MOP) at 300 ng/mL was incorporated to pooled Lew^{DA} cell suspension and 30 min later, cells were injected into UVA illumination bag XUV8501Q and then were illuminated with UV-A ($2J/cm^2$) in MacoGenic G2 system (MacoPharma). The haematocrit was <1% in all ECP batches produced. At the end of illumination, ECP product was collected from the illumination bag and concentrated to infuse immediately into Lewis rats according to the planned dose. An aliquot of each ECP batch was analyzed to define cell viability and proliferation capacity.

Analysis of the ECP Product

The viability of the ECP product and proliferative capacity in culture was analyzed using phytohemagglutinin (PHA-L) as a mitogen to trigger T-lymphocyte cell division. The viability of the ECP product was analyzed using Annexin V and viable dye stains. The proliferative capacity of ECP cells was determined by flow cytometry using carboxyfluorescein diacetate N-succinimidyl ester (CFSE) staining.

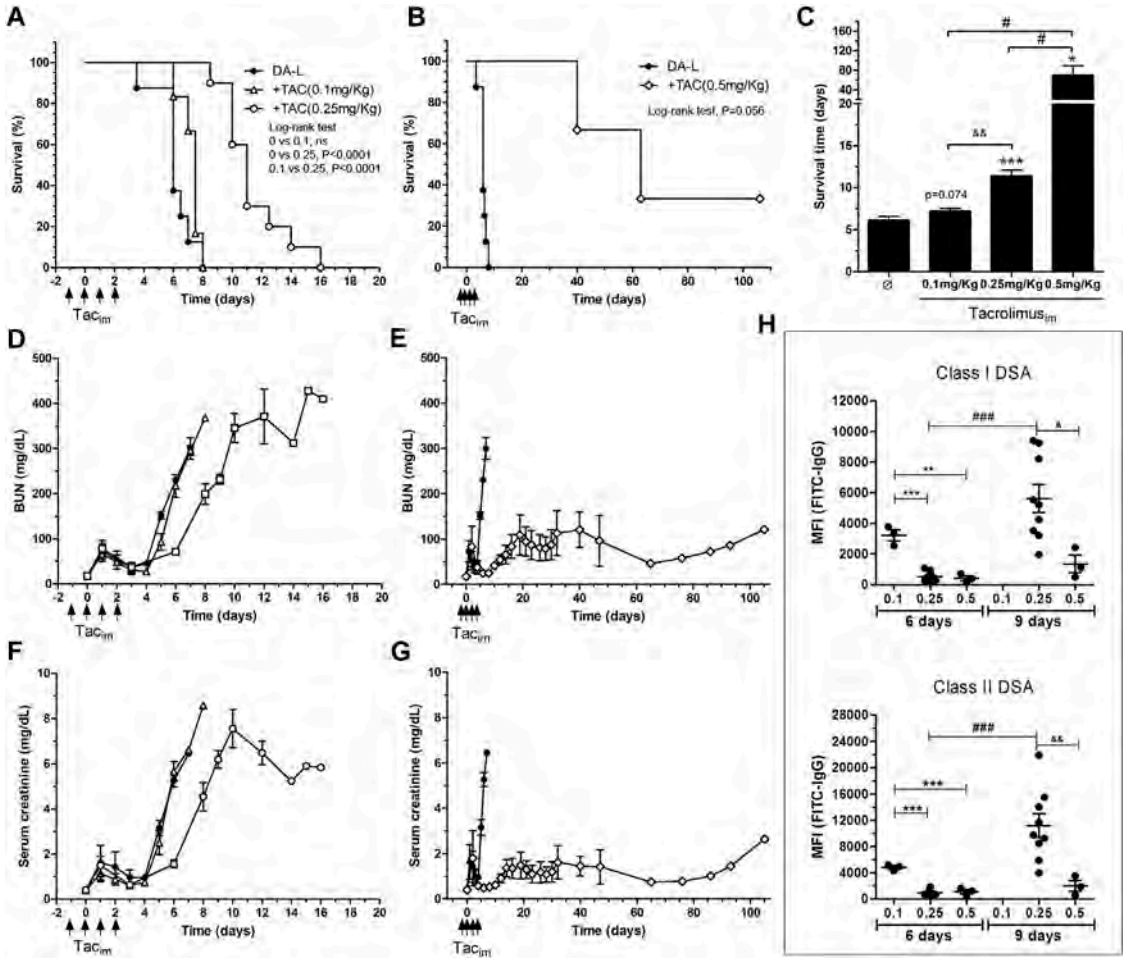


FIGURE 3 | Immunosuppresion set-up in DA-L rat kideny transplant model. **(A,B)** Rat survival analysis, DA-L without immunosuppresion ($n = 8$, circle), with TAC at 0.1 mg/kg ($n = 4$, triangle), with TAC at 0.25 mg/kg ($n = 10$, square) and with TAC at 0.5 mg/kg ($n = 3$, diamond). **(C)** Mean survival time analysis. *Significantly different when compared to none treated group (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). &Significantly different when compared to TAC dose of 0.25 mg/kg. #Significantly different when compared to TAC dose of 0.5 mg/kg. **(D–G)** Renal function analysis including blood urea nitrogen (BUN) and serum creatinine. **(H)** Quantification of class I and class II donor-specific antibodies (DSA) at 6 and 9 days after transplantation. *Significantly different when compared to TAC dose of 0.1 mg/kg (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). TAC dose of 0.1 mg/kg. &Significantly different when compared to TAC dose of 0.25 mg/kg. #Significantly different when compared each group a different time point. TAC_{im}, intramuscular Tacrolimus injection; MFI, mean fluorescence intensity.

Experimental Design

Lewis rat recipients were divided into four groups. All groups received intravenous TAC (0.25 mg/kg) for 4 days (−1, 0, +1 and +2 days with respect to transplantation). Group 1 (N = 10) received only TAC; group 2 (N = 5) in addition to TAC, received intravenous injections of 100×10^6 Lew^{DA} cells (negative control, no photopherized cells), whereas groups 3 (N = 4) and 4 (N = 9), in addition to TAC, received intravenous injections of 10×10^6 or 100×10^6 ECP cells respectively. In groups 2 to 4, seven doses of cells (Lew^{DA} or ECP) were administered in phosphate-buffered saline at −7, −3, 0, +2, +4, +7 and +14-day respect to transplantation (**Figure 1B**). Serum blood urea nitrogen (BUN) and serum creatinine levels were measured to determine kidney function.

Statistical Analysis

Statistical analysis was performed using the SPSS 21.0 statistics package. Univariate analysis using the log-rank test (Kaplan–Meier curves) was conducted to assess rat and graft survival (time from kidney transplantation to death). Values are given as mean ± SD. The Kruskal-Wallis or Mann-Whitney U tests were used where applicable.

Ethics

This study was approved by and conducted according to the guidelines of the local animal ethics committee (Comitè Ètic d'Experimentació Animal (CEEA) from Universitat de Barcelona, Decret 164/1998).

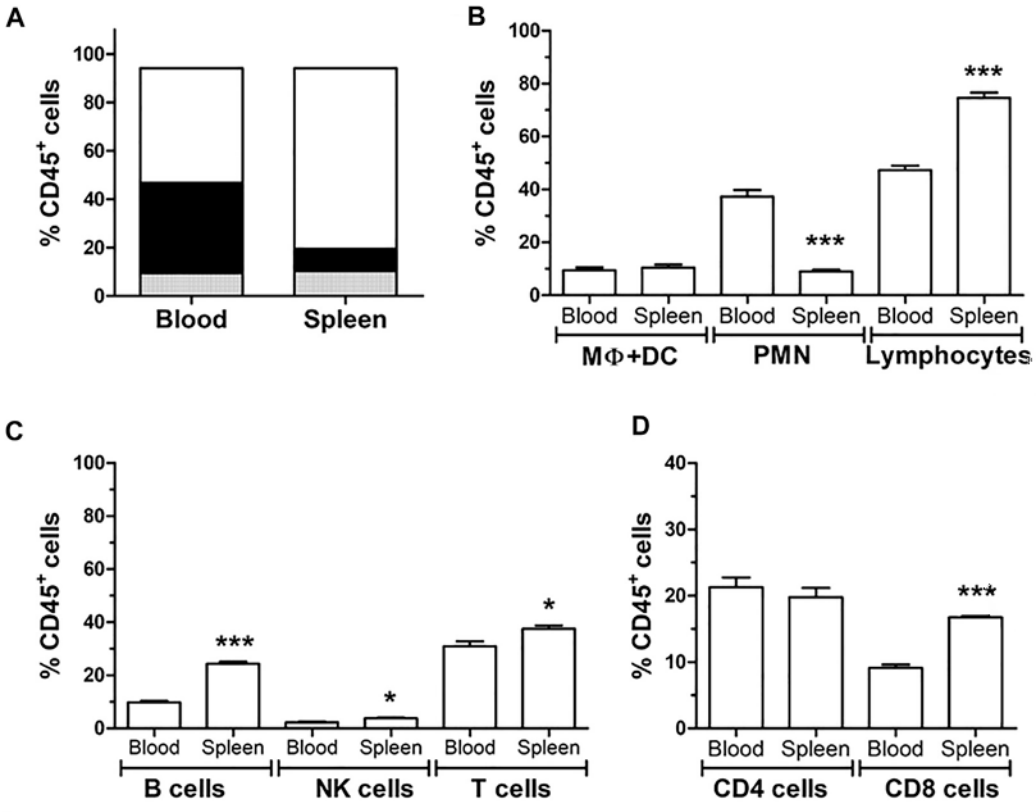


FIGURE 4 | Characterization of spleen and blood circulating leukocytes by flow cytometry. **(A)** Distribution of the different leukocyte cell types; polymorphonuclear cells (PMN, black), monocytes (grey) and lymphocytes (white) according to Forward scatter (FSC) and side scatter (SSC). **(B)** Quantification of the different leukocyte types **(C)** Quantification of the different lymphocyte; B cells, NK cells and T cells due to CD45R (HIS24) and CD161 and CD3, respectively. **(D)** Quantification of $CD3^+$ T cells in $CD4^+$ and $CD8^+$ cells. *Significantly different when compared to blood samples (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

RESULTS

We performed a full mismatch kidney transplantation rat model to determine ECP's immunomodulatory properties as an addon to immunosuppression therapy *in vivo*. The study primary outcomes were graft and rat survival.

Natural Course of the Allogeneic (DA-L) Kidney Transplantation Without Immunosuppression

All Lewis rats that received a DA kidney graft died within 8 days after transplantation (mean survival 6.12 ± 1.28 days), whereas Lewis rats that received an isogeneic kidney graft (Lewis rat donor) survived until the last day of experiment (established at D+15 after transplantation). The characterization of the allogeneic (DA-L) kidney transplantation is shown in **Figure 2**.

The DA-L kidney transplant model was characterized by a slight deterioration of renal function on day +1 post-transplantation followed by an improvement in renal function and subsequently, from day +4, a rapid decline of renal function with an increase of BUN and serum creatinine levels, graft loss and rat death (**Figures 2B,C**).

Donor-specific antibodies (DSA) anti-RT1A and anti-RT1B were detected since day three with a progressive increase until day 7 (**Figure 2D**).

Kidney graft histology at 6 days after transplantation was consistent with acute antibody-mediated rejection (ABMR). Histology showed cortical necrosis, with peritubular capillaritis, and thrombotic microangiopathy. The interstitial infiltrates were mostly characterized by polymorphonuclear leukocytes and macrophages, and C4d deposition on peritubular capillaries was strongly positive at immunohistochemistry (**Figure 2E**).

Impact of the TAC Therapy on the Allogeneic (DA-L) Kidney Transplantation

The recipients with low dose of TAC (0.1 mg/kg) had a mean survival time of 7.25 ± .69, not different from the transplant model without immunosuppression (6.12 ± 1.28; $p = 0.074$) (**Figures 3A,C**). Recipients with medium and high doses of TAC (0.25 and 0.5 mg/kg) showed a significant increase of rat survival when compared to recipients without immunosuppression, 11.4 ± 2.21 ($p = 0.0004$) and 70.0 ± 32.8 ($p = 0.0163$), respectively (**Figures 3A–C**). BUN and serum

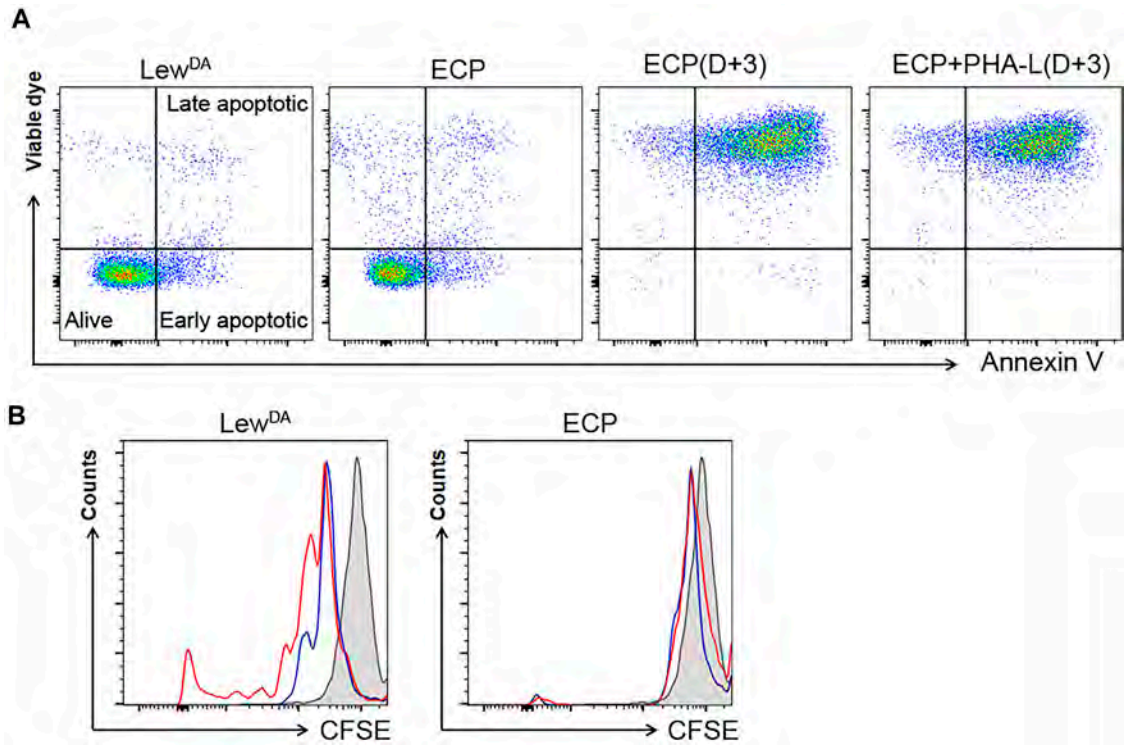


FIGURE 5 | Characterization of the extracorporeal photopheresis product. **(A)** Impact of ECP on the viability of Lew^{DA}. Representatives dot plots from Lew^{DA} cells, ECP cells, ECP cells cultured with full medium for 3 days and ECP cells cultured with phytohemagglutinin-L (PHA-L) for 3 days. In order to differentiate alive cells, early and late apoptotic cells, samples were stained with a viable die and Annexin V. **(B)** Impact of ECP on the proliferative capacity of Lew^{DA} splenonocytes and ECP product using CFSE staining. Left plot, Lew^{DA} splenocytes cultured for 3 days with or without PHA-L (red and blue lines, respectively). Right plot, ECP cells cultured for 3 days with or without PHA-L (red and blue lines, respectively). Gray shaded areas, cells stained with CFSE at Day 0.

creatinine levels, with a low TAC dose (0.1 mg/kg), were not different from the transplant model without immunosuppression. Whereas medium and high doses of TAC (0.25 and 0.5 mg/kg) preserved the renal function partially and showed a significant increase in survival (**Figure 3**).

The dose of 0.25 mg/kg of TAC was selected for the experimental design, and the low and high dose groups were discarded for the experimental model since they would make it difficult to show the effect of ECP treatment.

The effect of TAC doses on class I and II DSA levels is depicted in **Figure 3H**. On day 6 after transplantation, the dose of 0.1 mg/kg of TAC did not modify DSA levels compared with the model without immunosuppression (**Figures 2D**, **3H**). The groups with the intermediate and high doses of TAC (0.25 and 0.5 mg/kg) showed levels of DSA significantly lower compared to the baseline model at day 7 post-transplantation. On day +9, class I and class II DSA MFI were significantly lower in the TAC 0.5 mg/kg group compared to TAC 0.25 mg/kg.

Characterization of Lew^{DA} Cells

Lew^{DA} cells from peripheral blood and spleen cell suspension were characterized by flow cytometry (**Figure 4**). T and B cells, NK cells, monocytes Lew^{DA} cells from both cell suspensions were pooled before the photopheresis procedure, concretely 5.24 ± 1.20% of Lew^{DA} cells were from peripheral blood in our 27 batches.

Lymphocytes predominate in the spleen cells and the polymorphonuclear cells in peripheral blood. In spleen cells, B lymphocytes and NK cells were more frequent than in peripheral blood. The concentration of $CD4^+$ lymphocytes was similar between both origins, while $CD8^+$ lymphocytes predominate in the spleen. The proportion of dendritic cells and macrophages was not different between peripheral blood and spleen.

Characterization of ECP Cell Product

The ECP cell product's viability was assessed immediately after the photopheresis and after 3 days in culture with or without PHA-L as a mitogenic stimulus. ECP cell product seems to be alive (79.4 ± 1.4%) when the analysis was performed immediately after the photopheresis procedure compared to allogeneic Lew^{DA} cells (**Figure 5A**). However, when the ECP cell product was cultured for 3 days with or without PHA-L, about 95.2 ± 3.6% and 94.0 ± 0.2% of cells had to be considered apoptotic, respectively; moreover, just about 0.15 ± 0.06% and 0.14 ± 0.10% of cells remained alive, respectively (**Figure 5A**).

In order to evaluate the ECP cell product's proliferative capacity, a mitogenic stimulus was added in the cell culture media. The photopheresis procedure completely avoided the proliferation observed in Lew^{DA} cells (**Figure 5B**).

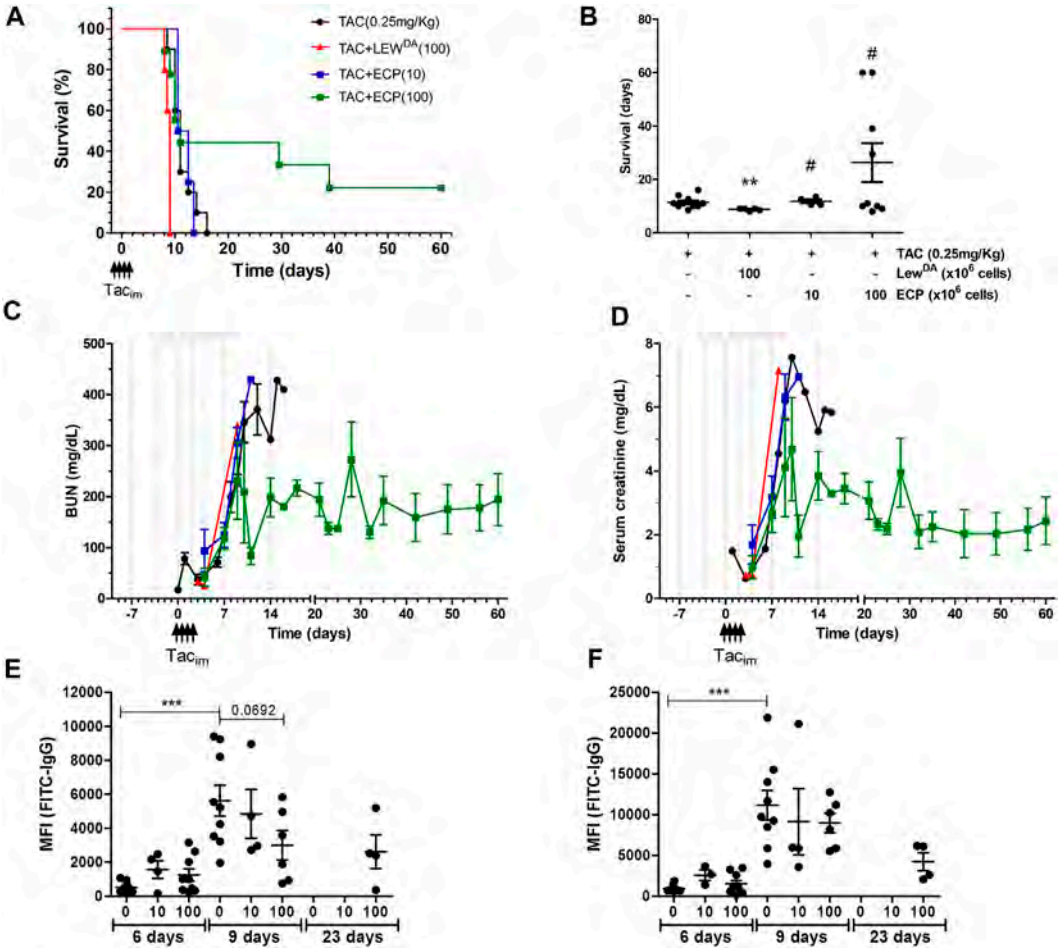


FIGURE 6 | Impact of ECP product on DA-L rat kidney transplant model. **(A)** Rat survival analysis of the following groups of rats: DA-L+TAC (0.25 mg/kg) (n = 10, black cicle), plus Lew^{DA} (100 × 10^6 cells, n = 5, red triangle), plus low dose of ECP (10 × 10^6 cells, n = 4, blue square), and plus high dose of ECP (100 × 10^6 cells, n = 9, green square) kidney transplantation. **(B)** Mean survival time analysis. *Statistical differences compare to TAC (0.25 mg/kg) group. **(C,D)** Renal function analysis including blood urea nitrogen (BUN) and serum creatinine. **(E,F)** Donor-specific antibodies analysis including rat MHC-class I (RT1A) and class II (RT1B). *Significantly different when compared to TAC dose of 0.25 mg/kg at 6 days. TAC$_{im}$, intramuscular Tacrolimus injection.

Impact of ECP on the Allogeneic (DA-L) Kidney Transplantation

The survival analysis is shown in **Figures 6A,B**. The infusion of Lew^{DA} cells from sensitized Lewis rats reduced the rat survival compared to TAC monotherapy group (8.7 ± 0.45 vs. 11.4 ± 2.21, p = 0.0012; Log-rank (Mantel-Cox) test, p = 0.0012). The illumination of Lew^{DA} cells, becoming ECP cells, blocks the acceleration of rejection observed in Lew^{DA} cells, all rats from the group TAC+ECP low dose (10 × 10^6 cells) had equal mean survival time than TAC monotherapy group (11.75 ± 1.5 vs. 11.4 ± 2.21, P=NS; Log-rank (Mantel-Cox) test, P=NS) and increased mean survival time than TAC+Lew^{DA} (11.75 ± 1.5 vs. 8.7 ± 0.45, p = 0.0175; Log-rank (Mantel-Cox) test, p = 0.0074), whereas 44% of rats in the TAC+ECP high dose (100 × 10^6) group survived until day 29 after transplantation. Mean survival time was prolonged in high doses of ECP until 26.28 ± 21.9 days being statistically different from TAC+Lew^{DA} group (p = 0.0308; Log-rank (Mantel-Cox) test, p = 0.0090).

Regarding renal function, BUN and creatinine were analyzed in **Figures 6C,D**, respectively. The infusion of Lew^{DA} cells accelerated the deterioration for kidney graft function compared to TAC monotherapy group. The high dose of ECP in combination with TAC stabilized the deterioration of kidney function in nearly 50% of rats (**Figures 6C,D** and **Supplementary Figure S2**), whereas a low dose of ECP did not confer any improvement in renal function compared to the TAC group.

The impact of ECP doses on class I and class II DSA is shown in **Figures 6E,F**. Both low and high ECP doses did not reduce the MFI of class I and class II DSA at day 6 after transplantation. The analysis at day 9 revealed that high doses of ECP in combination of TAC reduced partially class I DSA, concretely survivor rats had the lower class I and class II DSA levels. In the surviving rats at day 23 after transplantation, from TAC + ECP high dose group,

the MFI for class I and II DSA were 2620 ± 989 and 4241 ± 1102, respectively.

DISCUSION

In kidney transplantation, improving the management of antibody-mediated damage without increasing complications associated with immunosuppression is a crucial factor in graft and patient survival. At present, there are no FDA-approved treatments for acute or chronic antibody-mediated rejection, and plasma exchange with intravenous immunoglobulin constitutes the standard of care (34, 35). Even more, in chronic active ABMR, plasma exchange, and rituximab have been ineffective and are associated with a significant increase in severe infectious complications (36, 37). Rituximab has been also evaluated as induction therapy in kidney transplantation, however no convincing benefit was found and some safety concerns were identified (38, 39).

ECP is an attractive approach to prevent kidney graft rejection, given the absence of generalized immunosuppression or severe side effects.

DA-L kidney transplantation is a model characterized by early-onset antibody-mediated rejection with a rapid deterioration of renal function from day +4 after transplantation and short median survival of 6 days. To our knowledge, this is the first study to evaluate ECP in a full mismatch rat model of ABMR in kidney transplantation.

The efficacy of ECP on T-cell alloreactivity has long been proven, and in kidney transplantation, there are reports on the effectiveness of ECP in refractory T cell-mediated rejection (40). So, the efficacy of ECP on B- and plasma-cell mediated rejection mediatedantibody-mediated rejection is the remaining question. In the present study, *de novo* donor-specific antibodies (DSA) were detected from day three with a progressive increase, and kidney graft histology was consistent with ABMR. However, from an immune point of view, the development of high titers of DSA in 7 days, with the histological demonstration of antibody-mediated damage, fits better to a recall response. Although recipient rats have not been intentionally sensitized to donor antigens, we cannot rule out previous environmental exposure to cross-antigens with antigens from the donor rats.

In this model, a short course of four doses of TAC allows maintaining the graft's initial function, increasing survival with a median of 11 days. The application of Lew^{DA} cells from sensitized rats accelerated the rejection process (8.7 ± 0.45 days), whereas the addition of high dose of ECP cells at (100×10^6 cells, photopherized Lew^{DA} cells) prolonged mean survival time until 26.28 ± 21.9 days and stabilized the deterioration of kidney function in nearly 50% of rats. The addition of ECP to the treatment with a short course of TAC did not modify the expression of DSA in the initial period. However, on day 23 a decrease in DSA was evidenced compared to previous levels of MFI (day 9 after transplantation).

Previous evidence of ECP in solid organ transplantation, reported a significant decline in DSA and lung associated autoantibodies in pulmonary graft recipients with chronic allograft rejection in the form of the bronchiolitis obliterans (25). This decrease in antibodies occurred in conjunction with a reduction in pro-inflammatory cytokines and an increase in anti-inflammatory ones. A direct effect of ECP on DSA production cannot be ruled out, although indirect effects related to inflammatory factors appear to be involved. A decrease in B-cell activating factor (BAFF) levels and changes in B cell populations, has been identified as predictor of response to ECP treatment in GvHD (41, 42). Also, a mouse model of skin allograft showed that the infusion of splenocytes exposed to 8-MOP/UVA increased the number of IL-10^+ regulatory B cells in circulation and promoted survival of the graft (43).

It has to be highlighted that a high dose of ECP was decisive in the stabilization of renal function, whereas a low dose of ECP did not confer any improvement in kidney function or rat survival compared to TAC monotherapy treatment. Our study suggests a dose-response or at least the possibility of a dose threshold; in fact, some individuals' lack of response could be due to an insufficient dose.

Another debatable issue is the use of two pre-transplant doses. However, a point in favor of pre-transplant administration is the example of cellular therapies with mesenchymal cells (MSCs) where administration timing is of vital importance for their potency. Administration before the development of the inflammatory state increases the response to treatment (44).

This study has some limitations; immunosuppression was limited in time to highlight the effect of ECP in a small group of animals, making the model very aggressive. In humans, ECP therapy will be applied as add-on therapy to achieve immunomodulation. As usual in rat models, the cell source was mainly the spleen, while only 5.24% of ECP products were peripheral blood leukocytes. In contrast, ECP in humans is performed on peripheral blood leukocytes; the difference in the cell product can lead to different effects on the immune response that must be considered when the therapy moves to the clinic. In addition, the rate of antigen-specific cells in the ECP product should be known in future clinical trials, although we have not evaluated it in our study.

Another limitation of this study is the lack of evidence regarding ECP's mechanism in the renal rejection model. However, this is the first description of a positive effect of ECP in a full mismatch preclinical model that could teach us about the clinical application and mechanisms of ECP as an induction therapy in kidney transplantation. The mechanism underlying ECP immunomodulations is poorly known and sometimes contradictory. ECP induces apoptosis on leukocytes, as we shown in our approach; when these apoptotic cells return to the patient they generate an immunomodulatory effect (45, 46). Briefly, apoptotic cells are cleared by macrophages and dendritic cells (DC), which then upregulate suppressor factors (e.g., TGF-β, IL-10, IDO, HO-1, HLA-G, and PGE2) and downregulate costimulatory molecules (CD80 and CD86), resulting in tolerogenic DCs (TolDC). TolDCs suppress the effector T cell activity and induce the production of regulatory T cells (iTregs) (47–49). Recently, Pilon et al. demonstrated that human apoptotic cells induced by ECP (Apo-cells) can inhibit

allogeneic immune response that follows both direct and indirect alloantigen presentation (50).

Chronic antibody-mediated or cellular-mediated rejections are crucial in kidney transplant survival. However, it is necessary to develop new experimental models to address the utility of ECP in this particular problem.

In summary, ECP is an immunomodulatory therapy that appears to affect both the cellular and humoral arms of the immune response to the allograft. The established safety of ECP favours this approach as a potential treatment alternative in the setting of kidney transplantation. In our stringent model of acute kidney graft rejection, ECP was able to prolong kidney graft survival, preserving kidney function, although the effect of ECP on DSA production is not entirely clear. These promising results could pave the way for future clinical studies in kidney transplantation. In particular, our group is developing a clinical trial to elucidate the Impact of Photopheresis in the Prevention of Acute Rejection in Highly Sensitized *de Novo* Kidney Transplant Recipients (NCT04414735).

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The animal study was reviewed and approved by the Comitè Ètic d'Experimentació Animal (CEEA) from Universitat de Barcelona.

AUTHOR CONTRIBUTIONS

GP was involved in study conception, performing experiments, data analysis and writing of the manuscript. ML-R, MR-B, EB-M, NH-G, and EM-M were involved in performing experiments and data analysis. PV-A, ML, JC, DC, IR, EP, BB-G, and JMC were involved in data analysis and critical review of the manuscript. FD and JR were involved in study conception and critical review of the manuscript.

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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.2023.10840/full#supplementary-material

REFERENCES

1. Arend SM, Mallat MJ, Westendorp RJ, van der Woude FJ, van Es LA. Patient Survival after Renal Transplantation; More Than 25 Years Follow-Up. *Nephrol Dial Transpl* (1997) 12(8):1672–9. doi:10.1093/ndt/12.8.1672
2. Schnuelle P, Lorenz D, Trede M, Van Der Woude FJ. Impact of Renal Cadaveric Transplantation on Survival in End-Stage Renal Failure: Evidence for Reduced Mortality Risk Compared with Hemodialysis during Long-Term Follow-Up. *J Am Soc Nephrol* (1998) 9(11):2135–41. doi:10.1681/ASN.V9112135
3. Rao PS, Schaubel DE, Wei G, Fenton SS. Evaluating the Survival Benefit of Kidney Retransplantation. *Transplantation* (2006) 82(5):669–74. doi:10.1097/01.tp.0000235434.13327.11
4. Tennankore KK, Kim SJ, Baer HJ, Chan CT. Survival and Hospitalization for Intensive home Hemodialysis Compared with Kidney Transplantation. *J Am Soc Nephrol* (2014) 25(9):2113–20. doi:10.1681/ASN.2013111180
5. Bohmig GA, Eskandary F, Doberer K, Halloran PF. The Therapeutic challenge of Late Antibody-Mediated Kidney Allograft Rejection. *Transpl Int* (2019) 32(8):775–88. doi:10.1111/tri.13436
6. Dantal J, Soulillou JP. Immunosuppressive Drugs and the Risk of Cancer after Organ Transplantation. *N Engl J Med* (2005) 352(13):1371–3. doi:10.1056/NEJMe058018
7. Cheung CY, Tang SCW. An Update on Cancer after Kidney Transplantation. *Nephrol Dial Transpl* (2019) 34(6):914–20. doi:10.1093/ndt/gfy262
8. Sawitzki B, Harden PN, Reinke P, Moreau A, Hutchinson JA, Game DS, et al. Regulatory Cell Therapy in Kidney Transplantation (The ONE Study): a Harmonised Design and Analysis of Seven Non-randomised, Single-Arm, Phase 1/2A Trials. *Lancet* (2020) 395(10237):1627–39. doi:10.1016/S0140-6736(20)30167-7
9. Ezzelarab MB, Zahorchak AF, Lu L, Morelli AE, Chalasani G, Demetris AJ, et al. Regulatory Dendritic Cell Infusion Prolongs Kidney Allograft Survival in Nonhuman Primates. *Am J Transpl* (2013) 13(8):1989–2005. doi:10.1111/ajt.12310

10. Dreyer GJ, Groeneweg KE, Heidt S, Roelen DL, van Pel M, Roelofs H, et al. Human Leukocyte Antigen Selected Allogeneic Mesenchymal Stromal Cell Therapy in Renal Transplantation: The Neptune Study, a Phase I Single-center Study. *Am J Transpl* (2020) 20(10):2905–15. doi:10.1111/ajt.15910
11. Scarisbrick J. Extracorporeal Photopheresis: what Is it and when Should it Be Used? *Clin Exp Dermatol* (2009) 34(7):757–60. doi:10.1111/j.1365-2230.2009.03475.x
12. De Silvestro G. The Italian Registry of Therapeutic Apheresis - 2015. *Transfus Apher Sci* (2017) 56(1):75–81. doi:10.1016/j.transci.2016.12.024
13. Cid J, Carbasse G, Suarez-Lledo M, Moreno DF, Martinez C, Gutierrez-Garcia G, et al. Efficacy and Safety of One-Day Offline Extracorporeal Photopheresis Schedule Processing One Total Blood Volume for Treating Patients with Graft-Versus-Host Disease. *Transfusion* (2019) 59(8):2636–42. doi:10.1111/trf.15384
14. Edelson R, Berger C, Gasparro F, Jegasothy B, Heald P, Wintroub B, et al. Treatment of Cutaneous T-Cell Lymphoma by Extracorporeal Photochemotherapy. Preliminary Results. *N Engl J Med* (1987) 316(6): 297–303. doi:10.1056/NEJM198702053160603
15. Halle P, Paillard C, D'Incan M, Bordigoni P, Piguet C, De Lumley L, et al. Successful Extracorporeal Photochemotherapy for Chronic Graft-Versus-Host Disease in Pediatric Patients. *J Hematother Stem Cel Res* (2002) 11(3):501–12. doi:10.1089/15258160260090960
16. Rafei H, Kharfan-Dabaja MA, Nishihori T. A Critical Appraisal of Extracorporeal Photopheresis as a Treatment Modality for Acute and Chronic Graft-Versus-Host Disease. *Biomedicines* (2017) 5(4):60. doi:10.3390/biomedicines5040060
17. Rose EA, Barr ML, Xu H, Pepino P, Murphy MP, McGovern MA, et al. Photochemotherapy in Human Heart Transplant Recipients at High Risk for Fatal Rejection. *J Heart Lung Transpl* (1992) 11(4):746–50.
18. Sunder-Plassman G, Druml W, Steininger R, Honigsmann H, Knobler R. Renal Allograft Rejection Controlled by Photopheresis. *Lancet* (1995) 346(8973):506. doi:10.1016/s0140-6736(95)91355-6
19. Jaksch P, Knobler R. ECP and Solid Organ Transplantation. *Transfus Apher Sci* (2014) 50(3):358–62. doi:10.1016/j.transci.2014.04.006
20. Kirklin JK, Brown RN, Huang ST, Naftel DC, Hubbard SM, Rayburn BK, et al. Rejection with Hemodynamic Compromise: Objective Evidence for Efficacy of Photopheresis. *J Heart Lung Transpl* (2006) 25(3):283–8. doi:10.1016/j.healun.2005.10.004
21. Barr ML, Meiser BM, Eisen HJ, Roberts RF, Livi U, Dall'Amico R, et al. Photopheresis for the Prevention of Rejection in Cardiac Transplantation. Photopheresis Transplantation Study Group. *N Engl J Med* (1998) 339(24): 1744–51. doi:10.1056/NEJM199812103392404
22. Patel J, Klapper E, Shafi H, Kobashigawa JA. Extracorporeal Photopheresis in Heart Transplant Rejection. *Transfus Apher Sci* (2015) 52(2):167–70. doi:10.1016/j.transci.2015.02.004
23. Morrell MR, Despotis GJ, Lublin DM, Patterson GA, Trulock EP, Hachem RR. The Efficacy of Photopheresis for Bronchiolitis Obliterans Syndrome after Lung Transplantation. *J Heart Lung Transpl* (2010) 29(4):424–31. doi:10.1016/j.healun.2009.08.029
24. Jaksch P, Scheed A, Keplinger M, Ernst MB, Dani T, Just U, et al. A Prospective Interventional Study on the Use of Extracorporeal Photopheresis in Patients with Bronchiolitis Obliterans Syndrome after Lung Transplantation. *J Heart Lung Transpl* (2012) 31(9):950–7. doi:10.1016/j.healun.2012.05.002
25. Baskaran G, Tiriveedhi V, Ramachandran S, Aloush A, Grossman B, Hachem R, et al. Efficacy of Extracorporeal Photopheresis in Clearance of Antibodies to Donor-specific and Lung-specific Antigens in Lung Transplant Recipients. *J Heart Lung Transpl* (2014) 33(9):950–6. doi:10.1016/j.healun.2014.04.020
26. Hachem R, Corris P. Extracorporeal Photopheresis for Bronchiolitis Obliterans Syndrome after Lung Transplantation. *Transplantation* (2018) 102(7):1059–65. doi:10.1097/TP.0000000000002168
27. Kusztal M, Klak R, Krajewska M, Boratynska M, Patrzalek D, Klinger M. Application of Extracorporeal Photopheresis in Kidney Transplant Recipients: Technical Considerations and Procedure Tolerance. *Transpl Proc* (2011) 43(8): 2941–2. doi:10.1016/j.transproceed.2011.08.034
28. Tamain M, Sayegh J, Lionet A, Grimbert P, Philipponnet C, Hazzan M, et al. Extracorporeal Photopheresis for the Treatment of Graft Rejection in 33 Adult Kidney Transplant Recipients. *Transfus Apher Sci* (2019) 58(4):515–24. doi:10.1016/j.transci.2019.06.031
29. Lamioni A, Carsetti R, Legato A, Landolfo A, Isacchi G, Emma F, et al. Induction of Regulatory T Cells after Prophylactic Treatment with Photopheresis in Renal Transplant Recipients. *Transplantation* (2007) 83(10):1393–6. doi:10.1097/01.tp.0000261635.30578.d8
30. Dall'Amico R, Murer L, Montini G, Andreetta B, Zanon GF, Zacchello G, et al. Successful Treatment of Recurrent Rejection in Renal Transplant Patients with Photopheresis. *J Am Soc Nephrol* (1998) 9(1):121–7. doi:10.1681/ASN.V91121
31. Wolfe JT, Tomaszewski JE, Grossman RA, Gottlieb SL, Naji A, Brayman KL, et al. Reversal of Acute Renal Allograft Rejection by Extracorporeal Photopheresis: a Case Presentation and Review of the Literature. *J Clin Apher* (1996) 11(1):36–41. doi:10.1002/(SICI)1098-1101(1996)11:1<36::AID-JCA8>3.0.CO;2-C
32. Kumlien G, Genberg H, Shanwell A, Tyden G. Photopheresis for the Treatment of Refractory Renal Graft Rejection. *Transplantation* (2005) 79(1):123–5. doi:10.1097/01.tp.0000147197.24050.61
33. Rovira J, Ramirez-Bajo MJ, Banon-Maneus E, Lazo-Rodriguez M, Moya-Rull D, Hierro-Garcia N, et al. Tofacitinib Halts Progression of Graft Dysfunction in a Rat Model of Mixed Cellular and Humoral Rejection. *Transplantation* (2018) 102(7):1075–84. doi:10.1097/TP.0000000000002204
34. Velidedeoglu E, Cavaille-Coll MW, Bala S, Belen OA, Wang Y, Albrecht R. Summary of 2017 FDA Public Workshop: Antibody-Mediated Rejection in Kidney Transplantation. *Transplantation* (2018) 102(6):e257–e64. doi:10.1097/TP.0000000000002141
35. Montgomery RA, Loupy A, Segev DL. Antibody-mediated Rejection: New Approaches in Prevention and Management. *Am J Transpl* (2018) 18(3):3–17. doi:10.1111/ajt.14584
36. Moreso F, Crespo M, Ruiz JC, Torres A, Gutierrez-Dalmau A, Osuna A, et al. Treatment of Chronic Antibody Mediated Rejection with Intravenous Immunoglobulins and Rituximab: A Multicenter, Prospective, Randomized, Double-Blind Clinical Trial. *Am J Transpl* (2018) 18(4):927–35. doi:10.1111/ajt.14520
37. Pineiro GJ, De Sousa-Amorim E, Sole M, Rios J, Lozano M, Cofan F, et al. Rituximab, Plasma Exchange and Immunoglobulins: an Ineffective Treatment for Chronic Active Antibody-Mediated Rejection. *BMC Nephrol* (2018) 19(1): 261. doi:10.1186/s12882-018-1057-4
38. Macklin PS, Morris PJ, Knight SR. A Systematic Review of the Use of Rituximab as Induction Therapy in Renal Transplantation. *Transpl Rev (Orlando)* (2015) 29(2):103–8. doi:10.1016/j.trre.2014.12.001
39. van den Hoogen MW, Kamburova EG, Baas MC, Steenbergen EJ, Florquin S, Hj MK, et al. Rituximab as Induction Therapy after Renal Transplantation: a Randomized, Double-Blind, Placebo-Controlled Study of Efficacy and Safety. *Am J Transpl* (2015) 15(2):407–16. doi:10.1111/ajt.13052
40. Fernandez EJ, Lopez C, Ramirez A, Guerra R, Lopez L, Fernandez F, et al. Role of Photopheresis in the Treatment of Refractory Cellular Rejection in Kidney Transplantation. *Nefrologia* (2016) 36(3):327–8. doi:10.1016/j.nefro.2015.06.023
41. Whittle R, Taylor PC. Circulating B-Cell Activating Factor Level Predicts Clinical Response of Chronic Graft-Versus-Host Disease to Extracorporeal Photopheresis. *Blood* (2011) 118(24):6446–9. doi:10.1182/blood-2011-05-354019
42. Kuzmina Z, Greinix HT, Knobler R, Worel N, Kouba M, Weigl R, et al. Proportions of Immature CD19+CD21- B Lymphocytes Predict the Response to Extracorporeal Photopheresis in Patients with Chronic Graft-Versus-Host Disease. *Blood* (2009) 114(3):744–6. doi:10.1182/blood-2009-05-221028
43. Wei YX, Sun B, Xiao L, Shi BY. Extracorporeal Photopheresis: A Case of Immunotherapy Ahead of its Time. *Transplant Proc* (2018) 50:3906–35.
44. Pool M, Leuvenink H, Moers C. Reparative and Regenerative Effects of Mesenchymal Stromal Cells-Promising Potential for Kidney Transplantation? *Int J Mol Sci* (2019) 20(18):4614. doi:10.3390/ijms20184614
45. Poon IK, Lucas CD, Rossi AG, Ravichandran KS. Apoptotic Cell Clearance: Basic Biology and Therapeutic Potential. *Nat Rev Immunol* (2014) 14(3): 166–80. doi:10.1038/nri3607
46. Heshmati F. Updating ECP Action Mechanisms. *Transfus Apher Sci* (2014) 50(3):330–9. doi:10.1016/j.transci.2014.04.003
47. Gatza E, Rogers CE, Clouthier SG, Lowler KP, Tawara I, Liu C, et al. Extracorporeal Photopheresis Reverses Experimental Graft-Versus-Host

Disease through Regulatory T Cells. *Blood* (2008) 112(4):1515–21. doi:10.1182/blood-2007-11-125542

48. Spisek R, Gasova Z, Bartunkova J. Maturation State of Dendritic Cells during the Extracorporeal Photopheresis and its Relevance for the Treatment of Chronic Graft-Versus-Host Disease. *Transfusion* (2006) 46(1):55–65. doi:10.1111/j.1537-2995.2005.00670.x
49. Morelli AE. The Immune Regulatory Effect of Apoptotic Cells and Exosomes on Dendritic Cells: its Impact on Transplantation. *Am J Transpl* (2006) 6(2):254–61. doi:10.1111/j.1600-6143.2005.01197.x
50. Pilon C, Stehle T, Beldi-Ferchiou A, Matignon M, Thiolat A, Burlion A, et al. Human Apoptotic Cells, Generated by Extracorporeal Photopheresis, Modulate Allogeneic Immune Response. *Front Immunol* (2019) 10:2908. doi:10.3389/fimmu.2019.02908

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Experimental Assessment of Intestinal Damage in Controlled Donation After Circulatory Death for Visceral Transplantation

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There is an urgent need to address the shortage of potential multivisceral grafts in order to reduce the average time in waiting list. Since donation after circulatory death (DCD) has been successfully employed for other solid organs, a thorough evaluation of the use of intestinal grafts from DCD is warranted. Here, we have generated a model of Maastricht III DCD in rodents, focusing on the viability of intestinal and multivisceral grafts at five (DCD5) and twenty (DCD20) minutes of cardiac arrest compared to living and brain death donors. DCD groups exhibited time-dependent damage. DCD20 generated substantial intestinal mucosal injury and decreased number of Goblet cells whereas grafts from DCD5 closely resemble those of brain death and living donors groups in terms intestinal morphology, expression of tight junction proteins and number of Paneth and Globet cells. Upon transplantation, intestines from DCD5 showed increased ischemia/reperfusion damage compared to living donor grafts, however mucosal integrity was recovered 48 h after transplantation. No differences in terms of graft rejection, gene expression and absorptive function between DCD5 and living donor were observed at 7 post-transplant days. Collectively, our results highlight DCD as a possible strategy to increase multivisceral donation and transplantation procedures.

Keywords: experimental transplantation, organ procurement, donation after cardiac death, solid organ transplant, intestinal transplantation

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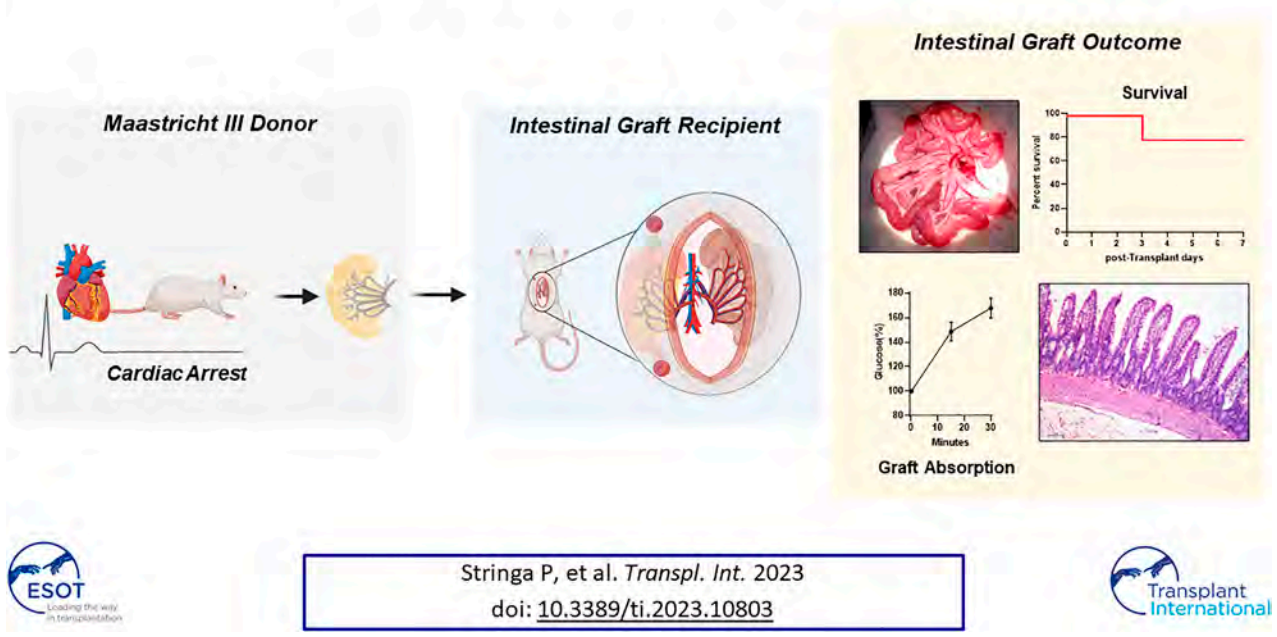
Abbreviations: BD, brain death; BDD, brain death donor; CA, cardiac arrest; CD, circulatory death; DCD, donations after circulatory death; DCD5, donation after 5 min of circulatory death; DCD20, donation after 20 min of circulatory death; GC, Goblet cell; ITx, intestinal transplantation; LD, conventional living donor; LLST, limitation of life support therapy; VLD, ventilated living donor; VT, visceral transplantation.



GRAPHICAL ABSTRACT |

INTRODUCTION

The universal shortage of organs has prompted the growing use of donations after circulatory death (DCD), which is expected to escalate further (1). Various organs including kidneys, liver, lungs, pancreas, and heart from DCD donors have been successfully employed for transplantation in several centers worldwide. The majority of the pitfalls and concerns regarding DCD donor use have been addressed by strict donor selection (2), improvements in normothermic regional perfusion (3), and *ex situ* machine perfusion devices (4). Although the actual impact of routine DCD use is challenging to assess, it has been estimated that organ donation has increased by 42% (5).

The valuable resource of DCD has been denied for intestinal grafts due to concerns regarding the ischemic susceptibility of this organ. Notably, experimental studies underwriting this veto are limited and underscored by heterogeneous methodology (6–8). Conversely, clinical evidence supports the use of these grafts in clinical settings. Moreover, there is an urgent need for grafts to cope with the mismatch between the waiting list for pediatric and adults intestinal transplantation (ITx) and scarcity of available organs. ITx candidates are predominantly those with longer waiting periods and higher mortality rates compared to other patients awaiting solid organ transplantation (9). Therefore, a thorough evaluation of the potential use of intestinal grafts from donors with DCD becomes highly relevant.

The aims of this study were therefore: 1) to develop an experimental model of Maastricht III DCD for solid organ transplantation in rodents, focusing on the viability of intestinal grafts; 2) to compare intestinal grafts quality from DCD to that in brain death donors (BDD) and living donors (LD) in the corresponding animal models and to assess the factibility of using intestinal graft from DCD in an experimental model of ITx; 3) to evaluate graft viability from DCD donors after ITx in a rat model.

MATERIALS AND METHODS

Animals

Adult (8–10 weeks) male Sprague-Dawley and Wistar rats were used in this study. The animals were group-housed in a climate-controlled room on a 12-h light–dark cycle at the animal facilities of our institution. Experimental protocols were performed in strict accordance with the recommendations of the *European Union Criteria for Animal Use in Scientific Experimentation* (63/2010 EU) and related Spanish legislation (RD 53/2013). The experimental procedures were approved by the local *Animal Welfare Ethics Committee* (PROEX 58.7/20).

Experimental Design

For the study of intestinal graft quality coming from different donation scenarios, animals were divided into five experimental groups (Five animals per group) (**Figure 1A**): 1. Conventional LD: i.v. organ perfusion; 2. Ventilated LD (VLD): mechanical ventilation for 2 h, i.v organ perfusion; 3. Donation after 5 min of

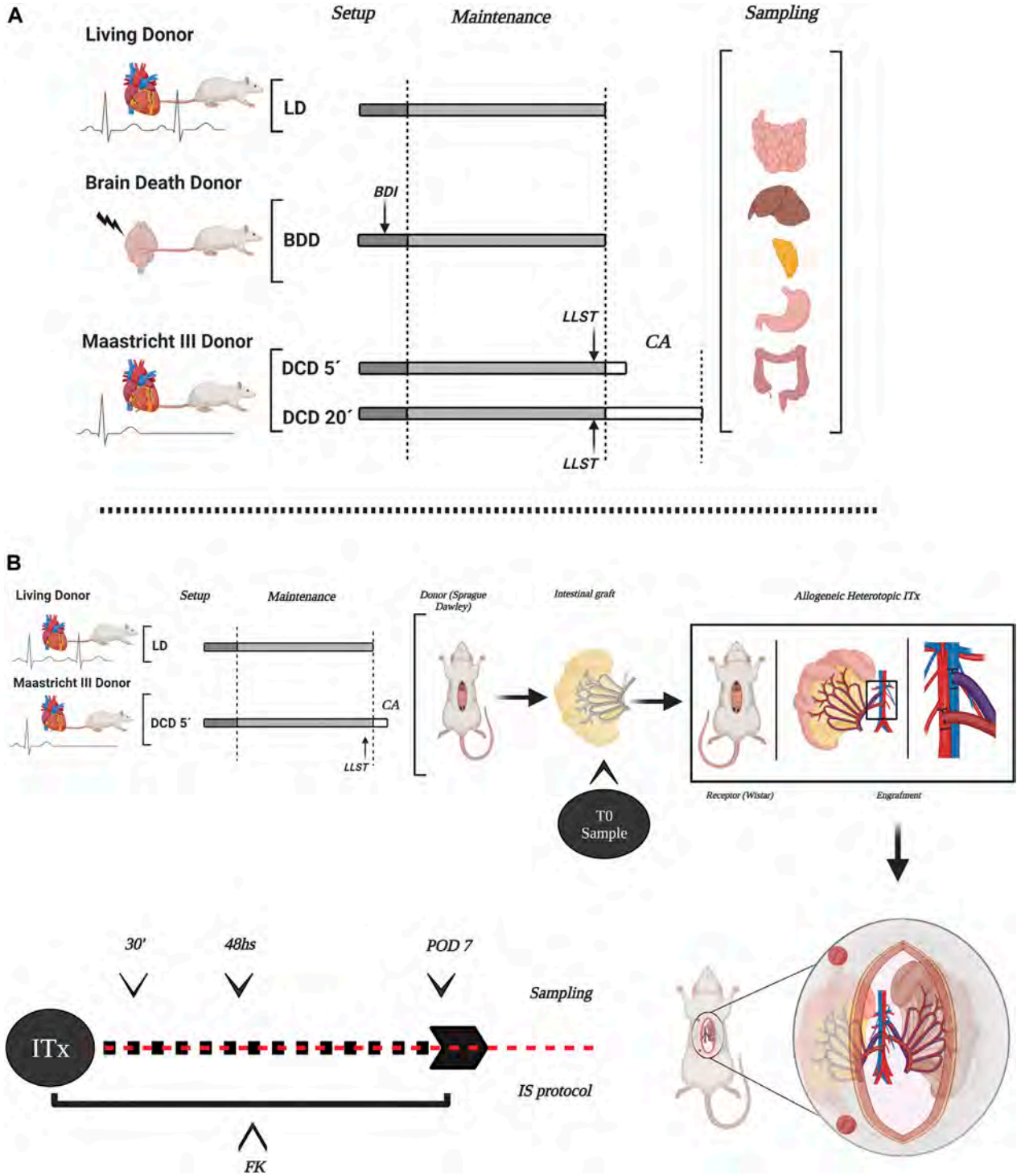


FIGURE 1 | Scheme of the experimental design in the different donation models and ITx. The study encompassed several types of organ donors in rats, representing the different stages and component organs of intestinal and multivisceral grafts **(A)**. Experimental design of allogeneic-heterotopic ITx model in rats. DCD5 and LD were considered to ITx. Transplanted intestines were sampled after transplantation to evaluate long-term outcome of the graft **(B)** LD, living donors; LVD, ventilated living donors; BDD, brain death donors; BDI, brain death induction; DCD, donation after circulatory death with two different times of CA (5 and 20 min); LLST, limitation of life support therapy; CA, cardiac arrest. This figure was generated with BioRender program (biorender.com).

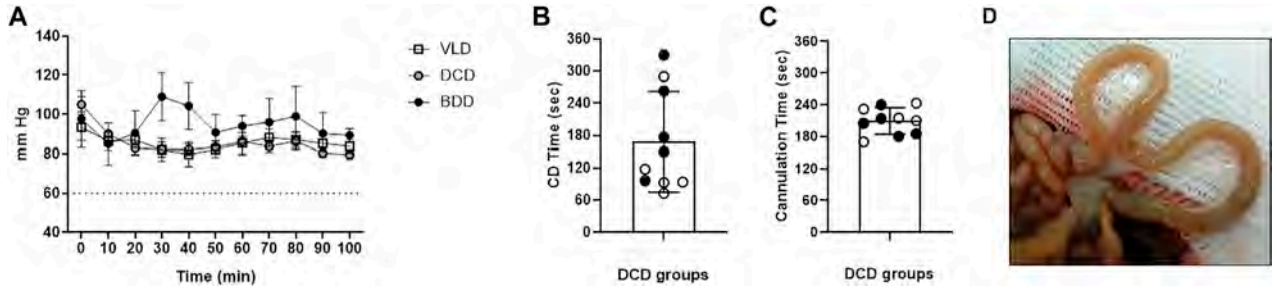


FIGURE 2 | Donor and graft monitoring during DCD. **(A)** MAP in living donor (LD), brain death (BD), and donation after circulatory death (DCD) groups exhibited normal values throughout the experimental procedure. No significant differences were observed between groups at any time-points. **(B)** Elapsed period (seconds) between limitation of life support therapy and CA diagnosis in the DCD groups. **(C)** After "no-touch period" cannulation time was measured from skin incision to the start of cold perfusion (black and white circles correspond to donation after 5 min of circulatory death [DCD5] and donation after 20 min of circulatory death [DCD20] groups, respectively). **(D)** Representative macroscopic image of an intestine from a DCD20 donor at the end of the experimental procedure. The pale appearance denoted correct washing of the graft.

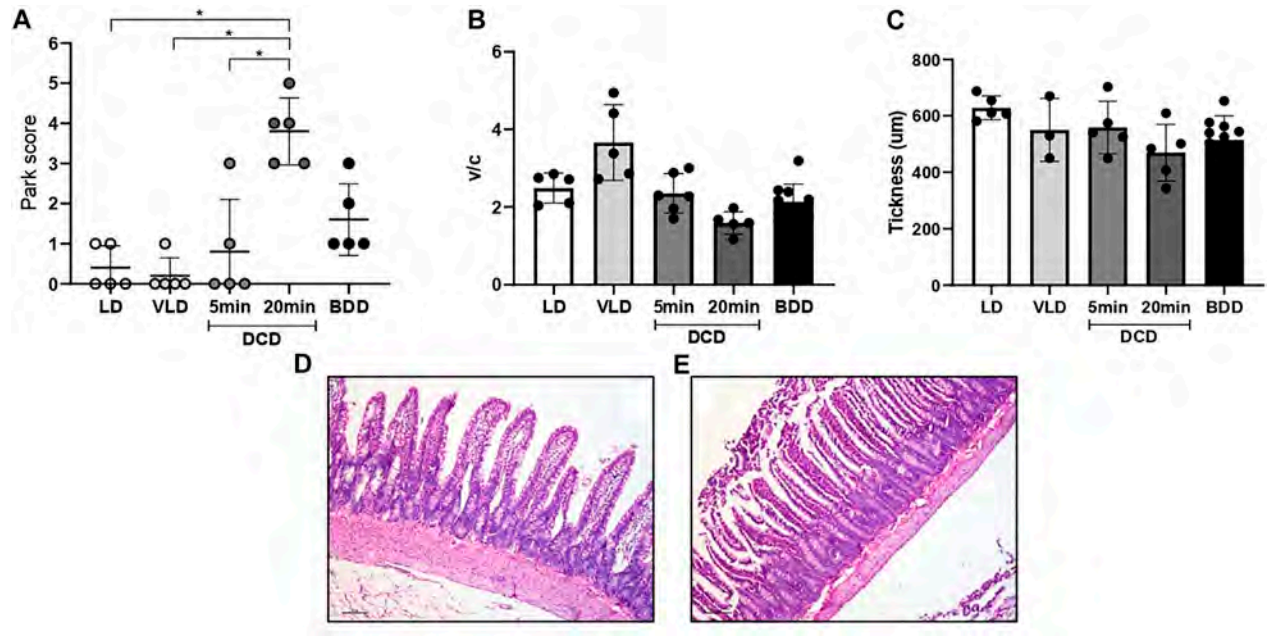


FIGURE 3 | Warm ischemia time impacts intestinal graft histology in donation after circulatory death (DCD). **(A)** Architecture in DCD5-group small bowel was similar to that of LD. Groups with the longest ischemia time presented with more severe damage based on Chiu/Park scores compared to the other donors, with significant differences vs. LD and DCD5 groups ($p < 0.05$). **(B,C)** Despite a worsening trend in the crypt villus index and intestinal mucosa thickness in the donation after 20 min of no-touch period (DCD20) group, no differences between groups in these morphometric studies were observed. Representative microphotographs from DCD5 **(D)** and DCD20 **(E)** group.

circulatory death (DCD5): mechanical ventilation for 2 h, 5 min of CD, i.v. organ perfusion 4. Donation after 20 min of circulatory death (CD): mechanical ventilation for 2 h, 20 min of CD, i.v. organ perfusion (10); 5. Brain Death (BD): BD and ventilation for 2 h, i.v organ perfusion (11, 12). Additional information from experimental procedures is available in **Supplementary Data S1**.

In all groups, samples of the different organs comprising the multivisceral graft (small bowel, stomach, pancreas, colon, and liver) were taken for histological analysis (13–20).

For long-term intestinal graft survival evaluation after allogeneic and heterotopic ITx, two experimental groups was performed (Five ITx per group) (**Figure 1B**): 1- LD + ITx, and 2: DCD5 + ITx. Additional information is available in **Supplementary Data S2**.

Samples of transplanted intestine were taken through intestinal ostomy at 0, 30 min, 48 h and 7 days after ITx. Histological and morphometric graft evaluation, Immunofluorescence staining, gene expression analysis and

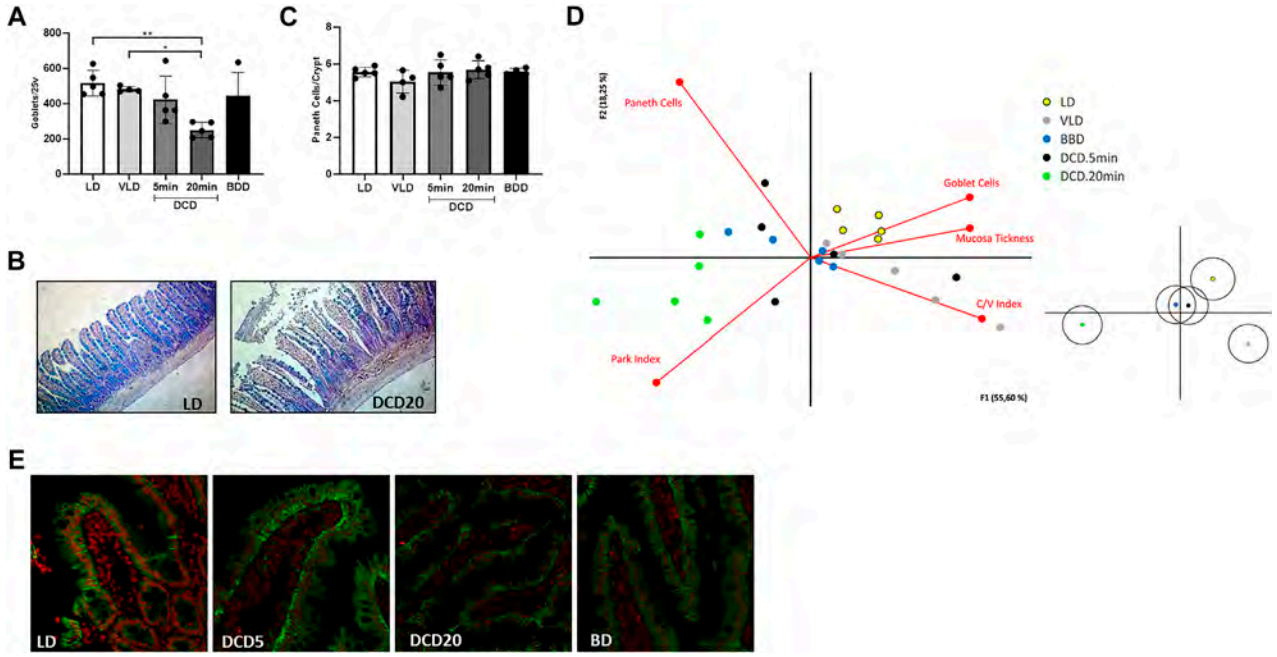


FIGURE 4 | Cell population study and principal component analysis of the intestinal graft in the different donation scenarios. **(A)** Goblet cell count using Alcian Blue staining. Significant differences between DCD20 and LD groups were observed (*$p < 0.05$; **$p < 0.01$). **(B)** Examples of high and low numbers of goblet cells in intestinal samples corresponding to LD and DCD20 groups, respectively. **(C)** All experimental groups exhibited similar numbers of Paneth cells. **(D)** Biplot visualizing correlations among variables. The DCD5 group closely resembled BDD (grafts used in human settings). Samples from the DCD20 group were the most distinct compared to the other experimental groups and were associated with histological damage variables based on Chiu/Park scores. Claudin-3 staining in different scenarios of intestinal procurement **(E)**.

graft functional evaluation was performed (21) (**Supplementary Datas S3–S7**).

RESULTS

Feasibility of Controlled Maastricht III DCD in Rats

The experimental model developed in this study enabled the replication of crucial phases of Maastricht III DCD (patient maintenance, donor limitation of life support therapy (LLST), diagnosis of CD, hypoperfusion phase, waiting period after cardiac arrest (no-touch period), cannulation time, washing, and multivisceral graft retrieval). The success of the Maastricht III model in rats for visceral transplantation (VT) was 83.3%. Two animals presented complications during the procedure and were excluded from the study (one case of prolonged hypotension and one case of massive hemorrhage through the artery cannula).

Successful donor monitoring was achieved in all experimental groups based on stable body temperature, O_2 saturation, and mean arterial pressure, which were established as inclusion parameters as described in the Materials and Methods section. BDD exhibited a slight increase in mean arterial pressure (MAP) at 30 and 40 min after BD induction, which was attributed to the norepinephrine infusion that was concurrently administered to this group (**Figure 2A**).

The time elapsed between the LLST and diagnosis of CD was 2.8 ± 1.4 min. No significant differences were observed between DCD5 and DCD20 (**Figure 2B**). CD temporal heterogeneity was not correlated with MAP at the time of LLST (data not shown). After "no-touch period" (5 or 20 min, according to experimental group), time required for opening, cross-clamp, cannulation and perfusion was 3.4 ± 0.35 min in DCD5 and 3.46 ± 0.35 in DCD20 groups (No significant differences between groups) (**Figure 2C**). Considering hypoperfusion phase, waiting period after cardiac arrest and cannulation, total warm ischemia time was 8.41 ± 0.35 min and 23.5 ± 0.34 for DCD5 and DCD20, respectively ($p < 0.05$).

At the time of sampling, the macroscopic appearance of intestinal grafts showed pale appearance in the five experimental groups, without traces of blood in the mesenteric vasculature or small intestinal wall, indicative of correct washing of the graft even in DCD20 (**Figure 2D**).

Morphological Characteristics of Multivisceral Grafts From DCD

The DCD group exhibited time-dependent small bowel histological damage. Prolonged CD generated substantial intestinal mucosal injury (3–5 based on the Park/Chiu score). Significant differences were observed between the DCD20 versus LD and DCD5 groups ($p < 0.05$, **Figure 3A**). However, no significant differences were observed in villus/crypt index and

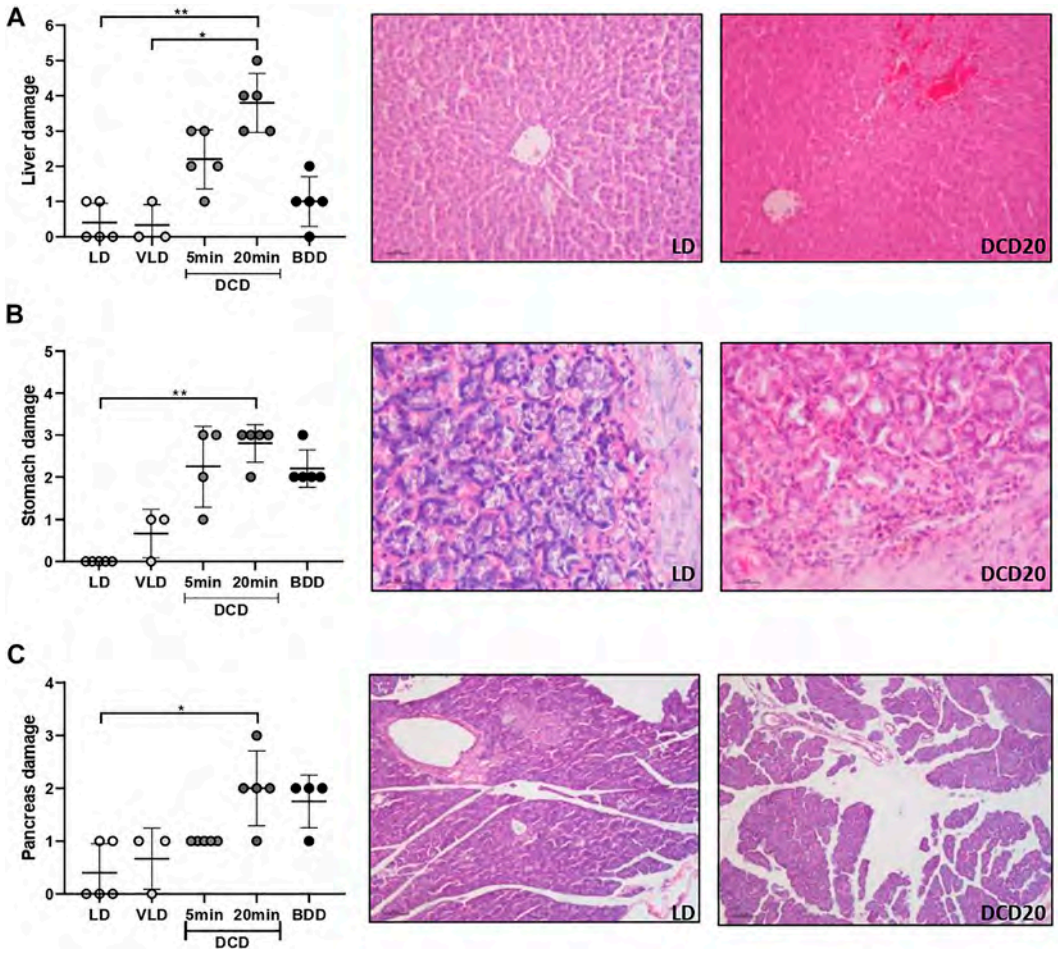


FIGURE 5 | Histological analysis of the remaining organs comprising multivisceral grafts in the different donation scenarios. Liver **(A)**, stomach **(B)**, and pancreas **(C)** analyses are presented. In all cases, significant differences were observed between the donation after 20 min of CA (DCD20) and LD groups (*$p < 0.05$; **$p < 0.01$). Representative microscopic images of low and high damage of the three organs are presented.

mucosal thickness between experimental groups (**Figures 3B,C**). Similar results were observed in all groups with the exception of DCD20 small bowel samples, in which GC count was considerably lower. A significant difference in Goblet cell (GC) analysis was noted between DCD20 and LD groups ($p < 0.05$, **Figures 4A,B**). Conversely, no differences were observed in Paneth cells per crypt count, with an average of approximately 5 Paneth cells in all study groups (**Figure 4C**). DCD20 group showed major alterations in tight junction proteins ZO-1 and claudin-1 expression (**Figure 4E**), whereas minor alterations were observed in DCD5 and BD groups.

The average variability explained by principal component analysis was 73% (**Figure 4D**, principal component 1, 55%; principal component 2, 18%). GC number, mucosal thickness, and villus-crypt index were directly correlated with LD and VLD groups, suggesting that higher GC number, thicker mucosa, and increased crypt-villus index were indicative of small bowel integrity. Park/chiu score was inversely correlated with these variables but was strongly correlated with the DCD20 group. Of note, despite data dispersion, BDD and DCD5 groups were clustered closer to the VD and VLD groups than to the DCD20 group.

In agreement with the trend observed for intestinal grafts, other organs were also affected by longer periods of DCD: DCD20 livers revealed significantly poorer morphology compared to LD (**Figure 5A**), with mild-to-moderate congestion and cytoplasmic vacuolization as the most relevant microscopic alterations (DCD20 vs. LD group, $p < 0.05$).

The DCD20 group presented more severe gastric injury, accompanied by glandular damage, epithelial desquamation, and cellular infiltrate of a mild-to-moderate degree ($p < 0.05$ vs. LD group). Similar but milder injuries were observed in DCD5 and BDD gruops (**Figure 5B**).

Moderate edema was the most common observation in the pancreas of BDD and DCD20 groups (**Figure 5C**). A significant difference between DCD20 and LD in pancreas histological damage was observed ($p < 0.05$).

Slight edema was observed in a subset of DCD and BDD colon samples. No alterations in this organ were detected in LD. No

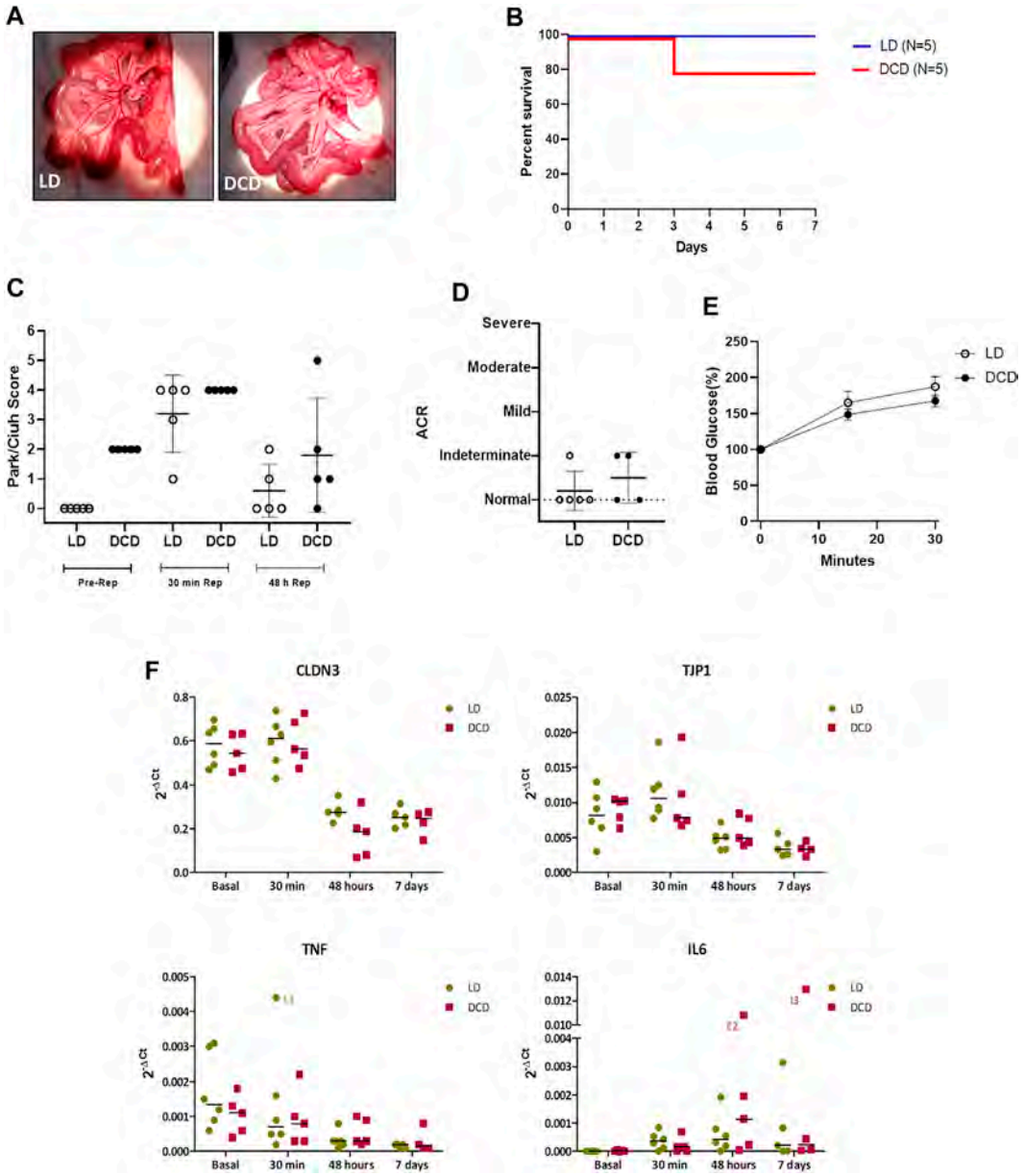


FIGURE 6 | Intestinal Graft monitoring from DCD after ITx. Macroscopic appearance of transplanted intestines during intra-surgical graft reperfusion (DCD5 and LD) **(A)**. Survival analysis of DCD and LD ITx recipients **(B)**. Histological analysis of Ischemia-Reperfusion injury **(C)** and acute cellular rejection **(D)** of the DCD5 and LD graft after ITx did not show significant differences between groups. Intestinal graft absorption **(E)** and gene expression of CLDN3, TJP1, TNF and IL-6 after transplantation were similar in DCD5 and LD animals **(F)**.

significant differences in the analysis on colon samples were observed between groups.

Long-Term Outcome of Allografts From DCD was Similar to the Gold Standard (LD) After Experimental ITx

Considering the results obtained in the graft characterization we decided to perform ITx using a heterotopic model previously described. In order to determine if DCD5 may affect the long-term transplant outcome, in spite of the lack of differences with LD graft. To this aim, two groups were subject to ITx using either DCD5 or LD grafts. Upon vascular anastomosis, DCD5 grafts reperfusion was fast and homogeneous, comparable to LD grafts (**Figure 6A**). Post-operative recipient recovery was appropriate and weight and clinical evolution was comparable in DCD5 and LD groups. Similar results were observed in survival curve (**Figure 6B**), only 1 animal from DCD group died in 3rd post-operative day, which was the same animal that showed extensive ischemia/reperfusion damage at 48 h post-transplant

(**Figure 6C**). Anyway, no statistical significant differences in mortality were observed between groups. All animals survived showing no signs of graft rejection until 7 post-operative days, when the protocol was ended. During ITx, serial samples of graft were obtained to evaluate ischemia/reperfusion damage (**Figure 6C**). Interestingly, although DCD5 donors have a Park/chiu score 2 points higher than LD in the pre-reperfusion sample, showing the impact of CD on intestinal histology as shown in previous experiments. Upon engraftment, the highest damage was observed in the 30 min post-reperfusion sampling, where both DCD5 and LD grafts showed similar damage (median Park/chiu score of 4 in both groups). Recovery from damage was evident in the 48 h post-reperfusion sample of LD and DCD5 groups. Histopathological analysis was performed on intestinal graft samples taken at day 7 post-operative. No signs of rejection were observed in either LD or DCD5 groups (**Figure 6D**). Furthermore, functional capacity of grafts was assessed at day 7 post-ITx using a glucose absorption test. Either LD or DCD5 grafts showed appropriate absorptive function, indicating that DCD procedure has not impacted in absorptive capacity of the graft (**Figure 6E**).

To determine comparative effects of DCD and LD on additional pathways, gene expression analysis was performed in graft samples taken at different times: just before the dissection procedure (basal samples) and 30 min, 48 h and 7 days after ITx (**Figure 6F**). ZO1 and claudin-3 were analyzed as markers of epithelial integrity, and IL6 and TNFα as reflection of pro-inflammatory condition. According to what observed in the histological analysis, no differences were observed between LD and DCD in any of the genes and time-points studied (**Figure 6F**). Both groups showed a high expression of claudin-3 in basal and 30′ samples which decreased significantly after 48 h (LD: $p = 0.002$, DCD: $p = 0.006$), this correlating with the Chiu-Park score normalization in the biopsies. A similar but smoother dynamic was observed in zonulin expression, showing the LD group a significant increase at 30' vs. basal samples ($p = 0.010$). Regarding the pro-inflammatory cytokines, TNFα showed the highest levels in basal samples, decreasing progressively afterwards. Nevertheless, IL6, which was almost undetectable at the beginning of the procedure, increased after reperfusion (**Figure 6F**). Overall, this experiment indicates that selection of appropriate DCD grafts results in a transplantation procedure with comparable outcomes to the LD experimental gold standard scenario.

DISCUSSION

Patients on solid organ transplantation waiting lists worldwide outnumber annual transplant procedures. Donor shortage is a main contributing factor in this regard, and the situation is expected to deteriorate unless clinical alternatives are identified (5, 22). DCD is a significant contributor to the donor pool in countries with legislation for DCD use. In some cases, DCD represents up to 40% of transplanted grafts. BD donation activity remains underdeveloped in several countries, and DCD is a valuable practical alternative for deceased organ donations (1, 23).

VT constitutes a notable challenge for DCD, as the liability of the intestinal barrier to ischemia and discouraging results from animal models have precluded its implementation in clinical practice (7). The availability of experimental models that recapitulate the main features of DCD will facilitate the development of novel interventions with promising results in preclinical settings (24). Consequently, our initial aim was to develop a model of experimental DCD that recapitulates the main features of the Maastricht III donation criteria.

In an interesting article, Søfteland et al have reported that rats and humans are more susceptible to intestinal ischemia-reperfusion injury than pigs, and laboratory animal characteristics must be considered in the experimental design of investigations. Following these conclusions, we consider that the selected specie to carry out the present work was adequate, and allows us to reliably study the impact of DCD procedure on multivisceral graft quality and ITx viability (25).

The experimental procedure of controlled DCD developed in the present work accurately reproduces the different phases of a Maastricht III donors used in the clinical practice. This model permits the characterization of distinct DCD-related features and provides a valuable tool for assessing different translational strategies to improve the quality of VT grafts. The maintenance period with mechanical ventilation and monitoring of vital parameters such as temperature and MAP in the DCD model constitute one of the strengths of the present work. In addition, the diagnosis of CD, the 5 min of no-touch period and cannulation maneuver before graft washing was based on the clinical practice of pediatric Maastricht III donors, highlighting the relevance of this model.

Our results demonstrated that the histopathological features of the small bowel from DCD with less warm ischemia time were similar to those of grafts from BDD in terms of Chiu/Park score, crypt-villus index, and intestinal mucosa thickness. We emphasize the good quality of the architecture of intestines from donors with CD, given the relatively short period of cardiac arrest prior to washing and organ removal. Roskott et al. reported that elevated expression of heat shock proteins occurs in the human intestine after DCD and BDD grafts exhibited higher IL6 expression levels compared with those in DCD (6). These data suggest that grafts for visceral transplantation warrant different pre-treatments in accordance with their immunological profiles. In this regard, our model unlocks a range of possibilities for evaluation in preclinical and clinical settings.

We observed greater histological damage in the small intestine than in colon samples, confirming the importance of studying the small bowel barrier as a more sensitive parameter of ischemic damage. This finding is in accordance with a previous study by Bresler et al. which described similar differences in ischemic damage between the small bowel and colon in an experimental model (20). With regard to specific cell populations, GC number was significantly lower in the experimental group with more severe histopathological damage (DCD20) but was similar between DCD5 and BDD. These results are consistent with previous reports about the impact of ischemic damage on GC (14, 25). Although Paneth cells exhibit differential sensitivity to Ischemia/reperfusion injury, we observed that warm ischemia in both DCD groups resulted in minor changes in the Paneth cell

population, which exhibited cell counts similar to those in BDD (16, 26).

Principal component analysis revealed that the intestines of the DCD5 and BDD groups were similar, whereas the DCD20 small bowel differed from that of the other experimental groups, which constitute encouraging results regarding intestinal graft quality. In addition to the intestine, visceral grafts may be composed of other organs, as previously stated. In our work, we assessed the impact of different types of donations on the stomach, pancreas, and liver. As shown in **Figure 5**, ischemic time negatively impacted donors with CD who experienced prolonged warm ischemic time. Notably, similarities in histological damage in the small intestine were noted between DCD5 and BDD groups. Considering these encouraging results, we also showed that DCD5 intestines can be engrafted using a small bowel rat heterotopic model with comparable results to LD intestine (**Figure 6**).

Decision to compare DCD-derived intestinal grafts with a no-touch waiting time of 5 min (similar to the clinical scenario of pediatric transplantation) with LD ITx procedures is based on two justifications: as reported by Oltean, in the experimental field of ITx in rodents, all published work where transplantation is performed use LD. Also, we considered that the great challenge for DCD was to compare it with the "gold-standard" donor situation regarding solid organ transplantation (27).

Notably similar rejection-free graft survival and graft function were observed in both groups and although a trend in increased ischemia-reperfusion damage was observed in DCD grafts, overall performance was similar, showing the feasibility of the use of DCD graft intestines at least in the condition tested. In agreement with reported warm ischemia period in solid organ transplantation and previous experimental experiences, we have selected a short warm ischemia time that allowed intestine to be transplanted without major damage. Cobianchi et al. have shown that using a pig small bowel transplantation model, having 20 min of cardiac arrest before DCD generates an intestinal damage that afterwards severely affects ITx outcome. In the rat model used, we observed that 20 min of cardiac arrest induce important changes in intestinal mucosa. However, having shorter periods allow the obtention of intestinal grafts that can be used in an ITx procedure without affecting the outcome. Current clinical practice has shifted towards normothermic perfusion in DCD donors with superior results to those achieved with traditional cold perfusion which is similar to the perfusion employed in the present work (28). Therefore, future work should implement the necessary devices to establish normothermic perfusion in this model.

In summary, the present work provides a detailed description of a novel experimental model of Maastricht III DCD that may contribute to evaluation of the feasibility of DCD for VT in the clinical practice. Our results suggest that the quality of visceral grafts from DCD and short ischemia times closely resemble those of BDD with comparable performance and preservation of functional capacity in a heterotopic intestinal transplant model, highlighting DCD as a possible solution to the clinical imbalance between the demand for organ donation and transplantation procedures.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The animal study was reviewed and approved by the CAM Animal Welfare Ethics Committee.

AUTHOR CONTRIBUTIONS

PS: participated in research design, participated in the writing of the paper, and participated in the performance of the research. LV: participated in the performance of the research and participated in research design. AE: participated in the performance of the research (IF analysis). MM: participated in the performance of the research (Histological analysis). RP-G: participated in the writing of the paper and data analysis. MP: participated in the performance of the research. JS: participated in research design. AA: participated in research design and participated in the writing of the paper. NL: participated in research design and critical revision of the manuscript. PT: participated in the performance of the research (gene expression analysis) and participated in writing of the paper. MR and FH: participated in research design and participated in the writing of the paper.

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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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REFERENCES

1. Smith M, Dominguez-Gil B, Greer DM, Manara AR, Souter MJ. Organ Donation after Circulatory Death: Current Status and Future Potential. *Intensive Care Med* (2019) 45(3):310–21. doi:10.1007/s00134-019-05533-0
2. Gozzini S, Perera MT, Mayer DA, Mirza DF, Kelly DA, Muiesan P, et al. Liver Transplantation in Children Using Non-heart-beating Donors (NHBD). *Pediatr Transpl* (2010) 14(4):554–7. doi:10.1111/j.1399-3046.2009.01280.x
3. Miñambres E, Suberviola B, Dominguez-Gil B, Rodrigo E, Ruiz-San Millan JC, Rodriguez-San Juan JC, et al. Improving the Outcomes of Organs Obtained from Controlled Donation after Circulatory Death Donors Using Abdominal Normothermic Regional Perfusion. *Am J Transpl* (2017) 17(8):2165–72. doi:10.1111/ajt.14214
4. Treckmann J, Moers C, Smits JM, Gallinat A, Maathuis MHJ, van Kasterop-Kutz M, et al. Machine Perfusion versus Cold Storage for Preservation of Kidneys from Expanded Criteria Donors after Brain Death. *Transpl Int* (2011) 24(6):548–54. doi:10.1111/j.1432-2277.2011.01232.x
5. Angelico R, Perera MTPR, Manzia TM, Parente A, Grimaldi C, Spada M. Donation after Circulatory Death in Paediatric Liver Transplantation: Current Status and Future Perspectives in the Machine Perfusion Era. *Biomed Res Int* (2018) 2018:1756069. doi:10.1155/2018/1756069
6. Roskott AM, van Haaften WT, Leuvenink HG, Ploeg RJ, van Goor H, Blokzijl T, et al. Histopathologic and Molecular Evaluation of the Organ Procurement and Transplantation Network Selection Criteria for Intestinal Graft Donation. *J Surg Res* (2014) 189(1):143–51. doi:10.1016/j.jss.2014.02.008
7. Cobianchi L, Zonta S, Vigano J, Dominioni T, Ciccocioppo R, Morbini P, et al. Experimental Small Bowel Transplantation from Non-heart-beating Donors: a Large-Animal Study. *Transpl Proc* (2009) 41(1):55–6. doi:10.1016/j.transproceed.2008.08.151
8. Guo M, Yao D, Li L, Lu C, Li Y, Li J. Intestinal Conditioning after Cardiac Arrest: The Use of Normothermic Extracorporeal Membrane Oxygenation in the Non-heart-beating Animal Model. *Artif Organs* (2016) 40(8):738–45. doi:10.1111/aor.12691
9. Lao OB, Healey PJ, Perkins JD, Reyes JD, Goldin AB. Outcomes in Children with Intestinal Failure Following Listing for Intestinal Transplant. *J Pediatr Surg* (2010) 45(1):100–7. doi:10.1016/j.jpedsurg.2009.10.019
10. Zhong X, Xiao Q, Liu Z, Wang W, Lai CH, Yang W, et al. TAK242 Suppresses the TLR4 Signaling Pathway and Ameliorates DCD Liver IRI in Rats. *Mol Med Rep* (2019) 20(3):2101–10. doi:10.3892/mmr.2019.10439
11. Kolkert JL, 't Hart NA, van Dijk A, Ottens PJ, Ploeg RJ, Leuvenink HG. The Gradual Onset Brain Death Model: a Relevant Model to Study Organ Donation and its Consequences on the Outcome after Transplantation. *Lab Anim* (2007) 41(3):363–71. doi:10.1258/002367707781282848
12. Cicora F, Stringa P, Guerrieri D, Vásquez D, Toniolo F, Roberti J, et al. Evaluation of Histological Damage of Solid Organs after Donor Preconditioning with Thymoglobulin in an Experimental Rat Model. *Transpl Immunol* (2013) 28(4):203–5. doi:10.1016/j.trim.2013.04.002
13. Hartog H, Brown RM, Neil DA, Sharif K, Gupte GL, Mirza DF, et al. Characterization of Ischemic Changes in Small Bowel after Normothermic Regional Perfusion: Potential to Consider Small Bowel Grafts from DCD Donors? *Transplantation* (2016) 100(12):e156–7. doi:10.1097/TP.0000000000001460
14. Søfteland JM, Casselbrant A, Akyürek LM, Hellstrom M, Oltean M. The Impact of Age and Luminal Preservation on the Development of Intestinal Preservation Injury in Rats. *Transplantation* (2020) 104(1):e8–e15. doi:10.1097/TP.0000000000002999
15. Johansson ME, Hansson GC. The Goblet Cell: a Key Player in Ischaemia-Reperfusion Injury. *Gut* (2013) 62(2):188–9. doi:10.1136/gutjnl-2012-302582
16. Kip AM, Ceulemans LJ, Hundscheid IHR, Canovai E, Hartog H, Brown RM, et al. Paneth Cell Alterations during Ischemia-Reperfusion, Follow-Up, and Graft Rejection after Intestinal Transplantation. *Transplantation* (2020) 104(9):1952–8. doi:10.1097/TP.0000000000003257
17. Suzuki S, Toledo-Pereyra LH, Rodriguez FJ, Cejalvo D. Neutrophil Infiltration as an Important Factor in Liver Ischemia and Reperfusion Injury. Modulating Effects of FK506 and Cyclosporine. *Transplantation* (1993) 55(6):1265–72. doi:10.1097/00007890-199306000-00011
18. Jahovic N, Erkanli G, Işeri S, Arbak S, Alican I. Gastric protection by Alpha-Melanocyte-Stimulating Hormone against Ethanol in Rats: Involvement of Somatostatin. *Life Sci* (2007) 80(11):1040–5. doi:10.1016/j.lfs.2006.11.036
19. Schmidt J, Rattner DW, Lewandrowski K, Compton CC, Mandavilli U, Knoefel WT, et al. A Better Model of Acute Pancreatitis for Evaluating Therapy. *Ann Surg* (1992) 215(1):44–56. doi:10.1097/00000658-199201000-00007
20. Bresler A, Ionac M, Oltean M. A Grading Score for Colon Preservation Injury in the Rat. *Transpl Proc* (2016) 48(2):521–4. doi:10.1016/j.transproceed.2015.08.048
21 . Stringa P, Romanin D, Lausada N, Papa Gobbi R, Zanuzzi C, Martin P, et al. Gut Permeability and Glucose Absorption Are Affected at Early Stages of Graft Rejection in a Small Bowel Transplant Rat Model. *Transpl Direct* (2017) 3(11):e220. doi:10.1097/TXD.0000000000000718
22. Andres AM, Ramos E, Hernandez F. Current Status of Pediatric Intestinal Transplant in Europe. *Curr Opin Organ Transpl* (2020) 25(2):183–8. doi:10.1097/MOT.0000000000000736
23. Gardiner D, Charlesworth M, Rubino A, Madden S. The Rise of Organ Donation after Circulatory Death: a Narrative Review. *Anaesthesia* (2020) 75(9):1215–22. doi:10.1111/anae.15100
24. Guo M, Lu C, Li L, Yao D, Li Y. Normothermic Extracorporeal Membrane Oxygenation Support: Improving the Function of Intestinal Grafts Obtained from Cardiac Death Donors. *Artif Organs* (2020) 44(10):1098–106. doi:10.1111/aor.13697
25. Søfteland JM, Casselbrant A, Biglarnia AR, Linders J, Hellstrom M, Pesce A, et al. Intestinal Preservation Injury: A Comparison between Rat, Porcine and Human Intestines. *Int J Mol Sci* (2019) 20(13):3135. doi:10.3390/ijms20133135
26. Grootjans J, Hodin CM, de Haan JJ, Derikx JPM, Rouschop KMA, Verheyen FK, et al. Level of Activation of the Unfolded Protein Response Correlates with Paneth Cell Apoptosis in Human Small Intestine Exposed to Ischemia/reperfusion. *Gastroenterology* (2011) 140(2):529–39. doi:10.1053/j.gastro.2010.10.040
27. Oltean M. Intestinal Transplantation: an Overview of the Recent Experimental Studies. *Curr Opin Organ Transpl* (2021) 26:240–4. doi:10.1097/MOT.0000000000000845
28. De Beule J, Vandendriessche K, Pengel LHM, Bellini MI, Dark JH, Hessheimer AJ, et al. A Systematic Review and Meta-Analyses of Regional Perfusion in Donation after Circulatory Death Solid Organ Transplantation. *Transpl Int* (2021) 34(11):2046–60. doi:10.1111/tri.14121

SUPPLEMENTARY MATERIAL

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ORIGINAL RESEARCH
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Subjective Cognition is Related to Patient-Reported Symptom Distress and Work Productivity Among Liver Transplant Recipients

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Cognitive decline may prevent liver transplant (LT) recipients from staying healthy and independent. This study examined associations of objective and subjective, rated by LT recipients and caregivers, cognitive decline with patient-reported physical and psychological symptom distress, ability to perform household tasks, and workplace productivity among LT recipients. Sixty pairs of LT recipients and caregivers participated in this cross-sectional study. Subjective cognition was measured by the Everyday Cognition. Objective cognition was assessed with four cognitive tests, including the Repeatable Battery for the Assessment of Neuropsychological Status. Patient-reported outcomes were assessed with the Rotterdam Symptom Checklist-Modified, Profile of Mood States-Short Form, Creative Therapy Consultants Homemaking Assessment, and Work Limitations Questionnaire. Linear regression analyses related objective and subjective cognition to the patient-reported outcomes. While objective cognitive decline was not associated with any patient-reported outcomes, subjective cognitive decline was significantly associated with the outcomes. Higher LT recipient self-rated cognitive decline was associated with higher physical symptom distress ($\beta = 0.30$, $p = 0.006$) and workplace productivity loss ($\beta = 14.85$, $p < 0.0001$). Higher caregiver-rated cognitive decline was associated with lower household tasks performance ($\beta = -18.55$, $p = 0.015$). Findings suggest to consider subjective cognition when developing an individualized post-transplant care plan.

Keywords: cognitive function, subjective cognition, patient-reported outcomes, symptom distress, work productivity, liver transplant recipients

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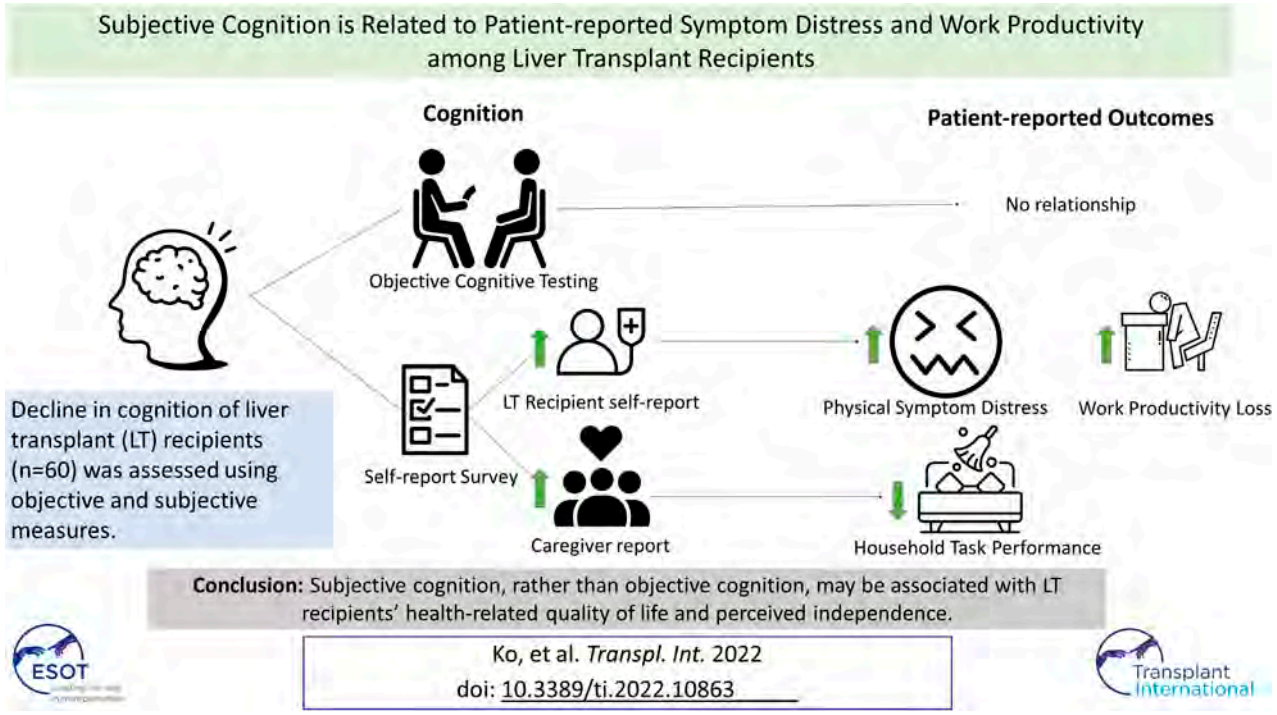
Abbreviations: CI, confidence intervals; CTC, creative therapy consultants; ECog, everyday cognition; LT, liver transplant; MELD-Na, model for end-stage liver disease with sodium; NAB, neuropsychological assessment battery; POMS-SF, profile of mood states-short form; RBANS, repeatable battery for the assessment of neuropsychological status; REDCap, research electronic data capture; RSCL-M, rotterdam symptom checklist-modified; SD, standard deviation; WLQ, work limitations questionnaire.



GRAPHICAL ABSTRACT |

INTRODUCTION

As more than 80% of liver transplant (LT) recipients survive beyond 5 years post-transplant (1), maintaining healthy and independent lives has become one of the top priorities for post-LT care (2). While some LT recipients tend to carry out activities to stay healthy and independent after LT, such as taking medication as prescribed or returning to employment, others do not. Up to 75% of LT recipients are non-adherent to their medication regimen (3–5), and less than 60% engage in either non-paid (e.g., homemakers or students) or paid work (6). Cognitive decline could be one of the potential factors that prevent successful performance of such activities. In fact, 9%–56% of LT recipients have objective impairment on formal cognitive testing (7–9).

Cognitive health is an important consideration for post-transplant recipients as cognitive decline after LT may affect recipients' abilities to maintain healthy and independent lives. Intact cognition, particularly in memory, attention, and executive function, is required for successful performance of tasks necessary to maintaining overall health and independence (10). For example, LT recipients should perform health maintenance tasks, such as monitoring and managing symptoms and side effects of immunosuppressants. However, recipients with cognitive decline in the above areas may have reduced abilities to monitor and manage symptoms (11, 12) that potentially lead to physical and psychological symptom distress. Furthermore, decline in cognition, including the aforementioned domains, may reduce work capacity and productivity (13, 14). Recipients with cognitive decline may not be able to successfully perform household tasks, return to work, or stay employed after LT. While cognitive health appears to relate to physical and psychological symptom distress, ability to perform household tasks, and workplace productivity, there is a paucity of literature examining these associations. Understanding cognitive decline in relation to these patient-reported outcomes may expand our knowledge regarding overall wellbeing and disease burden associated with cognitive decline in this population. Further, such knowledge may also inform how clinicians can facilitate LT recipients in achieving healthy and independent lives.

This study investigated the associations between cognition and patient-reported outcomes of physical and psychological symptom distress, ability to perform household tasks, and workplace productivity among LT recipients. Objective and subjective measures of cognition (global and multiple specific domains) were assessed in relation to the patient-reported outcomes. We included subjective cognitive measures, reported by LT recipients and caregivers, to assess the feasibility of using these self-report cognitive measures given the ease of use in clinical practice (15). We included caregivers in this study since caregivers may estimate LT recipients' cognition differently than recipients themselves (15). Subjective cognition reported by LT recipients and caregivers could be variously associated with patient-reported outcomes. In this study, caregivers were queried about perceived LT recipients'

cognition. We hypothesized that LT recipients with worse objective cognitive test scores or greater subjective cognitive decline reported by LT recipients or caregivers have worse symptom distress, poorer ability to perform household tasks, and decreased workplace productivity than those with better objective cognitive test scores or subjective cognitive status. This study further examined whether the above associations differ by post-transplant employment status because employment affects cognition (16).

PATIENTS AND METHODS

Population

A convenience sampling was used in this cross-sectional single center study to recruit participants between December 2018 and September 2019. LT recipients were eligible to participate in this study if they were over 18 years old, had received a LT at least more than 3 months ago (to minimize the potential influences of early post-operative complications) but not more than 2 years post-transplant, had caregivers who could answer questions about the recipients' cognition, and were able to speak and write English. LT recipients were excluded from this study if they had received any other organ transplant, such as kidney, had a history of a neurological disorder, such as Alzheimer's disease or stroke, had a history of head injury, or were not able to provide informed consent. Caregivers identified by participating LT recipients were included in this study if they were over 18 years old and able to speak and write English. Those who were not able to provide informed consent were excluded from this study. Recruitment process of participants are described elsewhere (15), but in short, a total of 207 LT recipients were invited to participate in the study, and 60 provided written informed consent.

Measures

LT recipients completed an online self-report survey assessing subjective cognition and patient-reported outcomes of physical and psychological symptom distress, ability to perform household tasks, and workplace productivity *via* Research Electronic Data Capture (REDCap) (17, 18). They then completed in-person, paper-and-pencil objective cognitive tests. Caregivers completed an online self-report survey assessing subjective cognition *via* REDCap.

Cognition

Subjective Cognition

The 39-item Modified Everyday Cognition (ECog) was used to assess LT recipient self-rated and caregiver-rated cognition in six domains: memory, language, visuospatial abilities, planning, organization, and divided attention (19, 20). LT recipients rated their current perceived difficulties in performing daily activities, and caregivers rated perceived difficulties that LT recipients currently have in performing daily activities from 1 (no difficulty) to 4 (severe difficulty/cannot do). "Don't know" was available and scored as 0. Mean total scores that represent global subjective cognition and mean domain scores are available with higher scores indicating greater perceived difficulties in performing daily activities (19). The ECog was found to be valid in differentiating cognitive decline from normal cognition (19). Cronbach's alpha coefficients in this study sample were good (0.89–0.98).

Objective Cognition

Global cognition was measured by the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) (21). The RBANS assesses five domains, attention, language, visuospatial/constructional abilities, and immediate and delayed memory, with 12 tests. Higher total scores indicate better global cognition (21). The RBANS has been shown to be effective in detecting mild cognitive impairment (22).

Cognition in three domains most commonly found to be impaired in the LT population (visuospatial abilities, executive function, and attention) (7, 23) were additionally assessed using reliable and valid cognitive tests. The Trail Making Test Parts A and B assess attention and executive function, respectively, and longer total time to complete the test indicates worse cognition (24). The Digit Span Backward test from the Neuropsychological Assessment Battery (NAB) assesses attention and executive function, and higher total scores are indicative of better cognition (25). The Rey-Osterrieth Complex Figure (Copy) assesses visuospatial constructional ability and executive function, and higher total scores are indicative of better cognition (26–28).

Patient-Reported Outcomes

Symptom Distress

The 28-item Rotterdam Symptom Checklist-Modified (RSCL-M) (29) asked LT recipients to rate the extent of physical symptom distress in the past week from 1 (Not at all) to 4 (Very much). Higher mean total scores indicate worse physical symptom distress (29). The validity of this questionnaire has been previously established (29). Cronbach's alpha of this questionnaire was 0.89 in the current study. The 37-item Profile of Mood States-Short Form (POMS-SF) (30) asked LT recipients to rate their psychological distress in six subscales (depression, vigor, confusion, tension, anger, and fatigue) in the past 2 weeks from 0 (Not at all) to 4 (Extremely). Higher mean total scores indicate worse psychological distress (30). The validity of POMS-SF was established in various populations, including the kidney transplant population (30). Cronbach's alpha of this questionnaire ranged from 0.82 to 0.95 in this study.

Ability to Perform Household Tasks

LT recipients completed the Creative Therapy Consultants (CTC) Homemaking Assessment, which was found to be reliable and valid to assess the performance of 29 household tasks in three categories: light (e.g., folding clothes), medium (e.g., washing dishes), and heavy (e.g., grocery shopping) tasks (31). Participants rated how much assistance they need to complete the tasks from 0 (Cannot complete without assistance) to 1 (Complete with no assistance). "Not applicable" was available

TABLE 1 | Participant characteristics.

Characteristics	Frequency (%) or mean (SD)		
	All LT recipients (N = 60)	LT recipients Employed (N = 17)	LT Recipients Not Employed (N = 43)
Age (years)	60.4 (6.9)	57.9 (5.9)	61.5 (7.0)
Sex			
Male	43 (71.7%)	12 (70.6%)	31 (72.1%)
Female	17 (28.3%)	5 (29.4%)	12 (27.9%)
Race			
Black	2 (3.3%)	0 (0.0%)	2 (4.7%)
White	56 (93.3%)	16 (94.1%)	40 (93.0%)
Other (e.g., aboriginal)	2 (3.3%)	1 (5.9%)	1 (2.3%)
Marital Status			
Single	10 (16.7%)	2 (11.8%)	8 (18.6%)
Single, living with partner	3 (5.0%)	2 (11.8%)	1 (2.3%)
Married	43 (71.7%)	12 (70.6%)	31 (72.1%)
Widowed/separated	4 (6.7%)	1 (5.9%)	3 (7.0%)
Education	14.1 (2.4)	14.5 (3.0)	13.9 (2.1)
Household income			
$20,000 or less	10 (20.0%)	0 (0.0%)	10 (27.7%)
$20,001 to $40,000	9 (18.0%)	1 (7.1%)	8 (22.2%)
$40,001 to $60,000	10 (20.0%)	2 (14.2%)	8 (22.2%)
Over $60,000	21 (42.0%)	11 (78.6%)	10 (27.8%)
Insurance			
Government insurance	23 (38.3%)	2 (11.8%)	21 (48.8%)
Non-government insurance	32 (53.3%)	13 (76.5%)	19 (44.2%)
Multiple	4 (6.7%)	1 (5.9%)	3 (7.0%)
None	1 (1.7%)	1 (5.9%)	0 (0.0%)
Cause of liver disease			
Non-alcoholic steatohepatitis	28 (46.7%)	6 (35.3%)	22 (51.2%)
Alcohol	10 (16.7%)	2 (11.8%)	8 (18.6%)
Hepatitis C	11 (18.3%)	5 (29.4%)	6 (14.0%)
Autoimmune/cholestatic	7 (11.7%)	3 (17.6%)	4 (9.3%)
Others	4 (6.7%)	1 (5.9%)	3 (7.0%)
History of pre-transplant hepatic encephalopathy			
No	17 (28.3%)	9 (52.9%)	8 (18.6%)
Yes	43 (71.7%)	8 (47.1%)	35 (81.4%)
Time since transplant (months)	12.9 (7.0)	11.8 (6.8)	13.4 (7.1)
MELD score	21.7 (10.0)	13.6 (6.0)	24.9 (9.4)

if participants do not engage in certain tasks and scored as 0. Higher total weighted scores indicate higher household work productivity (32). Cronbach's alpha for this questionnaire ranged between 0.84 and 0.92 in this study.

Workplace Productivity

LT recipients who were employed full-time or part-time at the time of survey administration completed the 25-item Work Limitations Questionnaire (WLQ) (33, 34). This questionnaire assesses the interference of health conditions in workplace productivity in four scales: Time, Physical, Mental-Interpersonal, and Output Demands. Participants rated difficulties at work in the past 2 weeks from 1 (None of the time) to 5 (All of the time) and rated their ability to work in the past 2 weeks from 1 (Able all of the time) to 5 (Able none of the time). "Does not apply to my job" was also available scoring as 0. Higher total scores indicate higher self-reported at-work productivity loss in the past 2 weeks (35). The WLQ has been validated with chronic disease populations (34). Cronbach's alpha for this questionnaire ranged from 0.79 to 0.96 in this study.

Demographic and Clinical Characteristics

LT recipients and caregivers completed a self-report demographic questionnaire. Clinical characteristics of LT recipients, such as the date of transplant and Model for End Stage Liver Disease-Sodium (MELD) score (36), were extracted from medical records.

Analysis

IBM SPSS Statistics version 26 (IBM, Armonk, NY, United States) and SAS Version 9.4 (SAS Institute, Cary, NC) were used to perform data analysis. Patient characteristics and scores for the study measures were described using means and standard deviations (SDs) for continuous measures and counts and proportions for categorical variables. Unadjusted and adjusted linear regression models were developed to examine the relationships of subjective and objective cognitive test scores with physical and psychological symptom distress and household tasks performance; adjusted models included LT recipients' age, education, and months since LT. Only an unadjusted regression model was reported for workplace productivity due to the small number of subjects who were

TABLE 2 | Summary of subjective and objective cognition, symptom distress, household tasks performance, and workplace productivity.

	All LT recipients (N = 60)	LT recipients employed (N = 17)	LT recipients not employed (N = 43)	*p*-value[a]
	Mean (SD)	Mean (SD)	Mean (SD)	
Ecog				
Global	1.5 (0.5)	1.2 (0.3)	1.6 (0.5)	**0.002**
Memory	1.7 (0.5)	1.4 (0.4)	1.8 (0.5)	**0.004**
Language	1.5 (0.6)	1.2 (0.3)	1.6 (0.6)	**0.004**
Visuospatial abilities	1.2 (0.4)	1.0 (0.1)	1.3 (0.5)	**0.000**
Planning	1.3 (0.5)	1.2 (0.3)	1.4 (0.5)	0.076
Organization	1.5 (0.7)	1.2 (0.3)	1.7 (0.7)	**0.001**
Divided attention	1.5 (0.7)	1.4 (0.6)	1.6 (0.7)	0.290
RBANS[b]				
Global	194.0 (25.5)[c]	210.9 (14.7)[d]	187.5 (25.9)[e]	**<0.0001**
Immediate memory index	40.1 (7.7)[c]	44.8 (5.0)[d]	39.3 (8.1)[e]	**0.003**
Visuospatial/constructional index	31.1 (4.5)[c]	32.3 (3.3)[d]	30.7 (4.8)[e]	0.154
Language index	28.6 (4.5)[c]	29.7 (4.6)[d]	28.1 (4.4)[e]	0.251
Attention index	48.9 (11.5)[c]	56.4 (6.8)[d]	46.0 (11.7)[e]	**0.000**
Delayed memory index	44.6 (5.7)[c]	47.6 (4.1)[d]	43.4 (5.9)[e]	**0.004**
Trail Making Test				
Part A	35.8 (17.1)[c]	29.1 (7.4)[d]	38.4 (19.1)[e]	**0.010**
Part B	90.0 (45.8)[c]	67.0 (16.5)[d]	98.9 (50.4)[e]	**0.001**
NAB Digit Span Backward	4.2 (2.0)[c]	4.9 (1.5)[d]	4.0 (2.0)[e]	0.071
Rey-Osterrieth Complex Figure (Copy)	27.6 (4.9)[c]	29.8 (4.3)[d]	26.7 (4.9)[e]	**0.025**
RSCL-M	1.6 (0.4)[c]	1.6 (0.5)[d]	1.6 (0.3)[e]	0.833
POMS-SF				
Total score	0.8 (0.5)	0.9 (0.5)	0.8 (0.5)	0.527
Depression	0.5 (0.7)	0.5 (0.7)	0.5 (0.7)	0.920
Vigor	1.6 (1.0)	1.9 (1.0)	1.6 (1.0)	0.289
Confusion	0.6 (0.6)	0.4 (0.6)	0.7 (0.6)	0.209
Tension	0.6 (0.7)	0.7 (0.7)	0.6 (0.7)	0.687
Anger	0.5 (0.6)	0.6 (0.6)	0.4 (0.6)	0.240
Fatigue	1.0 (1.0)	1.2 (1.1)	1.0 (0.9)	0.505
CTC Homemaking Assessment	90.5 (21.6)[f]	97.3 (7.9)	87.5 (24.9)[g]	**0.031**
WLQ				
Total Productivity Loss Score		3.4 (4.8)[d]		
Time Scale		17.0 (26.3)[d]		
Physical Scale		16.9 (24.1)[d]		
Mental-Interpersonal Scale		8.0 (14.2)[d]		
Output Scale		12.8 (21.5)[d]		

Note: Bold values denote statistical significance at the p < *0.05 level.*

[a]*Comparisons of cognition, symptom distress, household tasks performance, and workplace productivity between employed and non-employed LT recipients.*

[b]*Scores presented as raw scores.*

[c]*n = 57.*

[d]*n = 16.*

[e]*n = 41.*

[f]*n = 56.*

[g]*n = 39.*

working either full- or part-time at the time of survey administration (n = 17). Among the four objective cognitive tests, only the RBANS that indicates global cognition was included in the models given the small sample size. For all models, the coefficient estimates were tabulated along with 95% confidence intervals (CI) and p-values. Correlations between subjective and objective cognitive domain scores and patient-reported outcomes were examined using Pearson's correlation coefficient with 95% CIs to investigate which domains were specifically correlated with the outcomes. A two-tailed alpha of 0.05 was set as the level of statistical significance. Listwise deletion was used to deal with missing data.

This study performed additional analyses to examine differences in the findings by post-transplant employment status. Independent group t-tests, assuming unequal variance, were used to compare subjective and objective cognitive test scores and patient-reported outcomes of physical and psychological symptom distress and household tasks performance between employed and non-employed LT recipients. Unadjusted regression analyses and Pearson's correlation coefficients, comparing the same variables as above but stratified by post-transplant employment status, were also performed.

RESULTS

Participant Characteristics

Sixty pairs of LT recipients and their caregivers participated in this study. **Table 1** summarizes the

TABLE 3 | Associations between subjective and objective cognition and symptom distress, household tasks performance, and workplace productivity.

	Unadjusted			Adjusted[a]		
	N	β (95% CI)	*p*-value	N	β (95% CI)	*p*-value
RSCL-M						
LT recipient ECog	60	0.39 (0.22, 0.57)	**<0.0001**	54	0.30 (0.09, 0.51)	**0.006**
Caregiver ECog	60	0.29 (0.10, 0.48)	**0.004**		0.14 (–0.08, 0.36)	0.213
LT recipient RBANS	57	–0.00 (–0.01, 0.00)	0.172		0.00 (–0.00, 0.01)	0.661
POMS-SF						
LT recipient ECog	60	0.27 (0.02, 0.52)	**0.035**	54	0.25 (–0.04, 0.55)	0.091
Caregiver ECog	60	0.27 (0.01, 0.52)	**0.040**		0.18 (–0.13, 0.49)	0.245
LT recipient RBANS	57	0.00 (–0.00, 0.01)	0.255		0.00 (–0.00, 0.01)	0.210
CTC Homemaking Assessment						
LT recipient ECog	56	–7.34 (–19.20, 4.51)	0.220	50	2.09 (–12.54, 16.71)	0.775
Caregiver ECog	56	–13.24 (–24.89, –1.60)	**0.027**		–18.55 (–33.38, –3.73)	**0.015**
LT recipient RBANS	53	0.01 (–0.24, 0.27)	0.916		–0.26 (–0.55, 0.04)	0.090
WLQ[b]						
LT recipient ECog	16	14.85 (10.40, 19.30)	**<0.0001**			
Caregiver ECog	16	4.92 (–3.09, 12.94)	0.209			
LT recipient RBANS	15	–0.13 (–0.31, 0.05)	0.129			

Note: Bold values denote statistical significance at the p < *0.05 level.*

[a]*Adjusted for LT recipients' age, education, and months since transplant.*

[b]*Adjusted model for workplace productivity was not possible due to the small sample size (*n = *16).*

TABLE 4 | Correlations between the domains of subjective cognition and symptom distress, household tasks performance, and workplace productivity.

		RSCL-M		POMS-SF		CTC homemaking assessment		WLQ[a]	
		r (95% CI)	*p*-value	r (95% CI)	*p*-value	r (95% CI)	*p*-value	r (95% CI)	*p*-value
LT recipient ECog (*n* = 60)	Memory	0.58 (0.38, 0.72)	**<0.0001**	0.31 (0.06, 0.53)	**0.014**	–0.28[b] (–0.51, –0.02)	**0.032**	0.86 (0.63, 0.95)	**<0.0001**
	Language	0.40 (0.17, 0.60)	**0.001**	0.10 (–0.16, 0.34)	0.452	–0.21[b] (–0.45, 0.06)	0.123	0.68 (0.28, 0.88)	**0.002**
	Visuospatial abilities	0.17 (–0.09, 0.40)	0.203	0.02 (–0.24, 0.27)	0.879	–0.00[b] (–0.27, 0.26)	0.977	0.11 (–0.41, 0.57)	0.688
	Planning	0.48 (0.25, 0.65)	**<0.0001**	0.34 (0.09, 0.55)	**0.007**	–0.04[b] (–0.30, 0.22)	0.742	0.83 (0.56, 0.94)	**<0.0001**
	Organization	0.45 (0.22, 0.63)	**0.0002**	0.31 (0.06, 0.52)	**0.015**	–0.05[b] (–0.31, 0.22)	0.730	0.66 (0.24, 0.87)	**0.004**
	Divided attention	0.50 (0.28, 0.67)	**<0.0001**	0.39 (0.15, 0.59)	**0.002**	–0.19[b] (–0.43, 0.08)	0.168	0.87 (0.66, 0.96)	**<0.0001**
Caregiver ECog (*n* = 60)	Memory	0.42 (0.18, 0.61)	**0.001**	0.25 (0.00, 0.48)	0.050	–0.28[b] (–0.51, –0.02)	**0.033**	0.19 (–0.34, 0.63)	0.469
	Language	0.33 (0.09, 0.54)	**0.008**	0.15 (–0.11, 0.39)	0.240	–0.34[b] (–0.55, –0.08)	**0.010**	0.31 (–0.22, 0.70)	0.228
	Visuospatial abilities	0.11[c] (–0.16, 0.36)	0.435	0.10[c] (–0.17, 0.35)	0.474	–0.17[d] (–0.42, 0.11)	0.230	0.29 (–0.24, 0.69)	0.267
	Planning	0.28[e] (0.02, 0.50)	**0.034**	0.30[e] (0.04, 0.52)	**0.022**	–0.22[f] (–0.46, 0.05)	0.103	0.26 (–0.27, 0.67)	0.312
	Organization	0.31[g] (0.04, 0.54)	**0.023**	0.22[g] (–0.06, 0.46)	0.115	–0.26[h] (–0.50, 0.02)	0.064	0.33 (–0.24, 0.73)	0.233
	Divided attention	0.38[e] (0.14, 0.58)	**0.003**	0.35[e] (0.10, 0.55)	**0.007**	–0.26[f] (–0.50, 0.01)	0.053	0.40 (–0.12, 0.75)	0.112

Note: Bold values denote statistical significance at the p < *0.05 level.*

[a]*n = 16.*

[b]*n = 56.*

[c]*n = 55.*

[d]*n = 53.*

[e]*n = 58.*

[f]*n = 54.*

[g]*n = 52.*

[h]*n = 50.*

demographic of LT recipients. Most LT recipients were male (71.7%), white (93.3%), and married (71.7%). They had a mean age of 60.4 (SD = 6.9) and a mean of 14.1 (SD = 2.4) years of education, and mean time elapsed since LT was 12.9 months (SD = 7.0). The most common cause of liver failure was Non-alcoholic steatohepatitis (46.7%), and mean MELD score at LT was 21.7 (SD = 10.0; **Table 1**).

Seventeen of 60 LT recipients were employed full time (*n* = 15, 25.0%) or part time (*n* = 2, 3.3%) after LT. While the characteristics of employed were generally comparable to non-employed LT recipients, employed recipients were relatively younger (mean age = 57.9, SD = 5.9 versus mean age = 61.5, SD = 7.0) and had a higher annual household income (annual household income over $60,000: 78.6% *versus* 27.8%). A smaller number of employed recipients had pre-transplant hepatic

TABLE 5 | Unadjusted associations between subjective and objective cognition and symptom distress, household tasks performance, and workplace productivity stratified by post-transplant employment status.

	LT recipients employed			LT recipients not employed		
	N	**β (95% CI)**	***p*-value**	**N**	**β (95% CI)**	***p*-value**
RSCL-M						
LT recipient ECog	17	1.05 (0.37, 1.73)	**0.005**	43	0.36 (0.18, 0.54)	**0.0002**
Caregiver ECog	17	−0.04 (−0.82, 0.74)	0.917	43	0.35 (0.17, 0.53)	**0.0003**
LT recipient RBANS	16	−0.02 (−0.03, −0.00)	**0.018**	41	−0.00 (−0.01, 0.00)	0.504
POMS-SF						
LT recipient ECog	17	1.46 (0.80, 2.11)	**0.000**	43	0.20 (−0.07, 0.48)	0.140
Caregiver ECog	17	0.55 (−0.29, 1.40)	0.184	43	0.27 (−0.00, 0.54)	0.052
LT recipient RBANS	16	−0.01 (−0.03, 0.01)	0.177	41	0.00 (−0.00, 0.01)	0.133
CTC Homemaking Assessment						
LT recipient ECog	17	−14.96 (−28.06, −1.86)	**0.028**	39	−3.31 (−19.26, 12.63)	0.676
Caregiver ECog	17	−14.97 (−25.64, −4.30)	**0.009**	39	−11.15 (−26.50, 7.20)	0.150
LT recipient RBANS	16	0.07 (−0.24, 0.38)	0.642	37	−0.09 (−0.44, 0.25)	0.588

Note: Bold values denote statistical significance at the p < *0.05 level.*

TABLE 6 | Correlations between the domains of subjective cognition and symptom distress, household tasks performance, and workplace productivity stratified by post-transplant employment status.

		RSCL-M		POMS-SF		CTC homemaking assessment	
		r (95% CI)	***p*-value**	**r (95% CI)**	***p*-value**	**r (95% CI)**	***p*-value**
Employed (*n* = 17)							
LT recipient ECog	Memory	0.69 (0.32, 0.88)	**0.001**	0.74 (0.40, 0.90)	**0.0003**	−0.50 (−0.79, −0.02)	**0.035**
	Language	0.47 (−0.02, 0.77)	0.052	0.51 (0.04, 0.80)	**0.029**	−0.27 (−0.67, 0.24)	0.277
	Visuospatial abilities	0.04 (−0.45, 0.51)	0.876	0.16 (−0.35, 0.60)	0.528	−0.09 (−0.54, 0.41)	0.739
	Planning	0.63 (0.22, 0.85)	**0.004**	0.77 (0.46, 0.91)	<**0.0001**	−0.41 (−0.74, 0.09)	0.094
	Organization	0.54 (0.08, 0.81)	**0.021**	0.50 (0.03, 0.79)	**0.035**	−0.29 (−0.67, 0.23)	0.257
	Divided attention	0.47 (0.01, 0.78)	**0.048**	0.83 (0.58, 0.94)	<**0.0001**	−0.77 (−0.91, −0.47)	<**0.0001**
Caregiver ECog	Memory	−0.02 (−0.50, 0.46)	0.933	0.21 (−0.30, 0.63)	0.404	−0.37 (−0.72, 0.14)	0.139
	Language	−0.05 (−0.52, 0.44)	0.840	0.30 (−0.22, 0.68)	0.240	−0.60 (−0.84, −0.16)	**0.008**
	Visuospatial abilities	−0.10 (−0.56, 0.40)	0.693	0.23 (−0.28, 0.64)	0.372	−0.61 (−0.84, −0.18)	**0.006**
	Planning	−0.08 (−0.54, 0.41)	0.748	0.32 (−0.19, 0.70)	0.197	−0.58 (−0.83, −0.14)	**0.011**
	Organization	0.08[a] (−0.45, 0.57)	0.771	0.33[a] (−0.22, 0.72)	0.215	−0.58[a] (−0.84, −0.10)	**0.018**
	Divided attention	−0.05 (−0.52, 0.44)	0.849	0.41 (−0.09, 0.74)	0.095	−0.66 (−0.87, −0.26)	**0.002**
Non-employed (*n* = 43)							
LT recipient ECog	Memory	0.58 (0.34, 0.75)	<**0.0001**	0.24 (−0.06, 0.51)	0.115	−0.21[b] (−0.49, 0.12)	0.204
	Language	0.44 (0.16, 0.66)	**0.002**	0.06 (−0.25, 0.35)	0.711	−0.14[b] (−0.44, 0.18)	0.378
	Visuospatial abilities	0.21 (−0.10, 0.48)	0.173	0.05 (−0.25, 0.35)	0.748	0.08[b] (−0.24, 0.39)	0.611
	Planning	0.46 (0.19, 0.67)	**0.001**	0.27 (−0.03, 0.53)	0.075	0.04[b] (−0.28, 0.35)	0.823
	Organization	0.50 (0.23, 0.70)	**0.0004**	0.36 (0.06, 0.59)	**0.018**	0.04[b] (−0.28, 0.35)	0.787
	Divided attention	0.52 (0.25, 0.71)	**0.0003**	0.24 (−0.06, 0.51)	0.115	−0.09[b] (−0.39, 0.23)	0.582
Caregiver ECog	Memory	0.60 (0.37, 0.76)	<**0.0001**	0.31 (0.01, 0.56)	**0.040**	−0.24[b] (−0.52, 0.08)	0.135
	Language	0.48 (0.21, 0.68)	**0.001**	0.15 (−0.15, 0.43)	0.319	−0.28[b] (−0.55, 0.03)	0.075
	Visuospatial abilities	0.16[c] (−0.17, 0.46)	0.329	0.14[c] (−0.19, 0.44)	0.413	−0.09[d] (−0.41, 0.24)	0.595
	Planning	0.41[e] (0.12, 0.64)	**0.007**	0.34[e] (0.03, 0.58)	**0.030**	−0.16[f] (−0.46, 0.17)	0.335
	Organization	0.41[f] (0.10, 0.65)	**0.009**	0.22[f] (−0.12, 0.50)	0.196	−0.20[g] (−0.50, 0.14)	0.240
	Divided attention	0.58[e] (0.34, 0.75)	<**0.0001**	0.37[e] (0.07, 0.61)	**0.016**	−0.19[f] (−0.49, 0.14)	0.252

Note: Bold values denote statistical significance at the p < *0.05 level.*
[a]*n = 15.*
[b]*n = 39.*
[c]*n = 38.*
[d]*n = 36.*
[e]*n = 41.*
[f]*n = 37.*
[g]*n = 35.*

encephalopathy (47.1% *versus* 81.4%) and they had a lower MELD score at LT than the non-employed recipients (mean = 13.6, SD = 6.0 versus mean = 24.9, SD = 9.4; **Table 1**).

Most caregivers participated in this study were the spouse or significant other (73.3%) of LT recipients with a mean of 35 (SD = 12.6) years length of relationship. They tended to be female (80.0%), white (94.9%), and had a mean age of 57.1 (SD = 11.8) years and a mean of 13.9 (SD = 2.1) years of education. They reported that they spend a mean of 107 (SD = 56.8) hours per week with LT recipients.

Subjective and Objective Cognition

The ECog scores of LT recipients are summarized in **Table 2**. Summaries of caregivers' ECog scores were reported elsewhere (15) but briefly, scores of both LT recipients' and caregivers' ECog indicate mild perceived difficulties in performing daily activities (mean = 1.5, SD = 0.5; mean = 1.4, SD = 0.5).

See **Table 2** for the summary of objective cognitive performance.

Cognition and Post-Transplant Employment Status

Compared to LT recipients who did not return to work, employed LT recipients had lower global ECog scores (indicating less subjective concerns about cognition) and higher scores on global objective cognition and multiple domains (indicating higher levels of objective cognition; **Table 2**).

Patient-Reported Outcomes

Summaries of patient-reported outcomes are described in **Table 2**. Scores of the RSCL-M and the POMS-SF indicated that LT recipients in this study have mild physical (mean = 1.6, SD = 0.4) and psychological symptom distress (mean = 0.8, SD = 0.5). Scores of the CTC Homemaking Assessment indicate that LT recipients could perform approximately 90% (SD = 21.6) of household tasks (**Table 2**).

Patient-Reported Outcomes and Post-Transplant Employment Status

LT recipients who returned to work after LT reported that they experience 3.4% (SD = 4.8) of productivity loss at work (**Table 2**). While the time scale score was the highest (mean = 17.0, SD = 26.3) indicating that recipients perceive the greatest difficulties handling time and scheduling demands at work, the mental-interpersonal scale score was the lowest (mean = 8.0, SD = 14.2) indicating that recipients perceive the lowest difficulties when performing tasks that require cognitive or social skills at work. Employed LT recipients had higher CTC Homemaking Assessment scores than non-employed recipients, while no significant differences were found in RCSL-M and POMS-SF scores (**Table 2**).

Associations Between Subjective Cognition and Patient-Reported Outcomes

Higher total ECog scores of LT recipients were significantly associated with higher RSCL-M scores (β = 0.30, p = 0.006; **Table 3**). Domain analysis revealed that ECog scores in all domains except for visuospatial abilities were fair to moderately associated with RSCL-M scores (r = 0.40–0.58; **Table 4**). Higher total ECog scores of caregivers were significantly associated with lower CTC Homemaking Assessment scores (β = −18.55, p = 0.015; **Table 3**). Specifically, their ECog scores in memory and language were negatively correlated to CTC scores (r = −0.28 and −0.34, respectively; **Table 4**).

Higher LT recipients' total ECog scores were significantly associated with higher WLQ scores (β = 14.85, p < 0.0001; **Table 3**). Particularly, ECog scores in the domains of memory, language, planning, organization, and divided attention were correlated with higher scores on the WLQ (r = 0.66–0.87; **Table 4**). Caregiver ECog scores were not related to the WLQ, regardless of domain (**Tables 3**, **4**). Note, these associations were unadjusted for LT recipients' age, education, and months since transplant due to a small sample size.

Associations Between Subjective Cognition and Patient-Reported Outcomes by Post-Transplant Employment Status

This study further examined unadjusted associations between subjective cognition and patient-reported outcomes stratified by post-transplant employment status (**Tables 5**, **6**). In the employed group, higher LT recipient total ECog scores were associated with higher RSCL-M (β = 1.05, p = 0.005) and POMS-SF (β = 1.46, p = 0.0003; **Table 5**). Domain analysis revealed that higher ECog scores of LT recipient in the domains of memory, planning, organization, and divided attention moderately to strongly correlated with higher RSCL-M (r = 0.47–0.69) and POMS-SF (r = 0.50–0.83; **Table 6**). Higher total ECog scores of both LT recipient and caregiver were related to lower CTC scores (β = −14.96, p = 0.028; β = −14.97, p = 0.009, respectively; **Table 5**). While LT recipient ECog scores in memory and divided attention were related to lower CTC scores (r = −0.50 and −0.77, respectively), all caregiver ECog domain scores except for memory were moderately correlated with CTC Homemaking Assessment scores (r = −0.58–−0.66; **Table 6**). In the unemployed group, higher LT recipient and caregiver total ECog scores were associated with higher RSCL-M scores (β = 0.36, p = 0.0002; β = 0.35, p = 0.0003, respectively). Nearly all of the recipient and caregiver ECog domain scores, except visuospatial abilities, were fair to moderately correlated with higher RSCL-M scores (r = 0.44–0.58 in LT recipients and r = 0.41–0.60 in caregivers; **Table 6**). No statistically significant associations were noted with POMS-SF or CTC scores (**Table 5**).

Associations Between Objective Cognition and Patient-Reported Outcomes

Objective test scores of LT recipients were not associated with any patient-reported outcomes within the entire sample (**Table 3**). In employed recipients, higher global cognition (RBANS total score) was related to lower RSCL-M scores (β = −0.02, p = 0.018; **Table 5**).

DISCUSSION

This cross-sectional study, to our knowledge, is the first to examine relationships between subjective and objective cognition and patient-reported outcomes of physical and psychological symptom distress, ability to perform household tasks, and workplace productivity among LT recipients. We found higher LT recipient self-rated cognitive decline was associated with higher physical symptom distress and workplace productivity loss. Higher caregiver-rated cognitive decline was associated with lower household tasks performance. However, these associations appear to be related to post-transplant employment status of LT recipients (employed vs. not employed). Cognitive decline measured by objective cognitive tests was not significantly associated with any of the patient-reported outcomes. These findings may suggest that LT recipients' quality of life can be assessed with markers of subjective cognition, regardless of objective cognition.

Cognitive decline is one of the major health issues among LT recipients. Cognition, however, is not regularly assessed in transplant practice due to multiple reasons, including lengthy cognitive tests. Subjective cognition that is easily assessed by a valid and reliable self-report survey has potential to be used as a proxy for objective cognition at a busy transplant clinic. Most participants completed the ECog within 2 min (15). While we have shown that there are fair to moderate correlations between objective and subjective cognition among LT recipients (15), the present study highlights that these different cognitive measures may provide complementary information, particularly regarding patient-reported outcomes that require cognitive skills, such as workplace productivity.

Subjective cognition, not objective cognition, was associated with LT recipients' perceived health-related quality of life and independence. While objective cognitive decline may contribute to develop worse clinical outcomes, such as graft failure and mortality (37), subjective cognitive decline may affect LT recipients' overall quality of life (38). Recipient self-rated cognition, however, was not associated with psychological symptom distress. This finding was inconsistent with the literature in general (39) and chronic illness populations (40) that documented strong relationships between subjective cognitive decline and psychological symptoms. This unexpected finding may be related to the types of psychological symptoms assessed in this study. This study assessed overall mood state by measuring six different dimensions of mood (depression, vigor, confusion, tension, anger, and fatigue), while many previous studies were limited to the symptoms of depression and/or anxiety (39, 40). Additionally, this study recruited LT recipients regardless of their subjective cognitive status as opposed to intentionally recruiting individuals with cognitive complaints (39). Replication with a larger sample size that intentionally recruit LT recipients with cognitive complaints may advance the understanding of the associations between subjective cognition and psychological symptom distress in different dimensions of mood.

LT recipient self-rated cognition, not caregiver-rated cognition, was significantly correlated with physical symptom distress, indicating that LT recipients who experience subjective cognitive decline may also be experiencing higher levels of physical symptom distress. While the relationship between subjective cognition and physical symptoms, such as fatigue, has been documented in the chronic illness literature (41), the mechanism underlying this relationship has not been thoroughly examined. One possibility is that LT recipients who have subjective cognitive decline in memory and executive function, essential cognitive domains to self-manage, may believe that they are not capable to properly manage their physical symptoms. Their belief may reduce engagement in symptom monitoring and management, resulting in increased symptom distress.

Findings from this study may contribute to understanding the low employment rates among LT recipients. Consistent to previous studies in chronic disease populations (42), this study found a strong relationship between LT recipients' subjective cognitive decline and self-reported workplace productivity loss. Particularly, LT recipients' subjective ratings on memory and executive function, the core cognitive skills that are essential to perform job tasks, were correlated with the workplace productivity loss. Although these findings should be understood with caution due to a small sample size of employed LT recipients, they may suggest that subjective cognitive decline could be one of the barriers that prevent LT recipients from returning to work. Participating in work after LT is crucial to maintain independent and productive lives and reduce burden on recipients' families and communities. Nevertheless, fewer than 25% of LT recipients are employed in paid work within 2 years post-transplant (43, 44). Findings of this study suggest an in-depth examination of the impacts of subjective and objective cognition on employment rates and workplace productivity to tackle unemployment among LT recipients.

Finally, this study provides insight into to the planning of individualized treatment to advance patient's quality of life. Caregivers seem to be more accurate than patients themselves in estimating cognitive changes (15, 45). Their subjective ratings of LT recipients' cognition may provide essential information regarding functional independence of LT recipients that can be used in the development of an individualized rehabilitation therapy. Further, employment status seems alter the associations between subjective cognition and patient-reported outcomes. While employed LT recipients have better subjective and objective cognition than non-employed recipients, their subjective cognition is more broadly associated with patient-reported outcomes than non-employed recipients' subjective cognition. Such findings may imply that psychosocial circumstances of recipients, such as employment status, should be considered when planning a treatment for LT recipients.

A novel finding of this study is that subjective cognitive decline, not objective cognitive decline, is associated with LT recipients' overall wellbeing. This study also suggests subjective cognition could be associated with low employment rate in this population. However, a few limitations of this study should be noted. Because of the cross-sectional nature of this study, the observed associations do not indicate cause and effect. The small sample recruited at a single center limits the generalizability of the

study findings. Particularly, findings related to post-transplant employment status should be understood with caution due to the small sample size of employed LT recipients. Potential confounders that may affect the patient-reported outcomes, such as comorbidities and employment status before LT, were not included in the analysis.

In conclusion, LT recipients with subjective cognitive decline may benefit from extra support on improving their quality of life. In practice, clinicians may consider paying attention to LT recipients' complaints about their cognition as they may reflect LT recipients' poor quality of life. Clinicians may also consider including caregivers when developing an individualized post-LT care plan as these caregivers may provide supplemental information regarding LT recipients' cognition and functional independence. Future longitudinal studies with a larger diverse sample are suggested to investigate the underlying mechanism of the relationships between subjective cognition and patient-reported outcomes. Such findings may contribute to identify strategies to support recipients with subjective cognitive decline to optimize their health and retain independence after LT.

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 Vanderbilt University Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors contributed to the study design and acquisition of subjects and data. DK and KG were involved in the analysis and interpretation of the data and preparation of the manuscript. All authors read and approved the final manuscript.

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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.

AUTHOR DISCLAIMER

This project's contents are solely the responsibility of the authors and do not necessarily represent official views of the National Center for Advancing Translational Sciences or the National Institutes of Health.

REFERENCES

1. Kwong AJ, Kim WR, Lake JR, Smith JM, Schladt DP, Skeans MA, et al. OPTN/SRTR 2019 Annual Data Report: Liver. *Am J Transpl* (2021) 21(S2):208–315. doi:10.1111/ajt.16494
2. Serper M, Asrani S, VanWagner L, Reese PP, Kim M, Wolf MS. Redefining Success after Liver Transplantation: From Mortality toward Function and Fulfillment. *Liver Transpl* (2022) 28(2):304–13. doi:10.1002/lt.26325
3. Burra P, Germani G, Gnoato F, Lazzaro S, Russo FP, Cillo U, et al. Adherence in Liver Transplant Recipients. *Liver Transpl* (2011) 17:760–70. doi:10.1002/lt.22294
4. Mulder MB, Borgsteede SD, Darwish Murad S, Landman CS, Metselaar HJ, Hunfeld NGM. Medication-Related Problems in Liver Transplant Recipients in the Outpatient Setting: A Dutch Cohort Study. *Front Pharmacol* (2021) 12:637090. doi:10.3389/fphar.2021.637090
5. Leven EA, Annunziato R, Helcer J, Lieber SR, Knight CS, Wlodarkiewicz C, et al. Medication Adherence and Rejection Rates in Older vs Younger Adult Liver Transplant Recipients. *Clin Transpl* (2017) 31:e12981. doi:10.1111/ctr.12981
6. Huda A, Newcomer R, Harrington C, Keeffe EB, Esquivel CO. Employment after Liver Transplantation: A Review. *Transpl Proc* (2015) 47(2):233–9. doi:10.1016/j.transproceed.2014.10.022
7. Ko D, Bratzke LC. Cognitive Function in Liver Transplant Recipients Who Survived More Than 6 Months. *Prog Transpl* (2020) 30(4):335–41. doi:10.1177/1526924820958144
8. Ahluwalia V, Wade JB, White MB, Gilles HS, Heuman DM, Fuchs M, et al. Liver Transplantation Significantly Improves Global Functioning and Cerebral Processing. *Liver Transpl* (2016) 22(10):1379–90. doi:10.1002/lt.24498
9. Campagna F, Montagnese S, Schiff S, Biancardi A, Mapelli D, Angeli P, et al. Cognitive Impairment and Electroencephalographic Alterations before and after Liver Transplantation: What Is Reversible? *Liver Transpl* (2014) 20(8):977–86. doi:10.1002/lt.23909
10. Dolansky MA, Hawkins MAW, Schaefer JT, Sattar A, Gunstad J, Redle JD, et al. Association between Poorer Cognitive Function and Reduced Objectively Monitored Medication Adherence in Patients with Heart Failure. *Circ Heart Fail* (2016) 9(12):e002475. doi:10.1161/CIRCHEARTFAILURE.116.002475
11. Kaiser J, Dietrich J, Amiri M, Rüschel I, Akbaba H, Hantke N, et al. Cognitive Performance and Psychological Distress in Breast Cancer Patients at Disease Onset. *Front Psychol* (2019) 10:2584. doi:10.3389/fpsyg.2019.02584
12. Jung MS, Zhang M, Askren MK, Berman MG, Peltier S, Hayes DF, et al. Cognitive Dysfunction and Symptom burden in Women Treated for Breast Cancer: a Prospective Behavioral and fMRI Analysis. *Brain Imaging Behav* (2017) 11(1):86–97. doi:10.1007/s11682-016-9507-8
13. Glanz BI, Dégano IR, Rintell DJ, Chitnis T, Weiner HL, Healy BC. Work Productivity in Relapsing Multiple Sclerosis: Associations with Disability, Depression, Fatigue, Anxiety, Cognition, and Health-Related Quality of Life. *Value Health* (2012) 15(8):1029–35. doi:10.1016/j.jval.2012.07.010

14. Von Ah D, Storey S, Crouch A. Relationship between Self-Reported Cognitive Function and Work-Related Outcomes in Breast Cancer Survivors. *J Cancer Surviv* (2018) 12(2):246–55. doi:10.1007/s11764-017-0664-6
15. Ko D, Dietrich MS, Gifford KA, Ridner SH. Subjective Cognition Reported by Caregivers Is Correlated With Objective Cognition in Liver Transplant Recipients. *Liver Transpl* (2022) 28(2):269–79. doi:10.1002/lt.26213
16. Vance DE, Bail J, Enah C, Palmer J, Hoenig A. The Impact of Employment on Cognition and Cognitive reserve: Implications across Diseases and Aging. *Nurs Res Rev* (2016) 6:61–71. doi:10.2147/NRR.S115625
17. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research Electronic Data Capture (REDCap)—A Metadata-Driven Methodology and Workflow Process for Providing Translational Research Informatics Support. *J Biomed Inform* (2009) 42(2):377–81. doi:10.1016/j.jbi.2008.08.010
18. Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, O'Neal L, et al. The REDCap Consortium: Building an International Community of Software Platform Partners. *J Biomed Inform* (2019) 95:103208. doi:10.1016/j.jbi.2019.103208
19. Farias ST, Mungas D, Reed BR, Cahn-Weiner D, Jagust W, Baynes K, et al. The Measurement of Everyday Cognition (ECog): Scale Development and Psychometric Properties. *Neuropsychology* (2008) 22(4):531–44. doi:10.1037/0894-4105.22.4.531
20. Farias ST, Huss O, Chan ML, Ruiz R. Revisions and Updates to the Everyday Cognition (ECog) Scales. *Alzheimers Dement* (2020) 16(6):e043263. doi:10.1002/alz.043263
21. Randolph C, Tierney MC, Mohr E, Chase TN. The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS): Preliminary Clinical Validity. *J Clin Exp Neuropsychol* (1998) 20(3):310–9. doi:10.1076/jcen.20.3.310.823
22. Duff K, Hobson VL, Beglinger LJ, O'Bryant SE. Diagnostic Accuracy of the RBANS in Mild Cognitive Impairment: Limitations on Assessing Milder Impairments. *Arch Clin Neuropsychol* (2010) 25(5):429–41. doi:10.1093/arclin/acq045
23. Aceto P, Perilli V, Lai C, Ciocchetti P, Vitale F, Sollazzi L. Postoperative Cognitive Dysfunction after Liver Transplantation. *Gen Hosp Psychiatry* (2015) 37(2):109–15. doi:10.1016/j.genhosppsych.2014.12.001
24. Sánchez-Cubillo I, PeriáñEz JA, Adrover-Roig D, RodríGuez-SáNchez JM, RíOs-Lago M, Tirapu J, et al. Construct Validity of the Trail Making Test: Role of Task-Switching, Working Memory, Inhibition/interference Control, and Visuomotor Abilities. *J Int Neuropsychol Soc* (2009) 15:438–50. doi:10.1017/S1355617709090626
25. Waters GS, Caplan D. The Reliability and Stability of Verbal Working Memory Measures. *Behav Res* (2003) 35(4):550–64. doi:10.3758/BF03195534
26. McKinlay A. Rey Complex Figure Test. In: *Encyclopedia of Child Behavior and Development*. Boston, MA: Springer (2011). p. 1264–5. doi:10.1007/978-0-387-79061-9_2437
27. Strauss E, Sherman EMS, Spreen O. *A Compendium of Neuropsychological Tests*. third. New York: Oxford University Press (2006). p. 811.
28. Berry DTR, Allen RS, Schmitt FA. Rey-Osterrieth Complex Figure: Psychometric Characteristics in a Geriatric Sample. *Clin Neuropsychol* (1991) 5(2):143–53. doi:10.1080/13854049108403298
29. Stein KD, Denniston M, Baker F, Dent M, Hann DM, Bushhouse S, et al. Validation of a Modified Rotterdam Symptom Checklist for Use with Cancer Patients in the United States. *Pain Symptom Manage* (2003) 26(5):975–89. doi:10.1016/S0885-3924(03)00321-X
30. Curran SL, Andrykowski MA, Studts JL. Short Form of the Profile of Mood States (POMS-SF): Psychometric Information. *Psychol Assess* (1995) 7(1):80–3. doi:10.1037/1040-3590.7.1.80
31. Creative Therapy Consultants. *Evaluating a Homemaking Assessment for Broader Application to Practice* (2016). www.creativetherapyconsultants.ca/publications/# (Accessed April 28, 2018).
32. Creative Therapy Consultants. *The Creative Therapy Consultants Homemaking Assessment: Administration Guide* (2016). www.creativetherapyconsultants.ca/publications/# (Accessed April 28, 2018).
33. Lerner D, Amick BC 3rd, Lee JC, Rooney T, Rogers WH, Chang H, et al. Relationship of Employee-Reported Work Limitations to Work Productivity. *Med Care* (2003) 41(5):649–59. doi:10.1097/01.MLR.0000062551.76504.A9
34. Lerner D, Amick BC, Rogers WH, Malspeis S, Bungay K, Cynn D. The Work Limitations Questionnaire. *Med Care* (2001) 39(1):72–85. doi:10.1097/00005650-200101000-00009
35. Mapi Research Trust. *Work Limitations Questionnaire Version 1.0: Scaling and Scoring*. Lyon, France: Mapi Research Trust (2018).
36. Kim WR, Biggins SW, Kremers WK, Wiesner RH, Kamath PS, Benson JT, et al. Hyponatremia and Mortality Among Patients on the Liver-Transplant Waiting List. *N Engl J Med* (2008) 359(10):1018–26. doi:10.1056/NEJMoa0801209
37. Thomas AG, Ruck JM, Shaffer AA, Haugen CE, Ying H, Warsame F, et al. Kidney Transplant Outcomes in Recipients with Cognitive Impairment: A National Registry and Prospective Cohort Study. *Transplantation* (2019) 103(7):1504–13. doi:10.1097/TP.0000000000002431
38. Lycke M, Lefebvre T, Pottel L, Pottel H, Ketelaars L, Stellamans K, et al. Subjective, but Not Objective, Cognitive Complaints Impact Long-Term Quality of Life in Cancer Patients. *J Psychoso Oncol* (2019) 37(4):427–40. doi:10.1080/07347332.2018.1504154
39. Zlatar ZZ, Muniz M, Galasko D, Salmon DP. Subjective Cognitive Decline Correlates with Depression Symptoms and Not with Concurrent Objective Cognition in a Clinic-Based Sample of Older Adults. *J Gerontol B Psychol Sci Soc Sci* (2018) 73(7):1198–202. doi:10.1093/geronb/gbw207
40. D'hooghe MB, De Cock A, Van Remoortel A, Benedict RHB, Eelen P, Peeters E, et al. Correlations of Health Status Indicators with Perceived Neuropsychological Impairment and Cognitive Processing Speed in Multiple Sclerosis. *Mult Scler Relat Disord* (2019) 39:101904. doi:10.1016/j.msard.2019.101904
41. Nicol C, Ownsworth T, Cubis L, Nguyen W, Foote M, Pinkham MB. Subjective Cognitive Functioning and Associations with Psychological Distress in Adult Brain Tumour Survivors. *J Cancer Surviv* (2019) 13(5):653–62. doi:10.1007/s11764-019-00784-8
42. Chokka P, Bougie J, Rampakakis E, Proulx J. Assessment in Work Productivity and the Relationship with Cognitive Symptoms (AtWoRC): Primary Analysis from a Canadian Open-Label Study of Vortioxetine in Patients with Major Depressive Disorder (MDD). *CNS Spectr* (2019) 24(3):338–47. doi:10.1017/S1092852918000913
43. Huda A, Newcomer R, Harrington C, Blegen MG, Keeffe EB. High Rate of Unemployment after Liver Transplantation: Analysis of the United Network for Organ Sharing Database. *Liver Transpl* (2012) 18:89–99. doi:10.1002/lt.22408
44. Saab S, Wiese C, Ibrahim AB, Peralta L, Durazo F, Han S, et al. Employment and Quality of Life in Liver Transplant Recipients. *Liver Transpl* (2007) 13(9):1330–8. doi:10.1002/lt.21247
45. Miller LS, Brown CL, Mitchell MB, Williamson GM. Activities of Daily Living Are Associated with Older Adult Cognitive Status: Caregiver versus Self-Reports. *J Appl Gerontol* (2013) 32(1):3–30. doi:10.1177/0733464811405495

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ORIGINAL RESEARCH
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A Novel Online Calculator Predicting Acute Kidney Injury After Liver Transplantation: A Retrospective Study

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Acute kidney injury (AKI) after liver transplantation (LT) is a common complication, and its development is thought to be multifactorial. We aimed to investigate potential risk factors and build a model to identify high-risk patients. A total of 199 LT patients were enrolled and each patient data was collected from the electronic medical records. Our primary outcome was postoperative AKI as diagnosed and classified by the KDIGO criteria. A least absolute shrinkage and selection operating algorithm and multivariate logistic regression were utilized to select factors and construct the model. Discrimination and calibration were used to estimate the model performance. Decision curve analysis (DCA) was applied to assess the clinical application value. Five variables were identified as independent predictors for post-LT AKI, including whole blood serum lymphocyte count, RBC count, serum sodium, insulin dosage and anhepatic phase urine volume. The nomogram model showed excellent discrimination with an AUC of 0.817 (95% CI: 0.758–0.876) in the training set. The DCA showed that at a threshold probability between 1% and 70%, using this model clinically may add more benefit. In conclusion, we developed an easy-to-use tool to calculate the risk of post-LT AKI. This model may help clinicians identify high-risk patients.

Keywords: liver transplantation, risk factors, acute kidney injury, nomogram, online calculator

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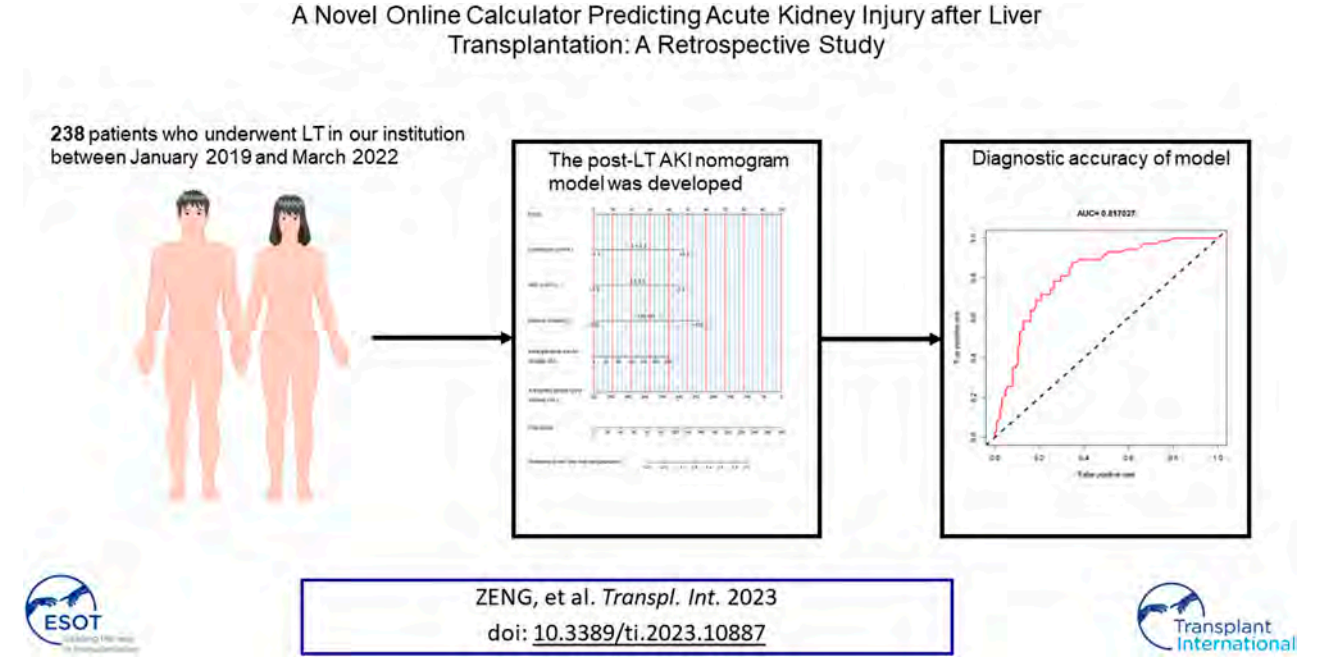
INTRODUCTION

Liver transplantation (LT) is the only definitive treatment for patients with end-stage liver disease, and the number of patients receiving LT has increased significantly during recent decades (1). Acute kidney injury (AKI) is a frequent but severe complication after LT and is associated with increased morbidity (2, 3). According to the current literature, which applied the 2012 Kidney Disease Improving Global Outcomes (KDIGO) criteria, the incidence of post-LT AKI is high, ranging from approximately 40%–60% (2, 4–8). For LT patients, there is no doubt that postoperative AKI is strongly associated with poor clinical outcomes, including increased graft rejection, longer hospital stay, more healthcare costs and shorter overall survival (2, 3). To avoid or reduce the occurrence of



GRAPHICAL ABSTRACT |

AKI after LT, discovering predisposing factors that can help us to identify high-risk patients is a pressing need.

The development of post-LT AKI is thought to be multifactorial (3). Several preoperative, intraoperative and postoperative variables have been reported, including serum creatinine, graft characteristics, intraoperative hemorrhage, urine output, cold ischemic time, calcineurin inhibitor nephrotoxicity and postoperative intensive care unit (ICU) stay (6, 9–11). Unfortunately, many studies used different postoperative AKI criteria and lack a standard definition; therefore, the above risk variables may not be sufficiently reliable among different studies. On the other hand, as we continue to learn more about the pathogenesis of post-LT AKI, new technique application and individualized treatment improved, and more new factors were identified. For instance, Pulitano and colleagues (12) confirmed that serum endothelin-1 and interleukin-18 levels were significantly predictive of post-LT AKI, which are involved in liver ischemia reperfusion injury (IRI).

A previous study reported that early diagnosis and treatment may improve the outcome of post-LT AKI patients (2). Therefore, constructing an accurate model to predict post-LT AKI becomes particularly meaningful. In this study, we applied the KDIGO criteria to define AKI after LT, identified potential risk factors and then developed a predictive model. We further constructed an easy-to-use online calculator to facilitate the calculation of the probability of AKI at the end of the transplant procedure. The objectives of our study are to help clinicians identify high-risk AKI patients, facilitate clinical decision-making and improve the prognosis of LT patients.

PATIENTS AND METHODS

Study Population

Inclusion criteria were patients who underwent LT in our institution between January 2019 and March 2022. Exclusion criteria were as following: 1) patients who were less than 18 years old; 2) received continuous renal replacement therapy (CRRT); 3) diagnosed with kidney diseases before surgery (including hepato-renal syndrome); 4) died within 48 h after surgery; 5) underwent combined liver-kidney transplantation or retransplantation and 6) missing important data. After the surgery, all patients were followed up daily until the discharge time. Besides, urine output and serum creatinine were used for routinely evaluation of renal function. This manuscript was prepared for publication using the applicable Equator guidelines for quality improvement studies.

Data Collection

We manually extracted preoperative, intraoperative and postoperative data from the electronic medical records by two independent investigators. Then, the patient cohort was randomly divided into two parts at a ratio of 7:3 for training and validation. Of note, we only used preoperative and intraoperative data to construct the model, and postoperative data were only applied to define the end point and outcome analysis. Besides, we failed to extract warm and cold ischemic time as the two variables were not available in our electronic systems. In this study, the liver was almost retrieved from deceased after brain death (DBD) donors.

The preoperative data included demographic information, preoperative comorbidities, etiologies, clinical manifestations, laboratory data, and hemodynamic parameters. Demographic

information included sex, age, body mass index (BMI), American Society of Anesthesiologists (ASA) score and personal lifestyle habits (smoking and alcoholism). The preoperative comorbidities included diabetes mellitus, hypertension, cardiovascular diseases (e.g., coronary heart disease) and respiratory diseases (e.g., chronic obstructive pulmonary disease). Etiologies of the studied population included viral hepatitis (e.g., hepatitis B virus), liver cancer (e.g., hepatocellular carcinoma) and others (e.g., alcohol-related liver disease and cholestatic liver disease). The clinical manifestations included symptoms of fluid overload (e.g., ascites) and major organ dysfunction (e.g., encephalopathy). The extent of ascites was graded according to the maximum depth of pelvic ascites measured by ultrasound: no ascites, non-severe ascites (<10 cm), and severe ascites (≥10 cm). Preoperative laboratory data included blood biochemistry, routine blood tests, and coagulation function, which was defined as the last measurement before surgery. The hemodynamic parameters included central venous pressure (CVP) and cardiac output (CO) on the day before surgery. We measured CVP *via* a central venous catheter, while the cardiac index was measured by the continuous thermodilution cardiac output technique. Meanwhile, the Child–Pugh score and the model for end-stage liver disease (MELD) score were also enrolled preoperatively.

Previous studies have indicated that intraoperative events have a crucial role in the development of post-LT AKI; therefore, we intended to collect as many intraoperative variables as possible (3, 13, 14). The intraoperative data in this study included the operation time, anhepatic time, inferior vena cava occlusion time, intraoperative hypotension, estimated blood loss, fluid administration (crystalloid and colloid), intraoperative transfusion [red blood cell (RBC), fresh frozen plasma (FFP) and platelet], intraoperative drug dosage (furosemide and insulin), total urine volume and anhepatic phase urine volume.

Postoperative data included the length of postoperative hospital stay, length of ICU stay, and in-hospital mortality. In addition, postoperative laboratory data [e.g., serum creatinine and aspartate aminotransferase (AST)] were also obtained.

Sample Size

As previously reported, the effective sample size for prediction generally suggests at least 10 events per variable, where events are defined as the proportion of cases in the least frequent of two outcome categories (15, 16). To our knowledge, the prevalence of AKI after LT, according to the KDIGO criteria, has been reported to be approximately 40%–60%, (4, 5) and we expected a 50% prevalence in our institution. Considering the accuracy and practicability of the nomogram model, 6 or fewer variables is thought to be appropriate, and therefore, 120 patients or more were required after calculation in the training set.

Outcome and Definition

All LT patients underwent a subcostal incision and midline extension under general anesthesia. The classic orthotopic LT was performed in the majority of patients, and we did not apply the veno-venous bypass technique during the anhepatic stage. The main outcome was the occurrence of AKI. Postoperative AKI was defined according to the 2012 KDIGO criteria as previously described, with follow-up limited to the first 7 postoperative days (17). AKI stage was determined for each patient using serum creatinine concentration- and urine output-based KDIGO definitions for stage 1 (≥0.3 mg/dL or 1.5-fold increase; $<0.5\ ml\ kg^{-1}\ h^{-1}$ for 6–12 h), stage 2 (2-fold increase; $<0.5\ ml\ kg^{-1}\ h^{-1}$ for ≥12 h) and stage 3 (≥4 mg/dL or 3-fold increase). The baseline serum creatinine was tested on the day before surgery. As any degree of AKI is associated with increased risk-adjusted mortality, we decided to include all stages of AKI in this study rather than only choosing severe AKI (stages 2 and 3), as previously reported (2, 7, 8, 18, 19). Hypertension and diabetes mellitus were defined according to standard criteria (20, 21). The definition of kidney diseases before surgery was kidney function damage or an estimated glomerular filtration rate (eGFR) < 60 ml/min/1.73 m^2. The MELD score was calculated based on the validated logarithmic index of serum bilirubin, creatinine and international normalized ratio (INR) as previously described (22). Intraoperative hypotension was defined as a systolic blood pressure <90 mmHg, a mean blood pressure <60 mmHg and/or a reduction in systolic blood pressure > 40 mmHg from baseline, as well as a duration greater than 15 min (23).

Statistical Analysis

All statistical analyses were performed with R software (version 4.2.0) and SPSS (version 23.0.0.0). Continuous variables are expressed as medians and interquartile ranges, while categorical variables are shown as case numbers and percentages. We applied the Mann-Whitney U test to compare the median of a continuous variable between two groups. To evaluate categorical data between two independent groups, a Chi-square test was used. A least absolute shrinkage and selection operating (LASSO) regression model was performed to select the optimal predictive factors, and the lambda within 1 standard error of the minimum was applied in this study. Those non-zero coefficients factors were then incorporated into the multivariable logistic regression model (24). A predictive nomogram model was developed with the training set based on the results of the multivariable logistic regression analysis. Subsequently, the performance of the model was evaluated by calibration and discrimination in the training and validation sets (25). The calibration ability of the predictive model was used by calibration curves (26). The discriminative ability of the model was determined by the area under the receiver operating characteristic (ROC) curve (AUC) (27, 28). Decision curve analysis (DCA) was also used to evaluate the clinical application value of this predictive model (29). In addition, an interactive web-based calculator based on this nomogram model was programmed with the Shiny package (https://CRAN.R-project.org/package=shiny). For analyses, a p-value < 0.05 was considered to be statistically significant in a 2-tailed test.

RESULTS

Overall Characteristics of Patients

From January 2019 to March 2022, a total of 238 patients who underwent LT in our hospital were enrolled. According to the

inclusion criteria, a total of 199 patients were identified in the final analysis, including 149 patients in the training set and 50 patients in the validation set, and the detailed flowchart is provided in **Figure 1**. The overall characteristics of patients in the two sets are summarized in **Table 1**. Fifty-three (37.9%) and twenty-one (35.6%) patients developed postoperative AKI in the training and validation cohorts, respectively.

To analyze the postoperative characteristics and outcomes, we divided the patients into two groups (AKI and non-AKI groups) (**Table 2**). The AKI group had a longer length of ICU stay ($p < 0.001$) and higher peak AST ($p < 0.001$) and peak serum creatinine levels ($p = 0.049$) than the non-AKI group. Notably, the in-hospital mortality of the AKI group was approximately 10 times greater than that of the non-AKI group ($p = 0.003$).

Variable Selection of AKI After LT

Using the LASSO algorithm, fifty-eight variables were reduced to 9 potential features, with a cross-validated error within 1 standard error of the minimum (**Figures 2A, B**). Five factors remained independently associated with the risks of AKI in LT patients after multivariate logistic regression analysis (**Table 3**). Preoperative variables (whole blood serum lymphocyte count, RBC count and serum sodium) and intraoperative variables (insulin dosage and anhepatic phase urine volume) were independent risk factors for post-LT AKI.

Model Construction and Online Calculator Development

We then established a simple-to-use nomogram model based on the above independent risk factors (**Figure 3A**). The nomogram showed that anhepatic phase urine volume was the most significant predictor for post-LT AKI, followed by preoperative serum sodium and whole blood lymphocyte count. To make this model more convenient for clinicians, we also developed an online calculator (**Figure 3B**). This online calculator is available at https://caobingbing.shinyapps.io/Nomogram_for_AKI_after_liver_transplantation/.

Performance and Validation of the Post-LT AKI Nomogram Model

The AUCs of the predictive nomogram were 0.817 (95% CI: 0.758–0.876) and 0.906 (95% CI: 0.831–0.981) for the training and validation sets, respectively (**Figures 4A, B**), indicating that this model demonstrates excellent accuracy in estimating the probability of AKI after LT. The calibration curve of the nomogram is presented in **Figures 5A, B**, which revealed good consistency between prediction and observation.

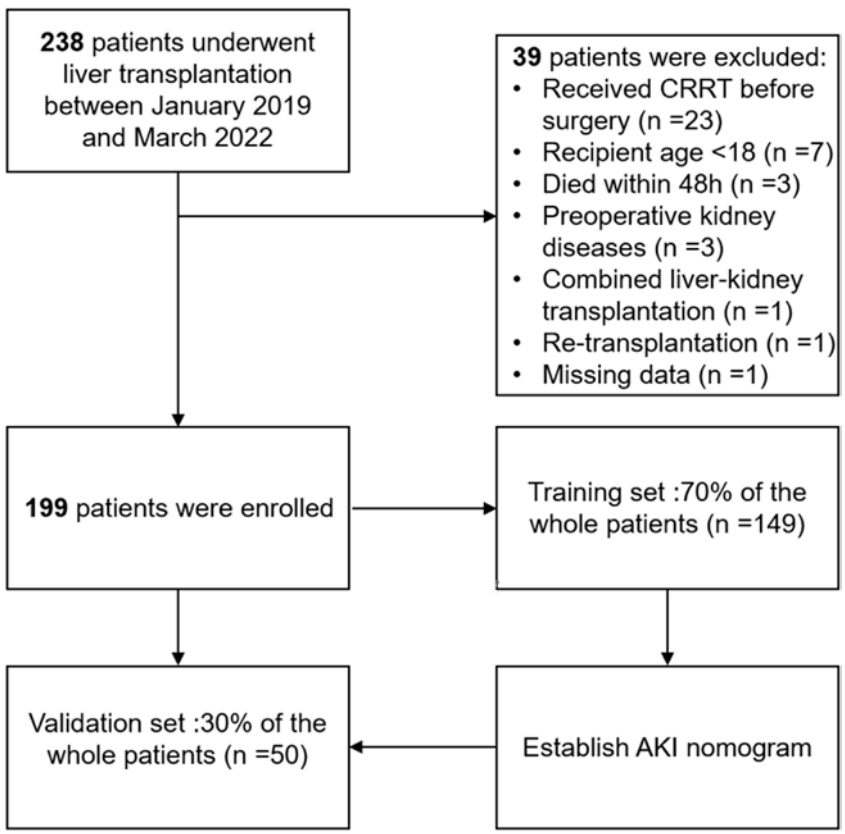


FIGURE 1 | The flowchart of our study.

TABLE 1 | Clinical characteristics of the study population in the training and validation sets.

Variables	Number (percentage, %) or median (IQRs)	
	Training set (*n* = 140)	**Validation set (*n* = 59)**
AKI after LT, yes	53 (37.9)	21 (35.6)
Sex, male/female	126/14 (90/10)	48/11 (81.4/18.6)
Age, <65/≥65 years old	124/16 (88.6/11.4)	54/5 (91.5/8.5)
BMI, <28/≥28 kg/m^2	126/14 (90/10)	51/8 (86.4/13.6)
ASA score, III/IV/V	43/91/6 (30.7/65/4.3)	16/40/3 (27.1/67.8/5.1)
Smoking	36 (25.7)	11 (18.6)
Alcoholism	38 (27.1)	14 (23.7)
Etiology of liver disease		
HBV hepatitis	57 (40.7)	24 (40.7)
HBV hepatitis + liver cancer	50 (35.7)	21 (35.6)
Liver cancer	12 (8.57)	2 (3.4)
Others	21 (15)	12 (20.3)
Preoperative comorbidities		
Diabetes mellitus	15 (10.7)	8 (13.6)
Hypertension	21 (15)	9 (15.2)
Cardiovascular diseases	7 (5)	2 (3.4)
Respiratory diseases	19 (13.6)	7 (11.9)
Previous abdominal surgery	58 (41.4)	18 (30.5)
Preoperative scores		
MELD score	13 (9–17)	13 (9–19)
Child-Pugh score	8 (6–9)	8 (6–10)
Encephalopathy	14 (10)	9 (15.2)
Ascites		
No	16 (11.4)	7 (11.9)
Non-severe ascites	109 (77.9)	45 (76.3)
Severe	15 (10.7)	7 (11.9)
Preoperative laboratory data		
WBC count, <4/4–10/>10 ×10^9/L	66/70/4 (47.1/50/2.9)	28/30/1 (47.5/50.8/1.7)
Neutrophils count, <1.8/1.8–6.3/>6.3 ×10^9/L	37/91/12 (26.4/65/8.6)	16/38/5 (27.1/64.4/8.5)
Lymphocyte count, <1.1/1.1–3.2/>3.2 ×10^9/L	101/38/1 (72.2/27.1/0.7)	38/20/1 (64.4/33.9/1.7)
RBC count, <3.5/3.5–5.5/>5.5 ×10^{12}/L	70/69/1 (50/49.3/0.7)	29/28/2 (49.2/47.5/3.2)
Hb level, g/L	106 (86.8–134.5)	111 (85–131.5)
Hct, <40%	103 (73.6)	45 (76.3)
PLT count, <40/40–100/>100 ×10^9/L	26/74/40 (18.6/52.9/28.5)	11/37/11
TP, <60 g/L	37 (26.4)	12 (20.3)
ALB, <35 g/L	70 (50)	23 (38.9)
TBIL, >21 μmol/L	110 (78.6)	47 (79.7)
DBIL, >6.8 μmol/L	107 (76.4)	48 (81.4)
IBIL, >17 μmol/L	93 (66.4)	39 (66.1)
AST, >40 U/L	81 (57.9)	38 (64.4)
ALT, >50 U/L	28 (20)	12 (20.3)
BUN, >7.2 mmol/L	24 (17.1)	9 (15.3)
sCr, >133 μmol/L	3 (2.1)	0 0)
PT, >14 s	100 (71.4)	43 (72.9)
INR, >1.15	100 (71.4)	42 (71.2)
aPTT, >32 s	85 (60.7)	40 (67.8)
TT, >21 s	34 (24.3)	12 (20.3)
FIB, ≤4 g/L	134 (95.7)	58 (98.3)
Potassium, <3.5/3.5–5.5/>5.5 mmol/L	25/115/0 (17.9/82.1/0)	13/45/1 (22/76.3/1.7)
Sodium, <135/135–145/>145 mmol/L	35/104/1 (25/74.3/0.7)	13/46/0 (22/78/0)
Chlorine, <100/100–110/>110 mmol/L	30/103/7 (21.4/73.6/5)	14/45/0 (23.7/76.3/0)
Preoperative CVP, cmH_2O	9 (7–12)	9 (8–13)
Preoperative CO, L/min	7 (5.4–8.4)	7 (5.9–8.1)
Preoperative eGFR, (mL/min/1.73 m^2)	99.4 (78.2–124.2)	100.1 (79.7–131.1)
Intraoperative data		
Operation time, minutes	425 (383.5–475)	443 (401.5–499)
Anhepatic time, minutes	60 (51.8–70)	64 (52–74.5)
IVC occlusion time, minutes	57.5 (47–63.2)	59 (48–69.5)
Intraoperative hypotension	45 (32.1)	17 (28.8)
Estimated blood loss, mL	600 (400–1,000)	600 (400–1,000)
Fluid administration		
Crystalloid, mL	2000 (1,200–2,800)	1875 (1,050–2,700)
Colloid, mL	300 (200–862.5)	300 (200–725)

(Continued on following page)

TABLE 1 | (*Continued*) Clinical characteristics of the study population in the training and validation sets.

Variables	Number (percentage, %) or median (IQRs)	
	Training set (*n* = 140)	**Validation set (*n* = 59)**
Intraoperative transfusion		
RBC, U	4 (0–7.63)	4 (1.5–6.8)
FFP, ml	600 (200–1,000)	600 (400–900)
PLT, U	1 (0–1)	1 (0–1)
Intraoperative drugs dosage		
Furosemide, mg	20 (0–20)	20 (0–35)
Insulin, IU	35 (0–60)	20 (0–80)
Total urine volume, ml	1,000 (697.5–1,512.5)	1,000 (725–1,375)
Anhepatic phase urine volume, ml	65 (20–200)	69 (40–150)

Abbreviations: IQR, interquartile range; AKI, acute kidney injury; LT, liver transplantation; BMI, body mass index; ASA, American Society of Anesthesiologists; HBV, hepatitis B virus; MELD, model for end-stage liver disease; WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; Hct, hematocrit; PLT, platelet; TP, total protein; ALB, albumin; TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; sCr, creatinine; PT, prothrombin time; INR, international normalized ratio; aPTT, activated partial thromboplastin time; TT, thrombin time; FIB, fibrinogen; CVP, central venous pressure; CO, cardiac output; eGFR, estimated glomerular filtration rate; IVC, inferior vena cava; FFP, fresh frozen plasma.

TABLE 2 | Postoperative characteristics and outcomes of the patients in AKI and non-AKI groups.

Variables	Number (percentage, %) or median (IQRs)		*p*-value
	AKI (*n* = 74)	**Non-AKI (*n* = 125)**	
Peak AST (U/L)	1326.5 (613.3–3459.5)	1,026 (591–1752)	<0.001
Peak sCr, μmol/L	136 (105–188.3)	93 (77–110)	0.049
Postoperative hospital stay (day)	26 (21–40.3)	25 (18–31)	0.057
Length of ICU stay (day)	7 (5–10)	5 (4–6)	<0.001
Died in hospital	7 (9.46)	1 (0.8)	0.003

Continuous variables are expressed as medians and interquartile ranges, while categorical variables are showed as case numbers and percentages. Abbreviations: AST, aspartate aminotransferase; sCr, creatinine; ICU, intensive care unit.

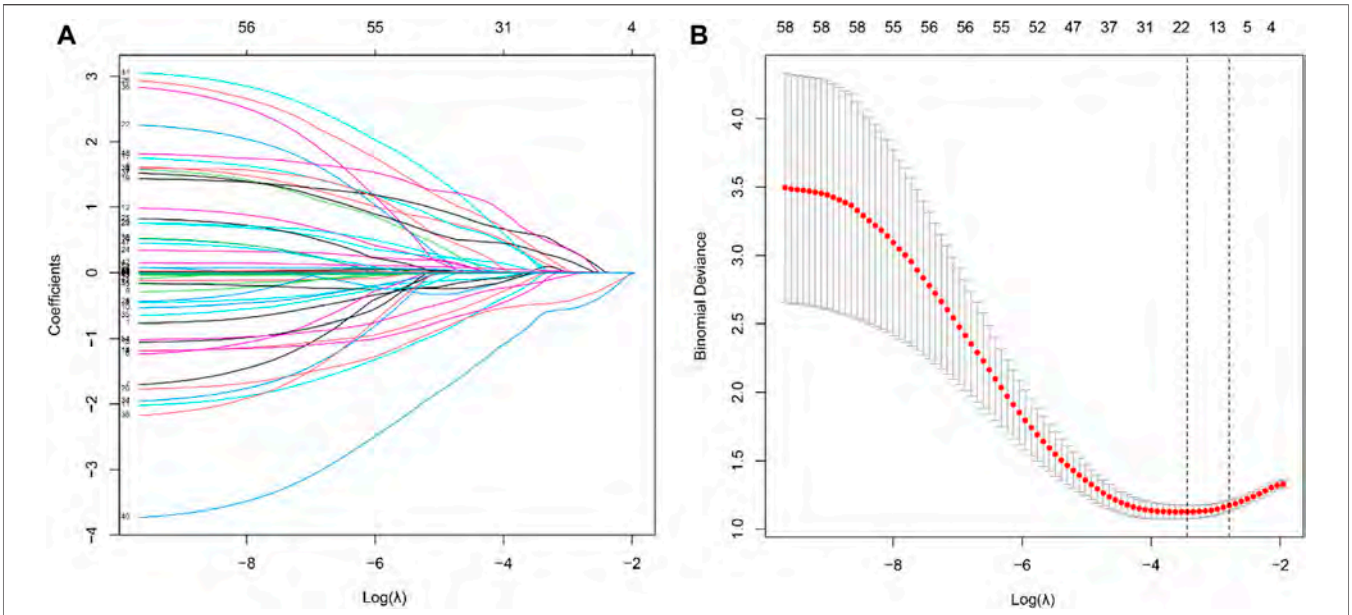


FIGURE 2 | Feature selection of LT patients using the LASSO logistic regression model. **(A)** A Lasso coefficient profile plot was built for the prediction of AKI after LT. **(B)** The optimal parameter (λ) was selected by the LASSO model using 10-fold cross-validation *via* 1 standard error of the minimum criteria.

TABLE 3 | Multivariable logistic regression analysis of predicting AKI after LT in the training cohort.

Variables	β	OR (95% CI)	*p*-value
Intercept	−0.397	0.673 (0.152–2.928)	0.596
Lymphocyte count, $\times10^9$/L	1.150	3.159 (1.496–7.031)	0.003
RBC count, $\times10^{12}$/L	−0.875	0.417 (0.185–0.904)	0.029
TT, s	0.596	1.815 (0.794–4.181)	0.157
Sodium, mmol/L	−1.108	0.330 (0.138–0.762)	0.010
Preoperative CVP, cmH_2O	0.055	1.056 (0.968–1.162)	0.234
Intraoperative hypotension	1.505	4.506 (0.755–33.105)	0.110
Intraoperative furosemide dosage, mg	0.018	1.018 (1.000–1.038)	0.059
Intraoperative insulin dosage, IU	0.009	1.009 (1.002–1.019)	0.016
Anhepatic phase urine volume, ml	−0.009	0.991 (0.986–0.996)	<0.001

β is the regression coefficient. Abbreviations: AKI, acute kidney injury; LT, liver transplantation; OR, odds ration; CI, confidence interval; RBC, red blood cell; TT, thrombin time; CVP, central venous pressure.

Clinical Use of the Post-LT AKI Nomogram Model

Finally, the DCA of AKI after the LT nomogram model is presented in **Figures 6A, B**. Compared with scenarios where no prediction model was used for a pretreatment decision, the nomogram model provided a favorable net benefit across a wide range, with a threshold probability across 1%–70% and 1%–80% for the training and validation sets, respectively. Overall, the above methods confirmed the clinical utility and reliability of our nomogram.

DISCUSSION

Herein, we describe a nomogram model to predict the probability of AKI in patients who underwent LT and then developed an online calculator for clinical use. In this study, we identified five risk factors as independent predictors for post-LT AKI, including whole blood serum lymphocyte count, RBC count, serum sodium, intraoperative insulin dosage and anhepatic phase urine volume. Moreover, we obtained several new findings in this study. First, the post-LT AKI nomogram model is a useful tool with good discrimination and calibration. Second, the AKI risk of individual patients who underwent LT can be calculated easily at the end of transplantation surgery by using our online calculator. Once clinicians identify high-risk patients, renal protection treatment (e.g., avoiding nephrotoxic medications and adjusting the dose of calcineurin inhibitors) is applied in a timely manner. Third, at a threshold probability between 1% and 70%, using our nomogram model may add more benefit to LT patients, indicating that the model described here may be widely applicable.

In the current study, we noted that AKI is a common issue for LT patients. According to the 2012 KDIGO criteria, we observed that the incidence of post-LT AKI in our center was 37.2%, which is slightly lower than the 40%–60% reported previously (4, 5, 7, 8). However, compared with the other surgical population (e.g., orthopedic and gastrointestinal surgery), which was reported to vary from 2.9% to 11.8%, LT patients have a much higher postoperative AKI incidence. (30, 31) In addition, we also observed that the in-hospital mortality of the AKI group was approximately 10 times greater than that of the non-AKI group. The length of ICU stay was significantly increased in the AKI group. Therefore, as reported in previous studies, we similarly demonstrate that post-LT AKI may lead to serious complications and cause worse prognosis (2, 4, 10, 13, 19, 30).

Many studies have demonstrated that several intraoperative factors can significantly contribute to postoperative AKI (14), and electrolyte/acid-base balance disorder, hypotension, blood or FFP transfusion, lactate concentration and urine output have been associated with an increased incidence of AKI. (4, 5, 7, 8) As we expected, two intraoperative risk factors (insulin dosage and anhepatic phase urine volume) were identified as independent predictors in this study. Regarding such intraoperative factors, clinicians can modify our intraoperative care and improve surgical procedures to reduce the risk of postoperative AKI.

The strongest risk factor in the nomogram model was anhepatic phase urine volume, which has not been reported before. The anhepatic phase is a unique and pivotal time during liver transplantation, and acid-base balance disorder, hemodynamic instability and renal congestion are much more pronounced (1, 14). Xu and colleagues (32) reported that low urine volume was significantly related to AKI, which reflects inadequate renal perfusion. A recent study demonstrated that an intraoperative urine volume < 0.5 ml kg^{-1} h^{-1} was associated with postoperative AKI in a major abdominal surgery population (33). Therefore, based on the results of our study, we can assume that a patient with more urine volume of the anhepatic phase during surgery may have better early renal function and a lower risk of postoperative AKI. Although there is debate about intraoperative urine volume reflecting renal function, we did provide a new idea for post-LT AKI. Further study is essential 1) to determine the potential association between anhepatic phase urine volume and AKI in LT patients; 2) to identify an optimal threshold of anhe-patic phase urine volume for predicting the postoperative AKI risk in patients receiving LT.

Another intraoperative factor we found in this study is the dosage of insulin, which is routinely used to maintain normoglycemia and correct acidosis. The intraoperative insulin requirement might reflect hyperglycemia and severe acid-base balance disorder, which can cause hypercoagulability, oxidative stress and endothelial dysfunction. As reported before,

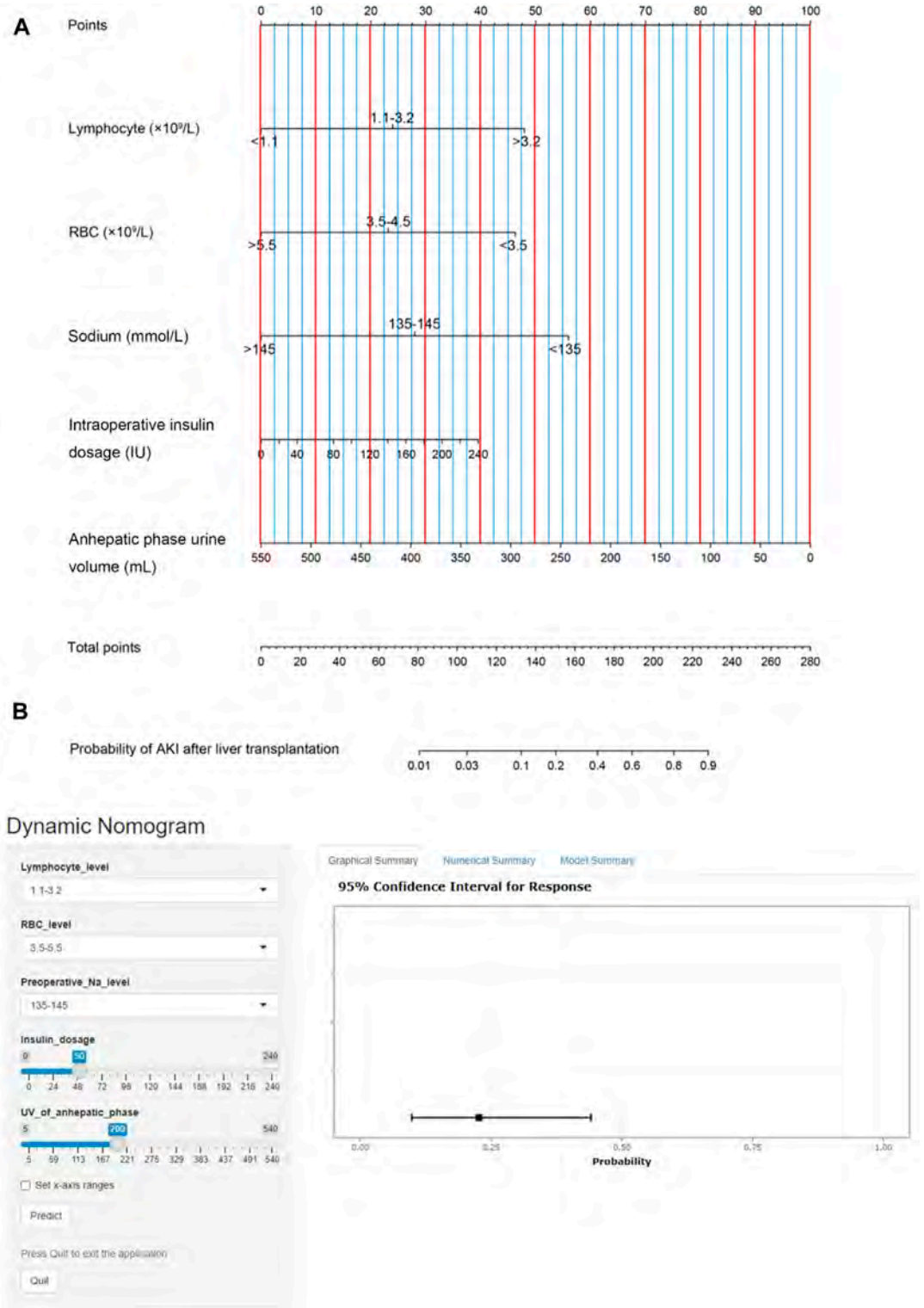


FIGURE 3 | (A) The post-LT AKI nomogram model was developed. This nomogram was constructed in the training set, with whole blood serum lymphocyte count, RBC count, sodium, intraoperative insulin dosage and anhepatic phase urine volume. **(B)** Online calculator predicting acute kidney injury after liver transplantation accessible at https://caobingbing.shinyapps.io/Nomogram_for_AKI_after_liver_transplantation/, depicting an example for predicting the probability of an LT patient with normal preoperative whole blood serum lymphocyte count, RBC count and serum sodium. The intraoperative insulin dosage was 50 IU, and anhepatic phase urine volume was 200 ml. Abbreviations: AKI, acute kidney injury; LT, liver transplantation; RBC, red blood cell.

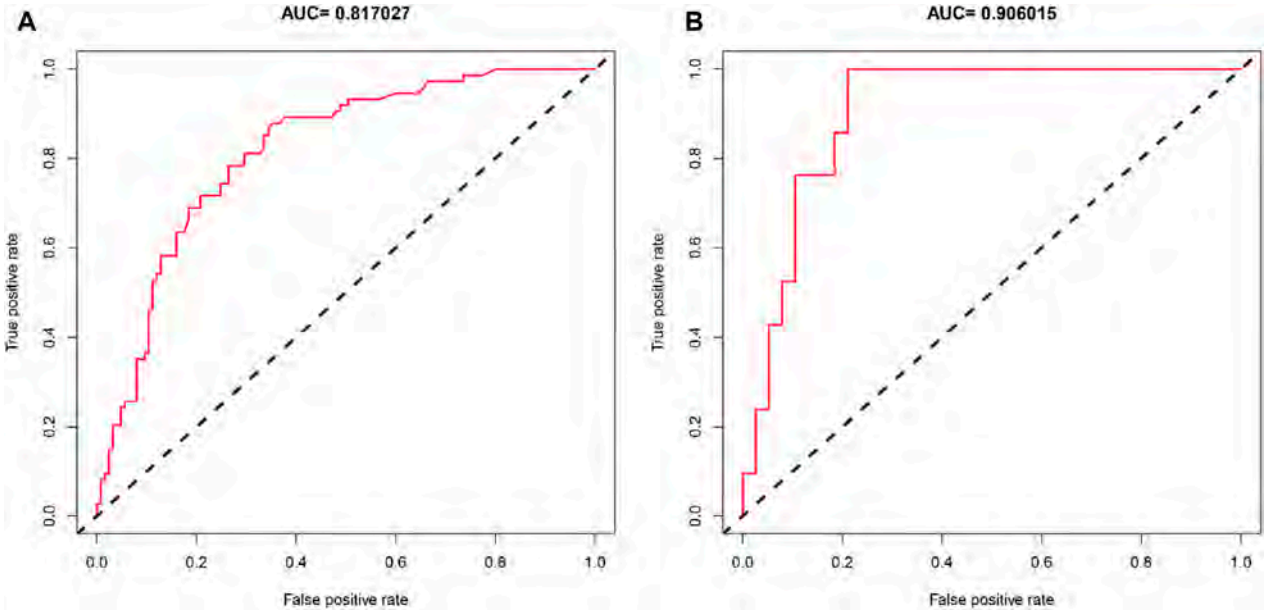


FIGURE 4 | Diagnostic accuracy of the post-LT AKI nomogram model in the training and validation sets. ROC curves showed AUCs of 0.817 and 0.906 in the training **(A)** and validation sets **(B)**.

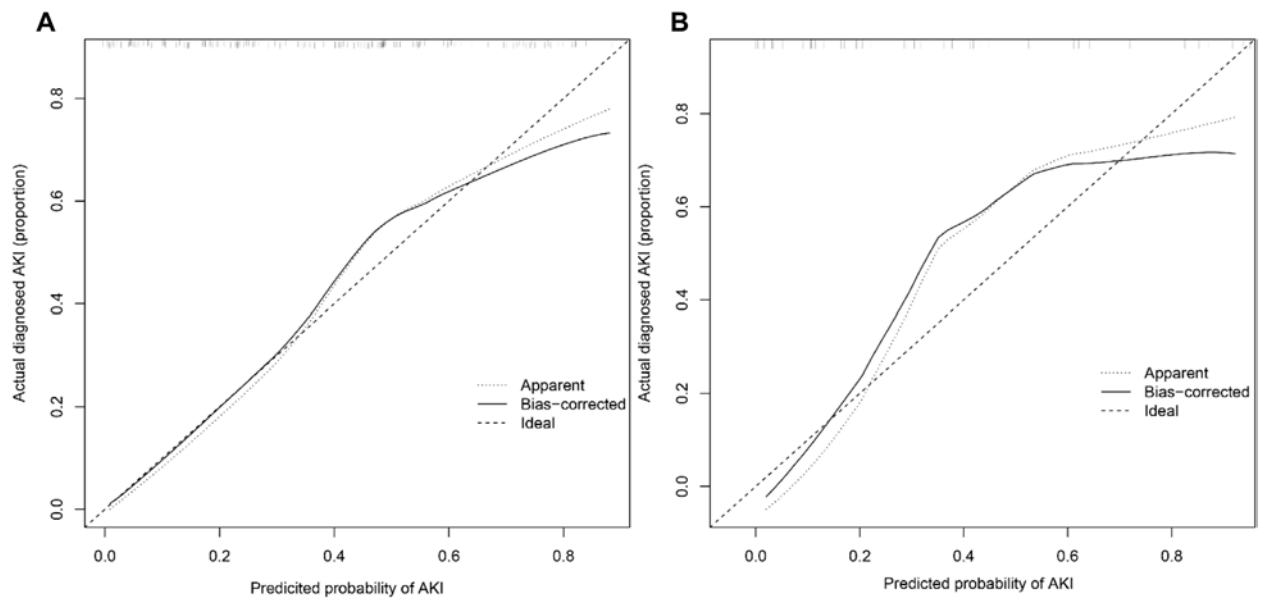


FIGURE 5 | Calibration curves of the post-LT AKI nomogram model in the training **(A)** and validation sets **(B)** (Bootstrap = 1,000 repetitions).

perioperative hyperglycemia has been suggested as a risk factor for post-LT AKI and is known to be associated with adverse outcomes in all inpatients, especially in sepsis (2). In this study, we found that insulin dosage, which has a positive correlation with AKI risk, is an important risk factor in LT patients and contributes to the development of post-LT AKI.

Three preoperative risk factors were identified as independent predictors, including whole blood lymphocyte count, RBC count and serum sodium. In general, the whole blood lymphocyte count is considered an indicator of the immune response in patients (34). In addition, lymphocytes, especially T cells, play a vital role in the whole evolution of kidney injury (35). In this study, we

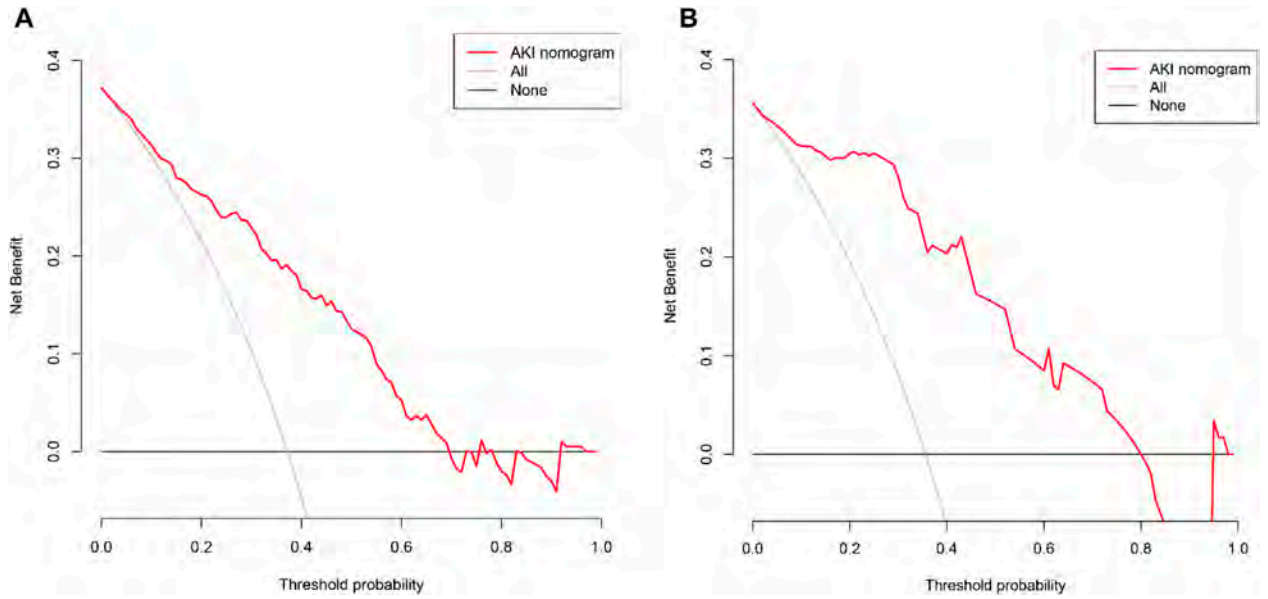


FIGURE 6 | Decision curve analysis of the nomogram for the estimation of post-LT AKI in the training set **(A)** and validation set **(B)**.

found that a higher whole blood lymphocyte count can increase the risk of post-LT AKI, rather than a low whole blood lymphocyte count. The possible explanation for this result may be that both dysregulation or overactivation of the immune response contribute to renal damage. Besides, the hepatitis infection preoperatively and the use of immunosuppressive medications during surgery have potential effect on the level of lymphocyte count. The preoperative whole blood RBC count was also significantly associated with post-LT AKI. The development of RBCs is regulated by erythropoietin, which is mainly produced by the kidneys, and the level of erythropoietin is de-creased in renal dysfunction patients. As preoperative renal dysfunction was previously reported as an independent predictor for post-LT AKI, we hypothesized that the patients in our study may have potential renal dysfunction (36). Meanwhile, a lower RBC count may be related to a higher risk of intraoperative hemorrhage and blood transfusion, which have been identified as risk factors for post-LT AKI. Overall, the fact that reduced RBC count is an interesting observation needing further studies to help us better understand the etio-pathogenesis of AKI. Additionally, we found that serum sodium was independently associated with post-LT AKI, which is consistent with a previous study (37). For patients awaiting liver transplantation, hyponatremia is associated with an increased frequency of complications (e.g., AKI) and reduced short-term survival (38). The occurrence of preoperative hyponatremia is often related to renal impairment, which is characterized by a decreased capacity for solute-free water excretion. Although the MELD score and recipient BMI have been previously reported to predict post-LT AKI, our study failed to show any association with postoperative AKI. This may be the result of our population having relatively low MELD scores, and the majority of recipients were not obese.

Measures should be taken to prevent AKI after identifying high-risk patients. One proposal to decrease the incidence of post-LT AKI is perioperative renal protection, such as promoting hemodynamic stability during the anhepatic phase (2). Another important measure is to reduce hepatic IRI, although this can be a difficult task. Extracorporeal donor liver perfusion is a new approach to reduce hepatic IRI and has been shown to be feasible (39). Taken together, this is not the first prediction nomogram model for post-LT AKI; however, it is the first to develop an online calculator for clinical use, and the discrimination and calibration of the model are excellent. Importantly, we applied the 2012 KDIGO criteria to define AKI instead of the old Risk, Injury, Failure, Loss, and End-stage criteria.

Our current study had several limitations. First, information on long-term survival was not available in this study, and this part of the work is now in progress. Additionally, due to the privacy protection policy, we failed to include donor data from electronic medical records. Next, selection bias may exist, as our study had a retrospective design. Finally, external validation in other populations or countries is needed to demonstrate its applicability. Indeed, a larger, more heterogeneous cohort at our institution is planned in the future to identify more predictors.

In conclusion, we present and validate an easy-to-use nomogram model to predict the probability of AKI in LT patients and then describe an online calculator to predict the AKI risk of each individual patient. This predictive model, including preoperative and intraoperative predictors, is a useful tool with good discrimination and calibration. The great advantage of our model is that it is available for clinicians to identify high-risk AKI patients at the end of surgery and avoid unnecessary postoperative renal injury.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

This work was approved by Sun Yat-sen Memorial Hospital of Sun Yat-sen University Ethics Committee (approval number: SYSKY-2022-041-01) and was registered in the China Clinical Trial Registration Center (trial registration number: ChiCTR2200059927). Given its retrospective design, the requirement for informed consent was waived by the institutional review board.

AUTHOR CONTRIBUTIONS

JZ participated in research design, review of patient charts and data interpretation; QL participated in research design and data collection; QW participated in data collection; LL participated in review of patient charts and data interpretation; XY participated in research design and statistical analysis; JL participated in research design and data analysis; BC participated in review of patient charts, data interpretation and analysis; all authors participated in the writing of the paper.

CONFLICT OF INTEREST

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

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REFERENCES

1. Adam R, Hoti E. Liver Transplantation: the Current Situation. *Semin Liver Dis* (2009) 29(1):3–18. doi:10.1055/s-0029-1192052
2. de Haan JE, Hoorn EJ, de Geus HRH. Acute Kidney Injury after Liver Transplantation: Recent Insights and Future Perspectives. *Best Pract Res Clin Gastroenterol* (2017) 31(2):161–9. doi:10.1016/j.bpg.2017.03.004
3. DellaVolpe J, Al-Khafaji A. Acute Kidney Injury before and after Liver Transplant. *J Intensive Care Med* (2019) 34(9):687–95. doi:10.1177/0885066618790558
4. Hilmi IA, Damian D, Al-Khafaji A, Planinsic R, Boucek C, Sakai T, et al. Acute Kidney Injury Following Orthotopic Liver Transplantation: Incidence, Risk Factors, and Effects on Patient and Graft Outcomes. *Br J Anaesth* (2015) 114(6):919–26. doi:10.1093/bja/aeu556
5. Trinh E, Alam A, Tchervenkov J, Cantarovich M. Impact of Acute Kidney Injury Following Liver Transplantation on Long-Term Outcomes. *Clin Transplant* (2017) 31(1):e12863. doi:10.1111/ctr.12863
6. Zhang Y, Yang D, Liu Z, Chen C, Ge M, Li X, et al. An Explainable Supervised Machine Learning Predictor of Acute Kidney Injury after Adult Deceased Donor Liver Transplantation. *J translational Med* (2021) 19(1):321. doi:10.1186/s12967-021-02990-4
7. Kalisvaart M, Schlegel A, Umbro I, de Haan JE, Polak WG, Ijzermans JN, et al. The AKI Prediction Score: a New Prediction Model for Acute Kidney Injury after Liver Transplantation. *HPB (Oxford)* (2019) 21(12):1707–17. doi:10.1016/j.hpb.2019.04.008
8. Guo D, Wang H, Lai X, Li J, Xie D, Zhen L, et al. Development and Validation of a Nomogram for Predicting Acute Kidney Injury after Orthotopic Liver Transplantation. *Ren Fail* (2021) 43(1):1588–600. doi:10.1080/0886022X.2021.2009863
9. Lee HC, Yoon SB, Yang SM, Kim WH, Ryu HG, Jung CW, et al. Prediction of Acute Kidney Injury after Liver Transplantation: Machine Learning Approaches vs. Logistic Regression Model. *J Clin Med* (2018) 7(11):428. doi:10.3390/jcm7110428
10. Tan L, Yang Y, Ma G, Zhu T, Yang J, Liu H, et al. Early Acute Kidney Injury after Liver Transplantation in Patients with normal Preoperative Renal Function. *Clin Res Hepatol Gastroenterol* (2019) 43(4):475–82. doi:10.1016/j.clinre.2018.07.009
11. He ZL, Zhou JB, Liu ZK, Dong SY, Zhang YT, Shen T, et al. Application of Machine Learning Models for Predicting Acute Kidney Injury Following Donation after Cardiac Death Liver Transplantation. *Hepatobiliary Pancreat Dis Int* (2021) 20(3):222–31. doi:10.1016/j.hbpd.2021.02.001
12. Pulitano C, Ho P, Verran D, Sandroussi C, Joseph D, Bowen DG, et al. Molecular Profiling of Postreperfusion Milieu Determines Acute Kidney Injury after Liver Transplantation: A Prospective Study. *Liver Transpl* (2018) 24(7):922–31. doi:10.1002/lt.25178
13. Berkowitz RJ, Engoren MC, Mentz G, Sharma P, Kumar SS, Davis R, et al. Intraoperative Risk Factors of Acute Kidney Injury after Liver Transplantation. *Liver Transplant* (2022) 28(7):1207–23. doi:10.1002/lt.26417
14. Durand F, Francoz C, Asrani SK, Khemichian S, Pham TA, Sung RS, et al. Acute Kidney Injury after Liver Transplantation. *Transplantation* (2018) 102(10):1636–49. doi:10.1097/TP.0000000000002305
15. Peduzzi P, Concato J, Kemper E, Holford TR, Feinstein AR. A Simulation Study of the Number of Events Per Variable in Logistic Regression Analysis. *J Clin Epidemiol* (1996) 49(12):1373–9. doi:10.1016/s0895-4356(96)00236-3
16. Vittinghoff E, McCulloch CE. Relaxing the Rule of Ten Events Per Variable in Logistic and Cox Regression. *Am J Epidemiol* (2007) 165(6):710–8. doi:10.1093/aje/kwk052
17. Kellum JA, Lameire N, KDIGO AKI Guideline Work Group. Diagnosis, Evaluation, and Management of Acute Kidney Injury: a KDIGO Summary (Part 1). *Crit Care* (2013) 17(1):204. doi:10.1186/cc11454
18. Joannidis M, Metnitz B, Bauer P, Schusterschitz N, Moreno R, Druml W, et al. Acute Kidney Injury in Critically Ill Patients Classified by AKIN versus RIFLE Using the SAPS 3 Database. *Intensive Care Med* (2009) 35(10):1692–702. doi:10.1007/s00134-009-1530-4
19. Barri YM, Sanchez EQ, Jennings LW, Melton LB, Hays S, Levy MF, et al. Acute Kidney Injury Following Liver Transplantation: Definition and Outcome. *Liver Transplant* (2009) 15(5):475–83. doi:10.1002/lt.21682
20. Erdine S, Ari O, Zanchetti A, Cifkova R, Fagard R, Kjeldsen S, et al. ESH-ESC Guidelines for the Management of Hypertension. *Herz* (2006) 31(4):331–8. doi:10.1007/s00059-006-2829-3
21. Alberti KG, Zimmet PZ. Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications. Part 1: Diagnosis and Classification of Diabetes Mellitus Provisional Report of a WHO Consultation. *Diabet Med* (1998) 15(7):539–53. doi:10.1002/(SICI)1096-9136(199807)15:7<539:AID-DIA668>3.0.CO;2-S
22. Kamath PS, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Kosberg CL, et al. A Model to Predict Survival in Patients with End-Stage Liver Disease. *Hepatology* (2001) 33(2):464–70. doi:10.1053/jhep.2001.22172
23. Guo F, Zhu G, Shen J, Ma Y. Health Risk Stratification Based on Computed Tomography Pulmonary Artery Obstruction index for Acute Pulmonary Embolism. *Sci Rep* (2018) 8(1):17897. doi:10.1038/s41598-018-36115-7
24. Sauerbrei W, Royston P, Binder H. Selection of Important Variables and Determination of Functional Form for Continuous Predictors in Multivariable Model Building. *Stat Med* (2007) 26(30):5512–28. doi:10.1002/sim.3148

25. Balachandran VP, Gonen M, Smith JJ, DeMatteo RP. Nomograms in Oncology: More Than Meets the Eye. *Lancet Oncol* (2015) 16(4):e173–80. doi:10.1016/S1470-2045(14)71116-7
26. Kramer AA, Zimmerman JE. Assessing the Calibration of Mortality Benchmarks in Critical Care: The Hosmer-Lemeshow Test Revisited. *Crit Care Med* (2007) 35(9):2052–6. doi:10.1097/01.CCM.0000275267.64078.B0
27. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the Areas under Two or More Correlated Receiver Operating Characteristic Curves: a Nonparametric Approach. *Biometrics* (1988) 44(3):837–45. doi:10.2307/2531595
28. Pencina MJ, D'Agostino RB. Overall C as a Measure of Discrimination in Survival Analysis: Model Specific Population Value and Confidence Interval Estimation. *Stat Med* (2004) 23(13):2109–23. doi:10.1002/sim.1802
29. Zhang Z, Rousson V, Lee WC, Ferdynus C, Chen M, Qian X, et al. Decision Curve Analysis: a Technical Note. *Ann translational Med* (2018) 6(15):308. doi:10.21037/atm.2018.07.02
30. Prowle JR, Forni LG, Bell M, Chew MS, Edwards M, Grams ME, et al. Postoperative Acute Kidney Injury in Adult Non-cardiac Surgery: Joint Consensus Report of the Acute Disease Quality Initiative and PeriOperative Quality Initiative. *Nat Rev Nephrol* (2021) 17(9):605–18. doi:10.1038/s41581-021-00418-2
31. Lankadeva YR, May CN, Bellomo R, Evans RG. Role of Perioperative Hypotension in Postoperative Acute Kidney Injury: a Narrative Review. *Br J Anaesth* (2022) 128(6):931–48. doi:10.1016/j.bja.2022.03.002
32. Xu X, Ling Q, Wei Q, Wu J, Gao F, He ZL, et al. An Effective Model for Predicting Acute Kidney Injury after Liver Transplantation. *Hepatobiliary Pancreat Dis Int* (2010) 9(3):259–63.
33. Myles PS, McIlroy DR, Bellomo R, Wallace S. Importance of Intraoperative Oliguria during Major Abdominal Surgery: Findings of the Restrictive versus Liberal Fluid Therapy in Major Abdominal Surgery Trial. *Br J Anaesth* (2019) 122(6):726–33. doi:10.1016/j.bja.2019.01.010
34. Zheng Z, Lin B, Zhang J, Yang Z, Xie H, Zhou L, et al. Absolute Lymphocyte Count Recovery at 1 Month after Transplantation Predicts Favorable Outcomes of Patients with Hepatocellular Carcinoma. *J Gastroenterol Hepatol* (2015) 30(4):706–11. doi:10.1111/jgh.12782
35. Cao C, Yao Y, Zeng R. Lymphocytes: Versatile Participants in Acute Kidney Injury and Progression to Chronic Kidney Disease. *Front Physiol* (2021) 12: 729084. doi:10.3389/fphys.2021.729084
36. Karapanagiotou A, Kydona C, Dimitriadis C, Sgourou K, Giasnetsova T, Fouzas I, et al. Acute Kidney Injury after Orthotopic Liver Transplantation. *Transplant Proc* (2012) 44(9):2727–9. doi:10.1016/j.transproceed.2012.09.096
37. Leithead JA, Rajoriya N, Gunson BK, Muiesan P, Ferguson JW. The Evolving Use of Higher Risk Grafts Is Associated with an Increased Incidence of Acute Kidney Injury after Liver Transplantation. *J Hepatol* (2014) 60(6):1180–6. doi:10.1016/j.jhep.2014.02.019
38. Ginès P, Guevara M. Hyponatremia in Cirrhosis: Pathogenesis, Clinical Significance, and Management. *Hepatology* (2008) 48(3):1002–10. doi:10.1002/hep.22418
39. Vogel T, Brockmann JG, Coussios C, Friend PJ. The Role of Normothermic Extracorporeal Perfusion in Minimizing Ischemia Reperfusion Injury. *Transpl Rev (Orlando)* (2012) 26(2):156–62. doi:10.1016/j.trre.2011.02.004

GLOSSARY

AKI acute kidney injury
ALT alanine aminotransferase
ALB albumin
aPTT activated partial thromboplastin time
ASA American Society of Anesthesiologists
AST aspartate aminotransferase
AUC area under the receiver operator characteristic curve
BMI body mass index
BUN blood urea nitrogen
CO cardiac output
CRRT continuous renal replacement therapy
CVP central venous pressure
DBD deceased after brain death
DCA decision curve analysis
eGFR estimated glomerular fil-tration rate
FFP fresh frozen plasma
GB globulin
Hb hemoglobin
HBV hepatitis B virus
Hct hematocrit
ICU intensive care unit
INR international normalized ratio
IQR interquartile range
IRI ischemia reperfusion injury
IVC inferior vena cava
KDIGO Kidney Disease Improving Global Outcomes
LASSO least absolute shrinkage and selection operator
LT liver transplantation
MELD model for End-Stage Liver Disease
PLT platelet
PT prothrombin time
RBC red blood cell
ROC receiver operator characteristic
sCr creatinine
TBil total bilirubin
TP total protein
TT thrombin time
WBC white blood cell

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Ipsilateral Aorto-Iliac Calcification is Not Directly Associated With eGFR After Kidney Transplantation: A Prospective Cohort Study Analyzed Using a Linear Mixed Model

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Aorto-iliac calcification (AIC) is a well-studied risk factor for post-transplant cardiovascular events and mortality. Its effect on graft function remains unknown. The primary aim of this prospective cohort study was to assess the association between AIC and estimated glomerular filtration rate (eGFR) in the first year post-transplant. Eligibility criteria were: ≥50 years of age or ≥30 years with at least one risk factor for vascular disease. A non-contrast-enhanced CT-scan was performed with quantification of AIC using the modified Agatston score. The association between AIC and eGFR was investigated with a linear mixed model adjusted for predefined variables. One-hundred-and-forty patients were included with a median of 31 (interquartile range 26–39) eGFR measurements per patient. No direct association between AIC and eGFR was found. We observed a significant interaction between follow-up time and ipsilateral AIC, indicating that patients with higher AIC scores had lower eGFR trajectory over time starting 100 days after transplant (p = 0.014). To conclude, severe AIC is not directly associated with lower post-transplant eGFR. The significant interaction indicates that patients with more severe AIC have a lower eGFR trajectory after 100 days in the first year post-transplant.

Keywords: kidney transplantation, kidney transplant recipients, peripheral artery disease, kidney transplant outcomes, chronic kidney disease

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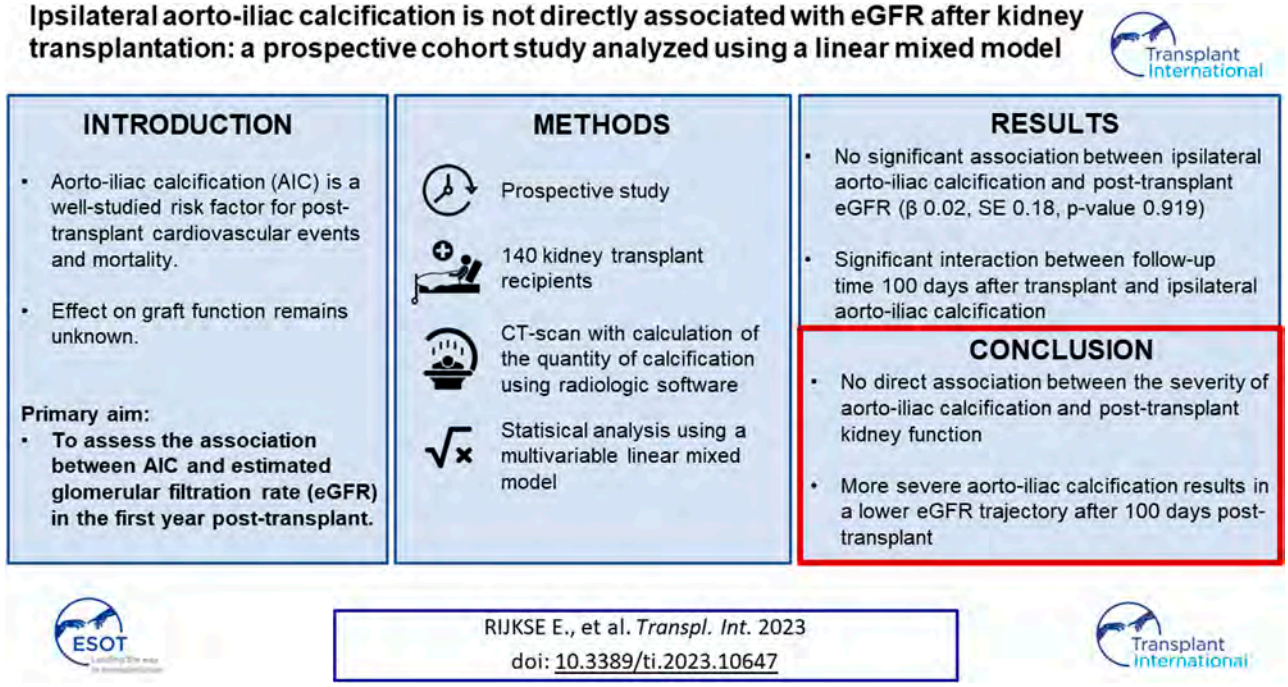
Abbreviations: AIC, aorto-iliac calcification; CKD, chronic kidney disease; DBD, donation after brain death; DCD, donation after circulatory death; DGF, delayed graft function; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; HLA, human leukocyte antigen; IQR, interquartile range; KDIGO, kidney disease improving global outcomes; PNF, primary non-function; PTA, percutaneous transluminal angioplasty; PTH, parathyroid hormone; vPRA, virtual panel reactive antibodies.



GRAPHICAL ABSTRACT |

INTRODUCTION

Vascular disease is prevalent in patients with end-stage renal disease (ESRD) due to the high incidence of traditional risk factors and chronic kidney disease (CKD) related factors, such as CKD-related mineral and bone disorder (1, 2). Vascular calcification was thought to occur primarily during ESRD, but recently it has been found that its development begins in earlier stages of CKD (1). The pathophysiological mechanism consists of both arteriosclerosis and atherosclerosis, of which arteriosclerosis is characterized by vascular stiffening and atherosclerosis by intimal wall thickening (3). Even though both subtypes of vascular disease are highly prevalent in CKD patients, arteriosclerosis is the most strongly linked to CKD (4). Vascular disease manifests clinically as coronary artery disease, cerebrovascular disease and peripheral arterial disease. As a result of this increased prevalence, mortality from cardiovascular disease is 10–20 times higher in patients with ESRD compared to the general population (5).

Vascular disease can also occur in the aorto-iliac arteries, resulting in an increased need for intra-operative vascular reconstructions (6). As a consequence, aorto-iliac vascular disease is the main reason for decline for kidney transplantation (7). Given that AIC is a manifestation of generalized vascular disease, it is not surprising that several studies have shown that AIC is associated with inferior survival and an increased risk of post-transplant cardiovascular events (8, 9). However, little is known about the association between ipsilateral AIC and graft function. This information is important, as a large retrospective cohort study found that 25% of all kidney transplant candidates presented with any degree of AIC (10).

Current studies that investigated the relationship between AIC and graft function have several limitations, from which the most important ones are a retrospective design, a subjective quantification method of aorto-iliac vascular disease which limits generalizability and the use of statistical methods that do not account for drop-out (10-16). To address these issues, we performed a prospective cohort study in which all patients underwent non-contrast-enhanced CT-scan for objective, quantitative assessment of AIC using an adaptation of the Agatston score. This score is widely used to quantify coronary artery calcification and has excellent inter-observer and inter-scanner agreement (17, 18). The primary aim of our study was to investigate the association of ipsilateral AIC with post-transplant estimated glomerular filtration rate (eGFR) trajectory in the first year post-transplant using a linear mixed model.

MATERIALS AND METHODS

Study Design and Eligibility Criteria

This prospective, single-center study was carried out in Erasmus Medical Center, the Netherlands, between 10 January 2019 and 13 August 2020. Power calculation for the study sample size can be found in the **Supplementary Material**. Patients who met the eligibility criteria were asked to participate upon admission for transplant. All patients 50 years or older were eligible for

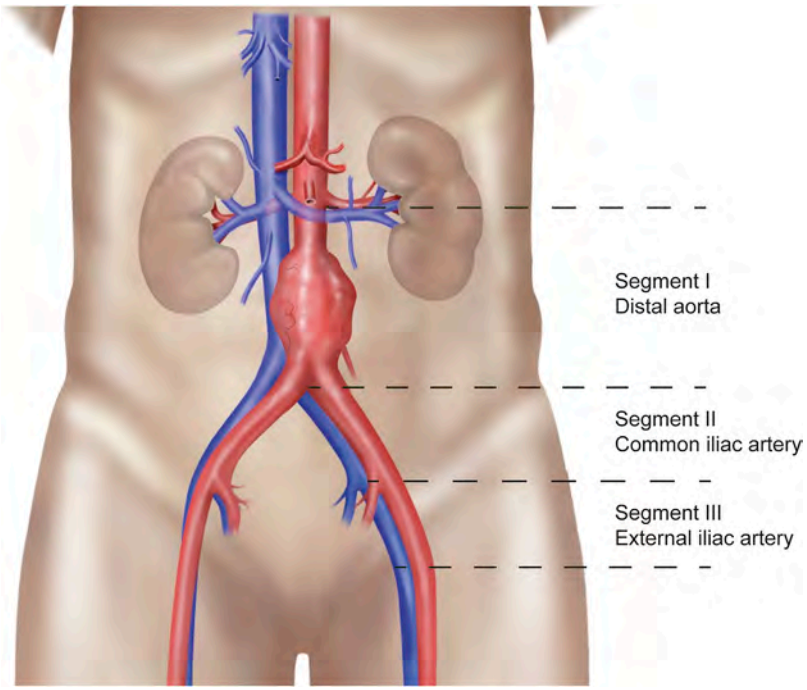


FIGURE 1 | Explanation of the anatomical segments.

inclusion. Patients from 30 years of age or older were eligible if they had at least one of the following risk factors for vascular disease: diabetes mellitus, 1 year or longer dialysis duration, smoking history of at least 10 pack years or a history with peripheral arterial disease, ischemic heart disease or a cerebrovascular accident (19). In addition, South-East Asian ethnicity was considered a risk factor as previous studies found that these patients were at increased risk of cardiovascular disease after adjustment for confounders (20). Combined liver-kidney transplant recipients, HLA incompatible recipients and dual transplant recipients were excluded from the study. The study was performed according to the Declaration of Helsinki and received approval from the local Medical Ethical Committee (MEC 2018-1401). The study was prospectively registered in the Netherlands Trial Register (NTR7641).

Study Procedure

Patients who gave written informed consent underwent a non-contrast-enhanced abdominal CT-scan upon admission for transplant. All scanners used were modern ≥128-multislice CT systems (Siemens Healthineers). A scanning protocol was developed specifically for the study to ensure that there would be no differences in scan parameters that could affect measurement of the calcification score. A low-dose CT-scan (at fixed 120 kVp) was performed and reconstructed to 3.0 mm slice thickness and 1.5 mm increment using a dedicated quantitative calcium scoring kernel (B35f or Qr36) without iterative reconstructions. Consequently, the scan was analyzed in Intellispace Portal (Philips) with the HeartBeat-CS application. This application is designed to calculate the Agatston score, which is a continuous quantification score for coronary calcification with a high specificity for the absence of coronary artery disease (21, 22). The calculation is based on the weighted density score given to the highest attenuation value multiplied by the area of the calcification speck. A CT attenuation threshold of 130 Hounsfield units (HU) is used for the detection of calcification, with only contiguous voxels totaling ≥1 mm^2 in area counted as lesions to reduce the influence of image noise(21). For calculation of the AIC score as an adapted version of the Agatston score, the aorto-iliac trajectory was divided into anatomical segments, as explained in **Figure 1**. These anatomical segments included the infrarenal aorta until the iliac bifurcation (segment I), the right and left common iliac artery until the internal iliac artery branch (segment II), and the right and left external iliac artery (starting from the internal iliac artery branch until Poupart's ligament) (segment III). The total AIC score was calculated as the sum of these separate calcification scores. The ipsilateral AIC score consisted of the sum of the aorta and ipsilateral common iliac artery, depending on the implantation side. The external iliac artery calcification score was not included in this score for 2 reasons. Firstly, the external iliac artery is not entirely in the inflow trajectory of the donor kidney, depending on whether the anastomosis is made with the proximal or distal part. Secondly, the transition from external iliac artery to common femoral artery was often unclear because of the use of a low-dose CT-scan which could result in misclassification.

Primary and Secondary Outcomes

The primary outcome of the study was eGFR in the first year post-transplant. All measurements of eGFR from day 1 until 400 days were used, as not all patients had their 1-year eGFR measurement exactly at 365 days post-transplant. eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula, which is standard in our hospital as recommended by the kidney disease improving global outcomes (KDIGO) guidelines (23, 24). Secondary outcomes were the incidence of delayed graft function (DGF), primary non-function (PNF), the presence and number of biopsy-proven acute rejection episodes in the first year post-transplant, uncensored and death-censored graft survival, and the need for a peri-operative or pre-operative vascular intervention.

Standard Transplantation Procedure

All donor kidneys were transplanted into the right or left iliac fossa by using the standard Gibson incision, depending on surgeon preference. Firstly, the anastomosis of the renal vein was performed end-to-side with the external iliac vein. Consequently, the arterial anastomosis was performed end-to-side with the external iliac artery using prolene 5.0 or 6.0. In the case of severe aorto-iliac calcification without a soft spot to implant the kidney, an endarterectomy could be performed with patch angioplasty prior to the arterial anastomosis. For the ureter, an extra-vesical anastomosis was performed as described by Lich-Gregoir. The ureter anastomosis was protected with an external splint or double J stent, depending on randomization arm of an ongoing randomized controlled trial investigating urologic complications. Standard immunosuppression regime of transplant recipients consisted of induction with basiliximab followed by triple-therapy with tacrolimus, mycofenolate mofetil and prednisone. Prednisone was gradually tapered and stopped after 4 months.

Statistical Analysis

Continuous variables were presented as median and interquartile range (IQR) for non-normally distributed variables and mean with standard deviation for normally distributed variables. Categorical variables were summarized as number and percentage. Baseline characteristics were compared between patients with a median or lower ipsilateral AIC score and patients with an above median AIC score. Categorical baseline characteristics were compared with chi-square tests or Fisher's exact test. Continuous baseline characteristics were compared using Mann-Whitney U test. Correlations between arterial segments were calculated using Spearman's correlation coefficient. The association between the ipsilateral AIC score as a continuous variable and eGFR trajectory was analyzed using a linear mixed model with random intercepts and random slopes. An unstructured covariance matrix was used because of unbalanced outcome data. We used a predefined model to correct for donor, recipient and transplant-related confounders. We included the following variables in our fixed effects: follow-up time, ipsilateral AIC score, recipient age, recipient sex, recipient diabetes, recipient smoking, a previous kidney transplant, total dialysis duration (including hemodialysis and peritoneal dialysis), coronary artery disease, peripheral arterial disease, donor type, donor age, donor diabetes, donor last creatinine, pre-emptive transplantation, cold

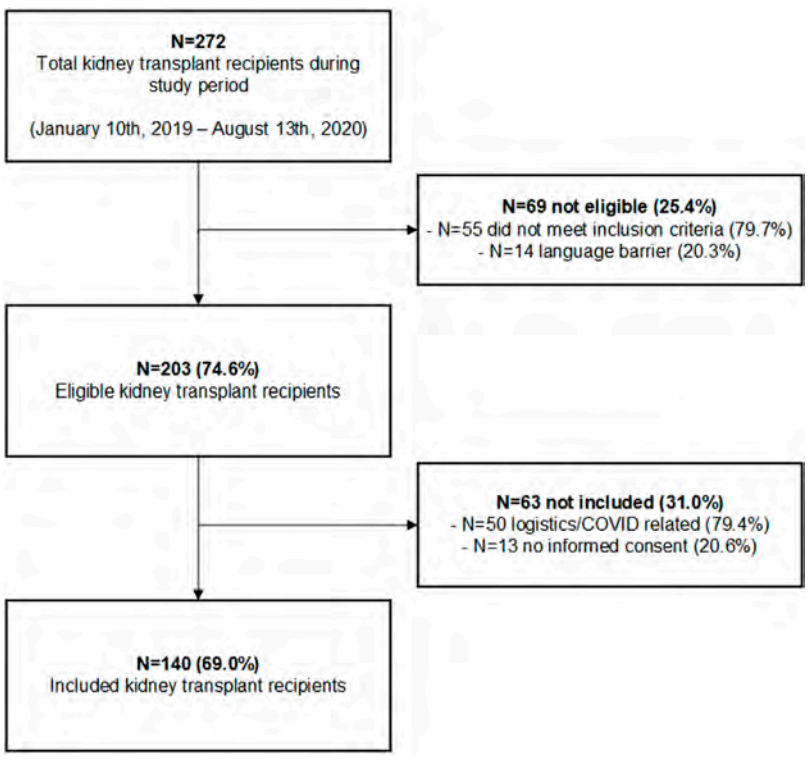


FIGURE 2 | Study flowchart (n = 140).

preservation technique (static cold storage or hypothermic machine perfusion), total human leukocyte antigen (HLA) mismatch, virtual panel reactive antibodies (vPRA), cold ischemic time, postoperative dialysis and 1 or more rejection episodes in the first year after transplant. The univariable analysis can be found in the **Supplementary Table S1**. In case of non-linearity for continuous variables, a natural cubic spline with 3 degrees of freedom was used (25). Knots for splines were selected based on the observed non-linear trajectories. Multicollinearity was investigated by calculating the generalized variance inflation factor (GVIF), where a value below 5 indicates no multicollinearity. The following clinically plausible interactions were tested with likelihood ratio tests: time and ipsilateral calcification score, time and donor type and time and delayed graft function. The main analysis included all eGFR measurements of the recipients, independent of graft failure. We performed one sensitivity analysis where we imputed an eGFR of 10 mL/min/1.73 m^2 after graft failure occurred if the measured eGFR was above 10 mL/min/1.73 m^2. Secondary outcomes were analyzed using unadjusted analyses due to a lack of statistical power for these outcomes. Differences in survival were calculated with the log-rank test. Median follow-up time was calculated with the reversed Kaplan-Meier method. R statistical software version 4.0.4. was used for data analysis (packages "nlme," "lme4," "splines2," "survival").

RESULTS

Selection of the Cohort

A total of 140 kidney transplant recipients were included in the study and received a non-contrast-enhanced CT-scan. The inclusion flowchart is shown in **Figure 2**. 272 patients received a kidney transplant during the inclusion period of which 69 did not meet the inclusion criteria. Two-hundred-and-three patients were eligible for inclusion with a consent rate of 69.0%. The consent rate was lower than expected due to logistical reasons and a mandatory study stop due to the COVID-19 pandemic. Median follow-up time in the cohort was 622 days (IQR 416-757).

Overall Calcification Scores

Median AIC scores per segment are illustrated in **Table 1**. The right and left external iliac artery had the lowest calcification scores with a median of 48 (IQR 0–412) and 52 (0–466), respectively. Scatter plots to identify correlations between calcification scores of separate arterial segments are presented in **Figure 3**. All AIC scores of arterial segments were highly correlated, but the highest correlation was found between the right and left common iliac artery (Spearman's $\rho = 0.90$, $p < 0.001$). The lowest correlation coefficients were found between the right external and common iliac artery (Spearman's $\rho = 0.70$, $p < 0.001$) and left external and common iliac artery (Spearman's $\rho = 0.65$, $p < 0.001$).

Baseline Characteristics

Baseline characteristics of the cohort are displayed in **Table 2**. To demonstrate differences between patients with a low or high ipsilateral AIC score, baseline characteristics were compared according to the median AIC score. Recipient age upon transplantation was significantly higher in recipients with a high ipsilateral calcification score (median age 66.5 (IQR 61.8–72.5) compared to 63.2 (IQR 54.7–68.7), $p = 0.005$). Patients with a high ipsilateral AIC score were more often current or former smokers (overall $p = 0.007$). Furthermore, patients with a high AIC score were more often presenting with a history of coronary artery disease ($p < 0.001$) or cerebrovascular disease ($p = 0.039$). Other baseline characteristics were not statistically different.

Longitudinal Trajectory of eGFR

Patients had a median of 31 (IQR 26–39) eGFR measurements available in the first year after transplant. **Figure 4** shows the spaghetti plot for eGFR trajectory, stratified according to donor type (**Figure 4B**) or AIC score quartile (**Figure 4C**). All AIC quartiles showed a similar pattern with a steep increase in the first period followed by a small decrease and stabilization phase. eGFR trajectories during the stabilization phase were visually different with a lower eGFR trajectory in the highest calcification quartile.

Linear Mixed Model for eGFR Trajectory After Transplant

To assess whether this visual difference in eGFR trajectory during the first year post-transplant could also be demonstrated when corrected for potential confounders, a linear mixed model was performed. The results of the linear mixed model are presented in **Table 3**. No direct association was observed between AIC score and post-transplant eGFR (β 0.02 (standard deviation (SD) 0.18), $p = 0.919$). Between day 50 till day 100 after transplant, eGFR increased significantly with 30.90 mL/min/1.73 m^2 (SD 3.59, $p < 0.001$). After 100 days follow-up time, eGFR increased significantly with 11.75 mL/min/1.73 m^2 (SD 1.80, $p < 0.001$). Patients who received a DCD graft had a lower eGFR (14.41 mL/min/1.73 m^2 lower (SD 4.918, $p = 0.003$). An increase in donor age led to a decrease in eGFR with 0.29 mL/min/1.73 m^2 per year of age (SD 0.08, $p < 0.001$). Furthermore, the need for postoperative dialysis and acute rejection in the first year were both associated with a lower eGFR with (9.11 mL/min/1.73 m^2 and 6.69 mL/min/1.73 m^2, respectively (SD 3.21, $p = 0.005$ and SD 2.82, $p = 0.019$, respectively)). The interactions between follow-up time and donor type and follow-up time and delayed graft function showed no significant addition to the model (likelihood ratio test: $p = 0.097$ and 0.134, respectively). However, a significant interaction was observed between follow-up time and ipsilateral AIC score (overall likelihood ratio test $p < 0.001$). The predicted values for eGFR during the follow-up time based on the mixed model are plotted in **Figure 5**, stratified according to ipsilateral AIC score quartile. This shows a sharp increase in eGFR measurements in the first period (day 0–50) for all values of ipsilateral AIC score. Further in the follow-up, it is shown that there is a relation between follow-up time and ipsilaterial AIC score; higher AIC scores show lower values of eGFR over time. The calculated GVIF values showed no important multicollinearity. Our sensitivity analysis, included in the **Supplementary Table S2**, showed similar results as our main analysis.

TABLE 1 | Calcification scores per segment in the whole cohort (n = 140).

Arterial segment		Left	Right
Aorta, median (IQR)	2,730 (754–7,135)		
Common iliac artery, median (IQR)		930 (154–2,288)	1,065 (152–2,211)
External iliac artery, median (IQR)		52 (0–466)	48 (0–412)
Total ipsilateral, median (IQR)	4,241 (1,144–10,221)		
Total, median (IQR)	5,451 (1,755–13,252)		

IQR, interquartile range.

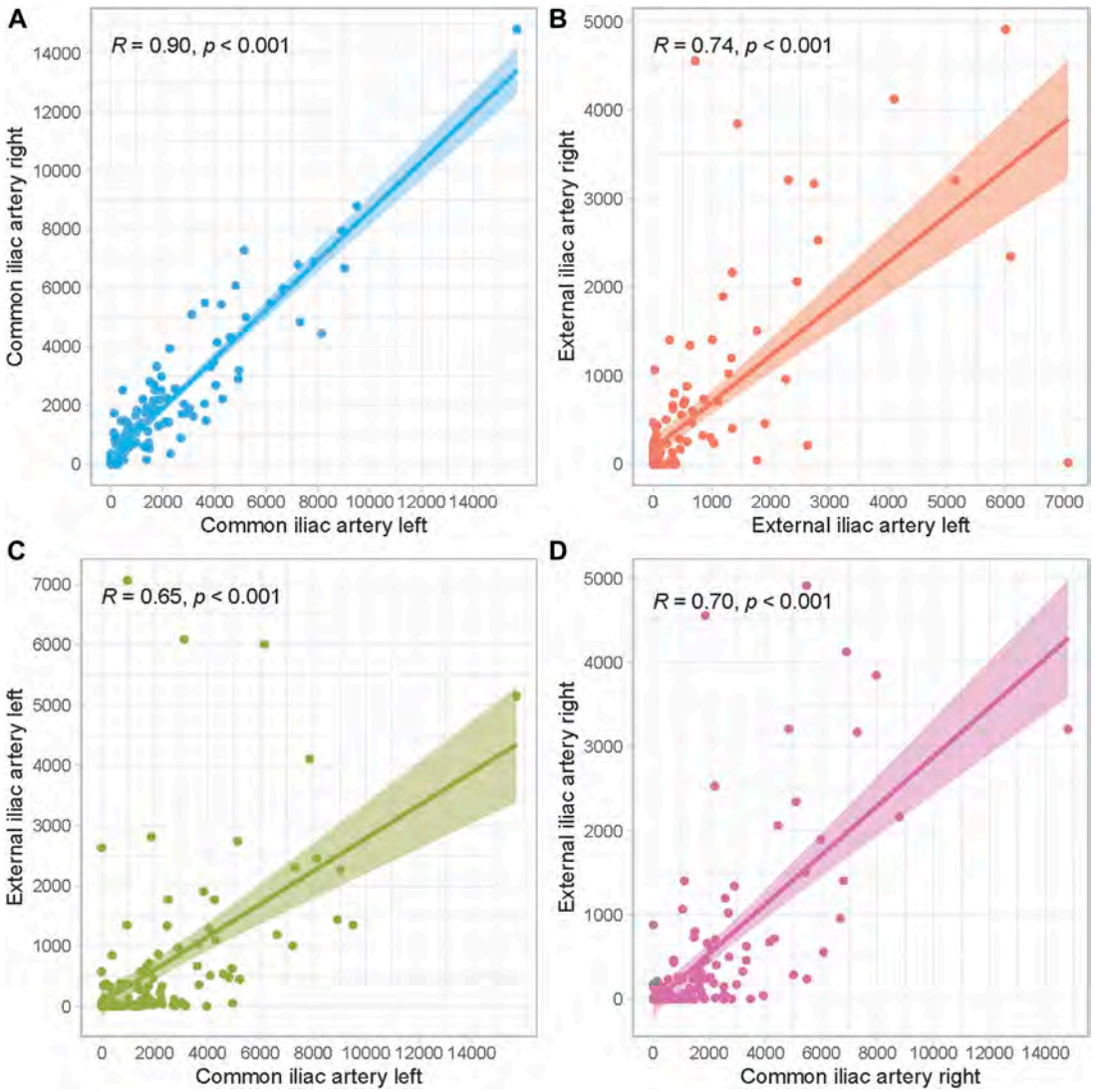


FIGURE 3 | Scatter plots to assess correlations between arterial segments. **(A)** Left and right common iliac artery, **(B)** Left and right external iliac artery, **(C)** Left common and external iliac artery, **(D)** Right common and external iliac artery.

Secondary Outcomes

The secondary outcomes are noted in **Table 4** and compared between patients with a high AIC score and patients with a low AIC score. No significant differences were observed for the incidence of DGF, PNF and acute rejection within the first year after transplant. Seven patients in the high AIC group received a perioperative endarterectomy with patch angioplasty compared to none in the low AIC group, which was a statistical significant difference (p = 0.013). One patient in the high

TABLE 2 | Baseline characteristics from the cohort, stratified according to the median ipsilateral AIC score.

	Low AIC score (≤4,241) N = 70	High AIC score (>4,241) N = 70	*p*-value
Recipient-related			
Age (years), median (IQR)	63.2 (54.7–68.7)	66.5 (61.8–72.5)	0.005
BMI (kg/m^2), median (IQR)	27.3 (23.9–30.8)	27.3 (24.5–32.5)	0.363
Sex			0.224
Male, n (%)	39 (55.7)	47 (67.1)	
Female, n (%)	31 (44.3)	23 (32.9)	
Smoking			0.007
Never, n (%)	35 (50.0)	17 (24.3)	
Currently, n (%)	9 (12.9)	15 (21.4)	
Former, n (%)	26 (37.1)	38 (54.3)	
Total dialysis duration (months), median (IQR)	7 (0–24.8)	11 (0–28.8)	0.247
Diabetes, n (%)	27 (38.6)	30 (42.9)	0.731
Hypertension, n (%)	58 (82.9)	62 (88.6)	0.469
Coronary artery disease			<0.001[a]
None, n (%)	67 (95.7)	39 (55.7)	
Single vessel, n (%)	0 (0.0)	14 (20.0)	
Double vessel, n (%)	1 (1.4)	7 (10.0)	
Triple vessel, n (%)	2 (2.9)	10 (14.3)	
Cerebrovascular disease, n (%)			<0.039[a]
None, n (%)	65 (92.9)	56 (80.0)	
TIA, n (%)	1 (1.4)	8 (11.4)	
CVA, n (%)	4 (5.7)	6 (8.6)	
Previous transplant, n (%)	12 (17.1)	11 (15.7)	1.000
COPD			0.245
No, n (%)	69 (98.6)	66 (94.3)	
GOLD I, n (%)	1 (1.4)	1 (1.4)	
GOLD II, n (%)	0 (0.0)	3 (4.3)	
Peripheral arterial disease, n (%)	1 (1.4)	7 (10.0)	0.063[a]
Donor-related			
Donor type			0.420
Living, n (%)	39 (55.7)	35 (50.0)	
DCD, n (%)	19 (27.1)	26 (37.1)	
DBD, n (%)	12 (17.1)	9 (12.9)	
Donor WIT (minutes), median (IQR)	3 (2-9)	4 (3-14)	0.129
Age (years), median (IQR)	56.0 (48.3–64.8)	63.0 (48.0–71.8)	0.079
BMI (kg/m^2), median (IQR)	25.0 (23.3–29.3)	26.0 (24.0–28.2)	0.695
Diabetes, n (%)	2 (2.9)	2 (2.9)	1.000
Hypertension, n (%)	14 (20.0)	22 (31.4)	0.176
Last creatinine (μmol/L), median (IQR)	69.0 (59.3–86.5)	72.0 (62.3–81.0)	0.793
Transplant-related			
Pre-emptive transplant, n (%)	37 (52.9)	49 (70.0)	0.056
HLA mismatch, median (IQR)	3 (2-5)	4 (3-5)	0.269
vPRA, median (IQR)	0 (0–24)	0 (0–0)	0.362
AB0i transplant, n (%)	2 (2.9)	1 (1.4)	1.000
Ureteral stent			0.729
Single J, n (%)	41 (58.6)	44 (62.9)	
Double J, n (%)	29 (41.4)	26 (37.1)	
Anastomosis time (minutes), median (IQR)	21.0 (16.3–25.8)	23.0 (16.0–28.0)	0.453
Cold ischemic time (minutes), median (IQR)	142.5 (114.3–656.8)	350.0 (130.3–656.8)	0.235

[a]*Fisher's exact test.*

AB0i, AB0 incompatible; BMI, body mass index; COPD, chronic obstructive pulmonary disease; CVA, cerebrovascular disease; DBD, donation after brain death; DCD, donation after circulatory death; GOLD, global initiative for obstructive lung disease; IQR, interquartile range; TIA, transient ischemic attack; vPRA, virtual panel reactive antibodies; WIT, warm ischemic time.

AIC group received a preoperative percutaneous transluminal angioplasty (PTA) with stenting compared to none in the below median AIC group (p = 1.000). Patient survival during the follow-up was inferior in the high AIC score group (log-rank test: p = 0.010). No difference was observed for death-censored graft survival.

DISCUSSION

This prospective cohort study found no direct association between AIC and eGFR after transplant. The results from this study are in line with earlier studies who did not find an

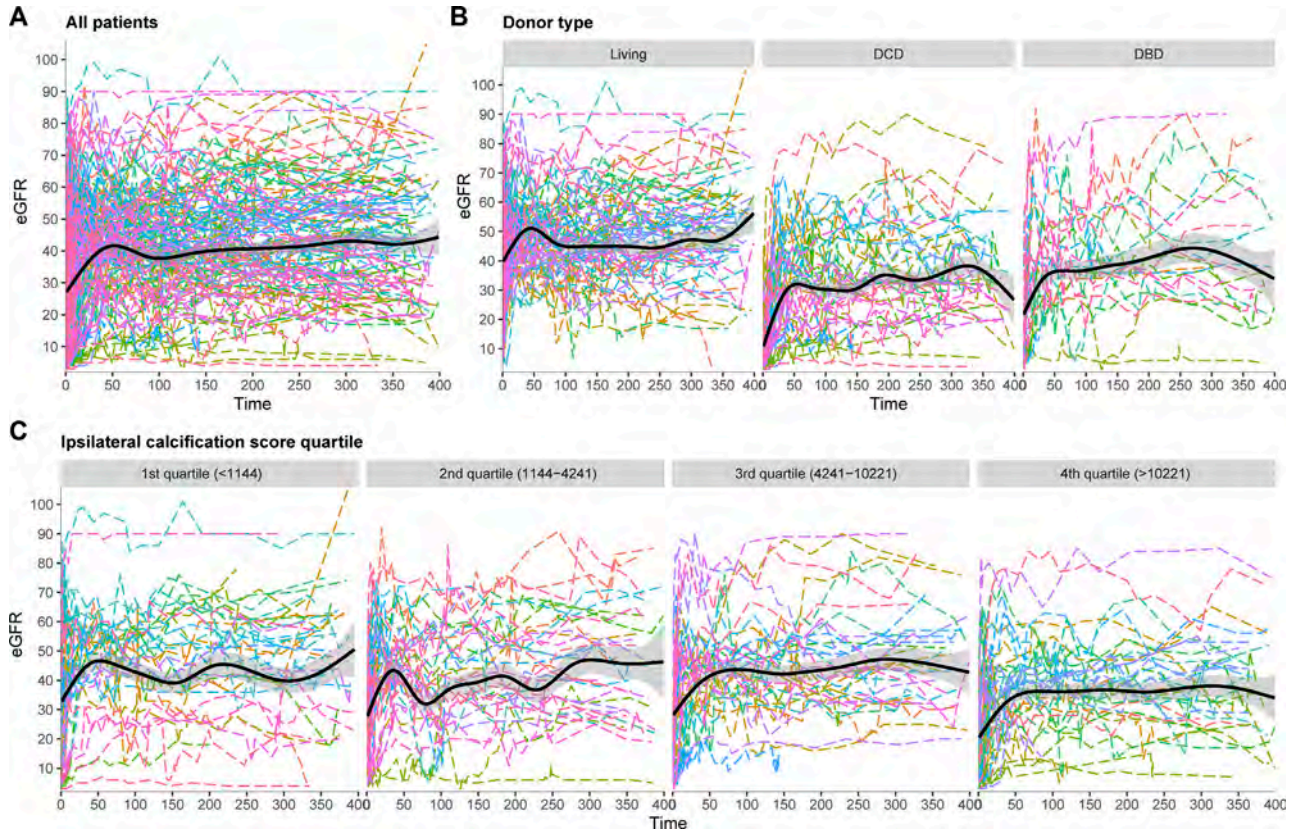


FIGURE 4 | Spaghetti plots for eGFR trajectory in the first year after transplant, **(A)** All patients, **(B)** Stratified according to donor type, **(C)** Stratified according to ipsilateral calcification score.

association between AIC and post-transplant graft function (11–14, 16). However, we found a significant interaction between time and ipsilateral calcification score, indicating that patients with a higher calcification score had a lower eGFR trajectory during the follow-up time. Other studies have not identified this interaction since none of these studies analyzed eGFR as a repeated measure over time by performing a linear mixed model. A linear mixed model is the statistical method of choice when analyzing post-transplant renal function (26). The most important reason to use this analysis is because other statistical methods do not account for drop-out. When studying renal function decline, initiation of dialysis, retransplantation and death with a function graft are the most common causes of drop-out. Because these are likely based on previously observed measurements of renal function, drop-out of the study is not completely random. Patients with high calcification scores are more likely to drop-out from the study due to death with a functioning graft because of their increased mortality risk (8, 9). Excluding these patients from the analysis results in biased estimates (26).

The pathophysiology behind the interaction that we found is not completely clear but could be speculated. Firstly, if atherosclerosis causes a hemodynamically significant stenosis with arterial lumen narrowing, this could lead to inflow problems resulting in allograft dysfunction. This has been proven in the case of transplant renal artery stenosis (27). As our study used non-contrast-enhanced CT-scan, we did not observe whether the calcification was causing a significant stenosis. Furthermore, CKD itself as well as dialysis are both important risk factors for accelerated atherosclerosis (2). Previous studies have shown that the progression of atherosclerosis slows down after transplant, but does not halt (28, 29). Therefore, it is possible that calcification progressed to a hemodynamically significant stenosis after transplant. It is also possible that intimal micro-calcification itself may already induce downstream silent ischemia and cellular necrosis to the graft, causing graft dysfunction. This mechanism has been described in studies regarding coronary artery calcification, showing that coronary artery atherosclerosis can cause significant myocardial ischemia in the absence of a hemodynamically significant stenosis due to endothelial and microvascular dysfunction (30). The last hypothesis is considered more likely because none of the patients presented with other symptoms of hemodynamically significant vascular disease during the follow-up time.

This is the first study to use a linear mixed model to assess the association between ipsilateral AIC as a continuous score and post-transplant eGFR. Most other studies used AIC as a binary

TABLE 3 | Linear mixed model for eGFR trajectory in the first year post-transplant.

	Value	Standard error	*p*-value	$(GVIF^{1/2df})^2$
(Intercept)	74.82	9.06	<0.001	
Time (days)				1.85
Day 1 – day 50	4.22	2.31	0.067	
Day 50 – day 100	30.90	3.59	<0.001	
After day 100	11.75	1.80	<0.001	
Recipient ipsilateral AIC score (per 1000 units)	0.02	0.18	0.919	2.37
Recipient age (per year)	−0.23	0.12	0.064	1.61
Recipient sex				1.36
Male	Ref			
Female	3.52	2.08	0.094	
Recipient diabetes				1.19
No	Ref			
Yes	0.33	1.93	0.865	
Recipient smoking				1.30
Never	Ref			
Currently	−0.04	2.92	0.988	
Quit	−0.08	2.25	0.973	
Previous kidney transplant				2.22
No	Ref			
Yes	0.43	3.51	0.902	
Total dialysis duration (per month)	0.03	0.06	0.621	2.54
Coronary artery disease				1.37
None	Ref			
Single vessel	5.67	3.45	0.103	
Double vessel	5.87	4.58	0.203	
Triple vessel	−2.81	3.75	0.455	
Peripheral arterial disease				1.38
No	Ref			
Yes	−4.51	4.41	0.309	
Donor type				3.24
Living	Ref			
DCD	−14.41	4.918	0.003	
DBD	−7.32	5.016	0.137	
Donor age (per year)	−0.29	0.08	<0.001	1.39
Donor diabetes				1.35
No	Ref			
Yes	−6.69	6.06	0.272	
Donor last creatinine (per μmol/l)	−0.04	0.03	0.150	1.74
Pre-emptive transplant				2.12
Yes	Ref			
No	−4.15	2.60	0.114	
Cold preservation				2.56
Static cold storage	Ref			
Hypothermic machine perfusion	0.10	3.00	0.972	
Total HLA mismatch (per 1 mismatch)	−0.21	0.63	0.744	1.23
vPRA (per %)	−0.02	0.04	0.634	2.32
Cold ischemic time (per minute)	0.00	0.01	0.917	4.87
Postoperative dialysis				2.60
No	Ref			
Yes	−9.11	3.21	0.005	
≥1 rejection episode				1.85
No	Ref			
Yes	−6.69	2.82	0.019	
Interaction time and ipsilateral calcification				1.96
Day 1 – Day 50: Ipsilateral calcification score	−0.32	0.23	0.179	
Day 50 – Day 100: Ipsilateral calcification score	−0.26	0.36	0.468	
After Day 100: Ipsilateral calcification score	−0.47	0.19	0.014	

ABOi, ABO incompatible; DBD, donation after brain death; DCD, donation after circulatory death; eGFR, estimated glomerular filtration rate; HLA, human leukocyte antigen; HLAi, human leukocyte antigen incompatible; vPRA, virtual panel reactive antibodies.

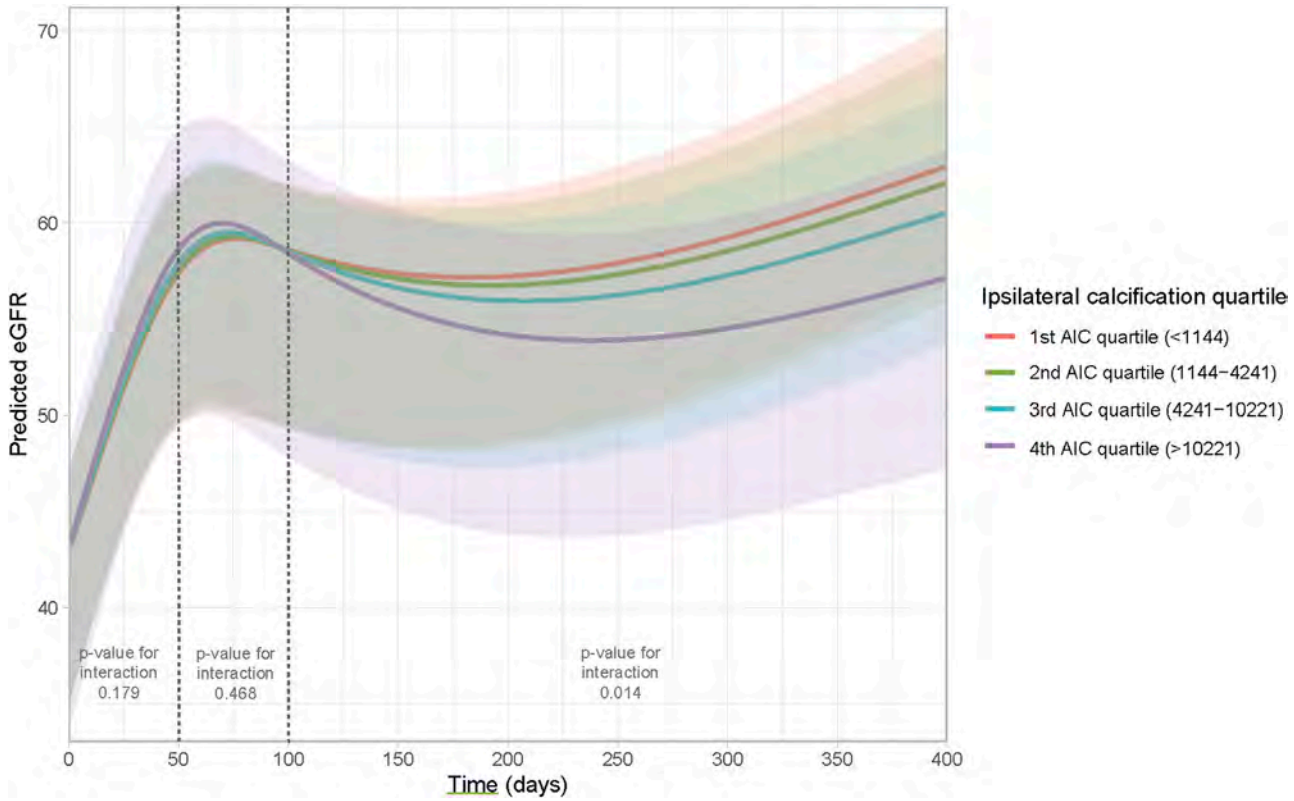


FIGURE 5 | Effect plot to visualize the interaction between time and ipsilateral AIC score. The Y-axis represents the predicted eGFR based on the model, the X-axis represents time.

TABLE 4 | Secondary outcomes.

Outcome	Low AIC score (≤4,241) N = 70	High AIC score (>4,241) N = 70	*p*-value
DGF, n (%)	10 (14.3)	19 (27.1)	0.094
PNF, n (%)	3 (4.3)	4 (5.7)	1.000[a]
Acute rejection <1 year, n (%)	15 (21.4)	17 (24.3)	0.841
Perioperative vascular procedure			**0.013[a]**
None, n (%)	70 (100)	63 (90.0)	
Endarterectomy, n (%)	0 (0)	7 (10.0)	
Preoperative vascular procedure			1.000[a]
None, n (%)	70 (100)	69 (98.6)	
PTA, n (%)	0 (0)	1 (1.4)	
1-year graft survival (death-censored), % (CI)	94.3 (89.0–99.9)	91.4 (85.0–98.2)	0.400[b]
1-year patient survival, % (CI)	100 (not estimable)	95.7 (91.1–100.0)	0.010[b]

[a]*Fisher's exact test.*

[b]*Log-rank test.*

CI, confidence interval; DGF, delayed graft function; IQR, interquartile range; PNF, primary non-function.

variable based on the presence or absence of any calcification, a categorical variable according to the severity of calcification, or a categorization of the modified Agatston score (10-12, 14-16). Even though categorization makes interpretation of results simple, it does not reflect the underlying biology where the severity of calcification can take any number between the minimum and maximum observed value. Furthermore, classification of calcification as minimal/moderate/severe is subjective and likely to result in low inter-observer agreement. This simplification also leads to a considerable loss of power with

an increased risk of a type II error (31). In our study, we used the modified Agatston score as an objective measure to quantify the amount of AIC. The Agatston score has shown before to have excellent inter-observer (spearman's $\rho \geq 0.99$) and inter-scanner agreement (spearman's $\rho \geq 0.97$) (17, 18). Our previous, dual-center, retrospective study also used the modified Agatston score to investigate a relationship between AIC and post-transplant outcomes (8). This study found an independent association between the modified Agatston score and uncensored graft survival, death with a functioning graft and cardiovascular events. Therefore, the modified Agatston score is also useful to identify patients at higher mortality risk post-transplant or patients that could benefit from more stringent cardiovascular monitoring (8).

Even though we did find a significant interaction between time and AIC, the impact of the difference in eGFR trajectory on long-term graft survival was not investigated. Prior studies found that eGFR is a good surrogate marker for graft survival (32). It can thus be stated that long-term graft survival outcomes may be inferior in patients with a high ipsilateral AIC score. The present study lacks the follow-up duration to evaluate the eGFR pattern and graft survival after 1 year. Further studies are needed to examine whether this statistical significant difference would ultimately lead to a clinically relevant difference in terms of graft survival.

Traditionally, the Agatston score has been performed by using semi-automatic software, which still requires marking of the calcified coronary artery lesions by a technician. However, a recent study showed that automatic, artificial intelligence based software had excellent correlation with the commonly used semi-automatic software (33). This allows easy calculation of the modified Agatston score on a simple, non-contrast-enhanced CT-scan. It was already shown that quantification of AIC could help identify patients at higher risk of cardiovascular mortality and events (8). The current study showed that ipsilateral AIC also negatively affects graft function over time. Therefore, standardized calculation of the modified Agatston score can help identify patients at higher risk of cardiovascular mortality and graft function decline. The simplicity of the calculation of this score, with the possibility to use an automatic algorithm, makes this score applicable in clinical practice.

Our study has several strengths and limitations. One strength is that our study is prospective, limiting selection bias. Non-contrast-enhanced CT-scan is the golden standard to measure vascular calcification which limits misclassification (34). The use of the modified Agatston score as an objective, continuous score, allowed precise quantification of AIC. A limitation of the study may be the somewhat low consent rate, which is largely due to the COVID pandemic, which caused a general study stop in our hospital. However, we do not expect that this had an impact on our results. Because we used a linear mixed model including all eGFR measurements, we had great statistical power, which allowed us to build a complex model adjusting for all factors that could potentially confound the association between calcification score and eGFR. A drawback of our linear mixed model is the complexity with the inclusion of interaction terms and splines making a direct interpretation of the estimates, or the calculation of a cut-off value, not possible.

In conclusion, this prospective cohort study found no direct relationship between ipsilateral calcification score and eGFR. However, a significant interaction between ipsilateral calcification score and follow-up time was observed, meaning that a higher calcification score is associated with a lower eGFR trajectory from 100 days after transplant. The focus should be to prevent AIC progression from an early CKD stage by promoting pre-emptive transplantation.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Medical Ethical Committee of Erasmus Medical Center, Rotterdam, Netherlands (2018-1401). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

ER: Study design, performance of the research, data analysis, writing of the paper. JR: Study design, supervision, review and editing of the paper. SB: Supervision of data analysis, review and editing of the paper. DB: Review and editing of the paper. MD: Development of scanning protocol, review and editing of the paper. HK: Study design, review and editing of the paper. JW: Review and editing of the paper. JIJ: Review and editing of the paper. RM: Study design, supervision, review and editing of the paper.

CONFLICT OF INTEREST

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

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.10647/full#supplementary-material

REFERENCES

1. Fang Y, Ginsberg C, Sugatani T, Monier-Faugere MC, Malluche H, Hruska KA. Early Chronic Kidney Disease-mineral Bone Disorder Stimulates Vascular Calcification. *Kidney Int* (2014) 85(1):142–50. doi:10.1038/ki.2013.271
2. Valdivielso JM, Rodriguez-Puyol D, Pascual J, Barrios C, Bermudez-Lopez M, Sanchez-Nino MD, et al. Atherosclerosis in Chronic Kidney Disease: More, Less, or Just Different? *Arterioscler Thromb Vasc Biol* (2019) 39(10):1938–66. doi:10.1161/ATVBAHA.119.312705
3. Drueke TB, Massy ZA. Atherosclerosis in CKD: Differences from the General Population. *Nat Rev Nephrol* (2010) 6(12):723–35. doi:10.1038/nrneph.2010.143
4. Amann K. Media Calcification and Intima Calcification Are Distinct Entities in Chronic Kidney Disease. *Clin J Am Soc Nephrol* (2008) 3(6):1599–605. doi:10.2215/CJN.02120508
5. Foley RN, Parfrey PS, Sarnak MJ. Clinical Epidemiology of Cardiovascular Disease in Chronic Renal Disease. *Am J Kidney Dis* (1998) 32(3):S112–9. doi:10.1053/ajkd.1998.v32.pm9820470
6. Werlin EC, Braun HJ, Walker JP, Freise JE, Amara D, Liu IH, et al. Utility of a Simplified Iliac Artery Calcium Scoring System to Guide Perioperative Management for Renal Transplantation. *Front Med (Lausanne)* (2021) 8:606835. doi:10.3389/fmed.2021.606835
7. Kianda MN, Wissing KM, Broeders NE, Lemy A, Ghisdal L, Hoang AD, et al. Ineligibility for Renal Transplantation: Prevalence, Causes and Survival in a Consecutive Cohort of 445 Patients. *Clin Transpl* (2011) 25(4):576–83. doi:10.1111/j.1399-0012.2010.01317.x
8. Benjamens S, Rijkse E, Te Velde-Keyzer CA, Berger SP, Moers C, de Borst MH, et al. Aorto-Iliac Artery Calcification Prior to Kidney Transplantation. *J Clin Med* (2020) 9(9):2893. doi:10.3390/jcm9092893
9. Rijkse E, van Dam JL, Roodnat JI, Kimenai H, Jnm IJ, Minnee RC. The Prognosis of Kidney Transplant Recipients with Aorto-Iliac Calcification: a Systematic Review and Meta-Analysis. *Transpl Int* (2020) 33(5):483–96. doi:10.1111/tri.13592
10. Hernandez D, Rufino M, Bartolomei S, Gonzalez-Rinne A, Lorenzo V, Cobo M, et al. Clinical Impact of Preexisting Vascular Calcifications on Mortality after Renal Transplantation. *Kidney Int* (2005) 67(5):2015–20. doi:10.1111/j.1523-1755.2005.00303.x
11. Aalten J, Dekker HM, van der Vliet JA, Hoitsma AJ. Does a plain X-ray of the Pelvis Predict Arterial Complications in Renal Transplantation? A Prospective Study. *Nephrol Dial Transpl* (2011) 26(6):2007–12. doi:10.1093/ndt/gfq705
12. Aitken E, Ramjug S, Buist L, Kingsmore D. The Prognostic Significance of Iliac Vessel Calcification in Renal Transplantation. *Transpl Proc* (2012) 44(10):2925–31. doi:10.1016/j.transproceed.2012.06.058
13. Benjamens S, Alghamdi SZ, Rijkse E, Te Velde-Keyzer CA, Berger SP, Moers C, et al. Aorto-Iliac Artery Calcification and Graft Outcomes in Kidney Transplant Recipients. *J Clin Med* (2021) 10(2):325. doi:10.3390/jcm10020325
14. Chavent B, Maillard N, Boutet C, Albertini JN, Duprey A, Favre JP. Prognostic Value of Aortoiliac Calcification Score in Kidney Transplantation Recipients. *Ann Vasc Surg* (2017) 44:245–52. doi:10.1016/j.avsg.2017.03.180
15. Hwang HS, Lim SW, Sun IO, Yang KS, Yoon HE, Chung BH, et al. Clinical Significance of Preexisting Microcalcification in the Iliac Artery in Renal Transplant Recipients. *Transplantation* (2015) 99(4):811–7. doi:10.1097/TP.0000000000000409
16. Munguia P, Caramelo R, Rubio MV, Sahdala L, Arnaudas L, Paul J, et al. Pre-Transplant Assessment of Vascular Calcification as a Risk Factor of Mortality, Graft Loss, and Cardiovascular Events in Renal Transplant Recipients. *Transpl Proc* (2015) 47(8):2368–70. doi:10.1016/j.transproceed.2015.08.038
17. Ghadri JR, Goetti R, Fiechter M, Pazhenkottil AP, Kuest SM, Nkoulou RN, et al. Inter-scan Variability of Coronary Artery Calcium Scoring Assessed on 64-multidetector Computed Tomography vs. Dual-Source Computed Tomography: a Head-To-Head Comparison. *Eur Heart J* (2011) 32(15):1865–74. doi:10.1093/eurheartj/ehr157
18. Mori S, Takaya T, Kinugasa M, Ito T, Takamine S, Fujiwara S, et al. Three-dimensional Quantification and Visualization of Aortic Calcification by Multidetector-Row Computed Tomography: a Simple Approach Using a Volume-Rendering Method. *Atherosclerosis* (2015) 239(2):622–8. doi:10.1016/j.atherosclerosis.2014.12.041
19. Menon V, Gul A, Sarnak MJ. Cardiovascular Risk Factors in Chronic Kidney Disease. *Kidney Int* (2005) 68(4):1413–8. doi:10.1111/j.1523-1755.2005.00551.x
20. Tillin T, Hughes AD, Mayet J, Whincup P, Sattar N, Forouhi NG, et al. The Relationship between Metabolic Risk Factors and Incident Cardiovascular Disease in Europeans, South Asians, and African Caribbeans: SABRE (Southall and Brent Revisited) -- a Prospective Population-Based Study. *J Am Coll Cardiol* (2013) 61(17):1777–86. doi:10.1016/j.jacc.2012.12.046
21. Blaha MJ, Mortensen MB, Kianoush S, Tota-Maharaj R, Cainzos-Achirica M. Coronary Artery Calcium Scoring: Is it Time for a Change in Methodology? *JACC Cardiovasc Imaging* (2017) 10(8):923–37. doi:10.1016/j.jcmg.2017.05.007
22. Cademartiri F, Maffei E, Palumbo A, Martini C, Seitun S, Tedeschi C, et al. Diagnostic Accuracy of Computed Tomography Coronary Angiography in Patients with a Zero Calcium Score. *Eur Radiol* (2010) 20(1):81–7. doi:10.1007/s00330-009-1529-9
23. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A New Equation to Estimate Glomerular Filtration Rate. *Ann Intern Med* (2009) 150(9):604–12. doi:10.7326/0003-4819-150-9-200905050-00006
24. Levin A, Stevens PE. Summary of KDIGO 2012 CKD Guideline: behind the Scenes, Need for Guidance, and a Framework for Moving Forward. *Kidney Int* (2014) 85(1):49–61. doi:10.1038/ki.2013.444
25. Gauthier J, Wu QV, Gooley TA. Cubic Splines to Model Relationships between Continuous Variables and Outcomes: a Guide for Clinicians. *Bone Marrow Transplant* (2020) 55(4):675–80. doi:10.1038/s41409-019-0679-x
26. Leffondre K, Boucquemont J, Tripepi G, Stel VS, Heinze G, Dunkler D. Analysis of Risk Factors Associated with Renal Function Trajectory over Time: a Comparison of Different Statistical Approaches. *Nephrol Dial Transpl* (2015) 30(8):1237–43. doi:10.1093/ndt/gfu320
27. Bruno S, Remuzzi G, Ruggenenti P. Transplant Renal Artery Stenosis. *J Am Soc Nephrol* (2004) 15(1):134–41. doi:10.1097/01.asn.0000099379.61001.f8
28. Alappan HR, Vasanth P, Manzoor S, O'Neill WC. Vascular Calcification Slows but Does Not Regress after Kidney Transplantation. *Kidney Int Rep* (2020) 5(12):2212–7. doi:10.1016/j.ekir.2020.09.039
29. Marechal C, Coche E, Goffin E, Dragean A, Schlieper G, Nguyen P, et al. Progression of Coronary Artery Calcification and Thoracic Aorta Calcification in Kidney Transplant Recipients. *Am J Kidney Dis* (2012) 59(2):258–69. doi:10.1053/j.ajkd.2011.07.019
30. Crea F, Camici PG, Bairey Merz CN. Coronary Microvascular Dysfunction: an Update. *Eur Heart J* (2014) 35(17):1101–11. doi:10.1093/eurheartj/eht513
31. Naggara O, Raymond J, Guilbert F, Roy D, Weill A, Altman DG. Analysis by Categorizing or Dichotomizing Continuous Variables Is Inadvisable: an Example from the Natural History of Unruptured Aneurysms. *AJNR Am J Neuroradiol* (2011) 32(3):437–40. doi:10.3174/ajnr.A2425
32. Clayton PA, Lim WH, Wong G, Chadban SJ. Relationship between eGFR Decline and Hard Outcomes after Kidney Transplants. *J Am Soc Nephrol* (2016) 27(11):3440–6. doi:10.1681/ASN.2015050524
33. Sandstedt M, Henriksson L, Janzon M, Nyberg G, Engvall J, De Geer J, et al. Evaluation of an AI-Based, Automatic Coronary Artery Calcium Scoring Software. *Eur Radiol* (2020) 30(3):1671–8. doi:10.1007/s00330-019-06489-x
34. Disthabanchong S, Boongird S. Role of Different Imaging Modalities of Vascular Calcification in Predicting Outcomes in Chronic Kidney Disease. *World J Nephrol* (2017) 6(3):100–10. doi:10.5527/wjn.v6.i3.100

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ORIGINAL RESEARCH
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Specificities of Meningitis and Meningo-Encephalitis After Kidney Transplantation: A French Retrospective Cohort Study

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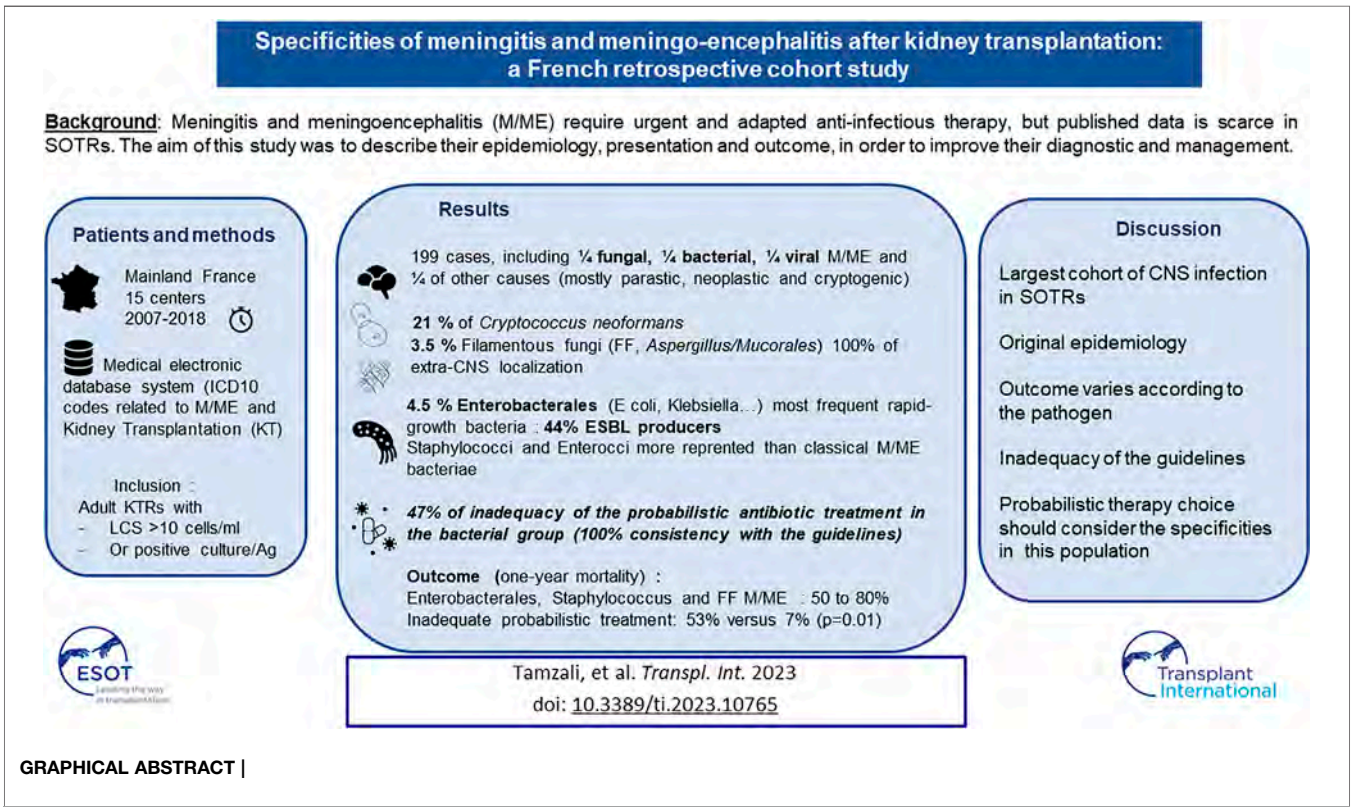
Kidney transplant recipients develop atypical infections in their epidemiology, presentation and outcome. Among these, meningitis and meningoencephalitis require urgent and adapted anti-infectious therapy, but published data is scarce in KTRs. The aim of this study was to describe their epidemiology, presentation and outcome, in order to improve their diagnostic and management. We performed a retrospective, multicentric cohort study in 15 French hospitals that included all 199 cases of M/ME in KTRs between 2007 and 2018 (0.9 case per 1,000 KTRs annually). Epidemiology was different from that in the general population: 20% were due to *Cryptococcus neoformans*, 13.5% to varicella-zoster virus,

5.5% to *Mycobacterium tuberculosis*, and 4.5% to Enterobacteria (half of which produced extended spectrum beta-lactamases), and 5% were Post Transplant Lymphoproliferative Disorders. Microorganisms causing M/ME in the general population were infrequent (2%, for *Streptococcus pneumoniae*) or absent (*Neisseria meningitidis*). M/ME caused by Enterobacteria, *Staphylococci* or filamentous fungi were associated with high and early mortality (50%–70% at 1 year). Graft survival was not associated with the etiology of M/ME, nor was impacted by immunosuppression reduction. Based on these results, we suggest international studies to adapt guidelines in order to improve the diagnosis and the probabilistic treatment of M/ME in SOTRs.

Keywords: kidney transplantation, immunosuppression, transplant infectious diseases, opportunistic infections, meningitis, encephalitis, *Cryptococcus neoformans*, enterobacteriales



GRAPHICAL ABSTRACT |

INTRODUCTION

Meningitis and meningo-encephalitis (M/ME) are potentially life-threatening infections with causes that are well described in immunocompetent hosts (1-3). According to current guidelines (4-7), clinical suspicion of M/ME implies the rapid initiation of high-dose, broad-spectrum probabilistic anti-infectious therapies while performing radiological and cerebrospinal fluid (CSF) biological work-up (8). Solid Organ Transplant Recipients (SOTRs) are subject to invasive infectious diseases, sometimes with atypical and severe presentation, and with a wider range of pathogens than the general population (5-11). A knowledge of the specific epidemiology of central nervous system (CNS) infections in this population is therefore critical to elicit the best probabilistic anti-infectious therapy. Non-etheless, few studies describe the epidemiology, clinical presentation and outcome of M/ME in SOTRs: retrospective cohorts of specific pathogens (12-15), mixed cohorts of liver, heart, and kidney transplant (KT) recipients (KTRs) (10-21) and case reports (22-27).

Here we describe the epidemiology, presentation, and outcome of M/ME that occurred in KTRs in France between 2007 and 2018, with a 2-year follow-up.

PATIENTS AND METHODS

Study Population

KTRs diagnosed with M/ME between 1st January 2007, and 31st December 2018, were identified in fifteen French Academic Hospitals with a kidney transplantation program. Participating centers were the university hospitals from the following French cities: Bordeaux, Lille, Limoges, Lyon, Marseille Montpellier, Nantes, Paris (Georges Pompidou, Henri Mondor, Necker, Pitié-Salpêtrière, Tenon), Rennes, Rouen and Toulouse.

The national electronic medical databases of these centers were screened for the International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10) codes for kidney transplantation and for codes related to CNS infections and their main etiologies (see **Supplementary Material**). When available, local clinical and microbiology databases were also screened.

The inclusion criteria were:

- Adult (≥18 years old) KTRs
- Diagnosis of M/ME between 1st January 2007 and 31st December 2018 defined by at least one of these observations:
  * CSF pleocytosis over 10 cells/mm^3
  * Positive CSF bacterial or mycological culture
  * Positive CSF antigen (*C. neoformans* [CrAg], *Aspergillus*, or *Streptococcus pneumoniae*).
- A functioning kidney graft at the diagnosis of M/ME

Consistent with these inclusion criteria, non-infectious meningitis were included.

Exclusion criteria:

- CSF with a positive polymerase chain reaction (PCR) for a pathogen without hypercellularity
- Cerebral abscess without CSF hypercellularity
- Subarachnoid hemorrhage
- High CSF protein concentration without hypercellularity

The following data were collected from the medical charts:

- Medical history, characteristics of the KT, immunosuppressive therapy and anti-infectious prophylaxis protocols.
- Clinical, biological, microbiological and radiological presentation at admission for M/ME.
- Therapeutic management, including anti-infectious and surgical treatments but also immunosuppressive treatment modulation, *i.e.*, a change of therapeutic class, the discontinuation of a drug, or a decrease of at least 25% of the trough level target.
- Clinical and biological outcome after meningitis, including patient and graft survival. The data was collected until the last available follow-up.

Meningitis was defined as the presence of cerebrospinal pleocytosis >10 elements/mm^3.

Encephalitic features were defined as the presence of one of the following: mental status or cognitive impairment, generalized seizures. Meningo-encephalitis was defined as the association of meningitis and encephalitic features.

A CSF was defined as lymphocytic or neutrophilic if its cellularity was made up of >50% of lymphocytes or neutrophils, respectively. It was defined as mixed if the difference between these percentages was below 10%.

Causative diagnosis was asserted by an infectious disease specialist based on specific chart review.

The "highly immunosuppressed status" was defined as the presence of at least one the following criteria:

- Recent KT (<6 months)
- A history of immunosuppressive therapy before KT
- A history of treatment of a rejection episode between last KT and the M/ME onset
- A recent (<2 years before M/ME) cytotoxic chemotherapy treatment
- Recent history of hemopathy (<5 years)

Statistical Analysis

The statistical analysis was performed using GraphPad PRISM® v9 (GraphPad Software, San Diego, CA, United States).

The annual incidence of M/ME was estimated by dividing the number of yearly cases by the number of living KTRs in the 15 participating centers during the same year.

Quantitative variables are presented as mean ± standard deviation or median (inter-quartile range, IQR) according to their normal or skewed distributions. Qualitative variables are presented as numbers (percentages). Data were compared using the Student's t-test, the Mann-Whitney test, or the χ^2 test as appropriate. In analysis including more than two groups, the data were compared using one-way ANOVA or Kruskal-Wallis test according to their normal or skewed distributions.

Survival analyses were performed using the Kaplan-Meier method. A Log-rank test was performed for the comparison between the groups, with a significant p-value of < 0.05.

Ethics

This study was approved by the Paris Public Hospitals ("Assistance Publique-Hôpitaux de Paris") Office of Data Protection (RGPD) and registered under project number 20181105112928.

RESULTS

Incidence of M/ME

Between 2007 and 2018, 199 cases of M/ME, caused by 200 pathogens were diagnosed in 194 patients (**Figure 1**), with a median follow-up of 3.58 IQR [10.0–69.0]. The mean annual incidence was 0.9 for 1,000 KTRs with no significant variation over the study period ($p = 0.81$).

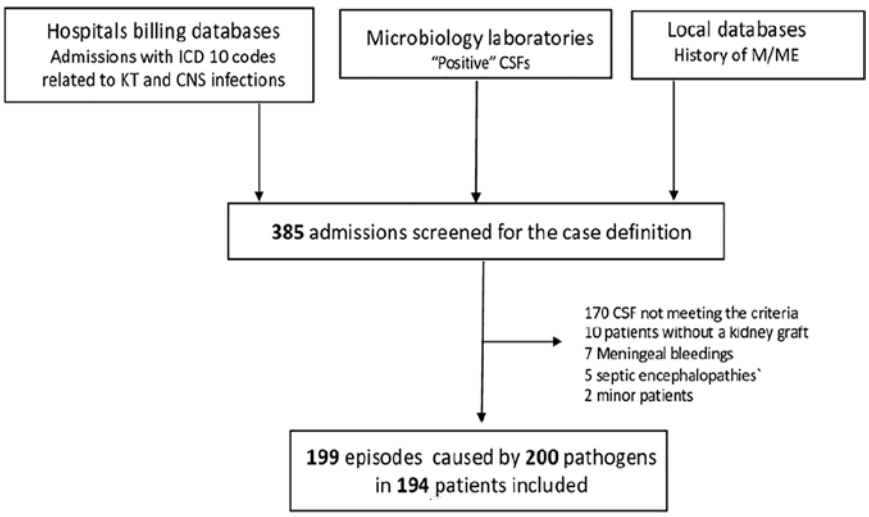


FIGURE 1 | Flow Chart. ICD, international classification of diseases; CNS, central nervous system; CSF, cerebrospinal fluid; M/ME, meningitis/meningo-encephalitis.

Population Characteristics

The characteristics of the patient population are shown in **Table 1**. M/ME mostly involved male patients (60.3%), born in mainland France (70.4%), at a mean age of 54.8 ± 14.4 years, and transplanted for the first time (82% of the cases). Thirty-six percent of the patients were also treated for diabetes mellitus, which was the most frequent cause of the initial nephropathy. Induction therapy consisted of anti-thymocyte therapy in two-thirds of the patients (consistently with previous French study reporting 41%–75% of anti-thymocyte therapy induction according to centers) (28) and almost half of the cohort (47%) matched our definition of highly immunosuppressed (see *Patients and Methods*). No patients had been treated with eculizumab or any other anti-complement therapy.

Etiologies of the M/ME in KTRs

Causes of M/ME were almost homogeneously distributed between Fungi, Bacteria, and Viruses (one-quarter each). The last quarter was divided into parasitic, non-infectious M/ME and M/ME of unknown cause (MUC) (**Figure 2A**).

Overall, the most frequent microorganisms were *C. neoformans* (20%), VZV, 13.5%, *Mycobacterium tuberculosis* (5.5%), Enterobacterales (4.5%) and filamentous Fungi (4.0%), **Figure 2B** and **Supplementary Table S2**. In 29 patients (totaling 14.5% of M/ME episodes) the cause remained unknown even after extensive investigation, making MUC the second-most frequent diagnosis. We compared the distribution of the main etiologies according to the level of immunosuppression (**Supplementary Table S4**). There was no striking difference except from filamentous fungi infections, all occurring in the highly immunosuppressed group (8 versus 0, $p = 0.002$).

Delay Between KT and M/ME

Half of the cases occurred within the first 3.4 (IQR [0.91–8.58]) years after KT. The earliest episode was diagnosed on the first day after KT and the latest case was diagnosed 43 years after transplantation.

The delay before M/ME onset varied with the group of etiology: viral M/ME occurred after a median delay of 2.5 years (QR 0.7–8.8), fungal M/ME after 2.8 years (IQR 0.7–5.8), bacterial M/ME after 3.5 years (IQR 1.3–8.8), parasitic M/ME after 4.9 years (IQR 2.0–9.8) and M/ME due to non-infectious causes after a median delay of 9.1 years (2.0–11.8).

The incidence of M/ME after KT was not linear for all microorganisms and varied according to the cause of MME. M/ME due to CMV and filamentous fungi occurred in the first 3 years after KT (**Figures 3A, C**), and Gram-negative rods (GNR) M/ME occurred in the first 4 years after KT in 90% of the cases (**Figure 3B**). PTLD (Post-Transplant Lymphoproliferative Disease) was the first cause of M/ME after 10 years post-transplantation (**Figure 3D**). The other etiologies, and especially *C. neoformans* and VZV did not seem to vary in risk in the years following KT.

Clinical and Biological Presentation at Diagnosis

The clinical presentation at admission is described in **Table 2**. The patients with a bacterial or a fungal M/ME presented more frequently with fever (85.4% and 87.5% versus 26%–72.1% in the other groups, $p = 0.0006$) and the patients with fungal M/ME more frequently with headaches (95.8% versus 42.8%–82.4%, $p = 0.001$). Neck stiffness was observed more frequently in bacterial and fungal infections (55% and 52%) than in viral infections (23%, $p = 0.08$).

Almost half of the patients presented clinical and/or encephalographic encephalitis. In more than half of the cases (53.3%), the clinical presentation included extra-CNS manifestations that could facilitate the diagnosis. The

TABLE 1 | Characteristics of the population.

Characteristic	*N* = 194
	n (%) or mean ± SD
Age (yr)	54.8 ± 14.4
Gender, (males)	117 (60.3)
Dialysis duration before KT, (yr)	4.9 ± 4.6
Previous KT	
None	159 (82.0)
1	31 (16.0)
≥2	4 (2.0)
Donor	
Deceased	173 (89.2)
Living	21 (10.8)
Country of birth	
Mainland France	136 (70.4)
North Africa	21 (10.6)
Subsaharian Africa	18 (9.0)
Other	18 (9.0)
Initial nephropathy	
Diabetes	29 (14.9)
CTIN	22 (11.3)
Undetermined	22 (11.3)
Polycystic Kidney Disease	18 (9.2)
Hypertensive nephropathy	18 (9.2)
IgA AN	17 (8.7)
Other	69 (35.6)
Diabetes	71 (36.6)
Pre-existing	41 (21.1)
Post-transplantation	30 (15.5)
Anti-infectious prophylaxis at M/ME onset	
Cotrimoxazole	41 (20.6)
Valaciclovir	32 (16.1)
Valganciclovir	7 (3.5)
Antifungal (azoles)	0 (0.0)
Immunity status	
Anti-thymocyte globulins for induction	113 (58.5)
HIV seropositivity	6 (3.0)
Highly immunosuppressed before M/ME[a]	91 (47.0)
History of treated rejection	51 (25.9)
Other	146 (74.1)
eGFR[b] at M/ME (ml/min/1.73 m^2)	46.5 ± 26.8

[a]*Defined as the presence of at least on the following: recent KT (<6 months), history of immunosuppressive therapy before KT, history of treatment of a rejection episode before M/ME onset, recent (<2 years) cytotoxic chemotherapy treatment, hemopathy.*
[b]*Most recent estimated glomerular filtration rate considered as stable before the onset of the M/ME, calculated according to the Modification of Diet in Renal Disease study equation.*
CTIN, Chronic Tubulo-interstitial Nephropathy; IgA AN, IgA associated nephropathy.

extra-neurologic involvements associated with the most frequent causes are summarized in **Supplementary Table S3**.

The number of total lymphocytes did not differ between the etiologic groups, but the $CD4^+$ lymphocyte count before onset was lower in the fungal, bacterial and viral group (when taken altogether) than in the parasitic and non-infectious group (**Table 2**; **Figure 4A**). Bacterial M/ME resulted in higher CSF cellularity, a higher CSF protein concentration, and a higher serum C-reactive protein concentration than the other M/ME (**Figures 4B–F**).

The CSF-to-blood glucose ratio was not significantly different between bacterial and fungal M/ME but was lower in bacterial M/ME as compared to Viral and Parasitic forms ($p = 0.004$) and in bacterial and fungal M/ME M/ME compared to the rest of the group ($p < 0.0001$). We assessed the efficiency of various ROC (receiver operating characteristic) curves of the blood to CSF glucose ratio to isolate specific etiologies. The best performance was achieved with a value of 0.5 in discriminating between bacterial and fungal forms vs. the others (area under the curve 0.70, 95% CI [0.63–0.78]) with a sensitivity of 72.5 95% CI [62.6–80.6] and specificity of 64.2 95% CI [54.2–73.1] (**Figure 4F**).

Viral and parasitic M/ME were lymphocytic in most of the cases, while bacterial M/ME was mostly neutrophilic (**Table 2**). There were no neutrophilic M/ME in the group of parasitic M/ME. The other groups of M/ME showed no specific cellularity.

Description of M/ME by Etiology

Viruses

Herpesviridae were responsible for 80% of the viral M/ME (**Figure 2D**), with 55% of VZV infection (all but one were recurrences). Only 4/39 (10%) of the patients with viral M/ME received valganciclovir (VGC) prophylaxis at meningitis onset. The 35 patients without VGC prophylaxis were successfully treated by intravenous acyclovir or ganciclovir, according to the Herpesviridae. Four patients were on VGC prophylaxis when the M/ME declared: one patient developed ganciclovir-resistant CMV infection and died of the meningoencephalitis, and three patients developed HSV or VZV acyclovir-sensitive M/ME, for which they were successfully treated.

Three patients developed EBV (Epstein-Barr Virus) meningo-encephalitis without lymphoproliferative disorder.

Bacteria

Slow-Growth Bacteria

Eleven patients were diagnosed with tuberculous meningitis, of which only two had a proven diagnostic (negative direct examination, positive culture). Among them, 6/11 (45%) were born outside of Western Europe (mostly in Africa) compared with 58/188 (30%) in the rest of the cohort ($p = 0.79$).

The eleven tuberculous meningitis were lymphocytic (100%), with a CSF-to-blood glucose ratio <0.5 in 72% of the cases and a protein concentration in the CSF >1 g/L in 72% of the cases.

One-year survival after presumptive treatment was 72%.

Three cases of Nocardia infection were included (*N. farcinica*, *N. nova*, and *N. paucivorans* infections). All three of them were brain-space occupying lesions. Two of them were associated with pulmonary lesions that helped the diagnosis. Treatment was medical only, with an association of meropenem and cotrimoxazole or levofloxacin.

Pyogenic Bacteria

All seven cases of M/ME occurring within the first year after KT were due to pyogenic bacteria. Listeria (in two patients), ESBL-producing enterobacterales (in two patients), Staphylococci (in

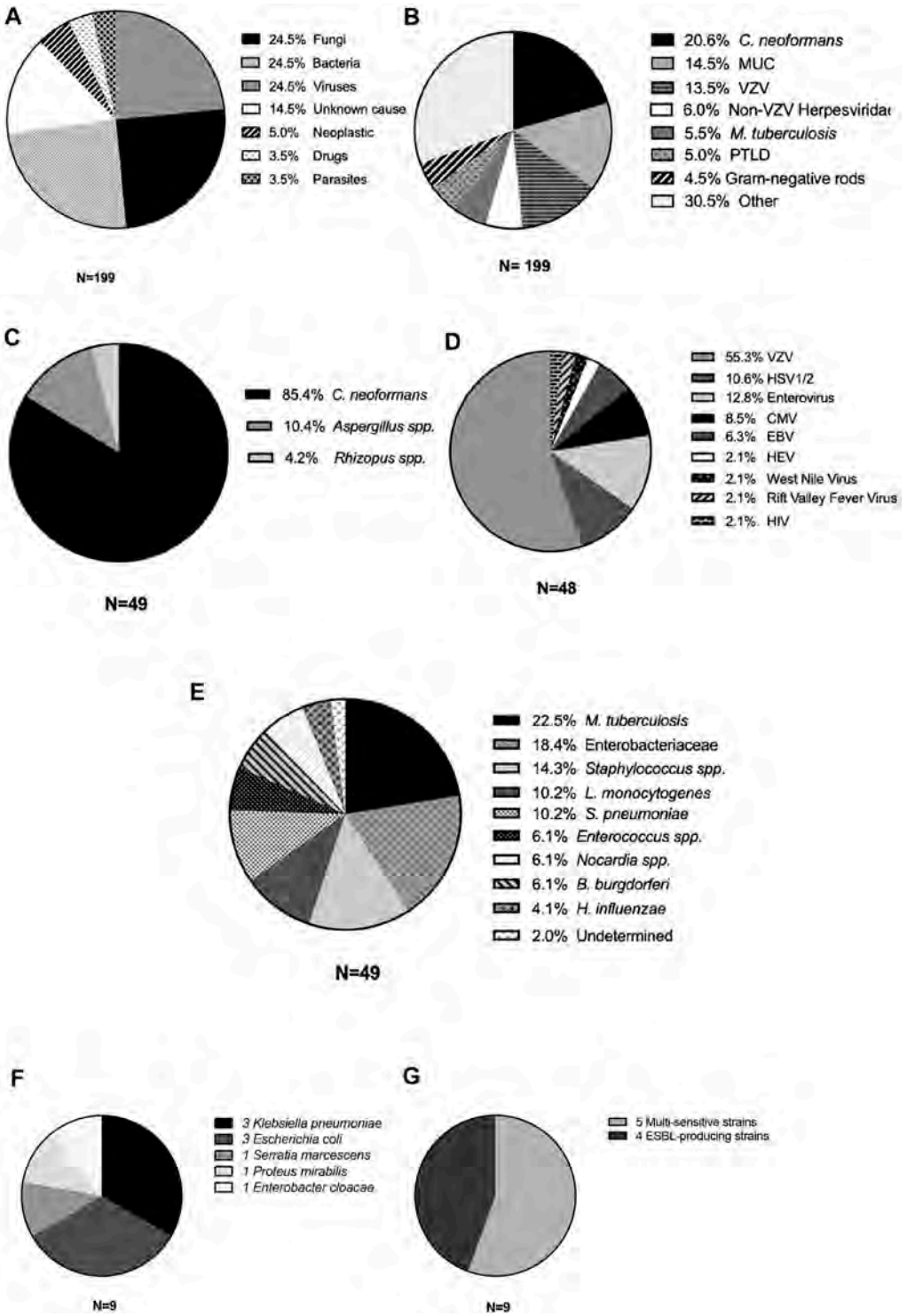


FIGURE 2 | Causes of meningitis and meningoencephalitis in kidney transplant recipients. Cases of M/ME are represented according to type of etiology **(A)**, main microorganism or cause **(B)**, type of Fungi **(C)**, virus **(D)**, or bacteria **(E)**. **(F)** All Enterobacteriales; **(G)** proportion of extended spectrum beta-lactamase producing Enterobacteriales. VZV, varicella-zoster virus; MUC, meningitis of unknown cause; VZV, varicella-zoster virus; PTLD, post-transplant lymphoproliferative disorder; HSV, herpes simplex virus; CMV, cytomegalovirus; EBV, Epstein-Barr virus; HEV, hepatitis E virus; HIV, human immunodeficiency virus; ESBL, extended-spectrum betalactamase.

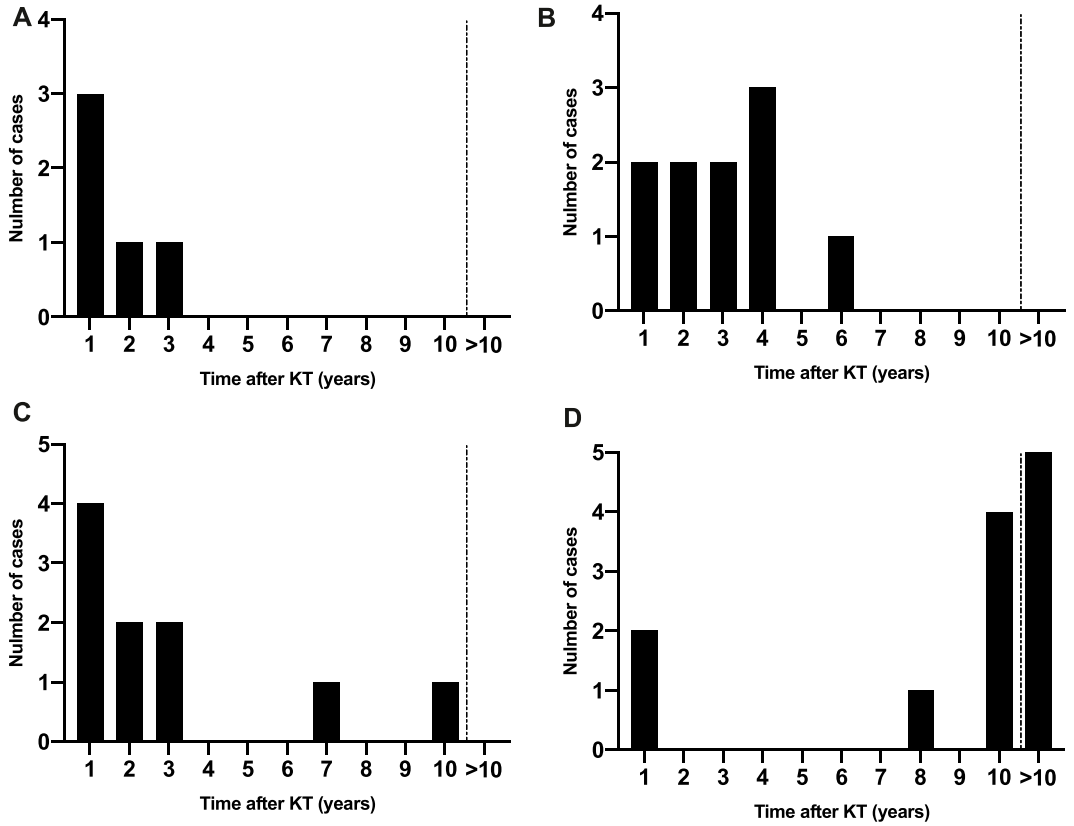


FIGURE 3 | Distribution of M/ME over time after KT according to the etiology. **(A)** Cytomegalovirus cases, **(B)** Gram-negative rod cases, **(C)** Filamentous fungi, **(D)** Post-transplant lymphoproliferative disorders.

two patients) and Entercococcus (one patient) were found as causative pathogens.

The most frequently found rapid-growth bacteria were GNRs (**Figure 2E**), found in nine patients (20% of bacterial M/ME and 4.5% of all M/ME), including three *Klebsiella pneumoniae*, three *Escherichia coli*, one *Serratia marcescens*, one *Enterobacter cloacae*, and one *Proteus mirabilis* (**Figure 2F**). Four of the strains (44%) expressed an Extended-Spectrum Beta-lactamase (**Figure 2G**).

Out of the nine patients with GNRs M/ME, three suffered a urinary tract infection with the same pathogen, and two had positive blood cultures. Three cases out of nine were hospital acquired infections (33%). Six- and 12-month mortality was 33%, and 44% respectively.

Two *Klebsiella* strains isolated from M/ME were retrospectively screened for specific virulence factors (29); none of the seven virulence factors tested were found.

All five cases of *S. pneumoniae* meningitis were related to the contiguous spread of a local infection (two otitis media, one mastoiditis, one ethmoidal sinusitis and one post-surgical breach).

Staphylococcus infections were mainly due to *S. aureus* (5/7, including three infective endocarditis). All cases of Staphylococcal M/ME were community acquired. No case of pyogenic M/ME initially presented with septic shock.

The thirty-two patients presenting a M/ME due to a rapid growth bacterium (i.e., excluding *Mycobacteria* and *Nocardia*) were initially all treated with a combination of a third-generation cephalosporin and amoxicillin. Among them, 15 patients (47%) were infected with a bacterium that was not sensitive to this combination. Mortality in the first month was 1/17 (6.7%) for the patients who received an effective treatment and 8/15 (53.3%) for the patients who presented a pathogen that was resistant to the initial therapy, $p = 0.01$.

Fungi

The most frequent fungus was *C. neoformans* (41 cases, 20.6% **Figures 2B, C**), with 95% of the cases presenting with headaches, 83% with fever and 49% with neck stiffness. Upon admission, C-reactive protein ranged from 3 to 287 mg/L, total leucocytes count from 2,300 to 17,800/mm^3 and CSF analysis showed lymphocytic hypercellularity in 49% of the cases and a cellularity ranging from 0 to 1,350 elements/mm^3 (median 41 IQR [13–139]).

CSF direct examination (India-Ink stain) had a 51% sensitivity, CSF culture 78% (blood 34%) and CSF Cryptococcal Antigen 94% (blood 93%).

Aspergillus and *Mucorales* CNS infections were associated with another localization (sinus and/or lung) in all the patients (**Supplementary Table S2**).

TABLE 2 | Clinical, biological and radiological characteristics of M/ME in the different etiological groups.

	All (194)	Fungi (49)	Viruses (48)	Bacteria (49)	Parasites (7)	Non-infectious (17)	*p*
	n/N (%) or median [IQR]	n/N (%) or median [IQR]	n/N (%) or median [IQR]	n/N (%) or median [IQR]	n/N (%] or median [IQR]	n/N [%] or median [IQR]	
Fever	146/193 (75.6)	41/48 (85.4)	31/43 (72.1)	43/49 (87.8)	2/7 (26.6)	9/17 (52.9)	0.0006
Headaches	151/192 (78.7)	46/48 (95.8)	32/43 (74.4)	39/49 (79.6)	3/7 (42.8)	14/17 (82.4)	0.009
Neck stiffness	73/193 (37.8)	23/48 (47.9)	10/43 (23.3)	11/49 (22.5)	1/7 (14.3)	6/17 (42.9)	0.03
Clinical encephalitis	94/193 (47.5)	26/48 (54)	26/43 (60.4)	35/49 (71.4)	3/7 (42.8)	4/17 (23)	0.01
Seizures	31/193 (16.0)	6/48 (12.5)	4/43 (9.3)	11/49 (22.4)	0/7 (0)	2/17 (11.8)	<0.0001
Abnormal EEG	12/90 (42.9)	6/16 (37.5)	16/23 (69.6)	14/20 (70)	3/4 (75)	4/6 (66.6)	0.23
Extra-CNS involvement	98/194 (53.3)	27/49 (55.1)	26/43 (60.5)	30/49 (61.2)	2 (26.6)	7/17 (41.2)	0.16
Last total lymphocyte count, elts/mm^3	880 [500–1,387]	690 [430–1,100]	1,199 [700–1,400]	880 [500–1,450]	750 [380–880]	1,360 [404–1,520]	0.08
Last CD4$^+$ count, elts/mm^3	234 [95–529]	158 [90–317]	278 [147–539]	225 [77–459]	448 [251–762]	527 [228–782]	0.02
Leucocyte count at admission, ×1,000 elts/mm^3	7.0 [3.9–10.0]	6.3 [3.6–9.7]	7.0 [4.6–102.5]	8.6 [6.0–14.8]	7.0 [4.0–7.6]	5.0 [3.0–7.1]	0.01
CRP at admission, mg/L	15.6 [5.0–82.0]	21 [5.0–80.5]	6.5 [2.5–27.8]	113 [56–209]	3.5 [3.0–7.0]	7 [5.0–17.5]	<0.0001
CSF cell count, elts/mm^3	53 [16–220]	45 [12–171]	6.5 [12–129]	113 [32–759]	11 [10–20]	84 [30–305]	0.0006
Lymphocytic	113 (60.1)	20 (45.5)	38 (90.5)	19 (39.6)	6 (85.7)	10 (58.8)	
Neutrophilic	57 (30.6)	19 (43.2)	3 (7.1)	26 (54.2)	0 0)	5 (29.4)	<0.001
Mixed	16 (8.1)	5 (11.3)	1 (2.3)	3 (6.1)	1 (14.3)	2 (11.8)	
CSF proteins, g/L	0.9 [0.6–1.8]	0.1 [10.6–2.0]	1.6 [0.5–1.4]	2.3 [0.7–3.5]	0.7 [0.4–1.1]	0.8 [0.5–1.0]	0.04
CSF blood/glucose ratio	0.5 [0.4–0.6]	0.5 [0.3–0.6]	0.6 [0.5–0.6]	0.4 [0.3–0.5]	0,7 [0.4–1.1]	0.5 [0.5–0.7]	0.0003
Abnormal findings on CT-scan	38/153 (25.2)	13/43 (30.2)	1/27 (3.7)	12/37 (32.4)	6/7 (85.7)	3/13 (23.1)	0.0005
Abnormal findings on MRI	93/151 (62.8)	26/40 (65.0)	20/34 (58.8)	22/32 (68.8)	7/7 (100)	8/13 (61.5)	0.8

EEG, electroencephalography; CRP, C-reactive protein; CSF, cerebro-spinal fluid; CT-Scan, computed tomography scanner; MRI, magnetic resonance imaging.

Parasites

Parasitic M/ME were all caused by *Toxoplasma gondii*, presenting as a pauci-cellular (<20 cells/mm^3) lymphocytic meningitis with a higher CSF-to-blood glucose ratio than in the case of other microorganisms (**Figure 4**). Cranial CT-scan was abnormal in 71% of the cases, and MRI in 100% of the cases, showing multiple cerebral abscesses.

Other Causes

Among the 17 non-infectious causes (**Figure 2**) 10 were neoplastic and 7 drug-related.

Neoplastic M/ME were characterized by a late onset (11 years after KT IQR [37–141]) mostly due to EBV-related PTLD (8/10).

Intravenous immunoglobulins (5/7), tacrolimus (1/7) and sirolimus were found as possible causes of drug-related meningitis, diagnosed after extensive etiological investigation (exclusion of other possible causes) and after a pharmacovigilance investigation.

Survival

One-year and 10-year post-M/ME patient survival were 74% and 70%, respectively (**Figure 5A**). There were significant differences in the outcome after M/ME according to the etiology Within the fungal group filamentous fungi were associated with the poorest outcome with a mortality of 75% at 36 months (**Figure 5B**). Within the bacterial group, pyogenic bacterial M/ME 1-year mortality was 57%. GNR and Staphylococcal meningitis were characterized by a particularly high and early mortality (55% and 70% at 6 months, respectively, **Figure 5C**). *Staphylococcus aureus* M/ME 1 year mortality reached 80%. One-year survival in the group of M/ME of unknown cause was 85%. There was no difference in matter of survival according to the level of immunosuppression.

One-year and 5-year death-censored graft survivals were 82% and 67%, respectively, in the overall population. Mortality was not significantly different between the different etiological groups, nor between the groups of patients undergoing immunosuppression minimization or not (**Figure 5D**).

DISCUSSION

We report the largest multicentric study of M/ME in KTRs, including nearly two hundred cases. We show that M/MEs are evenly caused by a wide array of bacteria, fungi and viruses, some of them characterized by specific clinical and/or biological parameters, most of them usually not found in immunocompetent hosts. We also show that the outcome principally depends on the etiology. For these reasons, we believe that the common guidelines for the treatment of M/ME in immunocompetent patients do not apply to KTRs. Consistently with a recent Swiss national study focusing on CNS in SOTs in general (21), M/ME is a relatively rare complication after KT.

Our study population did appeared as highly immunocompromised. We could not search for risk factors as we chose not to perform a controlled study to favor more numerous inclusions according to our goal.

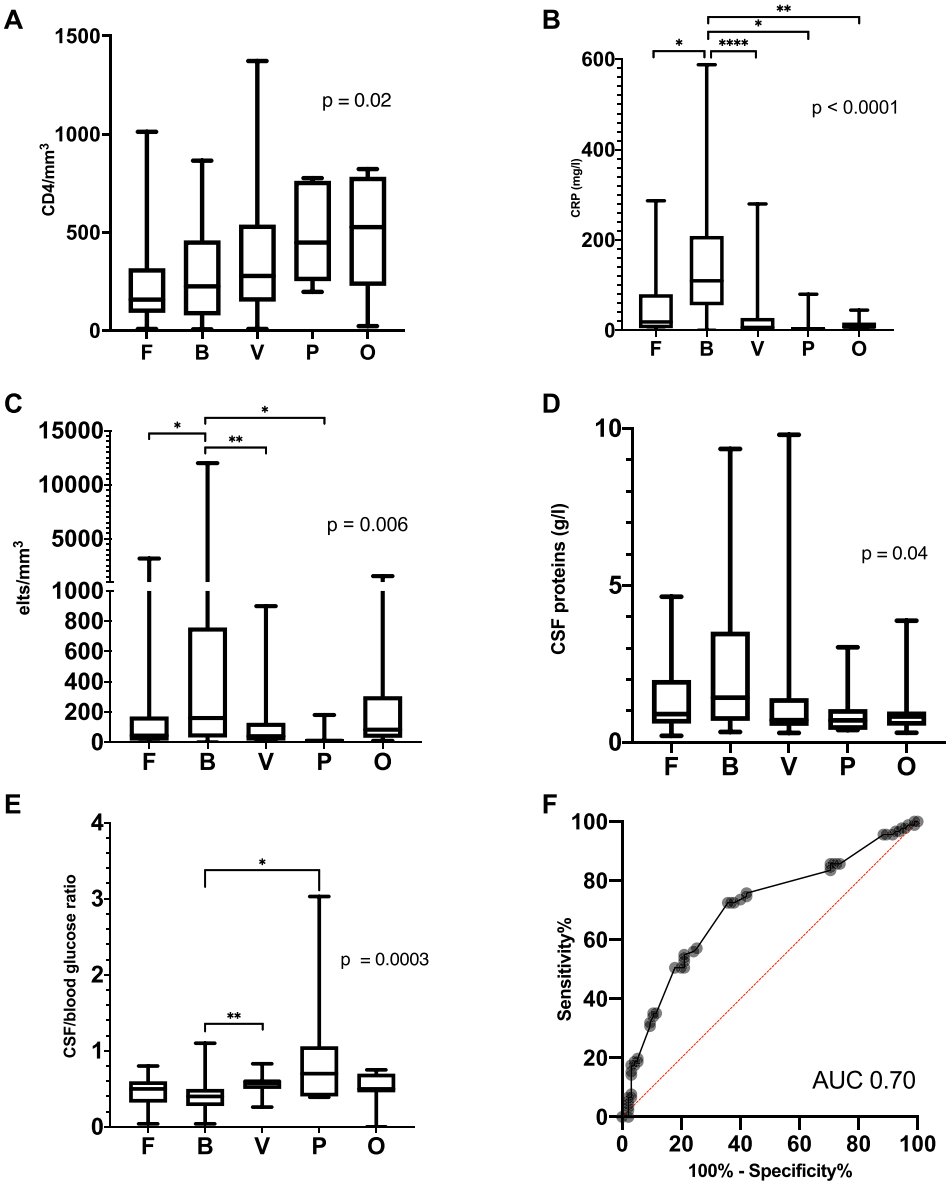


FIGURE 4 | Biological characteristics in the different groups of microorganisms or causes. **(A)** Last CD4$^+$ count before M/ME onset, **(B)** C-reactive protein at admission for M/ME, **(C)** CSF cellularity, **(D)** CSF protein level, **(E)** glucose CSF/blood ratio and **(F)** ROC Curve of the glucose CSF/blood ratio to discriminate bacterial and fungal M/ME from the other M/ME. Elts, elements; F, fungi; B, bacteria; V, viruses; P, parasites; O, other.

This study reveals several unique features of the M/ME epidemiology in KTRs. First of all, *C. neoformans*, VZV, Enterobacterales, and *Mycobacterium tuberculosis* totalize more than half of the cases. There were fewer than 4% of *S. pneumoniae* and *Haemophilus influenzae*, and no case of *Neisseria meningitidis*. Only five patients (2.5%) were diagnosed with *L. monocytogenes* infection. This strongly contrasts with the epidemiology of M/ME found in the general population (1, 2, 4).

Cotrimoxazole (CMX) prophylaxis could be one of the reasons for this strikingly different epidemiology (9). In KTRs receiving CMX prophylaxis, no *L. monocytogenes*, *Staphylococcus*, *S. pneumoniae* or *Nocardia* M/ME were observed. One case of GNR M/ME occurred while the patient was on CMX prophylaxis, but he was infected with a CMX-resistant ESBL-producing *Klebsiella pneumoniae* strain. One case of *Toxoplasma gondii* infection was also observed despite ongoing CMX prophylaxis.

We could not study the role of anti-pneumococcal vaccination in our population as this was optimized and recommended in France during the study period. In addition, its effectiveness remains controversial in SOTRs (30).

MUCs were frequent in our cohort (29 patients, 14.5% of the cases) consistent with what has been found previously (31). Most of these cases received a final diagnosis of a "possible viral origin"

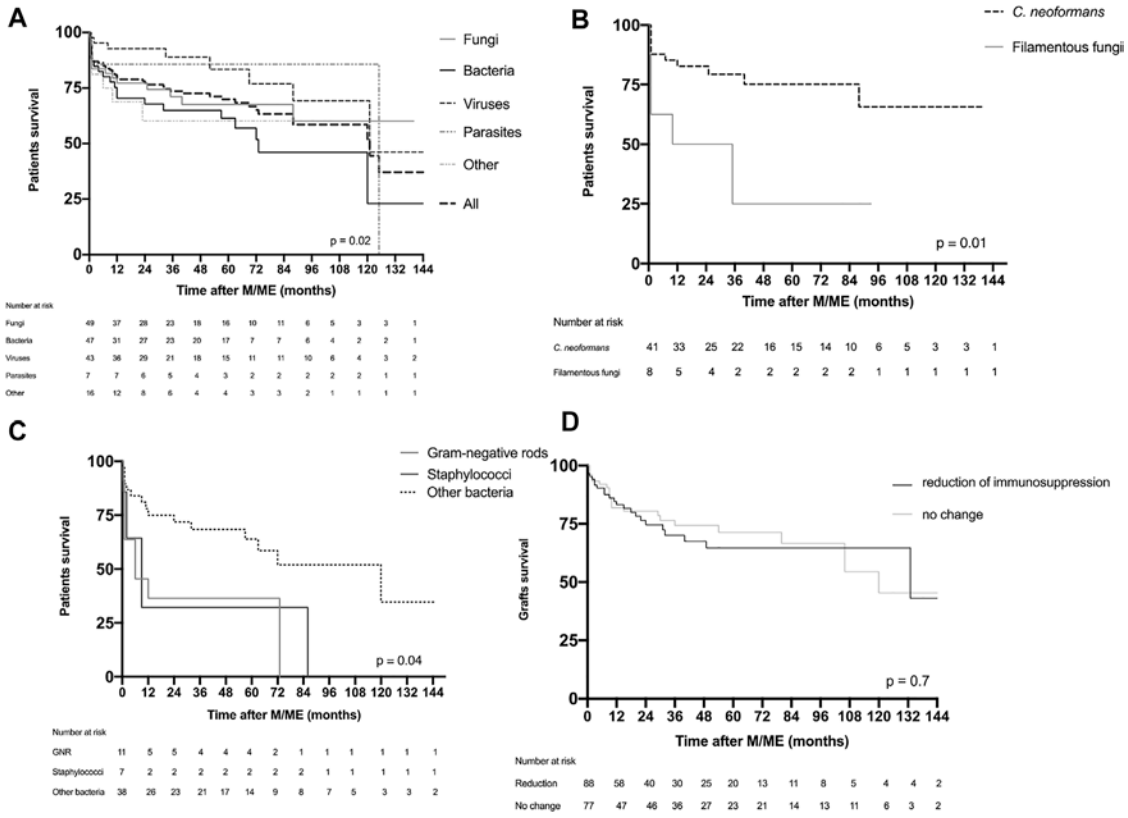


FIGURE 5 | Patient and graft survival. **(A)** Patient survival in the global population presented according to the group of causative microorganism or cause. **(B)** Survival of patients suffering fungal M/ME. **(C)** Survival of patients presenting with bacterial M/ME. **(D)** Graft survival according to reduction of immunosuppression of M/ME. F, fungi; B, bacteria; V, viruses; P, parasites; O, others; CN, *Cryptococcus neoformans*; FF, filamentous fungi; GNR, Gram-negative rods.

as they were frequently self-limiting. However, 72% of the patients received probabilistic anti-infectious therapy as recommended by the guidelines (consisting of an association of high dose cephalosporin 200 mg/kg/d, amoxicillin 200 mg/kg/d and acyclovir 15 mg/kg/8 h (3, 5, 6)). Eighteen percent of the patients with an unknown etiology died despite this anti-infectious regimen. The possible diagnosis are numerous: pathogens not systematically looked for like West-Nile Virus, which is recognized as an emerging disease causing CNS infections in transplant recipients (23, 32, 33), other viruses (3, 22, 26, 34), auto-immune diseases including paraneoplastic and post-infectious meningoencephalitis (35), undiagnosed fungal or parasitic infections, and undiagnosed neuro-meningeal tuberculosis. Metagenomic next-generation sequencing (mNGS) (36-38) should be used for an unbiased pathogen detection for cases of unknown origin as well as *M. tuberculosis* MTB/RIF Xpert® PCR testing (39).

C. neoformans was by far the first cause of M/ME (41 cases, 20% of all M/ME). This is consistent with *C. neoformans* infection being previously reported as the third-most frequent invasive fungal infection in a large mixed cohort of SOTRs (40). *C. neoformans* was also described as a rising pathogen in the SOTR population (41, 42) with a dose-dependent association with T-depleting induction treatments (43). This particularly high number of cryptococcal infections in M/ME is not surprising, given the tropism of *C. neoformans* for the CNS (where there is no cellular immunity) of immunocompromised hosts and the putative association of cryptococcal infections with chronic kidney failure (9, 42-44). The recently published series of CNS infections in SOTRs showed more Aspergillus than *Cryptococcus* infections: the difference can be explained by the inclusion of brain-space occupying lesions in that study, the inclusion of other types of SOTs or to antifungal prophylaxis, apparently given to a significant number of patients in this cohort (21). *C. neoformans* infection occurred at all times after KT consistent with a primary infections from the environment where it is ubiquitous (42, 45). *C. neoformans* should be specifically and systematically tested for in any case of neurological event in KTRs ([46) given the variability of the biological characteristics such as the cellularity of the CSF that can range from acellular to profuse pleocytosis of any type. Direct examination with India ink staining has a low sensitivity in SOTRs due to a weak fungal load (47). CrAg testing should be performed both in the peripheral blood and in the CSF in case of suspicion.

CNS infections caused by filamentous fungi were associated with an early occurrence and a very poor outcome, as already reported (9, 12, 21). All the cases also presented another site of

infection that could help with the diagnosis as previously reported (21).

VZV infection always presented as lymphocytic meningo-encephalitis, with an external (skin or eye) simultaneous recurrence in two-thirds of the cases. In VZV-seronegative KT candidates, VZV live-attenuated vaccine represents a very effective prevention and should be considered according to the guidelines (48, 49).

Bacterial meningitis was mostly due to *Mycobacterium tuberculosis*, GNRs and Staphylococci.

Interestingly, and for the first time, GNRs, Enterobacterales, were found as the first cause of pyogenic meningitis. GNR meningitis mostly occurred in the first 5 years after KT and manifested as a critical disease with a very high 1-year mortality. Half of the cases were not secondary to a urinary, blood or digestive infection. There was no case suspect of strongyloidiasis (all patients treated before transplantation with Ivermectin, no eosinophilia, no pulmonary involvement). We hypothesize that gut microbiota alterations in the pre- and post-transplantation setting can play a role. The combination of antibiotic treatment and the direct effects of immunosuppressive drugs on the gut microbiota result in an increase in proteobacteria (50, 51) associated with the development of infections, by immune dysregulation and the promotion of virulent strains (8, 52).

The CSF-to-blood glucose ratio did not appear as a reliable tool to identify bacterial M/ME. This may be explained by the frequency of fungal M/ME in immunosuppressed hosts as opposed to the general population. However, this ratio was frequently lower than 0.5 in patients suffering from a bacterial or a fungal M/ME.

Because third-generation cephalosporins and amoxicillin, recommended as a probabilistic therapy in case of M/ME in an immunocompromised population (5,6) do not cover ESBL-producing GNRs, *Staphylococci* nor *Enterococcus faecium*, we suggest a probabilistic therapy with drugs with a good blood barrier penetration, consisting in an association of high-dose parenteral meropenem and linezolid, that would cover all the rapid-growth bacteria not sensitive to the combination recommended in the guidelines. These antibiotics also cover *S. pneumoniae*, *N. meningitidis*, *L. monocytogenes*, and *H. influenzae* M/ME.

The interest of dexamethasone in KTRs MME is limited to *S. pneumoniae* and tuberculous meningitis, and should only be used in case of a strong suspicion (i.e., compatible direct examination or recent history of a local infection) as it can be associated with a poorer outcomes in other causes (5, 7).

Survival was very heterogenous depending on the diagnosis. Some bacterial (*Staphylococci* and GNRs) and some fungal (filamentous fungi) M/MEs were associated with the highest mortality and should be considered as the principal threat in case of M/ME in KTRs.

Some limitations should be however taken in consideration for external validity: first this study only included patients from France and some etiologies of M/ME tend to vary from one country to another (for instance tick-borne, Japanese, or Saint-Louis encephalitis are not to barely present on mainland France), second, the immunosuppression strategies can differ from one country to another and may result in a different epidemiology, and third the absence of a control group prevented us from identifying risk factors.

CONCLUSION

M/ME after KT encompass a wide range of causative diagnoses, mechanisms, and outcomes, of which our study provides a very detailed view. We show that the recommendations for the management of M/ME in the general population cannot be applied to KTRs in France. We believe that further studies should be performed in order to build specific guidelines for the management of M/ME in SOTRs.

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 APHP Est RGPD Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YT and JT designed the study, performed the analysis and wrote the article. PT helped designing the study. YT, TB, HH, CG, MG, SR, TN, MM collected the data. AS, TB, CG, MG, SR, TN, HH, VP, PT, HK, MH, VM, MM, VF, CL, MQ, JC, ER, DB, ET, SM, ES, BB, and NK helped on data collection and reviewed 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.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.10765/full#supplementary-material

REFERENCES

1. Brouwer MC, Tunkel AR, Beek DD. Epidemiology, Diagnosis, and Antimicrobial Treatment of Acute Bacterial Meningitis. *Clin Microbiol Rev* (2010) 23(3):467–92. doi:10.1128/CMR.00070-09
2. Brouwer MC, Beek DD. Epidemiology of Community-Acquired Bacterial Meningitis. *Curr Opin Infect Dis* (2018) 31(1):78–84. doi:10.1097/QCO.0000000000000417
3. Venkatesan A, Michael BD, Probasco JC, Geocadin RG, Solomon T. Acute Encephalitis in Immunocompetent Adults. *The Lancet* (2019) 393(10172): 702–16. doi:10.1016/S0140-6736(18)32526-1
4. Beek Dvan de, Cabellos C, Dzupova O, EspoSito S, Klein M, Kloek AT, et al. ESCMID Guideline: Diagnosis and Treatment of Acute Bacterial Meningitis. *Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis* (2016) 22(3): S37–62. doi:10.1016/j.cmi.2016.01.007
5. Tunkel AR, Hartman BJ, Kaplan SL, Kaufman BA, Roos KL, Scheld WM, et al. Practice Guidelines for the Management of Bacterial Meningitis. *Clin Infect Dis Oxford Acad* (2004) 39(9):1267–84. doi:10.1086/425368
6. Hoen B, de Debroucker T, Grimprel E, Wolff f M, Duval X, Varon E. *Management of Acute Community-Acquired Bacterial Meningitis*. (excluding newborns). Long version with arguments [Internet]. EM-Consulte. [cited 2020 Nov 6]. Available from: https://sci-hub.se/https://www.em-consulte.com/article/1312344/article/management-of-acute-community-acquired-bacterial-m.
7. Tunkel AR, Glaser CA, Bloch KC, Sejvar JJ, Marra CM, Roos KL, et al. The Management of Encephalitis: Clinical Practice Guidelines by the Infectious Diseases Society of America. *Clin Infect Dis* (2008) 47(3):303–27. doi:10.1086/589747
8. Fishman JA. Infection in Organ Transplantation. *Am J Transpl* (2017) 17(4): 856–79. doi:10.1111/ajt.14208
9. Singh N, Husain S. Infections of the central Nervous System in Transplant Recipients. *Transpl Infect Dis* (2000) 2(3):101–11. doi:10.1034/j.1399-3062.2000.020302.x
10. Coussement J, Lebeaux D, Delden Cvan, Guillot H, Freund R, Marbus S, et al. Nocardia Infection in Solid Organ Transplant Recipients: A Multicenter European Case-Control Study. *Clin Infect Dis Off Publ Infect Dis Soc Am* (2016) 63(3):338–45. doi:10.1093/cid/ciw241
11. Veen KEBvan, Brouwer MC, Ende Avan der, Beek Dvan de, vAn der Ende A. Bacterial Meningitis in Solid Organ Transplant Recipients: a Population-based Prospective Study. *Transpl Infect Dis* (2016) 18(5):674–80. doi:10.1111/tid.12570
12. Seok H, Huh K, Cho SY, Kang C-I, Chung DR, Huh WS, et al. Invasive Fungal Diseases in Kidney Transplant Recipients: Risk Factors for Mortality. *J Clin Med* (2020) 9:1824. doi:10.3390/jcm9061824[Internet][cited 2020 Nov 10]; (6). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7357124/.
13. Tamzali Y, Gras J, Denis B, Gits-Muselli M, Bretagne S, Peraldi MN, et al. Cryptococcus Neoformans Meningitis in Kidney Transplant Recipients: A Diagnostic and Therapeutic challenge. *Med Mycol Case Rep* (2021) 32:84–7. doi:10.1016/j.mmcr.2021.04.005
14. Yang Y, Chen M, Gu J, Zhu F, Xu X, Zhang C, et al. Cryptococcosis in Kidney Transplant Recipients in a Chinese university Hospital and a Review of Published Cases. *Int J Infect Dis* (2014) 26:154–61. doi:10.1016/j.ijid.2014.05.028
15. Khurana S, Batra N. Toxoplasmosis in Organ Transplant Recipients: Evaluation, Implication, and Prevention. *Trop Parasitol* (2016) 6(2):123–8. doi:10.4103/2229-5070.190814
16. Romero S, Montoro J, Guinot M, Almenar L, Andreu R, Balaguer A, et al. Post-Transplant Lymphoproliferative Disorders after Solid Organ and Hematopoietic Stem Cell Transplantation. *Leuk Lymphoma* 60 (2019). p. 142.
17. Baddley JW, Forrest GN. Cryptococcosis in Solid Organ Transplantation. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2013) 13(4):242–9. doi:10.1111/ajt.12116
18. Wiesmayr S, Tabarelli W, Stelzmueller I, Nachbaur D, Boesmueller C, Wykypiel H, et al. Listeria Meningitis in Transplant Recipients. *Wien Klin Wochenschr* (2005) 117(5–6):229–33. doi:10.1007/s00508-005-0311-5
19. Neofytos D, Fishman JA, Horn D, AnaissiE E, Chang CH, OlyAei A, et al. Epidemiology and Outcome of Invasive Fungal Infections in Solid Organ Transplant Recipients. *Transpl Infect Dis* (2010) 12(3):220–9. doi:10.1111/j.1399-3062.2010.00492.x
20. Gassiep I, McDougall D, Douglas J, Francis R, Playford EG. Cryptococcal Infections in Solid Organ Transplant Recipients over a 15-year Period at a State Transplant center. *Transpl Infect Dis* (2017) 19(1):e12639. doi:10.1111/tid.12639
21. Bogaart Lvan den, Lang BM, Rossi S, Neofytos D, Walti LN, Khanna N, et al. Central Nervous System Infections in Solid Organ Transplant Recipients: Results from the Swiss Transplant Cohort Study. *J Infect Elsevier* (2022) 85(1): 1–7. doi:10.1016/j.jinf.2022.05.019
22. Haneche F, Leparc-Goffart I, Simon F, Hentzien M, Martinez-Pourcher V, Caumes E, et al. Rift Valley Fever in Kidney Transplant Recipient Returning from Mali with Viral RNA Detected in Semen up to Four Months from Symptom Onset, France, Autumn 2015. Eurosurveillance. *Eur Centre Dis Prev Control* (2016) 21(18):30222.
23. ErtiLav M, Özkul A, ZeytiNoğlu A, Sen S, Sipahi S, Toz H, et al. Meningoencephalitis Caused by West Nile Virus in a Renal Transplant Recipient. *Mikrobiyol Bul* (2014) 48(4):674–82. doi:10.5578/mb.8212
24. Westhoff TH, Seibert FS, Bauer F, Stervbo U, Anft M, Doevelaar AAN, et al. Allograft Infiltration and Meningoencephalitis by SARS-CoV-2 in a Pancreas-Kidney Transplant Recipient. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2020) 20(11):3216–20. doi:10.1111/ajt.16223
25. Amirali MH, Liebenberg J, Pillay S, Nel J. Sporotrichosis in Renal Transplant Patients: Two Case Reports and a Review of the Literature. *J Med Case Rep* (2020) 14(1):79. doi:10.1186/s13256-020-02385-x
26. Tanveer F, Younas M, Fishbain J. Lymphocytic Choriomeningitis Virus Meningoencephalitis in a Renal Transplant Recipient Following Exposure to Mice. *Transpl Infect Dis* (2018) 20(6):e13013. doi:10.1111/tid.13013
27. Wright SE, Shaikh ZHA, Castillo-Lugo JA, Tanriover B. Aseptic Meningitis and Abducens Nerve Palsy as a Serious Side Effect of High Dose Intravenous Immunoglobulin Used in a Patient with Renal Transplantation. *Transpl Infect Dis* (2008) 10(4):294–7. doi:10.1111/j.1399-3062.2007.00294.x
28. Boucquemont J, Foucher Y, Masset C, Legendre C, Scemla A, Buron F, et al. Induction Therapy in Kidney Transplant Recipients: Description of the Practices According to the Calendar Period from the French Multicentric DIVAT Cohort. *Public Libr Sci* (2020) 15(10):e0240929. doi:10.1371/journal.pone.0240929
29. Compain F, Babosan A, Brisse S, Genel N, Audo J, Ailloud F, et al. Multiplex PCR for Detection of Seven Virulence Factors and K1/K2 Capsular Serotypes of *Klebsiella pneumoniae*. *J Clin Microbiol* (2014) 52(12):4377–80. doi:10.1128/JCM.02316-14
30. Dendle C, Stuart RL, Mulley WR, Holdsworth SR. Pneumococcal Vaccination in Adult Solid Organ Transplant Recipients: A Review of Current Evidence. *Vaccine* (2018) 36(42):6253–61. doi:10.1016/j.vaccine.2018.08.069
31. Glaser CA, Gilliam S, Schnurr D, Forghani B, Honarmand S, Khetsuriani N, et al. In Search of Encephalitis Etiologies: Diagnostic Challenges in the California Encephalitis Project, 1998-2000. *Clin Infect Dis Off Publ Infect Dis Soc Am* (2003) 36(6):731–42. doi:10.1086/367841
32. Yango AF, Fischbach BV, Levy M, Chandrakantan A, Tan V, Spak C, et al. West Nile Virus Infection in Kidney and Pancreas Transplant Recipients in the Dallas-Fort Worth Metroplex during the 2012 Texas Epidemic. *Transplantation* (2014) 97(9):953–7. doi:10.1097/01.TP.0000438621.81686.ab
33. Zanoni F, Alfieri C, Moroni G, Passerini P, Regalia A, Meneghini M, et al. Delayed Diagnosis of West Nile Virus Infection in a Kidney Transplant Patient Due to Inaccuracies in Commonly Available Diagnostic Tests. *Exp Clin Transpl Off J Middle East Soc Organ Transpl* (2020) 18(3):385–9. doi:10.6002/ect.2018.0107
34. Cordey S, Vu D-L, Schibler M, L'Huillier AG, Brito F, Docquier M, et al. Astrovirus MLB2, a New Gastroenteric Virus Associated with Meningitis and Disseminated Infection. *Emerg Infect Dis* (2016) 22(5):846–53. doi:10.3201/eid2205.151807
35. Graus F, Titulaer MJ, Balu R, Benseler S, Bien CG, Cellucci T, et al. A Clinical Approach to Diagnosis of Autoimmune Encephalitis. *Lancet Neurol* (2016) 15(4):391–404. doi:10.1016/S1474-4422(15)00401-9
36. Wilson MR, O'Donovan BD, Gelfand JM, Sample HA, Chow FC, Betjemann JP, et al. Chronic Meningitis Investigated via Metagenomic Next-Generation

Sequencing. *JAMA Neurol* (2018) 75(8):947–55. doi:10.1001/jamaneurol.2018.0463

37. Wilson MR, Zimmermann LL, Crawford ED, Sample HA, Soni PR, Baker AN, et al. Acute West Nile Virus Meningoencephalitis Diagnosed via Metagenomic Deep Sequencing of Cerebrospinal Fluid in a Renal Transplant Patient. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2017) 17(3):803–8. doi:10.1111/ajt.14058
38. Humières CD, Salmona M, Dellière S, Leo S, Rodriguez C, Angebault C, et al. The Potential Role of Clinical Metagenomics in Infectious Diseases: Therapeutic Perspectives. *Drugs* (2021) 81(13):1453–66. doi:10.1007/s40265-021-01572-4
39. Shen Y, Yu G, Zhao W, Lang Y. Efficacy of Xpert MTB/RIF Ultra in Diagnosing Tuberculosis Meningitis: A Systematic Review and Meta-Analysis. *Medicine (Baltimore)* (2021) 100(29):e26778. doi:10.1097/MD.0000000000026778
40. Pappas PG, Alexander BD, Andes DR, Hadley S, Kauffman CA, Freifeld A, et al. Invasive Fungal Infections Among Organ Transplant Recipients: Results of the Transplant-Associated Infection Surveillance Network (TRANSNET). *Clin Infect Dis* (2010) 50(8):1101–11. doi:10.1086/651262
41. Pasteur I. Rapports d'activité du CNR des Mycoses invasives et antifongiques. *Inst. Pasteur*. (2016) [cited 2018 Jun 28]. Available from: https://www.pasteur.fr/fr/sante-publique/CNR/les-cnr/mycoses-invasives-antifongiques/rapports-d-activite.
42. Vilchez RA, Fung J, Kusne S. Cryptococcosis in Organ Transplant Recipients: An Overview. *Am J Transpl* (2002) 2(7):575–80. doi:10.1034/j.1600-6143.2002.20701.x
43. Singh N, Dromer F, Perfect JR, Lortholary O. Cryptococcosis in Solid Organ Transplant Recipients: Current State-Of-The-Science. *Clin Infect Dis Off Publ Infect Dis Soc Am* (2008) 47(10):1321–7. doi:10.1086/592690
44. Singh N, Forrest G. Cryptococcosis in Solid Organ Transplant Recipients. *Am J Transpl* (2009) 9(4):S192–S198. doi:10.1111/j.1600-6143.2009.02911.x
45. Dromer F, Mathoulin S, Dupont B, Letenneur L, Ronin O, Group FCS. Individual and Environmental Factors Associated with Infection Due to Cryptococcus Neoformans Serotype D. French Cryptococcosis Study Group. *Clin Infect Dis Oxford Acad* (1996) 23(1):91–6. doi:10.1093/clinids/23.1.91
46. Liyanage DS, Pathberiya LPS, Gooneratne IK, Caldera MHPC, Perera PWS, Gamage R. Cryptococcal Meningitis Presenting with Bilateral Complete Ophthalmoplegia: a Case Report. *BMC Res Notes* (2014) 7:328. doi:10.1186/1756-0500-7-328
47. Maziarz EK, Perfect JR. *Cryptococcosis Infect Dis Clin North Am* (2016) 30(1):179–206. doi:10.1016/j.idc.2015.10.006
48. Pergam SA, Limaye AP. Varicella Zoster Virus in Solid Organ Transplantation: Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. *Clin Transplant* (2019) 33:e13622. doi:10.1111/ctr.13622 Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/ctr.13622.
49. Scemla A, Manda V. Infectious Complications after Kidney Transplantation. *Néphrologie Thérapeutique* (2019) 15:S37–S42. doi:10.1016/j.nephro.2019.03.010
50. Gabarre P, Loens C, Tamzali Y, Barrou B, Jaisser F, Tourret J. Immunosuppressive Therapy after Solid Organ Transplantation and the Gut Microbiota: Bidirectional Interactions with Clinical Consequences. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2021) 22:1014–30. doi:10.1111/ajt.16836
51. Fricke WF, Maddox C, Song Y, Bromberg JS. Human Microbiota Characterization in the Course of Renal Transplantation. *Am J Transpl* (2014) 14(2):416–27. doi:10.1111/ajt.12588
52. Tourret J, Willing BP, Dion S, MacPherson J, Denamur E, Finlay BB. Immunosuppressive Treatment Alters Secretion of Ileal Antimicrobial Peptides and Gut Microbiota, and Favors Subsequent Colonization by Uropathogenic *Escherichia coli*. *Transplantation* (2017) 101(1):74–82. doi:10.1097/TP.0000000000001492

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ORIGINAL RESEARCH
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Everolimus Based Immunosuppression Strategies in Adult Lung Transplant Recipients: Calcineurin Inhibitor Minimization Versus Calcineurin Inhibitor Elimination

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Everolimus (EVE) provides an alternative to maintenance immunosuppression when conventional immunosuppression cannot be tolerated. EVE can be utilized with a calcineurin inhibitor (CNI) minimization or elimination strategy. To date, clinical studies investigating EVE after lung transplant (LTx) have primarily focused on the minimization strategy to preserve renal function. The primary aim was to determine the preferred method of EVE utilization for lung transplant recipients (LTR). To undertake this aim, we compared the safety and efficacy outcomes of EVE as part of minimization and elimination immunosuppressant regimens. Single center retrospective study of 217 LTR initiated on EVE (120 CNI minimization and 97 CNI elimination). Survival outcomes were calculated from the date of EVE commencement. On multivariate analysis, LTR who received EVE as part of the CNI elimination strategy had poorer survival outcomes compared to the CNI minimization strategy [HR 1.61, 95% CI: 1.11–2.32, p=0.010]. Utilization of EVE for renal preservation was associated with improved survival compared to other indications [HR 0.64, 95% CI: 0.42–0.97, p=0.032]. EVE can be successfully utilized for maintenance immunosuppression post LTx, particularly for renal preservation. However, immunosuppressive regimens containing low dose CNI had superior survival outcomes, highlighting the importance of retaining a CNI wherever possible.

Keywords: lung transplantation, everolimus, calcineurin inhibitor, lung transplant recipients, mammalian-target-of-rapamycin inhibitor, lung transplant survival, nephrotoxicity

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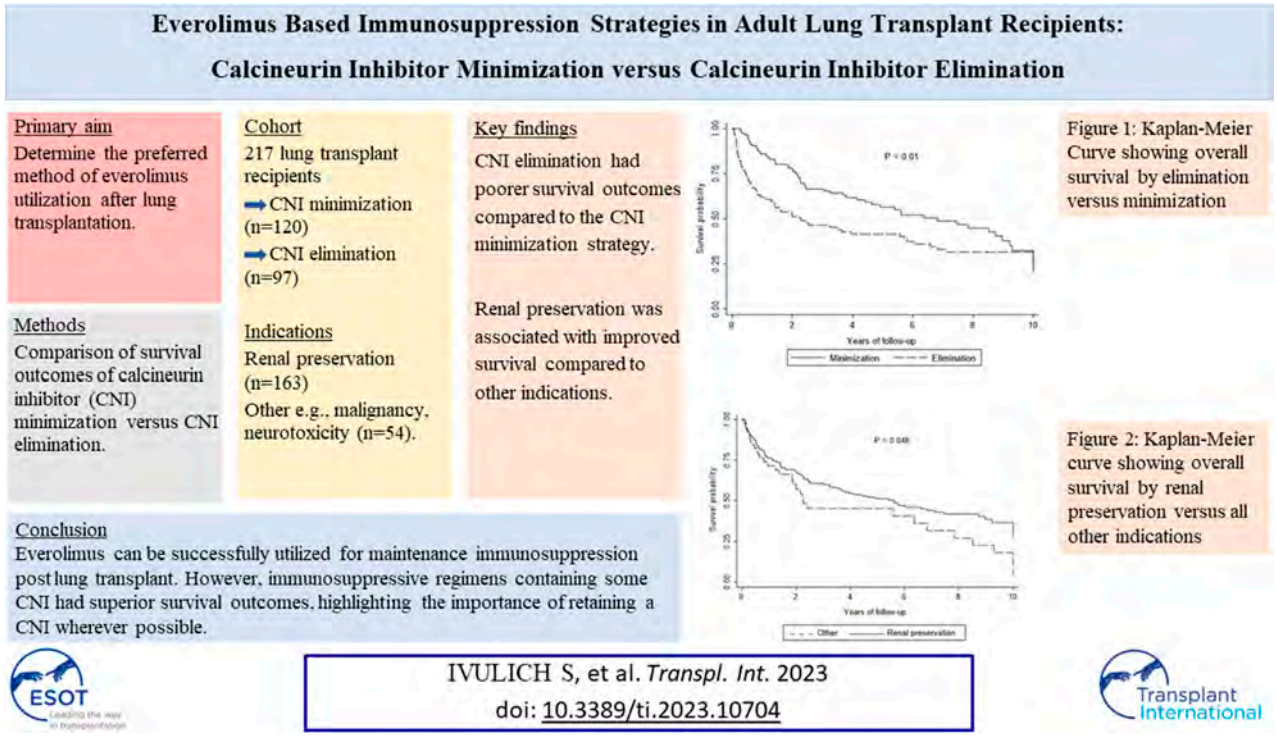
INTRODUCTION

Maintenance immunosuppressant regimens after lung transplantation (LTx) typically consist of a calcineurin inhibitor (CNI, tacrolimus or ciclosporin), an antiproliferative (mycophenolate or azathioprine) and a corticosteroid (1). Immunosuppressant regimens protect against the development of chronic lung allograft rejection (CLAD), the main barrier preventing better long-term outcomes (2).



GRAPHICAL ABSTRACT |

CNIs remain the cornerstone of immunosuppression post LTx to prevent allograft rejection, but their usage is limited by several side effects, predominantly nephron- and neurotoxicity. Antiproliferative immunosuppressants are associated with leukopenia, gastrointestinal side effects and hepatotoxicity (3, 4), and prednisolone is associated with diabetes mellitus and osteoporosis (5). Everolimus (EVE) provides a useful alternative when conventional maintenance regimens cannot be tolerated.

EVE has unique pharmacological effects distinct from other immunosuppressants. EVE may reduce the risk of malignancy or *Cytomegalovirus* (CMV) infection (6). However, EVE has its own limitations in the LTx setting with its use not recommended early post-LTx due to the risk of wound dehiscence and an association with pneumonitis, proteinuria, non-healing wounds, hematological and metabolic side effects (hyperlipidemia) (7).

The proportion of LTR not taking a CNI is rare with the International Society for Heart and Lung Transplantation (ISHLT) estimating that approximately 99% of lung transplant recipients (LTR) are taking a CNI at time of 1-year follow up (8). Reducing CNI exposure is a key component to reducing long-term CNI toxicity. To date, clinical studies investigating EVE after LTx have primarily focused on the minimization strategy to preserve renal function (9–13), with a paucity of evidence for elimination or other indications (e.g., malignancy, neurotoxicity). Most trials so far have demonstrated high rates of discontinuation, patient intolerance and significant side effects (9–11).

Given the limited evidence for CNI elimination after LTx and the implausibility of a randomized controlled trial, the primary aim of this study was to determine the preferred method of EVE utilization for LTR. To undertake this aim, we retrospectively compared the safety and efficacy outcomes of EVE as part of minimization and elimination immunosuppressant regimens.

MATERIALS AND METHODS

Between 2008 and 2020, 1,300 LTx were undertaken. In the current study, all recipients received standard triple immunosuppression with tacrolimus, an antimetabolite (azathioprine or mycophenolate mofetil) and corticosteroids. Ciclosporin was utilized as a second line CNI in settings where tacrolimus was withdrawn (e.g., CNI-neurotoxicity), but where the inclusion of a CNI was considered essential to maintain adequate immunosuppression. All individuals prescribed EVE were considered for inclusion with EVE being prescribed as a second line agent in 240 (18.5%). Excluded were those: lost to follow up, early discontinuation (duration of therapy <3 months), incomplete medical records or previous sirolimus therapy.

Therapeutic Drug Monitoring (TDM)

Therapeutic Drug Monitoring (TDM) for ciclosporin and tacrolimus trough concentrations were obtained using ACQUITY Ultraperformance Liquid Chromatography (Waters Corporation, Manchester, United Kingdom). Ciclosporin trough concentration

targets were 225–300 ng/mL in the first 3 months, 190–260 ng/mL between 3–12 months, and 150–225 ng/mL thereafter. Tacrolimus trough concentration targets were 10–12 ng/mL in first 6 months, 8–10 ng/mL between 6–12 months, and 4–8 ng/mL thereafter. Ciclosporin and tacrolimus targets were clinically modified in the presence of rejection episodes, significant renal impairment, or systemic sepsis (1, 14).

EVE Indications, Dosing, and Utilization Strategy

EVE was utilized for clinical LTx where the traditional initial immunosuppressive strategies were contraindicated (e.g., significant renal impairment, CNI-neurotoxicity, malignancy) (1, 14). Allocation to either strategy was determined by the degree of CNI intolerance at the time of initiation of EVE, and the decision to withdraw or reduce the CNI was based on clinical judgement. As per unit protocol, EVE was typically commenced at a moderate dose of 0.25–0.5 mg twice daily, with the CNI dose immediately halved (15). The dose was subsequently adjusted according to the target level for the strategy utilized.

All included LTR were subsequently divided into two groups: CNI minimization (Group A) or CNI elimination (Group B). For CNI minimization, when EVE was to be used in conjunction with a CNI (Group A), an EVE trough serum concentration of 3–5 ng/mL was targeted. If CNI cessation was planned, an EVE trough serum concentration of 5–7 ng/mL was targeted and the CNI was ceased once the EVE trough level was >3 ng/mL.

If a LTR remained on a CNI following introduction of EVE (Group A) for renal preservation and measured serum creatinine concentrations continued to increase or remained high, they would then typically transition to Group B and the CNI withdrawn. LTR who withdrew from a CNI within 90 days post EVE introduction were included as CNI elimination. Concomitant medication with azathioprine or mycophenolate plus prednisolone were continued according to local practice.

Donor Assessment, Recipient Selection, Transplantation Procedures and Postoperative Management

Our Alfred approach to lung donor referral, assessment and transplantation is described elsewhere (16, 17). Recipient selection is based on International and National Guidelines (18). Donor-recipient matching was generally undertaken according to our standard protocol as described elsewhere (19, 20). All patients received prophylactic antibiotics based on known or suspected donor and recipient microbiology results. CMV prophylaxis, monitoring and treatment strategies are described elsewhere (21). Surveillance bronchoscopy and transbronchial biopsies were performed as per hospital protocol at 2, 6-, 12-, 26- and 52-week post LTx.

Definition of Rejection

Acute cellular rejection (ACR) was defined as changes on transbronchial biopsy of $\geq$ ISHLT Grade 2, or in the absence of a biopsy an otherwise unexplained drop in lung function treated with intravenous corticosteroid (14, 22). CLAD was clinically diagnosed and defined by LTx clinic spirometry, and treated according to established practice and standard protocols at the time (14).

Pulmonary Toxicity

The onset of EVE induced pulmonary toxicity was diagnosed with the presence of clinical symptoms (e.g., dyspnoea, cough, or fever) and radiological signs of pulmonary involvement (pulmonary computed tomography scans or abnormal chest X-ray) not compatible with other diagnoses. Pulmonary symptoms would typically resolve after the discontinuation of EVE. Although histological diagnosis is considered the gold standard, this was not always undertaken (23). Without radiological findings or the exclusion of other pulmonary diseases, LTR were classified as suspected CLAD according to ISHLT criteria (24).

General Management Strategy for Renal Preservation

Induction therapy with the IL-2 receptor blocker, basiliximab was given as a CNI-sparing agent to LTR who were identified pre-transplant as being at higher risk of developing post-LTx renal dysfunction (n = 95). Subsequent strategies for LTR with renal impairment involved CNI reduction or elimination; control of hypertension, diabetes mellitus, and cholesterol; and initiation of EVE (25). Recipients of both strategies were routinely screened for evidence of proteinuria prior to conversion to EVE based immunosuppression. Proteinuria was not present in any LTR at baseline.

Study End Points

The primary endpoint for efficacy was survival, with secondary endpoints measured being incidence of CLAD and ACR. An additional secondary endpoint investigated was the impact of discontinuation on survival. The safety of the various strategies was assessed by measuring renal, hematological, and metabolic markers at baseline and 3 months after initiating EVE.

Statistical Analyses

Continuous data were summarized using means and standard deviations (SD) or medians and interquartile ranges (IQR) depending on distribution, and categorical data as counts and percentages. Comparisons between groups (minimization versus elimination strategy) were made using Student's t-test or Mann-Whitney U test as appropriate for continuous variables and chi-square or Fisher's exact test for categorical variables. Overall survival was defined as the time from the date of starting EVE to the date of death or last follow-up.

Univariate and multivariate analyses for overall survival were performed using Cox proportional hazards regression with results reported as hazard ratios (HR) and 95% confidence intervals (95% CI). Variables with a $p < 0.05$ on univariate analyses or those deemed clinically relevant were considered

TABLE 1 | Demographics

Characteristic	CNI minimization (n = 120)	CNI elimination (n = 97)	p-value
Age (yr), mean ± SD	52.8 ± 13.7	49.8 ± 15.0	0.12
Gender: male, (n%)	63 (52.5)	63 (64.9%)	0.07
Indications for transplantation, n (%)			
Chronic obstructive pulmonary disease	50 (41.7)	42 (43.3)	0.32
Cystic fibrosis/bronchiectasis	23 (19.2)	24 (24.7)	0.81
Interstitial lung disease	32 (26.6)	20 (20.6)	0.30
Pulmonary hypertension	9 (7.5)	6 (6.2)	0.70
Redo transplant	6 (5.0)	5 (5.2)	0.96
Transplantation type, n (%)			
Bilateral sequential lung	99 (82.5)	72 (74.2)	0.14
Single lung	16 (13.3)	19 (19.6)	0.21
Heart and lung	5 (4.2)	6 (6.2)	0.50
Early initiation of everolimus[a]	43 (35.7%)	33 (34%)	0.78
Indication for everolimus, (n, %)			
Renal Preservation (163)	87 (72.5)	76 (81.4)	0.32
Malignancy (28)			
Skin cancers	18 (15.0)	6 (6.2)	0.04
Other (PTLD, lung cancer)	1 (0.8)	3 (3.1)	
Neurotoxicity (18)			0.48
PRES	0 (0)	4 (4.1)	
Seizures	0 (0)	3 (3.1)	
Neurocognitive disorder	2 (1.7)	2 (2.1)	
Neuropathy (peripheral and optic)	2 (1.7)	1 (1.0)	
Tremor	3 (2.5)	0 (0)	
Migraines	1 (0.8)	0 (0)	
Other (8)			0.91[b]
Augment immunosuppression (6)	5 (4.2)	1 (1.0)	
Recurrent CMV (2)	1 (0.8)	1 (1.0)	

[a]*Everolimus started less than 1 year after lung transplant.*
[b]*Other includes other non-skin cancer malignancy.*
Abbreviations: CMV, Cytomegalovirus; *PTLD, post-transplant lymphoproliferative disorder; PRES, posterior reversible encephalopathy syndrome.*

for inclusion in the multivariable models. The Kaplan-Meier product-limit method was used to plot survival as a function of time after starting EVE, and comparisons between curves were made with the log-rank test. Changes in serum creatinine concentrations and eGFR from time of starting EVE to 3 months post were determined using paired t-test or Wilcoxon signed rank test as appropriate. All calculated p values were 2-tailed and a $p < 0.05$ indicated statistical significance.

Statistical analyses were performed with SAS version 9.4 (SAS Institute, Cary, NC, United States).

RESULTS

Patient Characteristics and Indications for EVE Use

The final cohort consisted of 217 LTR who started on EVE (120 CNI minimization and 97 CNI elimination). Baseline demographics are shown in **Table 1**. The most common indication for starting EVE was renal preservation (75%), followed by malignancy (13%) and neurotoxicity (8%) (**Table 1**). The median time from LTx to EVE initiation was 528 days [IQR: 240–1,460] with the median time of follow up for all LTR included being 1998 days [IQR: 938–3,770].

Efficacy

Overall Survival, CLAD and ACR

On multivariate analysis, LTR who received EVE as part of the CNI elimination strategy had poorer survival outcomes compared to the CNI minimization strategy [HR 1.61, 95% CI: 1.11–2.32, p = 0.010] (**Figure 1**). The median survival for the entire cohort was 1,797 days [IQR: 401–4,427] (CNI minimization: 2,491 days [IQR: 789–4,171] vs. CNI elimination: 840 days [IQR: 166–4,427]). Overall survival between the minimization and elimination groups at 1, 3 and 5 years was significant (1 year: 85.9% vs. 61.9%, $p \leq$ 0.0001, 3 years: 66.5% vs. 46.4%, p = 0.004, 5 years: 56.5% vs. 41.5%, p = 0.41). The incidence of ACR and CLAD was similar

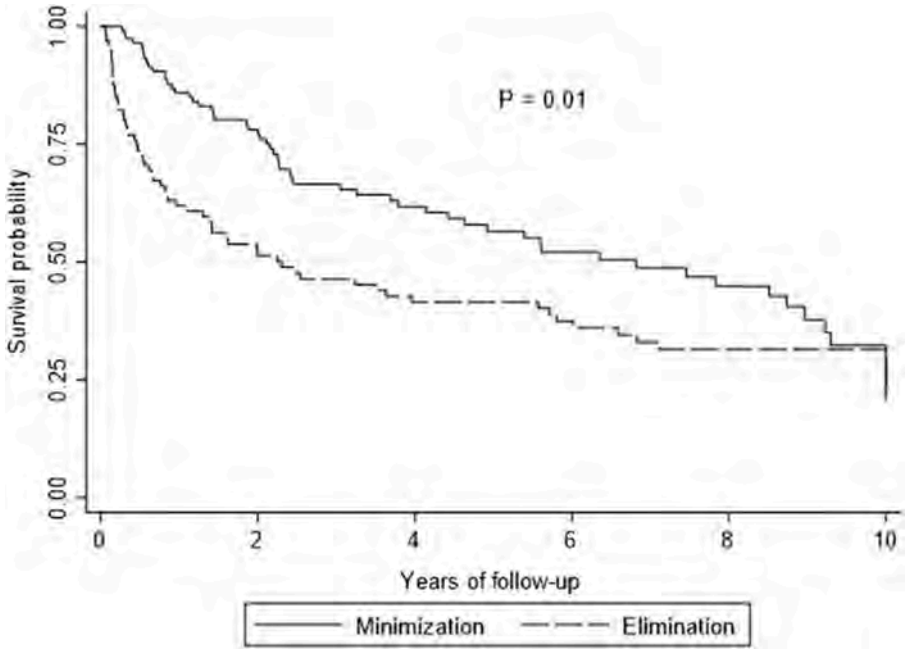


FIGURE 1 | Kaplan-Meier curve showing overall survival by elimination versus minimization. *p*-value is calculated from a log rank test comparing the entire survival experience between the two groups (minimization versus elimination).

between the groups (p = .76 and p = 0.83, respectively) (**Table 2**).

Preservation of Renal Function

On multivariate analysis, utilization of EVE for renal preservation was associated with improved survival compared to other indications [HR 0.64, 95% CI: 0.42–0.97, p = 0.032] (**Figure 2**). All LTR who started on EVE for renal preservation had an improvement in estimated glomerular filtration rate (eGFR) from 40 ± 16 mL/min/1.73 m^2 to 47 ± 19 mL/min/1.73 m^2 (p = 0.021) at 3 months after starting EVE.

The most significant improvements with renal function were demonstrated with the CNI elimination strategy at 3 months after commencing EVE. The mean serum creatinine concentration decreased from 164 ± 63 μmol/L to 136 ± 72 μmol/L (p = 0.0004) and eGFR improved from 42 mL/min/1.73 m^2 to 52 mL/min/1.73 m^2 ($p \leq 0.0001$). The improvements over 3 months for measured serum creatinine concentration and eGFR with the CNI minimization strategy were lower but also statistically significant (p = 0.021 and p = 0.019, respectively) (**Figure 3**). At 1-year after commencing EVE, measurements of kidney function (creatinine and eGFR) were comparable to those measured at 3 months after starting EVE (139 ± 67 μmol/L and 50 mL/min/1.73 m^2, respectively).

On multivariate analysis, high urinary protein levels measured at 3 months after starting EVE were associated with poorer survival outcomes [HR 1.21, 95% CI: 1.01–1.45, p = 0.033]. Proteinuria in the nephrotic range (≥3,000 mg/24 h) was present in four patients at 3 months after starting EVE, all occurring with the elimination strategy. At 3 months post conversion to EVE, the elimination strategy had a statistically higher level of measured urinary protein compared to the minimization strategy (CNI minimization: 0.075 g/L [IQR: 0.04–0.165] vs. CNI elimination: 0.16 g/L [IQR: 0.06–0.31], p = 0.001).

Malignancy

The survival outcomes for LTR prescribed EVE for malignancy were comparable to other indications (p = 0.631). A statistically higher proportion of LTR received EVE as part of the CNI minimization strategy for malignancy (p = 0.04), primarily for the management of skin cancers. EVE was introduced further from LTx (1,358 days [IQR: 743–1,967]), with EVE replacing azathioprine in 62.5% of LTR with malignancy.

Neurotoxicity

On univariate analysis, LTR who received EVE in the setting of neurotoxicity had poorer survival outcomes compared to other indications for EVE [HR 1.83, 95% CI: 1.05–3.18, p = 0.030], however this was not borne out in multivariate analysis. The median survival for LTR prescribed EVE for neurotoxicity as part of a CNI elimination strategy was markedly lower than the minimization strategy (CNI elimination: 292 days [IQR: 226–652] vs. CNI minimization: 1,725 days [IQR: 696–3,460]).

Safety

Discontinuation

Discontinuation of EVE due to adverse events was 39.2% for all LTR included in the study with a statistically higher number of

TABLE 2 | Clinical characteristics: Immunosuppression, theraputic drug monitoring, rejection and comorbidities.

Characteristic (n, %)	CNI minimization (*n* = 120)	CNI elimination (*n* = 97)	*p*-value
Immunosuppression			
Induction, n (%)			
Basiliximab	50 (41.7)	45 (46.4)	0.49
Maintenance Immunosuppression, n (%)[a]			
Tacrolimus	108 (90.0)	73 (75.3)	0.004
Ciclosporin	12 (10.0)	24 (24.7)	0.004
Mycophenolate	31 (25.8)	39 (40.2)	0.024
Azathioprine	33 (27.5)	27 (27.8)	0.96
No Antimetabolite	56 (47.6)	31 (32.0)	0.028
Therapeutic drug monitoring			
Tacrolimus			
Mean tacrolimus dose[b]	4.46 ± 2.94 mg		
Mean tacrolimus level[c]	5.46 ± 1.87 ng/mL		
Ciclosporin			
Mean ciclosporin dose[b]	120.84 ± 45 mg		
Mean ciclosporin concentration[c]	89.91 ± 63.62 ng/mL		
Everolimus			
Mean everolimus dose[b]	1.71 ± 0.91 mg	1.68 ± 1.11 mg	0.87
Mean everolimus concentration[c]	4.14 ± 2.12 ng/mL	5.58 ± 2.59 ng/mL	<0.0001
Rejection			
Rejection, n (%)			
Diagnosis of CLAD[d]	85 (70.8)	70 (72.2)	0.83
Acute Rejection—Pulse of Steroids[e]	18 (15.0)	16 (16.5)	0.76
Antithymocyte globulin (Equine or rabbit)	27 (22.5)	13 (13.4)	0.09
Laboratory results and comorbidities			
Hemoglobin[f], (mean ± SD)	115.8 ± 17.0 g/L	112.2 ± 21.4 g/L	0.07
White cell count[f], (mean ± SD)	6.77 ± 2.57 × 10^9/L	7.68 ± 3.63 10^9/L	0.032
Platelets[f], (mean ± SD)	236 ± 76.8 × 10^9/L	255 ± 123 × 10^9/L	0.16
Urinary protein[f] (median, IQR)	0.075 g/L (IQR: 0.04–0.165)	0.16 g/L (IQR: 0.06–0.31)	0.001
Hypertension[g]	74 (61.7)	56 (57.7)	0.56
Diabetes Mellitus[g]	47 (39.2)	37 (38.1)	0.88
Hyperlipidemia[g]	53 (44.2)	41 (42.3)	0.78
Cytomegalovirus reactivation[h]	30 (25.0)	32 (32.9)	0.20
Aspergillus colonization[h]	43 (35.8)	32 (33.0)	0.66

[a]*At the time of starting EVE.*
[b]*Total daily dose within 3 months after initiating.*
[c]*Within first 3 months after initiating.*
[d]*Diagnosis of CLAD pre or post starting on everolimus.*
[e]*Episode of ISHLT graded ≥2 ACR pre or post starting on everolimus.*
[f]*Measured at 3 months after starting everolimus.*
[g]*Comorbidity at 3 months after starting everolimus.*
[h]*CMV Reactivation or* Aspergillus *colonization at any time point after starting everolimus.*
Abbreviations: ACR, acute cellular rejection; CLAD, chronic lung allograft dysfunction; CMV, Cytomegalovirus*; ISHLT, International Society of Heart and Lung Transplantation.*

LTR discontinuing with the minimization cohort (CNI minimization: 48.3% versus CNI elimination: 29.9%, $p = 0.002$). On univariate analysis, LTR who discontinued EVE did not have poorer survival outcomes compared to those that remained on EVE [HR 1.06, 95% CI: 0.73–1.52, $p = 0.764$]. Pulmonary problems (18.4%), wound healing (5.1%) and edema (4.1%) were the most frequent causes of discontinuation (**Table 3**).

Pulmonary Toxicity

The most common reason for discontinuation of EVE was pulmonary related, with 18.4% of all patients discontinuing due to pulmonary toxicity or accelerated decline in spirometry/CLAD. LTR with pulmonary toxicity were more likely to discontinue EVE within the first year of initiating EVE (3-month discontinuation, 17.6% and 12-month discontinuation, 82.3%), with the median time of onset to pulmonary toxicity being 154 days [IQR: 107–304].

EVE trough concentrations were not supratherapeutic in LTR with pulmonary toxicity, with the mean trough concentration being 4.07 ± 1.52 ng/mL. All LTR had a full clinical recovery within 1 year of discontinuation with no fatalities attributed to pulmonary toxicity. Thirteen of the seventeen patients with pulmonary toxicity had confirmed CLAD at the time, confounding the diagnosis.

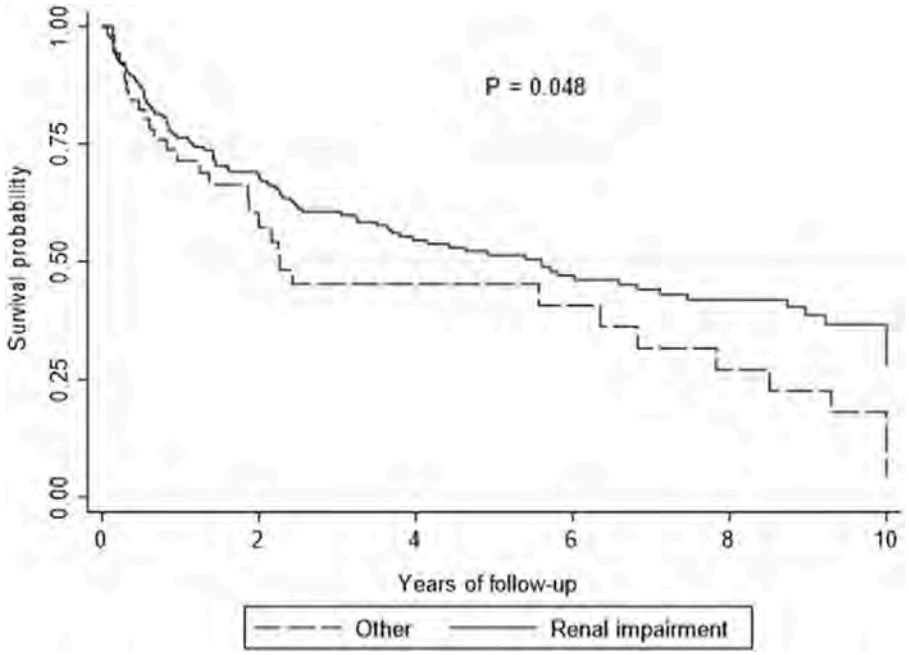


FIGURE 2 | Kaplan-Meier curve showing overall survival by renal preservation versus other indications.

Adverse Events and Laboratory Results

The incidence of adverse events was similar between the two treatment groups. Rates of hypertension, new-onset diabetes mellitus and hyperlipidemia were comparable at 3 months after starting EVE. The incidence of CMV reactivation or *Aspergillus* colonization was similar between the groups (**Table 2**).

Measured hemoglobin concentrations and platelet counts were comparable at 3 months after starting on EVE, whereas the CNI minimization group accounted for a statistically lower white cell count (p = 0.032) (**Table 2**).

Immunosuppression and Therapeutic Drug Monitoring

The most common immunosuppression regimens were EVE, tacrolimus, and prednisolone for CNI minimization (42.5%) and EVE, mycophenolate, and prednisolone for CNI elimination (40.2%). As expected, the mean EVE trough concentration within the first month after starting EVE was significantly lower with CNI minimization (mean trough concentration: 4.14 ± 2.12 ng/mL [Aim 3–5 ng/mL]) compared to CNI elimination (mean trough concentration: 5.58 ± 2.59 ng/mL [Aim 5–7 ng/mL], $p \leq 0.0001$). Key differences in immunosuppressive strategies are outlined in **Table 2**. Weaning dosage protocols after LTx for antimetabolite and prednisolone after LTx were comparable across the study groups.

On multivariate analysis, immunosuppressant regimens containing mycophenolate were associated with improved survival [HR 0.66, 95% CI: 0.44–0.99, p = 0.038]. All other immunosuppression and serum trough levels within the first month after starting EVE had no impact on survival (**Table 4**).

Cause of Death

There was no difference in CLAD-related mortality between the two cohorts (25.0% CNI minimization vs. 27.8% CNI elimination, p = 0.36). A statistically higher number of LTR died from infection within the elimination group (p = 0.036). Sepsis (bacterial n = 8, fungal n = 1), viral pneumonia (n = 1) and CMV infection (n = 1) were the most common reasons for infection-related mortality. Although the most common indication for starting EVE, death due to renal failure occurred in only six patients (**Table 5**).

DISCUSSION

Minimization Versus Elimination

We believe that this is the largest study investigating the feasibility of CNI-free maintenance regimens. Our study found that LTR receiving EVE as part of an elimination strategy had poorer survival outcomes compared to the minimization strategy. A potential explanation could be that the higher mortality rate for the elimination strategy reflects the clinical complexity of this group requiring complete CNI withdrawal. The elimination strategy included LTR with a higher measured serum creatinine, advanced malignancy, sepsis, or manifestations of neurotoxicity, such as seizures or PRES. Compared to ISHLT registry data, the 1 and 5-year survival outcomes for

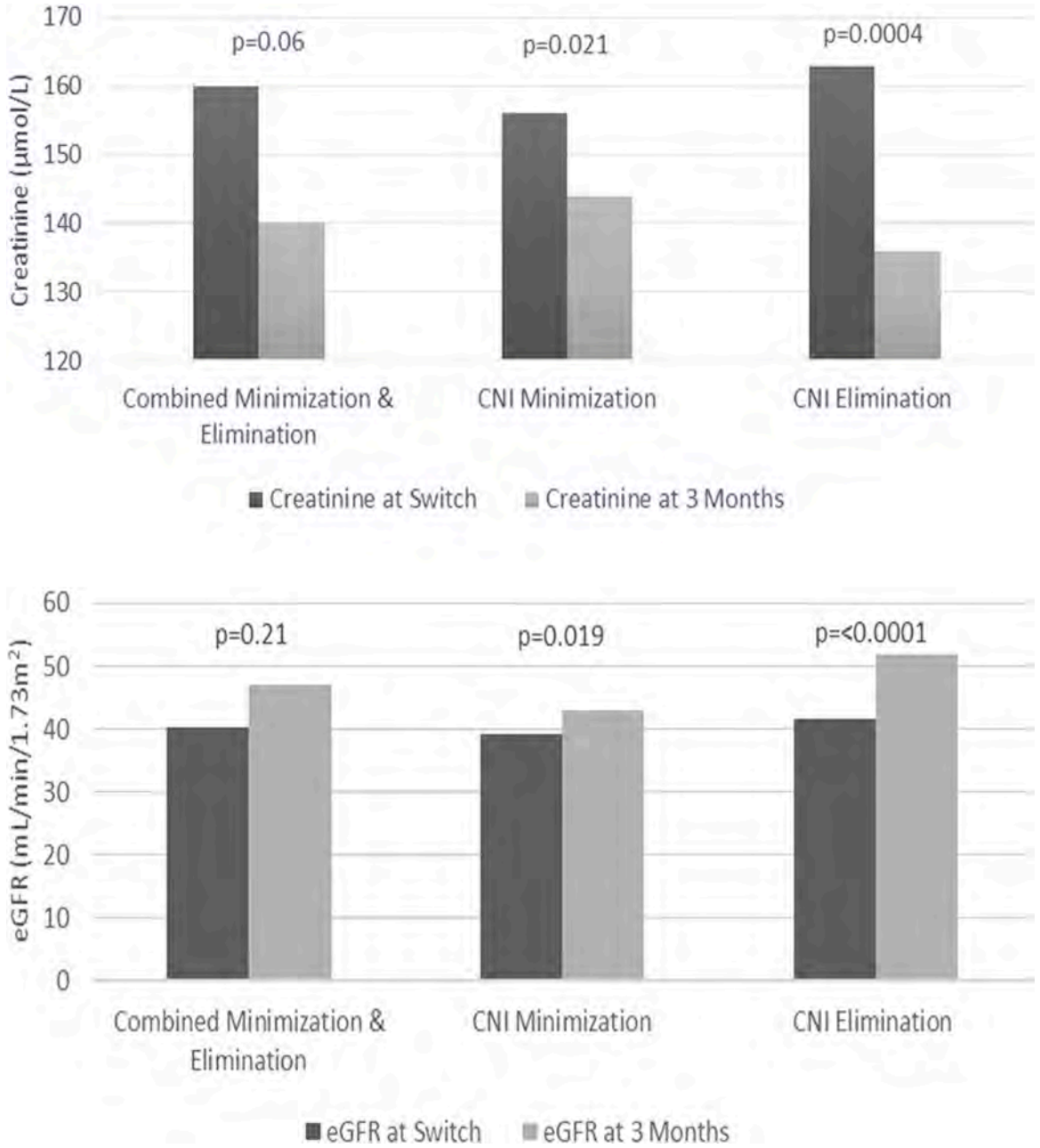


FIGURE 3 | Creatinine and estimated glomerular filtration rate (eGFR) changes from time to switch to 3 months.

both groups receiving EVE was markedly lower (26), indicating that the selection of our LTR receiving EVE may be representative of a clinically complex group of LTR. The incidence of CLAD between the two groups was also higher compared to ISHLT registry data, with CLAD being the leading cause of death for both strategies (27).

Nonetheless, CNIs remain the cornerstone of maintenance immunosuppressive regimens and our study provides further evidence that inclusion of CNIs remain paramount and long-term withdrawal may adversely impact survival. Considering the poorer survival outcomes for the elimination strategy, an assessment of the risks for a rechallenge with a lower dose CNI should be undertaken once stabilization of the LTR occurs, especially for those early after LTx. An alternative could be the incorporation of other CNI-sparing agents with EVE-based immunosuppression, such as intravenous immunoglobulin (IVIG) or belatacept to elevate survival outcomes to those comparable with CNI-based immunosuppression. This approach would require further investigation.

TABLE 3 | Everolimus discontinuation.

	CNI minimization	CNI elimination	*p*-value
(n, %)	*n* = 58 (48.3)	*n* = 27 (27.8)	0.002
Pulmonary (40)			
Drop in lung function/CLAD	18 (31)	5 (18.5)	0.23
Pulmonary toxicity	11 (19)	6 (22.2)	0.73
Peripheral oedema (9)	5 (8.6)	4 (14.8)	0.39
Hematological (7)	4 (6.9)	3 (11.1)	0.51
Wound healing (11)	7 (12.1)	4 (14.8)	0.72
Renal (9)			
Proteinuria	3 (5.2)	3 (11.1)	0.38
Dialysis/renal failure	2 (3.4)	1 (3.7)	1.00
Other (9)			
Dermatological (3)	2 (3.4)	1 (3.7)	1.00
Mouth ulcers (2)	2 (3.4)	0 (0)	1.00
Resolution of neurotoxicity (2)	2 (3.4)	0 (0)	1.00
Chronic Infection (1)	1 (1.7)	0 (0)	1.00
Leg pain (1)	1 (1.7)	0 (0)	1.00

Abbreviations: CLAD, chronic lung allograft dysfunction.

TABLE 4 | Univariate and multivariate analysis of factors influencing survival.

Summary of effects of different covariates on survival			
	Variable	**Hazard ratio (95% CI)**	***p*-value**
Univariate Analysis			
Demographics	Male	0.88 (0.61–1.26)	0.468
	Age	1.01 (0.99–1.02)	0.446
Everolimus Strategy	Early initiation of everolimus[a]	1.13 (0.77–1.67)	0.524
	Elimination strategy	1.54 (1.07–2.22)	0.017
Rejection	CLAD diagnosis[b]	1.83 (1.17–2.86)	0.007
Indication for Everolimus	Renal Preservation	0.64 (0.42–0.98)	0.034
	Neurotoxicity	1.83 (1.05–3.18)	0.030
	Skin cancer	0.83 (0.38–1.82)	0.631
Renal Indicators	Creatinine (Introduction of everolimus)	1.00 (1.00–1.01)	0.358
	Creatinine (3 months after introduction)	1.00 (1.00–1.00)	0.314
	Urinary protein	1.22 (1.03–1.46)	0.021
Immunosuppression	Basiliximab	1.31 (0.90–1.89)	0.148
	Tacrolimus	1.40 (0.87–2.25)	0.162
	Ciclosporin	0.72 (0.44–1.15)	0.162
	Mycophenolate	0.66 (0.45–0.99)	0.040
	Azathioprine	1.05 (0.71–1.55)	0.804
	No antimetabolite	1.46 (0.99–2.14)	0.051
Therapeutic Drug Monitoring[c]	Tacrolimus level[d]	1.07 (0.75–1.54)	0.704
	Everolimus level	1.01 (0.94–1.07)	0.863
Multivariate analysis			
Variable	Elimination strategy	1.61 (1.11–2.32)	0.010
	Renal preservation	0.64 (0.42–0.97)	0.032
	Urinary protein	1.21 (1.01–1.45)	0.033
	Mycophenolate	0.66 (0.44–0.99)	0.038

[a]*Everolimus started less than 1 year after lung transplant.*
[b]*Chronic lung allograft dysfunction pre or post starting everolimus.*
[c]*Average level within the first 3 months after starting everolimus.*
[d]*For minimization strategy.*
Abbreviations: ACR, acute cellular rejection; CLAD, chronic lung allograft dysfunction.

TABLE 5 | Cause of death.

Cause of death	CNI minimization	CNI elimination	*p*-value
(n, %)	*n* = 57 (47.5)	*n* = 61 (62.8)	0.024
CLAD	30 (25.0)	27 (27.8)	0.36
NSGF	11 (9.2)	9 (9.3)	0.51
Cancer	10 (8.3)	6 (6.2)	0.22
Infection	2 (1.7)	9 (9.3)	0.036
Renal failure	3 (2.5)	3 (3.1)	0.93
Coronary vascular accident	1 (0.8)	4 (4.1)	0.20
Other	0 (0)	3 (3.1)	0.09
Multi-organ failure			
Pancreatitis			
Hemophagocytic lymphohistiocytosis			

Abbreviations: CLAD, chronic lung allograft dysfunction; NSGF, non-specific graft failure.

Efficacy

Preservation of Renal Function

Renal impairment remains challenging post LTx, especially in the perioperative period with an increased risk of LTx-related morbidity and mortality (28). Management of renal impairment with EVE after LTx has been undertaken in several other studies (11, 13, 29, 30), and we demonstrated similar improvements with renal function, with no significant renal related mortality. We found that initiating EVE for renal preservation had superior survival outcomes compared to other indications (**Figure 3**).

Although CNI elimination was the most effective strategy at improving renal function, this was offset by the poorer survival outcomes compared to CNI minimization group. The benefits of the CNI elimination strategy on renal function need to be balanced against the risks of having a CNI-free regimen on survival. Based on the findings of our study, CNI minimization with close monitoring of creatinine would be the suggested approach when starting EVE for renal preservation. If creatinine continues to deteriorate, then temporary transition to the elimination strategy with future rechallenge with low dose CNI or the introduction with alternative immunosuppressants may be required.

Malignancy

Existing transplant literature has postulated that transitioning from a CNI to EVE may have beneficial effects in LTR with malignancy, due to its effect on cell metabolism and proliferation (31, 32). Our cohort of LTR were typically initiated with EVE for malignancy further from LTx, highlighting the long-term impact of immunosuppression on the emergence of skin malignancies. Although malignancy represented a relatively small cohort with the majority being non-melanoma skin cancers, the survival outcomes were successful with comparable survival to other cohorts with only two deaths from skin malignancy. CNI minimization appears to be the preferred immunosuppressive strategy for LTR with skin malignancies. Cessation of the antimetabolite should be undertaken pre-emptively at the earliest sign of skin malignancy for high risk LTR.

Neurotoxicity

CNI-induced neurotoxicity can manifest in severity from headaches or tremor to life-threatening complications such as seizures or posterior reversible encephalopathy syndrome (PRES). The transplant literature notes early major neurologic complications post LTx can result in substantive morbidity and mortality (33–35), and our center experienced similar challenges and outcomes.

On univariate analysis, LTR who received EVE for the management of neurotoxicity had the poorest survival outcomes. Most noteworthy were LTR with neurotoxicity allocated to the CNI elimination group with a median survival of only 292 days from the date of commencing EVE. Although unavoidable in some settings, our findings support the evidence that early CNI withdrawal is not recommended. Less severe manifestations of neurotoxicity, such as tremor or headaches may be resolved with the CNI minimization strategy, where improvement of symptoms may occur with lower target CNI target levels.

Safety

Discontinuation of Everolimus

The rate of discontinuation of EVE for our cohort was 39.6%, and these rates are consistent with other LTx studies ranging in incidence from 25%–55% (9–11). We hypothesized that high rates of discontinuation of EVE documented elsewhere are potentially destabilizing for immunosuppressant regimens with poorer survival outcomes. Our study found that the high rates of discontinuation of EVE did not contribute to poorer survival outcomes. A potential explanation is that alternative immunosuppressants would typically be substituted in LTR discontinuing EVE, lessening periods of subtherapeutic immunosuppression, and improving survival outcomes.

Impact on Pulmonary Function

One of the most serious side effects from EVE is pneumonitis that can result in life-threatening complications (36). EVE-induced pneumonitis has been limited to case reports after thoracic transplantation (7), and our study identified 17 potential cases, corresponding with an incidence of 8.2%. Most cases of pulmonary toxicity occurred within the first year, consistent

with the available literature (37). Although higher trough concentrations of EVE are associated with pulmonary toxicity (38), we found that with our cohort all had serum trough concentrations within the target range.

Unsurprisingly, all LTR had underlying pulmonary disease making it difficult to distinguish CLAD from pulmonary toxicity. LTR with pulmonary toxicity all had radiological changes, confirming organizing pneumonia or interstitial pneumonitis. A limitation of our study was that histopathological diagnosis was not undertaken in all suspected cases.

Adverse Events

Side effects from EVE reported in other studies in LTx remain high, ranging from 35%–82% (9, 10, 40). Cardiovascular risk factors, such as hyperlipidemia, hypertension and diabetes are all associated with immunosuppression regimens (5, 41). At 3 months after starting an EVE based regimen, we found no significant difference in the incidence of the cardiovascular risk factors. Hematology parameters suggested that bone marrow toxicity was not a safety concern with EVE for either regimen.

The most common non-pulmonary related reason for discontinuing was edema. Incidence of edema leading to discontinuation was consistent with other published data (42). Similarly, we found that patients who ceased due to edema tended to be earlier post LTx.

Immunosuppression and Therapeutic Drug Monitoring

Our study highlighted the key differences between the two groups and provided an insight into our immunosuppression strategies. The most significant finding was that mycophenolate containing immunosuppressant regimens were associated with improved survival. Given the potential survival benefits of mycophenolate, we would suggest including mycophenolate in regimens, especially in those where the CNI has been withdrawn.

Therapeutic drug monitoring of EVE after LTx is not well defined. Transplant literature has recommended a serum trough concentration ($C_{(0)}$ of 3–8 ng/mL or 3–12 ng/mL has been postulated as part of minimization regimens post LTx (13, 15). Our study demonstrated lower EVE concentration targets compared to the existing transplant literature. Considering the lagging survival outcomes, it would appear there is a potential to raise the serum trough concentrations of EVE for CNI minimization. However, this approach would need to proceed with caution as it could potentially increase the risk of further intolerance in a cohort with high rates of discontinuation. In addition, this may not be due to a dose-dependent effect as other underlying disease mechanisms may not be targeted by our current agents.

LIMITATIONS

There are several limitations to this study. Firstly, the study is retrospective with all data obtained from chart reviews. Additionally, the findings reflect a single center experience, our institution did not have a detailed protocol to guide usage, and usage has also evolved over time. Our study was non-randomized, and the cohort was heterogenous, with multiple indications for EVE being included in the two cohorts. Allocation to either strategy was dependent on clinical judgement at the time of starting on EVE. Although demographics between the two groups were similar, CNI withdrawal was more common in those acutely unwell potentially biasing the outcomes. Noting the difficulty of prospective randomized controlled studies, our study did not compare the survival outcomes for CNI minimization or elimination strategies against standard CNI based immunosuppressive regimens. Future studies could potentially investigate this approach.

CONCLUSION

EVE can be successfully utilized for maintenance immunosuppression post lung transplant, particularly where the indication is for renal preservation. However, immunosuppressive regimens containing some CNI had superior survival outcomes, highlighting the importance of retaining a CNI wherever possible. Future studies could potentially investigate the impact of lower CNI target levels in those with demonstrated previous intolerance and the subsequent impact on survival.

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 Alfred Hospital (252-12, 30/5/12) and Monash University (252-12, 19/4/17). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

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

CONFLICT OF INTEREST

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

REFERENCES

1. Ivulich S, Westall G, Dooley M, Snell G. The Evolution of Lung Transplant Immunosuppression. *Drugs* (2018) 78(10):965–82. doi:10.1007/s40265-018-0930-6
2. Penninga L, Penninga EI, Moller CH, Iversen M, Steinbruchel DA, Gluud C. Tacrolimus versus Cyclosporin as Primary Immunosuppression for Lung Transplant Recipients. *Cochrane Database Syst Rev* (2013) 5: CD008817. doi:10.1002/14651858.CD008817.pub2
3. Grass F, Schafer M, Cristaudi A, Berutto C, Aubert JD, Gonzalez M, et al. Incidence and Risk Factors of Abdominal Complications after Lung Transplantation. *World J Surg* (2015) 39(9):2274–81. doi:10.1007/s00268-015-3098-1
4. Bravo C, Gispert P, Borro JM, de la Torre M, Cifrian Martinez JM, Fernandez Rozas S, et al. Prevalence and Management of Gastrointestinal Complications in Lung Transplant Patients: MITOS Study Group. *Transpl Proc* (2007) 39(7):2409–12. doi:10.1016/j.transproceed.2007.07.054
5. Hackman KL, Bailey MJ, Snell GI, Bach LA. Diabetes Is a Major Risk Factor for Mortality after Lung Transplantation. *Am J Transpl* (2014) 14(2):438–45. doi:10.1111/ajt.12561
6. Pascual J, Royuela A, Fernandez AM, Herrero I, Delgado JF, Sole A, et al. Role of mTOR Inhibitors for the Control of Viral Infection in Solid Organ Transplant Recipients. *Transpl Infect Dis* (2016) 18(6):819–31. doi:10.1111/tid.12601
7. Ensor CR, Doligalski CT. Proliferation Signal Inhibitor Toxicities after Thoracic Transplantation. *Expert Opin Drug Metab Toxicol* (2013) 9(1): 63–77. doi:10.1517/17425255.2012.726219
8. Chambers DC, Cherikh WS, Harhay MO, Hayes D, Hsich E, Khush KK, et al. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: Thirty-Sixth Adult Lung and Heart-Lung Transplantation Report-2019; Focus Theme: Donor and Recipient Size Match. *J Heart Lung Transplant* (2019) 38(10):1042–55. doi:10.1016/j.healun.2019.08.001
9. Glanville AR, Aboyoun C, Klepetko W, Reichenspurner H, Treede H, Verschuuren EA, et al. Three-year Results of an Investigator-Driven Multicenter, International, Randomized Open-Label De Novo Trial to Prevent BOS after Lung Transplantation. *J Heart Lung Transplant* (2015) 34(1):16–25. doi:10.1016/j.healun.2014.06.001
10. Strueber M, Warnecke G, Fuge J, Simon AR, Zhang R, Welte T, et al. Everolimus versus Mycophenolate Mofetil De Novo after Lung Transplantation: A Prospective, Randomized, Open-Label Trial. *Am J Transplant* (2016) 16(11):3171–80. doi:10.1111/ajt.13835
11. Gottlieb J, Neurohr C, Muller-Quernheim J, Wirtz H, Sill B, Wilkens H, et al. A Randomized Trial of Everolimus-Based Quadruple Therapy vs Standard Triple Therapy Early after Lung Transplantation. *Am J Transplant* (2019) 19(6): 1759–69. doi:10.1111/ajt.15251
12. Snell GI, Valentine VG, Vitulo P, Glanville AR, McGiffin DC, Loyd JE, et al. Everolimus versus Azathioprine in Maintenance Lung Transplant Recipients: an International, Randomized, Double-Blind Clinical Trial. *Am J Transpl* (2006) 6(1):169–77. doi:10.1111/j.1600-6143.2005.01134.x
13. Gullestad L, Eiskjaer H, Gustafsson F, Riise GC, Karason K, Dellgren G, et al. Long-term Outcomes of Thoracic Transplant Recipients Following Conversion to Everolimus with Reduced Calcineurin Inhibitor in a Multicenter, Open-Label, Randomized Trial. *Transpl Int* (2016) 29(7): 819–29. doi:10.1111/tri.12783
14. Snell GI, Westall GP, Paraskeva MA. Immunosuppression and Allograft Rejection Following Lung Transplantation: Evidence to Date. *Drugs* (2013) 73(16):1793–813. doi:10.1007/s40265-013-0136-x
15. Kovarik JM, Snell GI, Valentine V, Aris R, Chan CK, Schmidli H, et al. Everolimus in Pulmonary Transplantation: Pharmacokinetics and Exposure-Response Relationships. *J Heart Lung Transpl* (2006) 25(4):440–6. doi:10.1016/j.healun.2005.12.001
16. Snell GI, Levvey BJ, Henriksen A, Whitford HM, Levin K, Paraskeva M, et al. Donor Lung Referrals for Lung Transplantation: A 'Behind the Scenes' View. *Heart Lung Circ* (2019) 29(5):793–9. doi:10.1016/j.hlc.2019.04.007
17. Snell GI, Levvey BJ, Levin K, Paraskeva M, Westall G. Donation after Brain Death versus Donation after Circulatory Death: Lung Donor Management Issues. *Semin Respir Crit Care Med* (2018) 39(2):138–47. doi:10.1055/s-0037-1615820
18. Orens JB, Estenne M, Arcasoy S, Conte JV, Corris P, Egan JJ, et al. International Guidelines for the Selection of Lung Transplant Candidates: 2006 Update-Aa Consensus Report from the Pulmonary Scientific Council of the International Society for Heart and Lung Transplantation. *J Heart Lung Transplant : official Publ Int Soc Heart Transplant* (2006) 25(7):745–55. doi:10.1016/j.healun.2006.03.011
19. Gabbay E, Williams TJ, Griffiths AP, Macfarlane LM, Kotsimbos TC, Esmore DS, et al. Maximizing the Utilization of Donor Organs Offered for Lung Transplantation. *Am J Respir Crit Care Med* (1999) 160(1):265–71. doi:10.1164/ajrccm.160.1.9811017
20. Snell GI, Griffiths A, Macfarlane L, Gabbay E, Shiraishi T, Esmore DS, et al. Maximizing Thoracic Organ Transplant Opportunities: the Importance of Efficient Coordination. *J Heart Lung Transplant : official Publ Int Soc Heart Transplant* (2000) 19(4):401–7. doi:10.1016/s1053-2498(00)00071-1
21. Westall GP, Cristiano Y, Levvey BJ, Whitford H, Paraskeva MA, Paul E, et al. A Randomized Study of Quantiferon CMV-Directed versus Fixed-Duration Valganciclovir Prophylaxis to Reduce Late CMV after Lung Transplantation. *Transplantation* (2020) 103:1005–13. doi:10.1097/TP.0000000000002454
22. Stewart S, Fishbein MC, Snell GI, Berry GJ, Boehler A, Burke MM, et al. Revision of the 1996 Working Formulation for the Standardization of Nomenclature in the Diagnosis of Lung Rejection. *J Heart Lung Transpl* (2007) 26(12):1229–42. doi:10.1016/j.healun.2007.10.017
23. Morelon E, Stern M, Israel-Biet D, Correas JM, Danel C, Mamzer-Bruneel MF, et al. Characteristics of Sirolimus-Associated Interstitial Pneumonitis in Renal Transplant Patients. *Transplantation* (2001) 72(5):787–90. doi:10.1097/00007890-200109150-00008
24. Verleden GM, Glanville AR, Lease ED, Fisher AJ, Calabrese F, Corris PA, et al. Chronic Lung Allograft Dysfunction: Definition, Diagnostic Criteria, and Approaches to Treatment-A Consensus Report from the Pulmonary Council of the ISHLT. *J Heart Lung Transplant* (2019) 38(5):493–503. doi:10.1016/j.healun.2019.03.009
25. Otani S, Levvey BJ, Westall GP, Paraskeva M, Whitford H, Williams T, et al. Long-term Successful Outcomes from Kidney Transplantation after Lung and Heart-Lung Transplantation. *Ann Thorac Surg* (2015) 99(3):1032–8. doi:10.1016/j.athoracsur.2014.11.023
26. Hayes D, Harhay MO, Cherikh WS, Chambers DC, Perch M, Khush KK, et al. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: Twenty-Fourth Pediatric Lung Transplantation Report - 2021; Focus on Recipient Characteristics. *J Heart Lung Transplant* (2021) 40(10):1023–34. doi:10.1016/j.healun.2021.07.018
27. Chambers DC, Cherikh WS, Goldfarb SB, Hayes D, Kucheryavaya AY, Toll AE, et al. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: Thirty-Fifth Adult Lung and Heart-Lung Transplant Report-2018; Focus Theme: Multiorgan Transplantation. *J Heart Lung Transplant* (2018) 37(10):1169–83. doi:10.1016/j.healun.2018.07.020
28. Puttarajappa CM, Bernardo JF, Kellum JA. Renal Complications Following Lung Transplantation and Heart Transplantation. *Crit Care Clin* (2019) 35(1): 61–73. doi:10.1016/j.ccc.2018.08.009
29. Schneer S, Kramer MR, Fox B, Rusanov V, Fruchter O, Rosengarten D, et al. Renal Function Preservation with the mTOR Inhibitor, Everolimus, after Lung Transplant. *Clin Transpl* (2014) 28(6):662–8. doi:10.1111/ctr.12353
30. Arora S, Gude E, Sigurdardottir V, Mortensen SA, Eiskjaer H, Riise G, et al. Improvement in Renal Function after Everolimus Introduction and Calcineurin Inhibitor Reduction in Maintenance Thoracic Transplant Recipients: the Significance of Baseline Glomerular Filtration Rate. *J Heart Lung Transpl* (2012) 31(3):259–65. doi:10.1016/j.healun.2011.12.010
31. Nair N, Gongora E, Mehra MR. Long-term Immunosuppression and Malignancy in Thoracic Transplantation: where Is the Balance? *J Heart Lung Transpl* (2014) 33(5):461–7. doi:10.1016/j.healun.2014.03.002
32. Fine NM, Kushwaha SS. Recent Advances in Mammalian Target of Rapamycin Inhibitor Use in Heart and Lung Transplantation. *Transplantation* (2016) 100(12):2558–68. doi:10.1097/TP.0000000000001432

33. Pizzi M, Ng L. Neurologic Complications of Solid Organ Transplantation. *Neurol Clin* (2017) 35(4):809–23. doi:10.1016/j.ncl.2017.06.013
34. Mateen FJ, Dierkhising RA, Rabinstein AA, van de Beek D, Wijdicks EF. Neurological Complications Following Adult Lung Transplantation. *Am J Transpl* (2010) 10(4):908–14. doi:10.1111/j.1600-6143.2009.02998.x
35. Shigemura N, Sclabassi RJ, Bhama JK, Gries CJ, Crespo MM, Johnson B, et al. Early Major Neurologic Complications after Lung Transplantation: Incidence, Risk Factors, and Outcome. *Transplantation* (2013) 95(6):866–71. doi:10.1097/TP.0b013e318280b359
36. Waldner M, Fantus D, Solari M, Thomson AW. New Perspectives on mTOR Inhibitors (Rapamycin, Rapalogs and TORKinibs) in Transplantation. *Br J Clin Pharmacol* (2016) 82(5):1158–70. doi:10.1111/bcp.12893
37. Baas MC, Struijk GH, Moes DJAR, Van Den Berk IAH, Jonkers RE, De Fijter JW, et al. Interstitial Pneumonitis Caused by Everolimus: A Case-Cohort Study in Renal Transplant Recipients. *Transpl Int* (2014) 27(5):428–36. doi:10.1111/tri.12275
38. Tedesco-Silva H, Saliba F, Barten MJ, De Simone P, Potena L, Gottlieb J, et al. An Overview of the Efficacy and Safety of Everolimus in Adult Solid Organ Transplant Recipients. *Transpl Rev* (2022) 36(1):100655. (no pagination). doi:10.1016/j.trre.2021.100655
40. Perbos E, Juinier E, Guidicelli G, Dromer C, Merville P, Billes MA, et al. Evolution of Donor-specific Antibodies (DSA) and Incidence of De Novo DSA in Solid Organ Transplant Recipients after Switch to Everolimus Alone or Associated with Low Dose of Calcineurin Inhibitors. *Clin Transplant* (2014) 28(9):1054–60. doi:10.1111/ctr.12418
41. Silverborn M, Jeppsson A, Martensson G, Nilsson F. New-onset Cardiovascular Risk Factors in Lung Transplant Recipients. *J Heart Lung Transpl* (2005) 24(10):1536–43. doi:10.1016/j.healun.2005.01.004
42. Gullestad L, Iversen M, Mortensen SA, Eiskjaer H, Riise GC, Mared L, et al. Everolimus with Reduced Calcineurin Inhibitor in Thoracic Transplant Recipients with Renal Dysfunction: A Multicenter, Randomized Trial. *Transplantation* (2010) 89(7):864–72. doi:10.1097/TP.0b013e3181cbac2d

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BRIEF RESEARCH REPORT
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Low HDL-Cholesterol Concentrations in Lung Transplant Candidates are Strongly Associated With One-Year Mortality After Lung Transplantation

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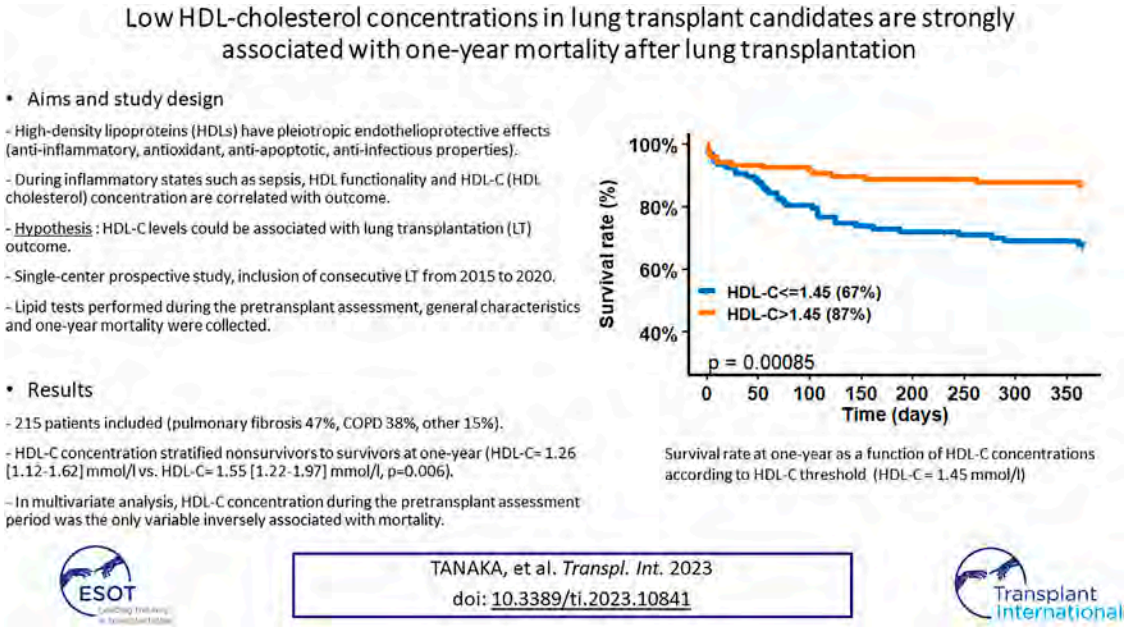
High-density lipoproteins (HDLs), whose main role is the reverse transport of cholesterol, also have pleiotropic anti-inflammatory, antioxidant, anti-apoptotic and anti-infectious properties. During sepsis, HDL cholesterol (HDL-C) concentration is low, HDL particle functionality is altered, and these modifications are correlated with poor outcomes. Based on the protective effects of HDL, we hypothesized that HDL-C levels could be associated with lung transplantation (LT) outcome. We thus looked for an association between basal HDL-C concentration and one-year mortality after LT. In this single-center prospective study including consecutive LTs from 2015 to 2020, 215 patients were included, essentially pulmonary fibrosis (47%) and chronic obstructive pulmonary disease (COPD) (38%) patients. Mortality rate at one-year was 23%. Basal HDL-C concentration stratified nonsurvivors to survivors at one-year (HDL-C = 1.26 [1.12–1.62] mmol/L vs. HDL-C = 1.55 [1.22–1.97] mmol/L, p = 0.006). Multivariate analysis confirmed that HDL-C concentration during the pretransplant assessment period was the only variable inversely associated with mortality. Moreover, mortality at one-year in patients with HDL-C concentrations ≤1.45 mmol/L was significantly higher (log-rank test, p = 0.00085). In conclusion, low basal HDL-C

Abbreviations: AIC, akaike information criterion; AUC, area under the curve; COPD, chronic obstructive pulmonary disease; HDL, high-density lipoprotein; HDL-C, HDL cholesterol; ICU, intensive care unit; LDL, low-density lipoprotein; LDL-C, LDL cholesterol; LT, lung transplantation; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristic; TC, total cholesterol; TG, triglyceride.

concentrations in candidates for LT are strongly associated with mortality after LT. To better understand this association, further studies in this field are essential and, in particular, a better characterization of HDL particles seems necessary.

Keywords: mortality, lung transplantation, outcome, HDL-cholesterol, lipoprotein



GRAPHICAL ABSTRACT |

INTRODUCTION

High-density lipoproteins (HDLs) belong to a family of nanoparticles whose main role is the reverse transport of cholesterol from tissues back to the liver (1). A high concentration of HDL-cholesterol (HDL-C) is negatively correlated with the occurrence of acute cardiovascular events such as ischemic stroke or acute coronary syndrome, conferring to this important scavenger role a strong endothelioprotective function (2, 3). Moreover, HDLs have pleiotropic properties that could play a protective role during acute inflammatory states, such as anti-inflammatory, antioxidant, anti-apoptotic and anti-infectious effects (4–7). For example, during sepsis, it has been shown that the HDL-C concentration is low, but HDL particle functionality is also altered, which could potentially impair endothelioprotective function (8–12). During sepsis, these quantitative and qualitative shifts in HDL are highly associated with morbidity and mortality (7–13).

In contrast with atherosclerosis or sepsis, the problematic lipoprotein and lipid levels in the specific case of lung transplantation (LT) have been less studied. Cottini et al. have shown that a low concentration of HDL-C was associated with more primary graft dysfunction (PGD) after LT (14). Moreover, in a retrospective study involving 172 consecutive LT, the same team demonstrated that the total cholesterol (TC)/HDL-C ratio was associated with mortality after LT (15).

The goal of the present study was to assess the lipid profile, particularly the HDL-C levels, in lung transplant candidates in our LT center and to determine a potential relationship with mortality after LT.

MATERIALS AND METHODS

Study Population

All consecutive patients who underwent LT at Bichat-Claude Bernard Hospital, Paris, from January 2015 to December 2020 were prospectively included in this observational, single-center analysis. As lipoprotein concentrations may be affected by liver dysfunction, patients scheduled for liver-lung transplantation were excluded from this study.

According to French law, the patient's absence of refusal was obtained before inclusion in the study. The

TABLE 1 | General characteristics, underlying disease and lipid profile of the overall population stratified by mortality at 1 year.

	Overall population (*n* = 215)	Alive at 1 year (*n* = 166)	Deceased at 1 year (*n* = 49)	p
General characteristics				
Age, years, median [IQR]	57 [51–62]	57 [51–62]	57 [47–62]	0.638
Male sex, *n* (%)	136 (63)	100 (60)	36 (73)	0.091
BMI (kg/m^2), median [IQR]	24 [20–27]	24 [20–27]	25 [21–28]	0.177
Diabetes mellitus, n (%)	19 (8.8)	12 (7.2)	7 (14)	0.151
Chronic coronary disease n (%)	10 (4.7)	8 (4.8)	2 (4.1)	>0.999
Statin use, n (%)	12 (5.6)	7 (4.2)	5 (10)	0.150
Aortic and peripheral vascular calcifications, n (%)	10 (4.7)	9 (5.4)	1 (2.0)	0.461
Mean pulmonary artery pressure (mmHg), median [IQR]	25 [20–30]	25 [20–30]	26 [20–34]	0.192
Underlying disease leading to LT				
COPD, n (%)	82 (38)	66 (40)	16 (33)	0.368
Pulmonary fibrosis, n (%)	100 (47)	76 (46)	24 (49)	0.693
Other pathologies, n (%)	34 (16)	24 (14)	10 (20)	0.316
Basal lipid profile				
Total cholesterol, mmol/L, median [IQR]	4.97 [4.34–5.70]	5.01 [4.36–5.69]	4.64 [4.33–5.75]	0.427
Triglycerides, mmol/L, median [IQR]	1.18 [0.89–1.65]	1.14 [0.87–1.59]	1.37 [0.97–1.86]	0.057
HDL-C, mmol/L, median [IQR]	1.45 [1.20–1.90]	1.55 [1.22–1.97]	1.26 [1.12–1.62]	0.006
LDL-C, mmol/L, median [IQR]	3 [2.42–3.54]	2.94 [2.40–3.49]	3.16 [2.55–3.55]	0.610

Continuous variables are expressed as the median and interquartile range (IQR) and were compared using the Mann–Whitney U test. Categorical variables are expressed as n (%) and were compared with Fisher's exact test. BMI, body mass index; COPD, chronic obstructive pulmonary disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LT, lung transplantation.

TABLE 2 | Multivariate analysis of risk factors for mortality at 1 year.

Pretransplant variables	Multivariate analysis of risk factors for mortality at 1 year		
	Odds ratio	95% CI	p
BMI > 25	1.15	0.55–2.37	**0.711**
Male sex	1.14	0.51–2.61	**0.752**
Diabetes mellitus	1.70	0.51–5.21	**0.359**
Statin use	1.42	0.33–5.50	**0.616**
Mean pulmonary artery pressure	1.04	0.99–1.09	**0.092**
Basal TG concentration	1.29	0.78–2.08	**0.303**
Basal HDL-C concentration	0.35	0.15–0.75	**0.008**

BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides.

Paris-North-Hospitals Institutional Review Board (Paris Diderot University, APHP, IRB No. 0006477) reviewed and approved the study.

Objectives

The main objective of this study was to investigate any potential association between the basal value of HDL-C and 1-year mortality. The secondary objectives were to assess the association between the basal value of HDL-C and 1-year mortality among patients with chronic obstructive pulmonary disease (COPD) and fibrosis, the two specific subgroups of our population.

Perioperative Management

The selection of lung transplant candidates (16) and perioperative care (17–20) was standardized for all patients according to current practices. During the intraoperative period, haemodynamic status was monitored using invasive arterial blood pressure, central venous and Swan Ganz catheters, and trans-oesophageal echocardiography. A venoarterial ECMO was implemented in cases of severe pulmonary arterial hypertension, SaO_2 <85%, SvO_2 <60%, cardiac output<1.5 L/min/m2 when clamping the pulmonary artery, if the patient did not tolerate single-lung ventilation (hypoxemia or hypercapnia), or in case of respiratory failure after transplantation of the first lung. After the surgical procedure, all patients were managed in a single intensive care unit (ICU). Care in ICU are performed according to international recommendations (21).

Data Collection

Demographic characteristics during the pretransplant assessment period were prospectively recorded. Mortality at 1 year was also prospectively collected. Occurrence of primary graft dysfunction (PGD), duration of vasopressor agent administration, need of per or postoperative ECMO support, acute kidney injury (AKI), occurrence of digestive complications such as acute mesenteric ischemia (AMI), duration of mechanical ventilation or length of stay in ICU were also collected. Simplified Acute Physiology Score II (SAPS-II) and sepsis-related organ failure assessment (SOFA) score were registered.

Lipid tests were performed during the pretransplant assessment period in the Biochemistry Laboratory of Bichat

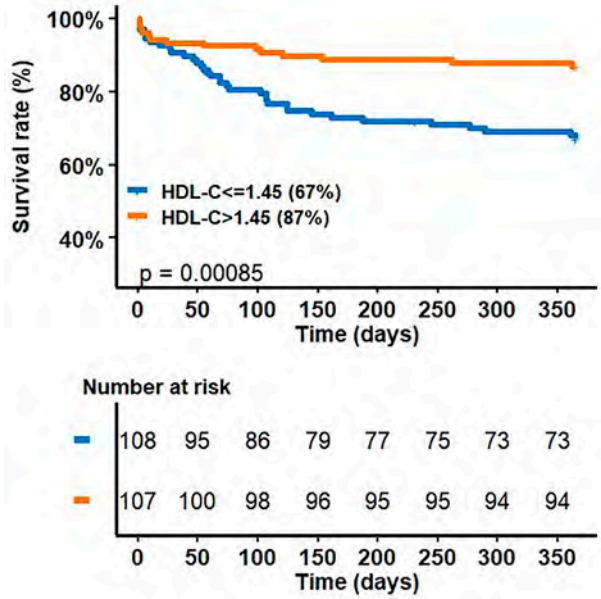


FIGURE 1 | survival rate at 1 year as a function of HDL-C concentrations according to HDL-C threshold (HDL-C = 1.45 mmol/L).

Claude-Bernard Hospital. This lipid assessment was performed in stable patients without any acute infectious episode. Total cholesterol (TC), HDL-C, LDL-C and triglyceride concentrations were determined by routine enzymatic assays (CHOL, HDLC, LDLC and TRIG methods, Dimension VISTA System, Siemens Healthineers). The reference values for these assays were HDL-C: >1.40 mmol/L; total cholesterol (TC): 4.40 < N < 5.20 mmol/L and triglycerides: 0.50 < N < 1.7 mmol/L. According to the European Society of Cardiology 2016 recommendations, LDL-C concentration targets have been established depending on vascular risk factors (22). All measurement methods were carried out in accordance with the guidelines.

Statistical Analysis

Continuous variables are expressed as medians with interquartile ranges (IQRs) and were compared with the Mann–Whitney U test. Categorical variables are expressed as counts and percentages and were compared with Fisher's exact test or the chi-square test, as appropriate. For 1-year mortality discrimination, receiver operating characteristic curve (ROC) analysis was performed, and the area under the curve (AUC) was calculated. The Youden index was used to determine the best threshold value of HDL-C.

Time-to-event analyses were estimated with Kaplan–Meier analyses. Multivariate associations were computed with binary logistic regression models; variables with nominal 2-tailed p values less than 0.2 were entered into the multivariate model, except for variables with obvious collinearity. The final models were selected using backward stepwise regression based on the Akaike information criterion (AIC). All statistical analyses were performed using R statistical software (https://www.r-project.org/). A p-value <0.05 was considered statistically significant.

RESULTS

Whole Population

General Characteristics

Between January 2015 and December 2020, 269 patients underwent LT in our institution. Three patients planned for liver-lung transplantation were excluded from the analysis. Fifty-one patients were excluded from the analysis because their lipid profile was not determined or was incomplete. Overall, 215 patients were finally included in this study. A total of 149 patients (69%) underwent double LT. The patient's general characteristics during the pretransplant assessment period are presented in **Table 1**. In this cohort, 10 (4.7%) patients had chronic coronary disease and all these patients underwent a percutaneous coronary intervention. No patient required coronary artery bypass graft (CABG). Moreover, only 10 (4.7%) patients had significant aortic and peripheral vascular calcifications. There was no difference in general characteristics between alive and deceased patients at 1 year.

Blood Lipid Profile

The median delay between the lipid test and LT was 303 [170–552] days. **Table 1** presents the lipid profile in the overall population. A comparison between patients alive or deceased at 1 year is also given. In the entire population, the median lipid concentrations were normal, according to the standard values (22). There was no difference between TC, LDL-C and TG concentrations between deceased and alive patients at 1 year. Interestingly, non-survivors had a lower HDL-C concentration than survivors at 1 year (HDL-C = 1.26 [1.12–1.62] mmol/L vs. HDL-C = 1.55 [1.22–1.97] mmol/L, p = 0.006).

Moreover, whereas there was no difference in mortality rates at 1 year between patients with and without chronic coronary disease (p > 0.999), HDL-C concentrations in patients with chronic coronary disease were lower than in patients without (HDL-C = 1.18 [1.02, 1.30] mmol/L vs. 1.50 [1.21, 1.96] mmol/L, p = 0.008). There was no difference in HDL-C concentrations when comparing patients with and without aortic and peripheral vascular calcifications (HDL-C = 1.69 [1.33, 2.30] mmol/L vs. 1.45 [1.20, 1.87] mmol/L, p = 0.138).

Outcome

The mortality rate at 1 year was 23%. Multivariate analysis with general characteristics during the pretransplant assessment period was performed. These results are expressed in **Table 2**. A high basal HDL concentration was predictive of good outcomes, suggesting a significant protective effect for 1-year mortality (odds ratio 0.35, 95% CI [0.15, 0.75], p = 0.008).

ROC curves were generated to assess the ability of lipid profiles (TC, HDL-C, LDL-C and TG) to discriminate 1-year mortality (**Supplementary Figure S1**). HDL-C had a higher AUC [0.63 (95% CI 0.54–0.71)]. The best threshold value of HDL-C was

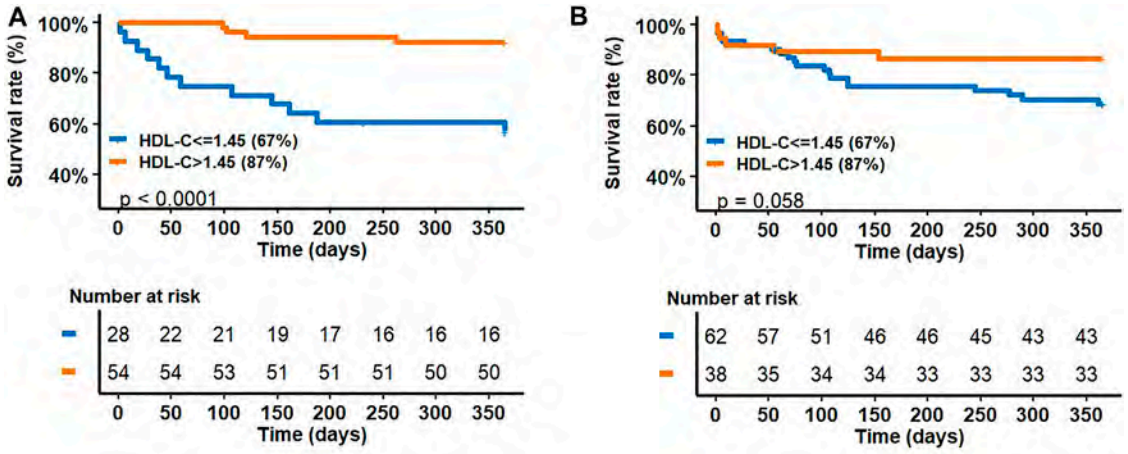


FIGURE 2 | survival rate at 1-year as a function of HDL-C concentrations according to HDL-C threshold (HDL-C = 1.45 mmol/L) in the COPD **(A)** and in fibrosis **(B)** sub-groups.

1.45 mmol/L (Youden index, sensitivity = 0.71, specificity = 0.56, positive predictive value (PPV) = 0.32, negative predictive value (NPV) = 0.86).

Figure 1 shows the survival rate at 1 year as a function of HDL-C concentrations according to the HDL-C threshold (HDL-C = 1.45 mmol/L). Survival at 1 year of patients with HDL-C concentrations ≤1.45 mmol/L was significantly lower (log-rank test, $p = 0.00085$).

Supplementary Table S1 shows the different values of outcome variables at 1-year mortality.

Subgroup of COPD Patients

- **Supplementary Table S2** describes the univariate and multivariate analysis of the general characteristics and lipid profile during the pretransplant assessment period and mortality at 1 year in the subgroup of COPD patients. As in the whole population, a high basal HDL-C concentration predicted a good outcome at 1 year in the subgroup of COPD patients (odds ratio 0.13, 95% CI [0.03–0.49], $p = 0.004$).

- ROC curves were constructed to assess the ability of lipid profiles (TC, HDL-C, LDL-C and TG) to discriminate 1-year mortality in the subgroup of COPD patients (**Supplementary Figure S2A**). HDL-C had a higher AUC [0.76 (95% CI 0.65–0.82)]. The best threshold value of HDL-C was 1.45 mmol/L (Youden index, sensitivity = 0.75, specificity = 0.76, positive predictive value (PPV) = 0.43, negative predictive value (NPV) = 0.93).

- **Figure 2A** shows the survival rate at 1 year as a function of HDL-C concentrations according to the HDL-C threshold (HDL-C = 1.45 mmol/L). The survival rate at 1 year of patients with HDL-C concentrations ≤1.45 mmol/L was significantly lower (log-rank test, $p < 0.0001$).

Subgroup of Patients With Fibrosis

- **Supplementary Table S3** shows univariate and multivariate analyses of the general characteristics during the pretransplant assessment period and the mortality at 1 year in the subgroup of fibrosis patients. Interestingly, in multivariate analysis, a high TG concentration was significantly associated with 1-year mortality (odds ratio 1.96, 95% CI [1.02–3.85], $p = 0.044$). No relationship between HDL-C concentration and mortality was found.

- ROC curves were generated to assess the ability of lipid profiles (TC, HDL-C, LDL-C and TG) to discriminate 1-year mortality in the subgroup of fibrosis patients (**Supplementary Figure S2B**). All lipid and lipoprotein values had low AUCs.

- **Figure 2B** shows the survival rate at 1 year as a function of HDL-C concentrations according to the HDL-C threshold (HDL-C = 1.45 mmol/L). There was no significant difference between survivors and non-survivors patients according to the HDL-C threshold (log-rank test, $p = 0.058$).

DISCUSSION

The main message of this manuscript is that low basal HDL-C concentration assessed during the pretransplant period is strongly associated with 1-year mortality after LT.

To our knowledge, our study is the first to show this link. Only one previous study has looked at the relationship between the lipid profile and mortality in the context of LT (15). Wenger et al., in a population of 144 LT patients, described a relationship between low basal HDL-C concentration and the occurrence of major cardiovascular events, but did not find any relationship with mortality (15). However, these authors showed that patients who died had a significantly higher TC/HDL-C ratio.

Unlike atherosclerosis or sepsis, where both HDL-C concentration and HDL particle functionality have been extensively studied (8–25), only a few studies have reported HDL-C levels in the context of respiratory disease and LT (26). A retrospective analysis of 126 consecutive individuals evaluated for LT with a diagnosis of COPD showed that HDL-C levels were increased and this was partially attributable to oral

steroid use (27). Interestingly, in this study, the HDL-C concentration was not associated with a reduced risk of coronary artery disease. This same team demonstrated that LT in COPD patients led to reductions in the serum levels of HDL-C (28). Moreover, when compared with other populations and, in particular, patients with fibrosis, the HDL-C concentration in COPD patients is very discriminating, which indirectly raises questions about the specific basal metabolism of this population when they are evaluated for LT.

Furthermore, in 69 patients with pulmonary arterial hypertension (PAH), a previous study reported that their HDL-C levels were significantly decreased and were associated with poor clinical outcomes independent of cardiovascular risk factors, insulin resistance and the severity of PAH (29). In patients with idiopathic pulmonary fibrosis (IPF), reduced amounts of apolipoprotein A-I, the major apolipoprotein comprising HDL particles, have been found in bronchoalveolar lavage fluid compared to normal controls (30). Interestingly, the data in our population of fibrosis patients did not highlight an association between basal HDL-C and mortality. The complexity of the fibrosis entity, sometimes occurring in relationship with systemic diseases, may explain, at least partially, the lack of associations. Barochia et al. showed in a study using nuclear magnetic resonance spectroscopy that high levels of small HDL particles (i.e., more functional HDL particles) were negatively correlated with lower mortality or LT (31). Beyond a modification of HDL-C concentration, structural changes of the particles may then influence the outcome.

Since our study was purely exploratory, our observations are only assumptions and it is thus impossible to conclude why we found a strong link between basal HDL-C concentrations and mortality. In light of our results, HDL-C appears to be a marker or an effector in the survival.

Nevertheless, during the per/postoperative periods of LT, acute inflammatory episodes are frequently described, such as systemic inflammatory response syndrome as well as sepsis (19–35). It has been reported that during these states, there is both a decrease in HDL-C and a functional modification of HDL particles with, in particular, a proinflammatory profile (11–42). Importantly, these shifts are associated with poor outcomes. Therefore, if the HDL-C concentration is low under basal conditions and there are both qualitative and quantitative changes during and after LT, the poor prognosis of patients can then be understood.

Our results motivate more powerful clinical studies and experimental studies: If clinical studies confirmed our result, the basal HDL-C concentration could be proposed for discriminating transplant candidates. Moreover, the functionalities of HDL particles before/during/after LT probably deserve additional investigation. Whereas during sepsis, functional and structural changes have been well described (7), no study has yet investigated these functionalities in LT. Studies, in particular -omics analyses, seem necessary to better characterize these LT candidates.

Our study has several limitations:

First, this investigation being purely observational, we have no element that can rationally explain our results. Second, the monocentric nature of our work with only 215 patients is a limitation, which can lead to recruitment bias. Third, the mixture of varied respiratory pathologies (COPD and fibrosis) can also bring about some biases. A focus on the specific COPD candidates could be very informative. Fourth, an analysis of HDL-C concentrations per and postoperatively would have been informative. Fifth, even if 1-year mortality is not a reliable indicator of LT transplant center performance (43), our mortality rate at 1 year (23%) seems to be slightly high and this could induce some bias. The high proportion of fibrosis and high emergency LT (18%) could possibly explain this elevated rate. Finally, whereas there was no difference in mortality rates at 1-year between patients with and without chronic coronary disease, HDL-C concentration in patients with chronic coronary disease were lower than in patients without. Even if this population is very small (n = 10), it could induce a bias.

In conclusion, our work showed that a low basal HDL-C concentration in candidates for LT was associated with increased mortality after LT. HDL-C appears to be a marker or an effector in the survival. To better understand this association, additional and more powerful studies are required. A better characterization of HDL particles is also a huge challenge.

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DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

According to French law, the patient's absence of refusal was obtained before inclusion in the study. The Paris-North-Hospitals Institutional Review Board (Paris Diderot University, APHP, IRB No. 0006477) reviewed and approved the study.

AUTHOR CONTRIBUTIONS

ST, CT, OM, and PhM contributed to study concept and design. ST and CT performed statistical analysis. ST, CT, AT-D, OM, BL-J, NZ, SJ-B, TR, TG, CG, YC, HM, PiM, EA, JM, and PhM were involved in data analysis and interpretation. ST, CT, OM, JM, PiM, AT-D, CG, EA, and PhM performed critical revision of the manuscript. All the authors read and approved the final manuscript.

CONFLICT OF INTEREST

CG reports having received grant support from Ohre Pharma, Pfizer, MSD, SOS Oxygène, ISIS Medical, Vivisol, Elivie and AstraZeneca, speaker fees, travel support from Pfizer, MSD and speaker fees for board memberships from SOS Oxygène and Pulmatrix. JM received congress reimbursement fees from Biotest and CSL Behring, Fingers Xed.

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

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.10841/full#supplementary-material

REFERENCES

1. Nofer JR, Levkau B, Wolinska I, Junker R, Fobker M, von Eckardstein A, et al. Suppression of Endothelial Cell Apoptosis by High Density Lipoproteins (HDL) and HDL-Associated Lysosphingolipids. *J Biol Chem* (2001) 276(37):34480–5. doi:10.1074/jbc.M103782200
2. Wilson PW, Abbott RD, Castelli WP. High Density Lipoprotein Cholesterol and Mortality. The Framingham Heart Study. *Framingham Heart Study Arteriosclerosis Déc* (1988) 8(6):737–41. doi:10.1161/01.atv.8.6.737
3. Cooney MT, Dudina A, De Bacquer D, Wilhelmsen L, Sans S, Menotti A, et al. HDL Cholesterol Protects against Cardiovascular Disease in Both Genders, at All Ages and at All Levels of Risk. *Atheroscler oct* (2009) 206(2):611–6. doi:10.1016/j.atherosclerosis.2009.02.041
4. Pirillo A, Catapano AL, Norata GD. HDL in Infectious Diseases and Sepsis. *Handb Exp Pharmacol* (2015) 224:483–508. doi:10.1007/978-3-319-09665-0_15
5. Tran-Dinh A, Diallo D, Delbosc S, Varela-Perez LM, Dang QB, Lapergue B, et al. HDL and Endothelial protection. *Br J Pharmacol Juin* (2013) 169(3):493–511. doi:10.1111/bph.12174
6. Ulevitch RJ, Johnston AR, Weinstein DB. New Function for High Density Lipoproteins. Their Participation in Intravascular Reactions of Bacterial Lipopolysaccharides. *J Clin Invest Nov* (1979) 64(5):1516–24. doi:10.1172/JCI109610
7. Tanaka S, Couret D, Tran-Dinh A, Duranteau J, Montravers P, Schwendeman A, et al. High-density Lipoproteins during Sepsis: from Bench to Bedside. *Crit Care* (2020) 24(1):134. doi:10.1186/s13054-020-02860-3
8. Chien JY, Jerng JS, Yu CJ, Yang PC. Low Serum Level of High-Density Lipoprotein Cholesterol Is a Poor Prognostic Factor for Severe Sepsis. *Crit Care Med* (2005) 33(8):1688–93. doi:10.1097/01.ccm.0000171183.79525.6b
9. Barlage S, Gnewuch C, Liebisch G, Wolf Z, Audebert FX, Gluck T, et al. Changes in HDL-Associated Apolipoproteins Relate to Mortality in Human Sepsis and Correlate to Monocyte and Platelet Activation. *Intensive Care Med* (2009) 35(11):1877–85. doi:10.1007/s00134-009-1609-y
10. Tanaka S, Stern J, Bouzid D, Robert T, Dehoux M, Snauwaert A, et al. Relationship between Lipoprotein Concentrations and Short-Term and 1-year Mortality in Intensive Care Unit Septic Patients: Results from the HIGHSEPS Study. *Ann Intensive Care* (2021) 11(1):11. doi:10.1186/s13613-021-00800-0
11. Tanaka S, Diallo D, Delbosc S, Genève C, Zappella N, Yong-Sang J, et al. High-density Lipoprotein (HDL) Particle Size and Concentration Changes in Septic Shock Patients. *Ann Intensive Care* (2019) 9(1):68. doi:10.1186/s13613-019-0541-8
12. Sharma NK, Tashima AK, Brunialti MKC, Ferreira ER, Torquato RJS, Mortara RA, et al. Proteomic Study Revealed Cellular Assembly and Lipid Metabolism Dysregulation in Sepsis Secondary to Community-Acquired Pneumonia. *Sci Rep* (2017) 7(1):15606. doi:10.1038/s41598-017-15755-1
13. Tanaka S, Labreuche J, Drumez E, Harrois A, Hamada S, Vigué B, et al. Low HDL Levels in Sepsis versus Trauma Patients in Intensive Care Unit. *Ann Intensive Care Déc* (2017) 7(1):60. doi:10.1186/s13613-017-0284-3
14. Cottini SR, Ehlers UE, Pagnamenta A, Brandi G, Weder W, Schuepbach RA, et al. Pretransplant Dyslipidaemia Influences Primary Graft Dysfunction after Lung Transplantation. *Interact Cardiovasc Thorac Surg Avr* (2016) 22(4):402–5. doi:10.1093/icvts/ivv295
15. Wenger U, Cottini SR, Noll G, Arndt S, Stehberger PA, Klinzing S, et al. Pretransplant Dyslipidaemia Determines Outcome in Lung Transplant Recipients. *Lipids Health Dis*. 23 avr 2013;12:53, doi:10.1186/1476-511X-12-53
16. Leard LE, Holm AM, Valapour M, Glanville AR, Attawar S, Aversa M, et al. Consensus Document for the Selection of Lung Transplant Candidates: An Update from the International Society for Heart and Lung Transplantation. *J Heart Lung Transpl nov* (2021) 40(11):1349–79. doi:10.1016/j.healun.2021.07.005
17. Elmaleh Y, De Tymowski C, Zappella N, Jean-Baptiste S, Tran-Dinh A, Tanaka S, et al. Blood Transfusion of the Donor Is Associated with Stage 3 Primary Graft Dysfunction after Lung Transplantation. *Clin Transpl sept* (2021) 35(9):e14407. doi:10.1111/ctr.14407
18. Atchade E, Younsi M, Elmaleh Y, Tran-Dinh A, Jean-Baptiste S, Tanaka S, et al. Intensive Care Readmissions in the First Year after Lung Transplantation: Incidence, Early Risk Factors and Outcome. *Anaesth Crit Care Pain Med Déc* (2021) 40(6):100948. doi:10.1016/j.accpm.2021.100948
19. Tanaka S, Geneve C, Tebano G, Grall N, Piednoir P, Bronchard R, et al. Morbidity and Mortality Related to Pneumonia and TRACHEOBRONCHITIS in ICU after Lung Transplantation. *BMC Pulm Med* (2018) 18(1):43. doi:10.1186/s12890-018-0605-9
20. Kao CC, Parulekar AD. Postoperative Management of Lung Transplant Recipients. *J Thorac Dis Sept* (2019) 11(14):S1782–S1788. doi:10.21037/jtd.2019.05.60
21. Marczin N, de Waal EEC, Hopkins PMA, Mulligan MS, Simon A, Shaw AD, et al. International Consensus Recommendations for Anesthetic and Intensive Care Management of Lung Transplantation. An EACTAIC, SCA, ISHLT, ESOT, ESTS, and AST Approved Document. *J Heart Lung Transpl nov* (2021) 40(11):1327–48. doi:10.1016/j.healun.2021.07.012
22. Catapano AL, Graham I, De Backer G, Wiklund O, Chapman MJ, Drexel H, et al. 2016 ESC/EAS Guidelines for the Management of Dyslipidaemias. *Eur Heart J* (2016) 1437(39):2999–3058. doi:10.1093/eurheartj/ehw272

23. Abbott RD, Garrison RJ, Wilson PW, Epstein FH, Castelli WP, Feinleib M, et al. Joint Distribution of Lipoprotein Cholesterol Classes. The Framingham Study. *Framingham Study Arteriosclerosis Juin* (1983) 3(3):260–72. doi:10.1161/01.atv.3.3.260
24. Rohatgi A, Khera A, Berry JD, Givens EG, Ayers CR, Wedin KE, et al. HDL Cholesterol Efflux Capacity and Incident Cardiovascular Events. *N Engl J Med* (2014) 371(25):2383–93. doi:10.1056/NEJMoa1409065
25. Vaisar T, Pennathur S, Green PS, Gharib SA, Hoofnagle AN, Cheung MC, et al. Shotgun Proteomics Implicates Protease Inhibition and Complement Activation in the Antiinflammatory Properties of HDL. *J Clin Invest Mars* (2007) 117(3):746–56. doi:10.1172/JCI26206
26. Gordon EM, Figueroa DM, Barochia AV, Yao X, Levine SJ. High-density Lipoproteins and Apolipoprotein A-I: Potential New Players in the Prevention and Treatment of Lung Disease. *Front Pharmacol* (2016) 7:323. doi:10.3389/fphar.2016.00323
27. Reed RM, Iacono A, DeFilippis A, Eberlein M, Girgis RE, Jones S. Advanced Chronic Obstructive Pulmonary Disease Is Associated with High Levels of High-Density Lipoprotein Cholesterol. *J Heart Lung Transpl juin* (2011) 30(6):674–8. doi:10.1016/j.healun.2010.12.010
28. Reed RM, Hashmi S, Eberlein M, Iacono A, Netzer G, DeFilippis A, et al. Impact of Lung Transplantation on Serum Lipids in COPD. *Respir Med Déc* (2011) 105(12):1961–8. doi:10.1016/j.rmed.2011.10.003
29. Heresi GA, Aytekin M, Newman J, DiDonato J, Dweik RA. Plasma Levels of High-Density Lipoprotein Cholesterol and Outcomes in Pulmonary Arterial Hypertension. *Am J Respir Crit Care Med* (2010) 182(5):661–8. doi:10.1164/rccm.201001-0007OC
30. Kim TH, Lee YH, Kim KH, Lee SH, Cha JY, Shin EK, et al. Role of Lung Apolipoprotein A-I in Idiopathic Pulmonary Fibrosis: Antiinflammatory and Antifibrotic Effect on Experimental Lung Injury and Fibrosis. *Am J Respir Crit Care Med* (2010) 182(5):633–42. doi:10.1164/rccm.200905-0659OC
31. Barochia AV, Kaler M, Weir N, Gordon EM, Figueroa DM, Yao X, et al. Serum Levels of Small HDL Particles Are Negatively Correlated with Death or Lung Transplantation in an Observational Study of Idiopathic Pulmonary Fibrosis. *Eur Respir J Déc* (2021) 58(6):2004053. doi:10.1183/13993003.04053-2020
32. Meilhac O, Tanaka S, Couret D. High-Density Lipoproteins Are Bug Scavengers. *Biomolecules.* (2020) 10(4):598. doi:10.3390/biom10040598
33. Pietrantoni C, Minai OA, Yu NC, Maurer JR, Haug MT, Mehta AC, et al. Respiratory Failure and Sepsis Are the Major Causes of ICU Admissions and Mortality in Survivors of Lung Transplants. *Chest Févr* (2003) 123(2):504–9. doi:10.1378/chest.123.2.504
34. Chambers DC, Perch M, Zuckermann A, Cherikh WS, Harhay MO, Hayes D, et al. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: Thirty-Eighth Adult Lung Transplantation Report - 2021; Focus on Recipient Characteristics. *J Heart Lung Transpl* (2021) 40(10):1060–72. doi:10.1016/j.healun.2021.07.021
35. Shepherd HM, Gauthier JM, Li W, Krupnick AS, Gelman AE, Kreisel D. Innate Immunity in Lung Transplantation. *J Heart Lung Transpl juill* (2021) 40(7):562–8. doi:10.1016/j.healun.2021.03.017
36. Hacquebard M, Ducart A, Schmartz D, Malaisse WJ, Carpentier YA. Changes in Plasma LDL and HDL Composition in Patients Undergoing Cardiac Surgery. *Lipids Déc* (2007) 42(12):1143–53. doi:10.1007/s11745-007-3114-9
37. Tanaka S, De Tymowski C, Zappella N, Snauwaert A, Robert T, Lortat-Jacob B, et al. Lipoprotein Concentration in Patients Requiring Extracorporeal Membrane Oxygenation. *Sci Rep* (2021) 11(1):17225. doi:10.1038/s41598-021-96728-3
38. de la Llera Moya M, McGillicuddy FC, Hinkle CC, Byrne M, Joshi MR, Nguyen V, et al. Inflammation Modulates Human HDL Composition and Function *In Vivo*. *Atheroscler juin* (2012) 222(2):390–4. doi:10.1016/j.atherosclerosis.2012.02.032
39. Vaisar T, Tang C, Babenko I, Hutchins P, Wimberger J, Suffredini AF, et al. Inflammatory Remodeling of the HDL Proteome Impairs Cholesterol Efflux Capacity. *J Lipid Res Août* (2015) 56(8):1519–30. doi:10.1194/jlr.M059089
40. Tanaka S, De Tymowski C, Assadi M, Zappella N, Jean-Baptiste S, Robert T, et al. Lipoprotein Concentrations over Time in the Intensive Care Unit COVID-19 Patients: Results from the ApoCOVID Study. *PLoS One* (2020) 15(9):e0239573. doi:10.1371/journal.pone.0239573
41. Begue F, Tanaka S, Mouktadi Z, Rondeau P, Veeren B, Diotel N, et al. Altered High-Density Lipoprotein Composition and Functions during Severe COVID-19. *Sci Rep* (2021) 11(1):2291. doi:10.1038/s41598-021-81638-1
42. Sharma NK, Ferreira BL, Tashima AK, Brunialti MKC, Torquato RJS, Bafi A, et al. Lipid Metabolism Impairment in Patients with Sepsis Secondary to Hospital Acquired Pneumonia, a Proteomic Analysis. *Clin Proteomics* (2019) 16:29. doi:10.1186/s12014-019-9252-2
43. Wakeam E, Thumma JR, Bonner SN, Chang AC, Reddy RM, Lagisetty K, et al. One-year Mortality Is Not a Reliable Indicator of Lung Transplant Center Performance. *Ann Thorac Surg* (2022) -4975(22):22500292–232. doi:10.1016/j.athoracsur.2022.02.028

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BRIEF RESEARCH REPORT
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A Novel Three-Dimensional Approach Towards Evaluating Endomyocardial Biopsies for Follow-Up After Heart Transplantation: X-Ray Phase Contrast Imaging and Its Agreement With Classical Histopathology

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Endomyocardial biopsies are the gold standard for surveillance of graft rejection following heart transplantation, and are assessed by classical histopathology using a limited number of previously stained slices from several biopsies. Synchrotron propagation-based X-ray phase contrast imaging is a non-destructive method to image biological samples without tissue preparation, enabling virtual 2D and 3D histopathology. We aimed to show the feasibility of this method to assess acute cellular rejection and its agreement to classical histopathology. Right ventricular biopsies were sampled from 23 heart transplantation recipients (20 males, mean age 54±14 years) as part of standard follow-up. The clinical diagnosis of potential rejection was made using classical histopathology. One additional study sample was harvested and imaged by X-ray phase contrast imaging, producing 3D datasets with 0.65 µm pixel size, and up to 4,320 images per sample. An experienced pathologist graded both histopathological and X-ray phase contrast images in a blinded fashion. The agreement between methods was assessed by weighted kappa, showing substantial agreement (kappa up to 0.80, $p < 0.01$) between X-ray phase contrast imaging and classical histopathology. X-ray phase contrast imaging does not require tissue processing, allows thorough analysis of a full myocardial sample and allows identification of acute cellular rejection.

Keywords: heart transplantation, synchrotron, histopathology, graft rejection, X-ray phase contrast imaging

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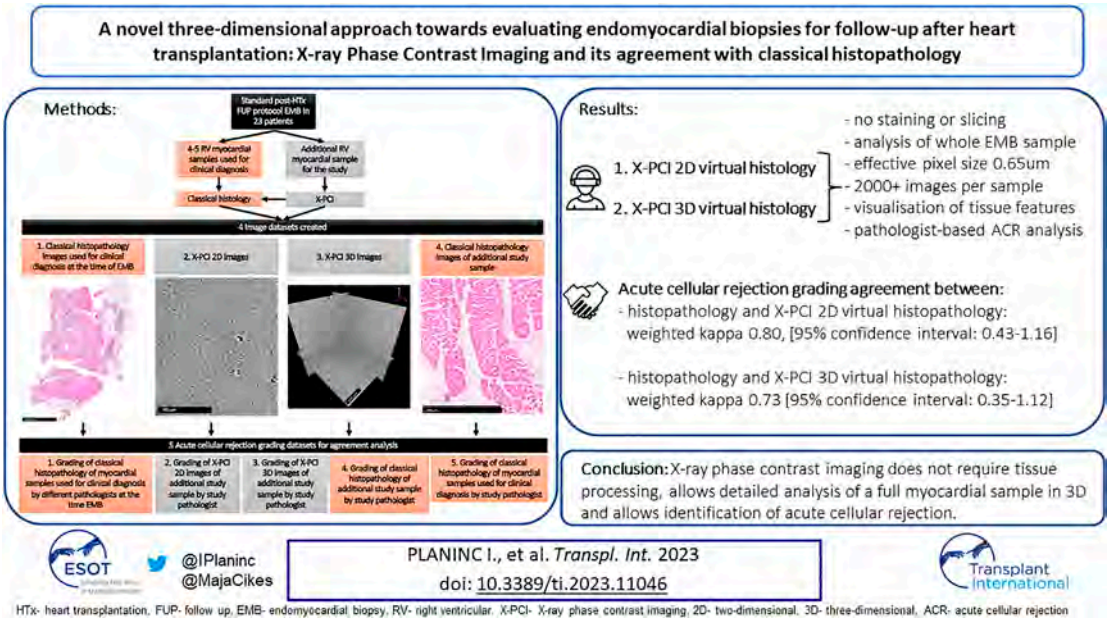

GRAPHICAL ABSTRACT |
A novel three-dimensional approach towards evaluating endomyocardial biopsies for follow-up after heart transplantation: X-ray Phase Contrast Imaging and its agreement with classical histopathology.

INTRODUCTION

Close monitoring and follow-up of heart transplantation (HTx) recipients is essential for timely recognition of post-transplantation complications, such as acute cellular rejection (ACR) (1, 2). Echocardiography or cardiac magnetic resonance imaging are powerful in the detection of global and regional cardiac dysfunction allowing for indirect identification of fibrosis, but are unable to specifically diagnose rejection (3). "Liquid biopsies" based on cell-free DNA technology are emerging tools in recognition of ACR, however still without wide acceptance in everyday clinical practice (4). Therefore, histopathological analysis of endomyocardial biopsy (EMB) samples remains the standard of care in rejection surveillance (5).

Synchrotron radiation-based X-ray Phase Contrast Imaging (X-PCI) has become a well-accepted technique in soft tissue research. In X-PCI, advantage is taken of the refractive properties of X-rays when traveling through soft tissues to increase the contrast of resulting images. Given the need for highly coherent X-ray beams, synchrotrons (large scale research facilities) are currently primarily used for X-PCI, providing three-dimensional (3D) high resolution (<1 μm pixel size) imaging with excellent contrast.

In cardiovascular research, X-PCI has so far been utilized to study heart architecture *ex-vivo* in animal models, and human hearts (fetal and adult), both healthy and diseased (6–13).

In this pilot study, we aimed to show the potential of X-PCI to assess features of ACR in full 3D volumes of EMB samples, and its agreement with clinical histopathology.

METHODS

Patients

We included 23 HTx recipients that underwent scheduled EMBs per Institutional protocol. The first 20 patients were included in consecutive manner, and the remaining three patients with known high-grade rejection (2R or 3R) were included from the Institutional archives to enrich the initial sample that was lacking such findings. The proportion of added high-grade rejection samples was based on the occurrence of graft-rejection requiring treatment of around 7%–10% of surveillance EMBs (2, 14). Patient medical data were collected retrospectively. The study was approved by the institutional Ethics review board (Approval of the Ethics Committee of the University Hospital Centre Zagreb, Croatia; Class: 8.1-17/137-2, No: 02/21 AG), and all of the patients signed an informed consent.

Endomyocardial Biopsy

The EMB was performed following a standardized clinical procedure, and according to the technical recommendations proposed by ISHLT (**Supplementary Material**) (4). Besides 3–4 myocardial samples used for histopathological diagnosis in

the clinical setting, an additional sample was taken for the purposes of this study; all of the samples were initially placed in formalin solution.

X-Ray Phase Contrast Imaging Acquisition and Visualization

Synchrotron radiation-based X-PCI acquisition was performed at the TOMCAT beamline of the Swiss Light Source (Paul Scherrer Institute, Switzerland). With no further tissue preparation, the samples (at propagation distance of 20 cm between sample and detector) were fully illuminated by a monochromatic X-ray beam with an energy of 20 keV. X-rays were converted to visible light, amplified by 10x objective, and recorded with an effective pixel size of 0.65 µm. (**Supplementary Figure S1**) (7). For each tomogram, a total of 2,501 projections, 20 darks and 50 flats were acquired with a exposure time of 200 ms, resulting in approximately 10 min acquisition time followed by approximately 3 min for reconstruction of 3D datasets. Reconstructed 3D datasets were obtained from projections using the Gridrec algorithm (7, 15). Depending on the true size of the imaged myocardial tissue sample, one or several tomograms were made, and X-PCI datasets consisted of 2,160–4,320 images of 1.66 mm × 1.66 mm size and 0.65 µm thickness (**Supplementary Material**). The whole process of sample positioning, X-ray imaging, and dataset production required approximately 20 min per usual sized EMB (one tomogram per biopsy).

The identification of the histopathological features of ACR on the obtained X-PCI images, as well as image optimization with contrast and brightness adjustment was done using the open-source software Fiji (version of the program: ImageJ 1.51s, Wayne Rasband, National Institute of Health, United States) (16, 17).

Histopathology

Samples were stained by hematoxylin and eosin (H&E) and fixated on glass slides. At least 10 sections were analyzed by light microscopy for diagnosis of ACR according to ISHLT 2004. Recommendations, while immunohistochemistry was performed as part of the clinical patient management, but not on the study samples for which the focus was on ACR (5).

Classical histopathology was initially done on the 3–4 myocardial samples for routine clinical diagnosis of ACR grade, together with routine immunohistochemistry.

Research Protocol and Datasets for Comparative Graft Rejection Analysis

For comparative ACR analysis, the X-PCI images were presented in two ways: firstly, X-PCI 2D datasets mimicked the ISHLT recommendations for analysis of classical histopathological EMB samples for ACR (5). From the full 3D dataset originally containing up to 4,320 images, 10 images were randomly selected for analysis, maintaining the original superior-inferior sample orientation. If the distance between the slices was too large to adequately assess histopathological features, additional consecutive images from the 3D dataset were obtained.

Secondly, in the X-PCI 3D dataset, the pathologist could use any of the images (up to 4,320) from the sample (Graphical Abstract).

An experienced pathologist assessed ACR grades in both histopathological and X-PCI images, blinded to the identity and any clinical data of the patient. The same pathologist also re-assessed the original samples harvested for clinical diagnosis at the time of the routine follow-up, again in a blinded fashion (Graphical Abstract) (5, 18).

In total, for ACR grading agreement analysis, five distinctive datasets were used (Graphical Abstract; **Supplementary Figure S2**).

Statistical Analysis

Baseline patient characteristics are expressed as means and standard deviations for normally distributed, and as median with interquartile range for non-normally distributed continuous variables. Categorical variables are expressed as counts and percentages.

Agreement between methods was assessed by weighted kappa, with weights being assigned to the grades of rejection according to how they would influence patient management (in terms of ACR: grades 2R and 3R carried increased magnitude of weight in the calculations) (19, 20). A p-value of < 0.05 was considered statistically significant. Statistical analysis was performed using STATA (Stata/IC 13.1 for Mac, Statacorp, Texas, United States).

RESULTS

Patient Characteristics

The patient characteristics are shown in **Supplementary Table S1**. The majority were males, with mean age 54 ± 14 years, and median time from HTx of 24.6 months (IQR 4.9–35.6 months). Most of the patients were transplanted due to non-ischaemic dilated cardiomyopathy (52.2%), with arterial hypertension, hyperlipoproteinemia and diabetes mellitus as the most common comorbidities at the time of EMB harvesting (in 65.2%, 34.8% and 26.1% of the patients, respectively). Overall, the patients had normal left ventricular dimensions, preserved left-ventricular ejection fraction (LVEF) and did not have significant pulmonary hypertension. The most commonly used immunosuppressive regimen was the combination of mycophenolate with a calcineurin inhibitor. The clinical histopathological diagnoses are shown in **Supplementary Table S2**.

Detailed individual patient characteristics are shown in **Supplementary Table S3**.

Agreement Between X-Ray Phase Contrast Imaging and Classical Histopathology

Figures 1A1–A4 show an example of an X-PCI image used in analysis, its digitally colored version (**Figures 1A5–A7**), and for comparison, the conventional histopathological image of the similar area of the same EMB sample is also shown (**Figure 1B**).

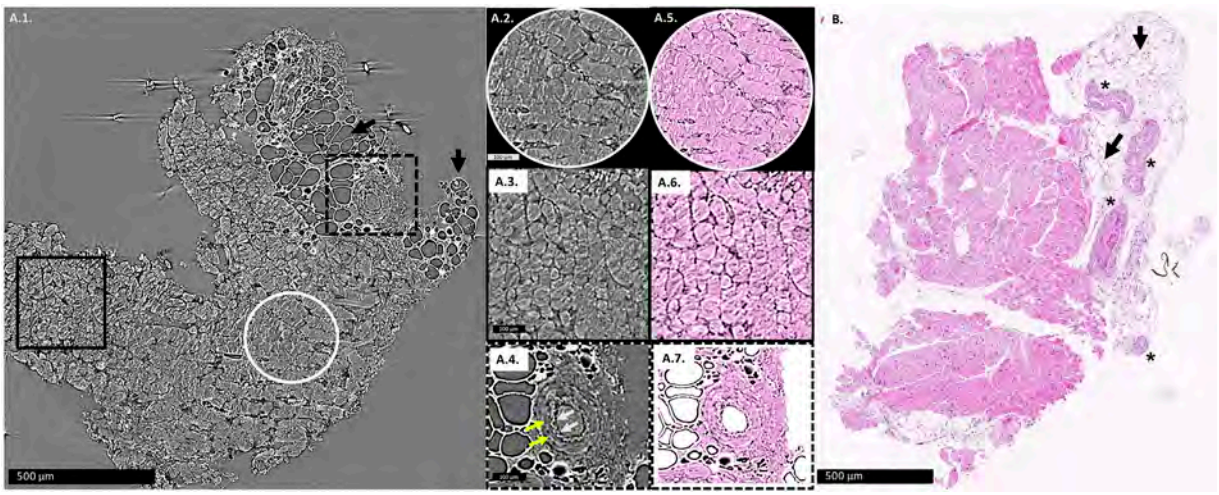

FIGURE 1 | (A1): X-PCI image of the EMB sample with no rejection (0R), where arteries, adipocytes (arrows) and cardiomyocytes can similarly be distinguished. Enlarged longitudinal cut of cardiomyocyte aggregates is shown in the white circle **(A2)**, while cardiomyocyte aggregates virtually transversely cut are shown in the black rectangle **(A3)**. **(A4)** Enlarged view of an artery, with white arrows showing the wavy contour of the tunica intima, while yellow arrows indicate the muscular tunica media. **(A5–A7)** the same X-PCI images shown in **(A2–A4)** digitally colorized to imitate H&E staining in classical histopathology. **(B)**: Histopathological slice of a similar area of the same sample, where arteries (*), adipocytes (arrows) and cardiomyocytes can be distinguished.

TABLE 1 | Agreement between different study datasets in the ACR grading according to the ISHLT 2004. Grading system. All the weighted kappa's printed in bold have $p < 0.01$.

References vs. comparison method	Weighted kappa	95% CI
Clinical histopathological diagnosis vs.		
Study sample histopathology	**0.69**	0.27–1.10
X-PCI 2D virtual histopathology	**0.80**	0.43–1.16
X-PCI 3D virtual histopathology	**0.73**	0.35–1.12
Study sample histopathology vs.		
X-PCI 2D virtual histopathology	**0.80**	0.42–1.17
X-PCI 3D virtual histopathology	**0.73**	0.34–1.11
Clinical histopathological diagnosis by one observer vs.		
Clinical histopathological diagnosis	**0.93**	0.56–1.31
Study sample histopathology	**0.61**	0.26–0.97
X-PCI 2D virtual histopathology	**0.73**	0.38–1.08
X-PCI 3D virtual histopathology	**0.65**	0.30–1.0

When using the clinical diagnosis made by classical histopathology as the reference method, X-PCI 2D and 3D virtual histopathology showed substantial agreement with the reference method [weighted kappa 0.80, (95% confidence interval: 0.43–1.16), and 0.73 (95% confidence interval: 0.35–1.12)] (**Table 1**). When using classical histopathology of the X-PCI imaged sample as the reference, a substantial agreement was achieved with both X-PCI 2D and 3D virtual histopathology as well [weighted kappa 0.80, (95% confidence interval: 0.42–1.17), and 0.73 (95% confidence interval: 0.34–1.11)]. The best agreement between the different methods was achieved in ruling out ACR that required treatment (**Table 1**).

When samples used for clinical diagnosis at the time of EMB were reassessed by the dedicated study pathologist, the agreement between the histopathological diagnosis made by this observer and the X-PCI 2D and 3D virtual histopathology remained substantial [weighted kappa 0.73, (95% confidence interval: 0.38–1.08), and 0.65 (95% confidence interval: 0.30–1.00)]. The agreement between the originally determined clinical diagnosis by classical histopathology (graded by different random pathologists) and the diagnoses reassessed by the study pathologist, i.e., inter-observer agreement was excellent [weighted kappa 0.93 (95% confidence interval: 0.56–1.31)].

Figure 2 shows an illustrative comparison between histopathology slices and X-PCI data of a similar area of the same sample for each of the different rejection grades.

DISCUSSION

In this study, we have used, for the first time, X-PCI to image human samples from EMBs. We have described the similarities between the features typical for normal myocardium and different stages of rejection, as well as inferences for its diagnostic use, as compared with routine assessment by clinical histopathology. We have shown that X-PCI allows for non-destructive visualization (effective isotropic pixel size of 0.65 µm) of the entire EMB sample in full-volume, allowing for the 3D dataset to be analyzed in any direction, without the use of staining agents. We have demonstrated that it is feasible for an experienced pathologist to successfully assess X-PCI images even for ACR grades, and with substantial agreement with classical histopathology.

Routine clinical follow-up protocols use EMB for the assessment of ACR by classical histology since no other method of structural tissue analysis has been proven as clinically relevant in the care of HTx recipients. Electron microscopy (EM) has been used for research purposes in

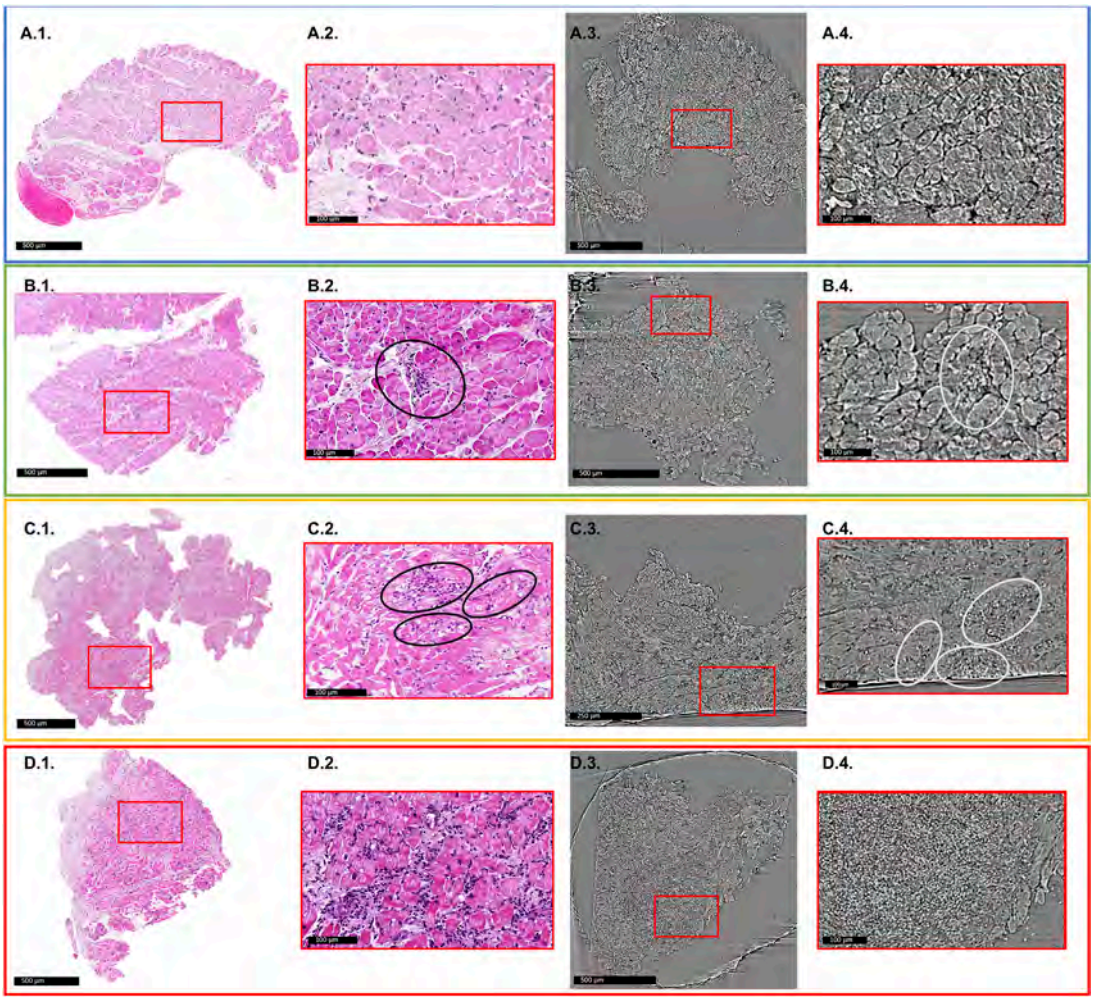

FIGURE 2 | Comparison of histology slices with X-PCI images of a similar region of the same sample for each rejection grade. Red rectangles indicate areas of enlarged images shown in red boxes. **(A1–A4)** 0R grade. No cellular infiltrate present. **(A1,A2)** light microscopy; **(A3,A4)** XPCI. **(B1–B4)** 1R grade. Mild rejection (1R) is defined as cellular infiltrate with 1 area of cardiomyocyte injury. **(B2)** Black ellipse indicates area of perivascular cellular infiltrate, while in the **(B4)** area indicated with white ellipse shows interstitial infiltrate that replaces aggregates of cardiomyocytes indicating injury. This sample also shows signs of fibrosis most probably due to previous rejection episodes in this patient. **(C1–C4)** 2R grade. Moderate rejection is defined with two or more areas of cellular infiltrate with associated cardiomyocyte injury. **(C2)** Black ellipses show several interstitial infiltrates invading myocardium and replacing cardiomyocyte aggregates. The same is indicated in image **(C4)** with white ellipses. **(D1–D4)** 3R grade. Severe rejection is defined with diffuse cellular infiltration with diffuse damage to cardiomyocytes. **(D2)** shows extensive cellular infiltrates and diffuse disruption of tissue architecture. **(D4)** Cellular infiltrates completely replace the cardiomyocytes, and only a diffuse accumulation of cells is seen in this image.

patients with allograft rejection, and it did not add to the clinical decision process for these patients (21, 22). Conversely, EM is routinely used in the analysis of kidney biopsies, both for establishing the etiology of disease, as well as in surveillance of renal graft rejection (23). Although high-resolution and high-contrast optical confocal laser scanning microscopy (CM) provides substantial contrast, achieves axial resolution of around 800 nm, and allows for acquisition of 3D image datasets, it requires special fluorescence stains that may be unevenly distributed and hinder quantification attempts (24). A pilot-study by White et al. described and imaged features of ACR in human EMB samples of HTx recipients using optical CM, but did not compare ACR grading versus classical histopathology (25).

The latest studies performed with X-PCI show some clear advantages for tissue imaging: it is a non-destructive technique providing macro and/or micrometer scale details with high spatial resolution. Recently, possibilities of X-PCI for obtaining a comprehensive and 3D representation of a rat heart as a whole (organ level analysis at 5.8 um), but also at the cellular level were reported (cardiomyocyte analysis at 0.65 µm pixel size) (7, 8). Furthermore, X-PCI has successfully been utilized for the imaging of fetal human hearts in a similar fashion (9). It has thus been proven as a suitable technique to capture the

TABLE 2 | Comparison of advantages and disadvantages of classical histopathology by light microscopy versus X-PCI virtual 2D/3D histopathology.

Classical histopathology by light microscopy	X-PCI virtual 2D/3D histopathology
Readily available in any pathology laboratory	Currently mainly limited to synchrotron facilities.
Destructive sample preparation	Sample remains intact.
Once already cut, the slice cannot be cut in different direction	No limitations in ways or number of times of reslicing.
Typically 10 or 20 slices	2,000+ slices (depending on the sample size).
Microtome slices of 3–4 um	Slice thickness in our setting 0.65 um.
Resolution limit around 0.2 um	Resolution limit ≈0.2 um. Pixel size was 0.65 um, but can be reduced (requires longer scan time).
Staining for basic structure differentiation	No contrast agents or stains used.
Cellular infiltrates may be characterized through specific staining	Cellular infiltrates identified, but can only be characterized from geometry or X-ray absorption coefficient resulting in grey level difference.
Vasculature assessment only in prepared slices	Vasculature assessment in the whole sample.

morphology of the cardiac tissue, such as the organization of cardiomyocytes, vasculature, and collagen matrix.

Cardiomyocytes, interstitial spaces, cellular infiltrates, vascular structures, or adipocytes can easily be identified on X-PCI images (**Figures 1**, **2**; **Supplementary Figure S3**). In addition, an experienced pathologist could discern the presence and extent of cellular infiltrate and its influence on tissue architecture, which is the basis of the ACR grading process (**Figure 2**). Propagation-based X-PCI images arranged according to recommendations for histopathological analysis of H&E sections (2D X-PCI virtual histology), yielded similar levels of agreement in ACR grading as was the case with 3D X-PCI virtual histology, in comparison to classical histopathology. Although we showed its potential in revealing features of ACR, it is important to understand that currently X-PCI cannot discern antibody mediated rejection (AMR), besides identification of cardiomyocyte destruction or architecture distortion.

Major benefit of 3D X-PCI virtual histology is its feature of providing detailed examination of the whole sample by producing dataset of several thousands of images.

In this study, the pathologist was provided with only one plane of the 3D structure to analyze the samples, but using simple open source imaging software such as Fiji (16, 17), one can virtually generate image slices in any desired direction, which could lead to an even better comprehension of the structural relations within the sample (**Supplementary Figure S3**; **Supplementary Video S1**).

The overall concordance between pathologists assessing ACR has been shown to be relatively low. One of the largest studies showed total agreement in ACR grading of only around 70%, and it was mainly based on concordance on samples with no signs of rejection (26). Indeed, the best agreement between methods in our study was achieved in ruling out ACR that required treatment. Moreover, a multicenter study on 827 EMBs showed the greatest variability in agreement between pathologists in grade 2 of the 1990 ISHLT grading system, having great importance since grade 2 and 3A (1R and 2R according to the 2004. ISHLT grading system) differ in treatment approach (26, 27).

Due to the obvious problems with the methodology, initiatives are under way to define ACR not solely on histopathology, but to combine it with clinical and laboratory parameters, or to completely change the paradigm by moving to the concept of "liquid biopsies" using the cell free DNA approach (4). However, classical histopathology remains the routine method worldwide. Besides integration of structural with clinical or laboratory data, one of the proposed directions to advance the area is switching to histopathology image digitalization, which is inherent to X-PCI.

LIMITATIONS

At this point, X-PCI is a research tool confined to highly specialized synchrotron facilities, capable to image EMB samples at the required resolution and in a high throughput way. Nevertheless, major engineering advances are being undertaken to miniaturize the equipment in the translation of synchrotron techniques into laboratory-compatible setups, with the aim of their integration in the clinical setting (28–31). Therefore, parallel studies of the clinical feasibility and utility of this technique such as the one we present are needed in order to move forward this rapidly evolving field in an interdisciplinary manner.

The process of EMB grading with X-PCI at the synchrotron beam setup used in this study was dedicated to the identification of cellular infiltrates, currently not focusing on the identification of AMR, nor further analysis of cellular organelles such as nuclei. Furthermore, only one experienced pathologist was trained to grade the X-PCI samples.

This study has the limitation of its observational nature and small study sample, limiting its generalizibility, however still including the same ratio of high-grade to low-grade rejection samples as seen in everyday clinical practice. The study sample represents significantly more males, a trend that is also seen in the demographics of HTx recipients in large registries. However, based on these pivotal data, a full validation of the method additionally strengthened by a prospective design with larger study sample, and more observers, is foreseen in the future.

CONCLUSION

We have demonstrated that X-ray based imaging, specifically exploiting the use of phase-contrast imaging, allows to evaluate

ACR from EMBs. We compared X-PCI ACR grading with classical histopathology, showing a substantial agreement between the two methods. The main advantages of X-PCI include: 1) visualization of the whole EMB in a full-volume without need for slice selection, 2) a 3D dataset that can be analyzed in any direction, while 3) being a non-destructive method, not requiring any staining nor slicing (**Table 2**).

While the technique is currently mainly limited to synchrotrons, we do consider the study relevant for both a wider transplant and pathology community, since it introduces a non-destructive three-dimensional imaging method of tissue samples that could be utilized beyond heart biopsies. Ongoing developments should soon allow its transfer to hospital facilities, presenting X-PCI as a potential aid to routine clinical workflows.

We particularly highlight the potential of future translation of X-PCI from previous animal studies to a clinical application, thus setting the direction for future research to better understand the pathophysiological processes in the cardiac graft and its failure.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the institutional Ethics review board (Approval of the Ethics Committee of the University Hospital Centre Zagreb, Croatia; Class: 8.1-17/137-2, No: 02/21 AG; Approval of the Ethics Committee of the University of Zagreb School of Medicine). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

IP, II, HD, PG-C, MS, BB, AB, DM, HG, and MC designed the study and planned the experiments; IP, II, HD, PG-C, BB, AB, and MC performed the imaging experiments, reconstructed the images; IP collected the patient data and performed the statistical analysis; II performed histological and X-PCI analysis and interpretation; BS and HJ performed endomyocardial biopsies and tissue collection; All authors critically revised the manuscript and gave their final approval of the content.

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CONFLICT OF INTEREST

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

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11046/full#supplementary-material

REFERENCES

1. McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Bohm M, et al. 2021 ESC Guidelines for the Diagnosis and Treatment of Acute and Chronic Heart Failure. *Eur Heart J* (2021) 42:3599–726. doi:10.1093/eurheartj/ehab368
2. Khush KK, Cherikh WS, Chambers DC, Harhay MO, Hayes D, Hsich E, et al. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: Thirty-Sixth Adult Heart Transplantation Report- 2019; Focus Theme: Donor and Recipient Size Match. *J Heart Lung Transpl* (2019) 38:1056–66. doi:10.1016/j.healun.2019.08.004
3. Olymbios M, Kwiecinski J, Berman DS, Kobashigawa JA. Imaging in Heart Transplant Patients. *JACC Cardiovasc Imag* (2018) 11:1514–30. doi:10.1016/j.jcmg.2018.06.019
4. Agbor-Enoh S, Shah P, Tunc I, Hsu S, Russell S, Feller E, et al. Cell-Free DNA to Detect Heart Allograft Acute Rejection. *Circulation* (2021) 143:1184–97. doi:10.1161/CIRCULATIONAHA.120.049098
5. Stewart S, Winters GL, Fishbein MC, Tazelaar HD, Kobashigawa J, Abrams J, et al. Revision of the 1990 Working Formulation for the Standardization of Nomenclature in the Diagnosis of Heart Rejection. *J Heart Lung Transpl* (2005) 24:1710–20. doi:10.1016/j.healun.2005.03.019
6. Gonzalez-Tendero A, Zhang C, Balicevic V, Cardenes R, Loncaric S, Butakoff C, et al. Whole Heart Detailed and Quantitative Anatomy, Myofibre Structure and Vasculature from X-ray Phase-Contrast Synchrotron Radiation-Based Micro Computed Tomography. *Eur Heart J Cardiovasc Imaging* (2017) 18:732–41. doi:10.1093/ehjci/jew314
7. Dejea H, Garcia-Canadilla P, Cook AC, Guasch E, Zamora M, Crispi F, et al. Comprehensive Analysis of Animal Models of Cardiovascular Disease using Multiscale X-ray Phase Contrast Tomography. *Sci Rep* (2019) 9:6996. doi:10.1038/s41598-019-43407-z
8. Planinc I, Garcia-Canadilla P, Dejea H, Ilic I, Guasch E, Zamora M, et al. Comprehensive Assessment of Myocardial Remodeling in Ischemic Heart Disease by Synchrotron Propagation Based X-ray Phase Contrast Imaging. *Sci Rep* (2021) 11:14020. doi:10.1038/s41598-021-93054-6
9. Garcia-Canadilla P, Dejea H, Bonnin A, Balicevic V, Loncaric S, Zhang C, et al. Complex Congenital Heart Disease Associated with Disordered Myocardial Architecture in a Midtrimester Human Fetus. *Circ Cardiovasc Imaging* (2018) 11:e007753. doi:10.1161/CIRCIMAGING.118.007753
10. Garcia-Canadilla P, Cook AC, Mohun TJ, Oji O, Schlossarek S, Carrier L, et al. Myoarchitectural Disarray of Hypertrophic Cardiomyopathy Begins Pre-birth. *J Anat* (2019) 235:962–76. doi:10.1111/joa.13058
11. Baličević V, Lončarić S, Cardenes R, Gonzelez-Tendero A, Bruno P, Crispi F, et al. Assessment of Myofiber Orientation in High Resolution Phase-Contrast CT Images. In: *Functional Imaging and Modeling of the Heart*. Maastricht, The Netherlands: Springer International Publishing (2015). p. 111–9.
12. Dejea H, Bonnin A, Cook AC, Garcia-Canadilla P. Cardiac Multi-Scale Investigation of the Right and Left Ventricle *Ex Vivo*: a Review. *Cardiovasc Diagn Ther* (2020) 10:1701–17. doi:10.21037/cdt-20-269
13. Dejea H, Garcia-Canadilla P, Stampanoni M, Zamora M, Crispi F, Bijnens B, et al. Microstructural Analysis of Cardiac Endomyocardial Biopsies with Synchrotron Radiation-Based X-Ray Phase Contrast Imaging. In: M. Pop, G. Wright, editors. *Functional Imaging and Modelling of the Heart*, 10263. Cham: Springer (2017). FIMH 2017. Lecture Notes in Computer Science.
14. Crespo Leiro MG, Zuckermann A, Bara C, Mohacsi P, Schulz U, Boyle A, et al. Concordance Among Pathologists in the Second Cardiac Allograft Rejection Gene Expression Observational Study (CARGO II). *Transplantation* (2012) 94:1172–7. doi:10.1097/TP.0b013e31826e19e2
15. Marone F, Stampanoni M. Regridding Reconstruction Algorithm for Real-Time Tomographic Imaging. *J Synchrotron Radiat* (2012) 19:1029–37. doi:10.1107/S0909049512032864
16. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, et al. Fiji: an Open-Source Platform for Biological-Image Analysis. *Nat Methods* (2012) 9:676–82. doi:10.1038/nmeth.2019
17. Schindelin J, Rueden CT, Hiner MC, Eliceiri KW. The ImageJ Ecosystem: an Open Platform for Biomedical Image Analysis. *Mol Reprod Dev* (2015) 82:518–29. doi:10.1002/mrd.22489
18. Billingham ME, Cary NR, Hammond ME, Kemnitz J, Marboe C, McCallister HA, et al. A Working Formulation for the Standardization of Nomenclature in the Diagnosis of Heart and Lung Rejection: Heart Rejection Study Group. The International Society for Heart Transplantation. *J Heart Transpl* (1990) 9:587–93.
19. Watson PF, Petrie A. Method Agreement Analysis: a Review of Correct Methodology. *Theriogenology* (2010) 73:1167–79. doi:10.1016/j.theriogenology.2010.01.003
20. McHugh ML. Interrater Reliability: the Kappa Statistic. *Biochem Med* (2012) 22:276–82. doi:10.11613/bm.2012.031
21. Revelo M, Miller DV, Stehlik J, Hammond E, Kfoury B. Can Electron Microscopy of Endomyocardial Biopsies Define Chronic AMR? *J Card Fail* (2016) 22(8):S46. doi:10.1016/j.cardfail.2016.06.139
22. Mudhar HS, Wagner BE, Suvarna SK. Electron Microscopy of Myocardial Tissue. A Nine Year Review. *J Clin Pathol* (2001) 54:321–5. doi:10.1136/jcp.54.4.321
23. De Kort H, Moran L, Roufosse C. The Role of Electron Microscopy in Renal Allograft Biopsy Evaluation. *Curr Opin Organ Transpl* (2015) 20:333–42. doi:10.1097/MOT.0000000000000183
24. Crossman DJ, Ruygrok PN, Feng Hou Y, Soeller C. Next-generation Endomyocardial Biopsy: the Potential of Confocal and Super-resolution Microscopy. *Heart Fail Rev* (2015) 20:203–14. doi:10.1007/s10741-014-9455-6
25. White R, Crossman DJ, Isaacson M, Gibbs H, Ruygrok PN. Confocal Scanning Microscopy in Assessment of Cardiac Allograft Rejection-a Pilot Study. *Transpl Proc* (2015) 47:2513–6. doi:10.1016/j.transproceed.2015.08.010
26. Marboe CC, Billingham M, Eisen H, Deng MC, Baron H, Mehra M, et al. Nodular Endocardial Infiltrates (Quilty Lesions) Cause Significant Variability in Diagnosis of ISHLT Grade 2 and 3A Rejection in Cardiac Allograft Recipients. *J Heart Lung Transpl* (2005) 24:S219–26. doi:10.1016/j.healun.2005.04.001
27. Duong Van Huyen MF, Fedrigo M, Fishbein GA, Leone O, Neil D, Marboe C, et al. The XVth Banff Conference on Allograft Pathology the Banff Workshop Heart Report: Improving the Diagnostic Yield from Endomyocardial Biopsies and Quilty Effect Revisited. *Am J Transpl* (2020) 00:3308–18. doi:10.1111/ajt.16083
28. Viermetz M, Birnbacher L, Willner M, Achterhold K, Pfeiffer F, Herzen J. High Resolution Laboratory Grating-Based X-ray Phase-Contrast CT. *Sci Rep* (2018) 8:15884. doi:10.1038/s41598-018-33997-5
29. Vila-Comamala J, Romano L, Jefimovs K, Dejea H, Bonnin A, Cook AC, et al. High Sensitivity X-ray Phase Contrast Imaging by Laboratory Grating-Based Interferometry at High Talbot Order Geometry. *Opt Express* (2021) 29:2049–64. doi:10.1364/OE.414174
30. Otendal M, Tuohimaa VU, Hertz HM. A 9keV Electron-Impact Liquid-Gallium-Jet X-ray Source. *Rev Sci Instrum* (2008) 79:016102. doi:10.1063/1.2833838
31. Eggl E, Schleede S, Bech M, Achterhold K, Loewen R, Ruth RD, et al. X-ray Phase-Contrast Tomography with a Compact Laser-Driven Synchrotron Source. *Proc Natl Acad Sci USA* (2015) 112:5567–72. doi:10.1073/pnas.1500938112

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LETTER TO THE EDITOR
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Novel Therapeutic Strategies for Dyslipidemia: First Report of Inclisiran Therapy in a Kidney Transplanted Patient

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Keywords: kidney transplantation, renal transplantation, dyslipidemia, inclisiran, LDL

Dear Editors,

Kidney transplant recipients are high-risk cardiovascular patients and cardiovascular events are the most common cause of death after kidney transplantation [1]. Management of cardiovascular risk factors, which includes adequate lowering of LDL cholesterol (LDLC) to the recommended levels, is difficult to achieve after renal transplantation or is not implemented consistently often enough [2]. This is partly because immunosuppressive therapies such as tacrolimus, prednisolone, or everolimus themselves have adverse effects on lipid levels and partly because there are incompatibilities and interactions between statins and immunosuppressive drugs i.e., ciclosporin A that limit adequate statin therapy and ezetimibe administration [3, 4].

Therefore, novel and highly efficient therapies such as inclisiran (SmPC Leqvio, Novartis, Germany) may contribute to better LDLC management in this patient population. Inclisiran is a small interference-RNA against protein convertase subtilisin/kexin type 9 (PCSK9), preventing LDL receptor degradation [5]. It is injected subcutaneously at month 0 and 3 and every 6 months thereafter and results in ~50% LDLC reduction [6]. Inclisiran was first approved in the European Union in December 2020 for the treatment of primary hypercholesterolemia or mixed dyslipidemia in combination with a statin or other lipid-lowering therapies in patients who do not achieve LDLC goals with the maximum tolerable statin dose, or alone or in combination with other lipid-lowering therapies in patients with statin intolerance or for whom a statin is contraindicated.

To our knowledge, there is no data about the use of inclisiran in kidney transplant recipients yet. Therefore, we present for the first time a case of a patient treated with inclisiran after renal transplantation.

Our 79-year-old male patient received a deceased donor kidney transplant 12 years prior to the first inclisiran administration. End-stage renal disease was caused by right-sided nephrectomy due to renal cell carcinoma and unspecified nephrosclerosis of the left kidney. The immunosuppressive regimen at the time reported consisted of everolimus and prednisolone, due to a history of CMV disease. Serum creatinine was 2.44 mg/dL with an estimated GFR of 24 mL/min/m^2 (CKD4A2T, CKD EPI). The patient has a distinct cardiovascular risk profile. In addition to male sex and older age, he suffers from metabolic syndrome (mixed dyslipidemia, arterial hypertension, post-transplant diabetes mellitus, BMI of 25 kg/m^2) with hyperuricemia and has a history of smoking (approximately 13 pack years). This has led to progressive peripheral artery disease (Fontaine IIB) and coronary artery disease.

Serum lipids were inadequately controlled during therapy with atorvastatin 80 mg and ezetimibe 10 mg daily (total cholesterol 5.18 mmol/L, LDLC 2.46 mmol/L, HDLC 2.12 mmol/L

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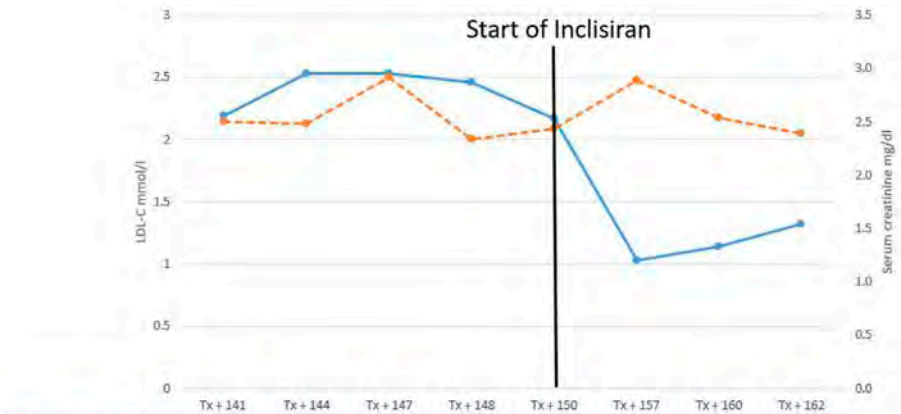

	Tx + 141	Tx + 144	Tx + 147	Tx + 148	Tx + 150	Tx + 157	Tx + 160	Tx + 162
Creatinine mg/dl	2.5	2.48	2.92	2.34	2.44	2.89	2.54	2.39
LDL-C mmol/l	2.19	2.53	2.53	2.46	2.17	1.03	1.14	1.32
Creatinine-kinase U/l NR <174 U/l	76	52	93	140	68	89	69	52

FIGURE 1 | Serum-creatinine (dotted line) and LDL-cholesterol (line) before and after introduction of inclisiran therapy. Values of creatinine, LDL-cholesterol and creatinine-kinase are shown in the table. NR, normal range.

and triglycerides 1.79 mmol/L). For our very high-risk patient, the 2019 ESC/EAS guidelines on the treatment of dyslipidaemia recommend a target LDLC of < 1.4 mmol/L and an LDLC reduction >50% from baseline values [7]. Therapeutic options were discussed with the patient and the patient opted for inclisiran therapy for optimal therapy adherence.

Inclisiran (284 mg i.m.) was administered at 0 and 3 months and then every 6 months while continuing atorvastatin and ezetimib. LDLC was significantly lowered to 1.03, 1.14, and 1.32 mmol/L after 6, 9 and 12 months, respectively (**Figure 1**).

During the 1-year follow-up, renal function was stable after 12 months (serum creatinine 2.39 mg/dL, eGFR 25 mL/min/m^2; **Figure 1**). We did not observe relevant side effects, or increase in proteinuria, creatinine-kinase or change in everolimus level.

The case presented demonstrates that inclisiran can be safely and conveniently administered with a profound effect on LDLC levels after renal transplantation. Further research needs to be conducted to demonstrate efficacy on cardiovascular death in transplanted patients.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: clinical data. Requests to access these datasets should be directed to the corresponding author.

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. 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

LU and SR wrote the manuscript. LU, SR, and KG contributed data. UJ, HP, and KG revised the manuscript.

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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.

REFERENCES

1. Awan AA, Niu J, Pan JS, Erickson KF, Mandayam S, Winkelmayer WC, et al. Trends in the Causes of Death Among Kidney Transplant Recipients in the United States (1996-2014). *Am J Nephrol* (2018) 48(6):472–81. doi:10.1159/000495081
2. Thölking G, Schulte C, Jehn U, Schütte-Nütgen K, Pavenstädt H, Suwelack B, et al. The Tacrolimus Metabolism Rate and Dyslipidemia after Kidney Transplantation. *J Clin Med* (2021) 10(14):3066. doi:10.3390/jcm10143066

3. Ichimaru N, Yamanaka K, Kato T, Kakuta Y, Abe T, Imamura R, et al. Risk Factors and Incidence for Lipid Abnormalities in Kidney Transplant Patients. *Transpl Proc* (2015) 47(3):672–4. doi:10.1016/j.transproceed.2014.12.029
4. Simonson SG, Raza A, Martin PD, Mitchell PD, Jarcho JA, Brown CD, et al. Rosuvastatin Pharmacokinetics in Heart Transplant Recipients Administered an Antirejection Regimen Including Cyclosporine. *Clin Pharmacol Ther* (2004) 76(2):167–77. doi:10.1016/j.clpt.2004.03.010
5. Lambert G, Charlton F, Rye KA, Piper DE. Molecular Basis of PCSK9 Function. *Atherosclerosis* (2009) 203(1):1–7. doi:10.1016/j.atherosclerosis.2008.06.010
6. Wright RS, Ray KK, Raal FJ, Kallend DG, Jaros M, Koenig W, et al. Pooled Patient-Level Analysis of Inclisiran Trials in Patients with Familial Hypercholesterolemia or Atherosclerosis. *J Am Coll Cardiol* (2021) 77(9): 1182–93. doi:10.1016/j.jacc.2020.12.058
7. Authors/Task Force MembersESC Committee for Practice Guidelines CPGESC National Cardiac Societies. 2019 ESC/EAS Guidelines for the Management of Dyslipidaemias: Lipid Modification to Reduce Cardiovascular Risk. *Atherosclerosis* (2019) 290:140–205. doi:10.1016/j.atherosclerosis.2019.08.014

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LETTER TO THE EDITOR
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Cytokine and Chemokine Secretome and Risk of CMV Infection Following Discontinuation of Valganciclovir Prophylaxis

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Dear Editors,

The advent of interferon (IFN)-γ release assays (IGRAs) to quantify cytomegalovirus (CMV)-specific cell-mediated immunity (CMV-IMC) has represented a major step in the effort to individualize preventive strategies after kidney transplantation (KT). We have recently shown, however, that the QuantiFERON®-CMV (QTF-CMV) assay at the time of discontinuation of antiviral prophylaxis exhibits suboptimal accuracy (sensitivity of 77.4%, specificity of 34.3%, positive [PPV] and negative predictive values [NPV] of 64.1% and 50.0%, respectively) to predict protection among KT recipients that had received induction therapy with antithymocyte globulin (ATG) (1). The assessment of IFN-γ production by IGRAs is aimed at recapitulating the Th1-polarized CMV-IMC. Nevertheless, $CD4^+$ T-cell functions are also mediated through other lymphocyte subsets (such as Th2 or Th17), each of which secretes a distinct cytokine profile. A comprehensive profiling of the cytokine and chemokine responses upon CMV-specific stimulation may improve the performance of the QTF-CMV assay (2).

Samples collected from patients recruited in a previous study were used for the present analysis (1). Consecutive CMV-seropositive KT recipients receiving ATG induction at our institution between April 2015 and June 2018 underwent CMV-CMI monitoring by the QTF-CMV assay at months 2, 3, 4 and 5. A 3-month course of valganciclovir prophylaxis was scheduled in all of them. The QTF-CMV assay was performed according to the manufacturer's instructions. We selected those samples obtained at the time of discontinuation of prophylaxis (±3 weeks). The following 27 cytokines and chemokines were measured in stimulated ("CMV tube") and unstimulated ("nil tube") plasma samples by means of analyte-specific capture beads coated with target-specific capture antibodies in a Luminex® 200 instrument: IFN-γ, interleukin [IL]-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-17A, IL-18, IL-21, IL-22, IL-23, IL-27, tumor necrosis factor [TNF]-α, eotaxin (CCL11), GRO α (CXCL1), IP-10 (CXCL10), SDF-1α (CXCL12), MCP-1 (CCL2), MCP-2 (CCL8), MIP-1α (CCL3), MIP-1β (CCL4) and RANTES (CCL5) (Th1/Th2 Cytokine & Chemokine 20-Plex ProcartaPlex™ Panel 1, Th9/Th17/Th22 Cytokine 7-Plex ProcartaPlex™ Panel, and MCP-2 ProcartaPlex™ Simplex kits, all from Thermo Fisher Scientific, Waltham, MA). The study outcome was the occurrence of clinically significant (DNAemia >1,000 IU/mL) CMV infection (asymptomatic viremia or clinical disease) from the discontinuation of prophylaxis to post-transplant month 12. Further details are provided in Supplementary Methods.

We included 78 KT recipients (**Supplementary Table S1**, **Supplementary Results**), 13 of which developed clinically significant CMV infection following discontinuation of valganciclovir

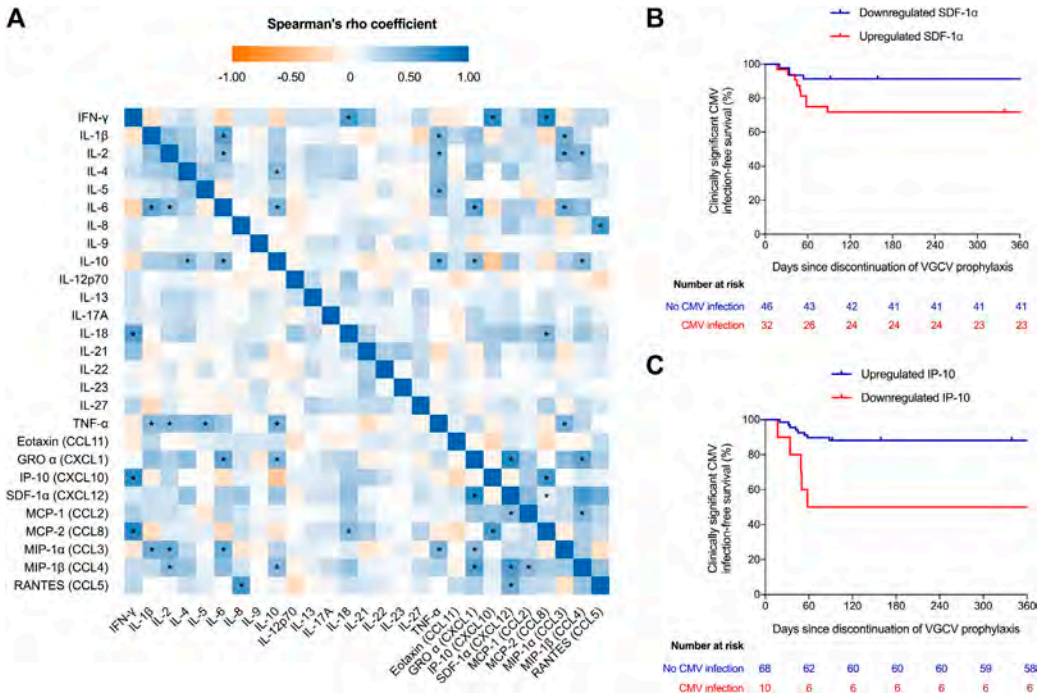

FIGURE 1 | (A) Heatmap of the correlation between analyzed cytokines and chemokines. Values obtained in unstimulated plasma samples were subtracted from CMV peptide-stimulated samples ("CMV tube" minus "nil tube"). **(B)** Clinically significant (DNAemia >1,000 IU/mL) CMV infection-free survival after the discontinuation of valganciclovir prophylaxis according to expression (upregulation or downregulation) of IP-10 and **(C)** SDF-1α upon whole blood stimulation with CMV-specific viral peptides (log-rank *p*-values = 0.001 and 0.027, respectively). Three patients were censored at days 92, 159 and 339 because of graft loss (rejection), loss to follow-up and death (gastric adenocarcinoma), respectively. The criteria for classifying a given recipient in the upregulated or regulated categories was that the increment (Δ) in plasma IP-10 or SDF-1α levels from unstimulated baseline samples to CMV peptide-stimulated samples ("CMV tube" minus "nil tube") was positive (Δ >0) or zero or negative (Δ ≤0), respectively. * *p*-value < 0.00007 (Bonferroni-corrected threshold for statistical significance). CMV, cytomegalovirus; GRO α, growth-related onconge α; IFN, interferon; IL, interleukin; IP-10, interferon-γ-inducible protein 10; MCP, monocyte chemoattractant protein; MIP-1, macrophage inflammatory protein 1; RANTES, regulated upon activation, normal T-cell expressed and presumably secreted; SDF-1α, stromal cell-derived factor 1α; TNF, tumor necrosis factor-α; VGCV, valganciclovir.

prophylaxis (12-month incidence: 17.9%). The median interval between the timing of sampling and the end of prophylaxis, on one hand, and the occurrence of CMV infection, on the other hand, were 35.0 (interquartile range [IQR]: 24.0–70.0) and 45.0 (IQR: 33.0–56.0) days, respectively. The analysis of the blood secretome after CMV-specific stimulation revealed detectable levels in the majority of patients, since only 5 cytokines (IL-5, IL-9, IL-17A, IL-22, IL-23 and IL-27) were detected in less than 50% of specimens. The comparison of cytokine/chemokine expression at baseline and following stimulation ("CMV tube" minus "nil tube") showed a significant increase (Δ >0) of IFN-γ, IL-18, IP-10 and MCP-2, whereas IL-1β, IL-6, IL-10, TNF-α and MIP-1α were downregulated (Δ ≤0) (**Supplementary Table S2**). The heatmap of cytokines/chemokines correlations—once unstimulated samples were subtracted from the CMV peptide-stimulated samples—is shown in **Figure 1A**. The highest correlations were found between IFN-γ and MCP-2 and IP-10 levels (Spearman's rho coefficients = 0.766 and 0.726, respectively; *p*-values < 0.00001).

We analyzed the blood secretome according to the occurrence of clinically significant CMV infection after cessation of prophylaxis. Upregulation of IP-10 and IL-2 and downregulation of SDF-1α were significantly associated with a lower risk of this outcome, with the strongest associations observed for IP-10 and SDF-1α (**Supplementary Table S3**). The 12-month clinically significant CMV infection-free survival was higher in recipients in which IP-10 levels increased (hazard ratio [HR]: 0.188; 95% confidence interval [CI]: 0.061–0.577; *p*-value = 0.003) (**Figure 1B**) and SDF-1α levels decreased (HR: 0.288; 95% CI: 0.09–0.937; *p*-value = 0.039) following stimulation (**Figure 1C**). Older patient age (54.6 ± 11.5 versus 43.9 ± 9.1 years; *p*-value = 0.006) (**Supplementary Table S4**) and lower number of ATG doses (median: 4.7 [IQR: 1.5–6.6] versus 5.6 [IQR: 1.7–6.9] doses; *p*-value = 0.016) (**Supplementary Table S5**) were associated with IP-10 upregulation and SDF-1α downregulation, respectively. By applying IP-10 upregulation as diagnostic criteria, the sensitivity, specificity, PPV and NPV to predict effective protection from clinically significant CMV infection were 92.3%, 38.5%, 88.2% and 50.0%, respectively.

We have characterized the cytokine and chemokine secretome in whole blood samples from seropositive KT

recipients stimulated with a pool of CMV peptides contained in the commercial QTF-CMV assay. Not surprisingly, IL-18, IP-10 and MCP-2 levels were found to be upregulated and strongly correlate with IFN-γ. Indeed, IL-18 is a potent inducer of IFN-γ production (3), whereas IP-10 expression is activated by the IFN-γ-signaling in several cell types. Of note, IP-10 circulates at much higher levels than IFN-γ and plays a role in the generation and function of effector T-cells (4). Therefore, it has been proposed that the detection of IP-10 may serve as a convenient alternative to IGRA for the diagnosis of latent tuberculosis infection (5, 6).

In our experience, the demonstration of IP-10 upregulation in response to CMV peptides yielded a better diagnostic accuracy in terms of sensitivity and PPV to predict immune protection than the cut-off for IFN-γ proposed by the manufacturer (≥0.2 IU/mL) in the QTF-CMV assay (1). Since specificity and NPV improved only marginally, it is likely that pathways different from the IFN-γ/IP-10 axis may be involved in conferring protection against CMV. In addition, a relatively high proportion of patients were apparently protected (only 17.9% developed the outcome), which lowers the NPV. By using a similar approach, Lisboa et al. also found that MCP-2 and IP-10 were the cytokines/chemokines showing the highest expression increase upon CMV peptide stimulation, with close correlation with IFN-γ production. These authors reported an excellent discriminatory capacity to predict spontaneous CMV viremia clearance for both chemokines (2). Differences in analyzed outcomes and CMV serostatus may explain the discordance regarding the predictive role of MCP-2 between the study by Lisboa et al. (2) and ours.

In addition, we report the novel observation that SDF-1α downregulation is predictive of protection against CMV. Often considered a homeostatic chemokine, inflammatory activities have been attributed to SDF-1 (CXCL12) (7). Polymorphisms in the *CXCL12* gene are associated with the occurrence of CMV reactivation after allogeneic hematopoietic stem cell transplantation (8), whereas elevated plasma levels identify poor immune reconstitution in HIV patients (9). SDF1 mainly signals *via* the CXCR4 receptor, and it has been shown that CMV enhances SDF-1/CXCR4 signaling during infection through the product of the *UL111A* gene (which encodes a viral ortholog of human IL-10) (10). It may be hypothesized that the downregulation of SDF-1α expression would partially abrogate this immune evasion mechanism, leading to a decreased host susceptibility to CMV replication.

Due to the small sample size of our study, the present results should be confirmed in a larger prospective cohort, as well as its potential application to the clinical decision-making process.

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 Clinical Research Ethics Committee of University Hospital "12 de Octubre" (reference 14/245). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MF-R designed research; PP, TR-M, and NR performed laboratory procedures; TR-M obtained patient samples; MF-R, IR-G, and AA collected clinical data; MF-R analyzed data and wrote the letter; and NR, IR-G, AA, and JMA contributed to the concept of the study and critically reviewed the manuscript. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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CONFLICT OF INTEREST

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

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.10979/full#supplementary-material

REFERENCES

1. Fernández-Ruiz M, Rodríguez-Goncer I, Parra P, Ruiz-Merlo T, Corbella L, López-Medrano F, et al. Monitoring of CMV-specific Cell-Mediated Immunity with a Commercial ELISA-Based Interferon-γ Release Assay in Kidney Transplant Recipients Treated with Antithymocyte Globulin. *Am J Transpl* (2020) 20:2070–80. doi:10.1111/ajt.15793
2. Lisboa LF, Egli A, Fairbanks J, O'Shea D, Manuel O, Husain S, et al. CCL8 and the Immune Control of Cytomegalovirus in Organ Transplant Recipients. *Am J Transpl* (2015) 15:1882–92. doi:10.1111/ajt.13207
3. Dinarello CA, Novick D, Puren AJ, Fantuzzi G, Shapiro L, Mühl H, et al. Overview of Interleukin-18: More Than an Interferon-Gamma Inducing Factor. *J Leukoc Biol* (1998) 63:658–64. doi:10.1002/jlb.63.6.658
4. Liu M, Guo S, Hibbert JM, Jain V, Singh N, Wilson NO, et al. CXCL10/IP-10 in Infectious Diseases Pathogenesis and Potential Therapeutic Implications. *Cytokine Growth Factor Rev* (2011) 22:121–30. doi:10.1016/j.cytogfr.2011.06.001
5. Ruhwald M, Bodmer T, Maier C, Jepsen M, Haaland MB, Eugen-Olsen J, et al. Evaluating the Potential of IP-10 and MCP-2 as Biomarkers for the Diagnosis of Tuberculosis. *Eur Respir J* (2008) 32:1607–15. doi:10.1183/09031936.00055508
6. Qiu X, Tang Y, Yue Y, Zeng Y, Li W, Qu Y, et al. Accuracy of Interferon-γ-Induced Protein 10 for Diagnosing Latent Tuberculosis Infection: a Systematic Review and Meta-Analysis. *Clin Microbiol Infect* (2019) 25:667–72. doi:10.1016/j.cmi.2018.12.006
7. Janssens R, Struyf S, Proost P. Pathological Roles of the Homeostatic Chemokine CXCL12. *Cytokine Growth Factor Rev* (2018) 44:51–68. doi:10.1016/j.cytogfr.2018.10.004
8. Vallejo M, Muñiz P, Kwon M, Solán L, Bailén R, Carbonell D, et al. Risk Prediction of CMV Reactivation after Allogeneic Stem Cell Transplantation Using Five Non-HLA Immunogenetic Polymorphisms. *Ann Hematol* (2022) 101:1567–76. doi:10.1007/s00277-022-04841-8
9. Yeregui E, Viladés C, Domingo P, Ceausu A, Pacheco YM, Veloso S, et al. High Circulating SDF-1and MCP-1 Levels and Genetic Variations in CXCL12, CCL2 and CCR5: Prognostic Signature of Immune Recovery Status in Treated HIV-Positive Patients. *EBioMedicine* (2020) 62:103077. doi:10.1016/j.ebiom.2020.103077
10. Tu CC, O'Connor CM, Spencer JV. Identification of a Novel Signaling Complex Containing Host Chemokine Receptor CXCR4, Interleukin-10 Receptor, and Human Cytomegalovirus US27. *Virology* (2020) 548:49–58. doi:10.1016/j.virol.2020.06.006

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